Department of Civil Engineering

School of Engineering

Investigation of Biofilms Contribution to Chloramine Decay in Pilot Scale System

Rekha Aryal Adhikari

This thesis is presented for the Degree of

Master of Engineering

of

Curtin University

November 2011

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: …….

Date: …….

ABSTRACT

Many drinking water distribution systems are using chloramine as a secondary disinfectant instead of chlorine because of its greater stability. However, the use of chloramine as a disinfectant has some drawbacks. The main demerits are auto-decomposition of chloramine, direct chemical reaction with water born constituents, sudden onset of nitrification and subsequent acceleration of chloramine decay. Traditionally, nitrification indicators such as ammonia, nitrite and nitrate are monitored to understand the status of a system. This monitoring does not provide information on what controls the chloramine decay in the system.

In long distribution system, water can spend greater proportion of time. As a result, microbiological or chemical reactions take place in pipes that can be broadly categorised in terms of biofilms, sediments and bulk water. The chloramine decay resulted from the bulk water, biofilms and sediments in full scale system can be quantified by using microbiological factor (F_m) and a reservoir acceleration factor (F_{Ra}) method. Chemical and microbial decay in bulk water can be separated by F_m methods. Similarly, impact of biofilms in combination with sediments on chloramine decay can be quantified from (F_{Ra}) method. However, impact of biofilms and sediments on chloramine decay in individual still remained unexplored. Therefore, this research aims to understand the contribution of biofilms and sediments separately on chloramine decay in drinking water distribution system by studying a pilot scale system.

A pilot scale reactor consisting of a series of HDPE tanks fed with chloramine containing water was studied in detail. The reactor was run to create different nitrifying and chloramine containing conditions. To understand the impact of biofilm, it was grown on PVC and HDPE coupons (cylindrical pieces of pipes) over a long period (115 days) in continuous systems. Then, the samples were taken to conduct batch test. Chloramine decay was monitored to understand the impact of biofilms under various conditions such as different age, grown under different nitrifying conditions, different materials and subjected them to different temperature and different initial chloramine concentration during the batch test.

Experimental results indicated that impact of biofilms grown under severely nitrifying condition is significantly higher than the mildly nitrifying condition although further investigation is needed prior to reach concrete conclusion. However, the impact of biofilms on chloramine decay could not be changed with different topped up chloramine residuals.

The age of biofilms showed a prominent effect on chloramine decay. It was also found that there is a cycle on formation of biofilms and goes through the process of formation and sloughing consequently affecting the chloramine decay. Further, the cycle was found to be dependent on the material used. Investigation on PVC coupons showed shorter cycle compared to HDPE coupons. Similarly, effect of biofilms also increased with increase in temperature within the tested range. These experimental evidence showed the substantial effect of biofilms on chloramine throughout the distribution system while investigating in terms of chloramine decay. However, further study is recommended in order to understand the mechanism behind the impact of biofilms on chloramine decay by quantifying the carbohydrate and protein present in it and by repeating the experiments.

Keywords: chloramine, bulk water, biofilms, sediment, nitrification

ACKNOWLEDGMENTS

I am heartily thankful to my supervisor A/Prof. Arumugam Sathasivan, whose encouragement, guidance and support from the initial to the final level enabled me to develop an understanding in this subject. It is my great opportunity and pleasure to share your valuable experience. Furthermore, I am grateful for your understanding and efforts to help me out with my financial struggle during the study period.

I would also like to extend my appreciation to the Department of Civil Engineering. Special thanks are given to Professor Hamid Nikarz, the Head of the Department. Many staff in the Department of Engineering deserves my thanks for their kindness. It was pleasure to work with you.

My appreciation goes to Curtin University for funding this research and George Kastl who designed the system which is used for this experiment when he was an employee of Sydney Water Corporation. Acknowledgements are also due to Sydney Water Corporation for allowing us to use the design. The experimental help provided by Bal Krishana K C is also acknowledged.

At the end, I would like to thank to my parents and my husband. Your constant encouragement and considerate help inspired to overcome any obstruction in my work and to go forward.

Rekha Aryal Adhikari

CHAPTER 1

INTRODUCTION

1.1 Background

Chloramine is increasingly employed in the United States and Australia as an alternative secondary disinfectant for chlorine in drinking water supply systems. This is because chloramine reacts less with organic matters than chlorine which result in long lasting residuals in the distribution system. While it controls microbial growth in finished water, it reduces the formation of chlorinated disinfection by products (DBPs), especially trihalomethanes (THM) and haloacetic acids (Gregory, 2004). However, in long distribution systems like Goldfield and Agricultural Water Supply System (G&AWSS) in Western Australia, where water is piped over 650 km from Mundaring to Kalgoorlie and consisting of further 7000 km of pipe system, various factors accelerate chloramine decay. Conditions further worsen during summer in this above ground system as the outside temperature rises up to 50 °C. Thus, the role of various factors such as temperature, biofilms bacterial activities, bulk water bacterial activities, and sediments need to be clearly understood to quantify the chloramine decay properly.

In chloraminated water, monochloramine decays as a result of two principal mechanisms; microbial and chemical process (Sathasivan et al., 2005). Nitrification is an important factor to the loss of chloramine in long drinking water distribution system. During nitrification the free ammonia, introduced to the distribution system during chloramine formation, eventually serves as an energy source for ammonia oxidizing bacteria (AOB). Further growth and decay of microorganisms produce nitrite and organic compounds, which exert a chloramine demand. Eventually, it supports the growth of nitrite-oxidizing bacteria (NOB) and heterotrophs.

Apart from them, biofilm also plays a prominent role on chloramine decay and water quality deterioration in chloraminated drinking water. Biofilm is a collection of microorganisms surrounded by the slime. Attachment to either an inert or a living

surface provides favourable shelter and nutrients (organic and inorganic materials) for bacteria and other microorganisms.

Biofilms is widely reported to be present in chloraminated reservoir and water distribution systems. They contribute to the loss of disinfectant decay, either through the direct disinfectant demand imposed by biofilm matrix or through nitrification in chloraminated system. In addition, several factors enhance biofilm formation in distribution systems such as temperature, nutrients, pipe materials and sediments (Momba et al., 2000). However, their subsequent effect in drinking water distribution system still remains unexplored and poorly understood. Thus, this research will be unique and is likely to be the first comprehensive research on contribution of biofilm to chloramine decay in drinking water distribution systems.

In order to understand the mechanism of biofilms formation, respective effect on disinfectant and further fundamentals associated with it, a detailed literature review has been conducted. It then provides a detailed description of experimental work performed. Pilot scale experimental set up simulating an actual distribution system and biofilms coupons inserted into them were used to understand the impact of biofilms on chloramine decay. Conclusion and Recommendation for Further Research follows the Results and Discussion.

1.2 Research Problem and Objective

The objective of this research is to undertake an in-depth assessment of chloramine decay resulting from biofilms in drinking water distribution system. To fulfil the major objective of finding the effect of biofilms on chloramine decay, the research will investigate the following aspects;

1. Evaluation of the effect of biofilms on chloramine decay under mildly and severely nitrifying conditions.

2. Cyclic growth of biofilms with respect to time and thus the impact of it on chloramine decay using biofilms coupons.

3. Investigation of impact of biofilms on chloramine decay under different temperature and initial chloramine concentrations.

4. Quantification of impact of biofilms on chloramine decay in the continuous flow pilot scale system.

1.3 Research Significance

The purpose of water supply distribution system is to deliver safe drinking water to each consumer that is adequate in quantity and stringent in quality. The management of water quality in distribution systems is a major technological challenge to the water utilities. Distribution of safe drinking water to the community has been a major global concern for many centuries. One of the major concerns is presence of microorganisms, especially pathogens. It can be divided into three types: bacteria, viruses and parasitic protozoa. Bacteria and viruses can exist in both surface and ground water whereas parasitic protozoa can be found mainly in surface water. Therefore, water utilities use disinfectants to kill the pathogenic microorganisms to provide safe drinking water. Thus, disinfection of drinking water is important for the maintenance of water quality in transmission and distribution system.

Chlorine is widely used for the disinfection of treated water prior to introducing into the transmission system. However, it is challenging to maintain detectable residual at extremities of long distribution systems, as chlorine usually reacts faster. Thus, to minimize the potential growth of waterborne organisms, most of the water utilities have switched to chloramine to maximize disinfection stability and minimize DBPs. However, chloramine as a disinfectant is also not completely immune to microbial growth resulting from the available substrate such as carbon, nitrogen, phosphorous.

Further, bacteria also start to consume ammonia becoming available from decay of chloramine itself as their source of energy leading to a rampant growth of microbial community and bring biofilms into existence in the drinking water distribution system. These biofilms further jeopardize the stability of chloramine and degrade the water quality in the distribution system. Indeed, numerous researches have been

conducted on biofilms and many more that investigates on biofilms-related issues. However, the biofilms development due to various reasons in the distribution system and its impact on disinfectant decay still remains poorly understood. Therefore, this research will be unique and is likely to be the first comprehensive research on contribution of biofilms to chloramine decay in drinking water distribution system. In this context, it aims to investigate the effect of biofilms in drinking water distribution by using a laboratory system. The expected outcome of this research is a better understanding of biofilms contribution in accelerating chloramine decay within the distribution system.

1.4 Composition of the Thesis

This research was undertaken with the aim of investigating the contribution of biofilms on chloramine decay in a pilot scale reactor.

Chapter 1 begins with a discussion on disinfectant (chloramine and chlorine). This is followed by the introduction of biofilms and its impact on chloramine decay in water distribution system. The significance of the research is also highlighted in this chapter.

Chapter 2 provides a detailed literature review in order to understand the biofilms formation in water supply systems, especially chloraminated drinking water systems.

Chapter 3 provides a detail on sample sources and general methodology adopted for different experiments undertaken in this research.

Chapter 4 discusses the results on impact of biofilms under various conditions, such as temperature, different ages of biofilms. Similarly, this will further investigate the impact of biofilms that was grown under various conditions such as different chloramine residuals and nitrification conditions.

As a remedial measure to minimize the impact of biofilms on distribution system, a further investigation was carried out with various topped up chloramine at various doses.

Chapter 5 summarizes the achievements of the research along with practical implications and suggestions for future work.

The thesis ends with a list of references.

CHAPTER 2

LITERATURE REVIEW

2.1 Chloramine in Water Distribution System

Chloramine is most commonly formed when ammonia is added to chlorine to provide secondary disinfection to drinking water. There are three types of inorganic chloramine; monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and trichloramine (NCl_3). Formation of monochloramine consists of set of reaction;

$$Cl_2 + H_2O \rightarrow HOCl + HCl \quad (2.1)$$

The concentration of hypochlorous acid and hypochlorite ion depends on the pH. Both HOCl and OCl are good disinfecting agents, but HOCl is more effective (Wolfe et al., 1984)

$$HOCl \leftrightarrow H^+ + OCl^- \quad (2.2)$$

Both of chlorine species in the above reaction are powerful oxidant, capable of reacting with many substance presents in water. When ammonia is introduced under the appropriate condition, it reacts with the hypochlorous acid to produce monochloramine:

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O \quad (2.3)$$

The typical purpose of chloramine is to provide a longer - lasting disinfectant as the water moves through reservoirs and pipes to consumers. Monochloramine became more popular in the last few decades because of their ability to reduce the trihalomethanes and other DBPs in the distribution system (Brodtmann et al., 1979; Cotruvo, 1981). However, its stability presents some additional challenges to water utilities.

Monochloramine decay depends on various factors such as bulk water reaction, auto - decomposition, nitrification, sediments presence, corrosion and biofilms. Further, bulk water goes through chemical and microbial decay. In microbial decay, bacteria play a prime role on chloramine loss (Sathasivan et al., 2005).

An auto decomposition of chloramine follows a complex set of reactions. During this process, ammonia in monochloramine is oxidised to nitrogen gas with smaller quantities of nitrate along with some other unknown products (Valentine et al., 1987; Vikesland et al., 1996). Similarly, nitrite (NO_2) present in raw water or produced from partial nitrification in drinking water exerts chloramine demand. The stoichiometry of Equation 2.6 can be attributed to a direct reaction between monochloramine and nitrite or to a reaction between nitrite and hypochloric acid produced by hydrolysis of monochloramine. The reaction rate between monochloramine and nitrite is slow, but it accelerates significantly in the presence of bromide (Valentine, 1985; Margerum et al., 1994; Vikesland et al., 2001).

$$3NH_2Cl \rightarrow N_2+NH_3+3Cl^- + 3H^+ \quad (2.4)$$

$$4NH_2Cl +3H_2O \rightarrow 3NH_3+ NO_3 +4Cl + 5H^+ \quad (2.5)$$

$$NH_2Cl + NO_2+ H_2O \rightarrow NO_3 +NH_4 + Cl \quad (2.6)$$

These chemical reactions release total free ammonia nitrogen as an energy source for nitrifiers, resulting in nitrification. It is a two-step microbiological process: first ammonia is oxidised to nitrite by AOB, and then nitrite is oxidized to nitrate by NOB. The presence of AOB only, without NO_2 – N oxidizers, is the most commonly encountered situation in water distribution systems and is the water utility's greatest challenge to maintain good water quality in distribution systems.

Previous studies observed nitrification in both low and high chloramine residual up to 6.0 mg/L in distribution systems (Lieu et al., 1993; Skadsen, 1993; Odell et al., 1996; Wilczak et al., 1996). High chloramine residuals may not stop nitrification and appears ineffective once nitrifiers are established in the distribution system (Wilczak et al., 1996). Nitrifying bacterial activities can be estimated by increased NOx (nitrite + nitrate) levels. This is because in a chloraminated environment, nitrite is oxidized by chloramine or NOB to nitrate but there is only one mechanism to convert ammonia to nitrite, which is by AOB. It is a result of free ammonia, which is produced by various reasons such as chloramine decay and overdosing of ammonia during chloramine formation or naturally occurring ammonia in groundwater

(Wilczack et al., 1996; Norton and LeChevallier, 1997; Wolfe and Lieu, 2001; Sathasivan et al., 2005; Zhang et al., 2009).

There are many factors which directly affect nitrification process in the distribution system such as, chloramine residual, chlorine to ammonia-N ratio, distribution system hydraulics, distribution system pipes, temperature and pH (Cohen et al., 2001). Chloramine disinfection in drinking water networks and water reservoirs is often associated with nitrification. Using higher chloramine residual provides higher free ammonia to AOB. Also, once nitrification is established in the distribution system, it is hard to control even with high chloramine residual (up to 8.0 mg/L). Thus, increased free ammonia in chloraminated waters is one of the primary reasons for high nitrification rate and accelerated chloramine loss (Cohen et al., 2001).

Similarly, it is believed that detention time and pipe materials also play an important role in nitrification. Longer the detention time, the more likely it is for nitrification to govern, as nitrifying bacteria grows slowly and higher water age correlates to lower disinfectant residual. In addition, nitrification occurs in conduits of any material and is believed that certain pipe materials may provide more favourable conditions. For example, tuberculated unlined cast-iron pipes may provide a good environment for nitrifying bacteria growth. It occurs in chloraminated water systems over a wide pH range from 6.5 to 10 and preferentially at temperatures above 15 °C (Wolfe et al., 1990; Cohen et al., 2001; Lipponen et al., 2002).

The rate of decomposition of chloramine depends on temperature. The ideal temperature for nitrifying bacterial regrowth is 25 °C to 30 °C (Sathasivan et al., 2009). Therefore, the amount of chloramine loss in summer is higher than winter and then more ammonia, released from combined form, is available for AOB. Summer temperatures usually hover around optimum levels and hence higher bacterial growth can be expected.

The rate of nitrification also varies with pH. In general, the pH will decrease during nitrification. At a lower pH, the release of free ammonia increases and as rate of chloramine decay increases with formation of di-chloramine and tri-chloramine. The physical and operational factors, such as the water treatment processes and distribution system conditions largely affect nitrification (Wolfe et al., 1990;

Lipponen et al., 2002). In addition to nitrification, nitrifying bacteria can secrete organic compounds that stimulate the growth of bacteria detected by heterotrophic plate count (HPC) (Watson et al., 1989).

Accumulated material or particles in reservoirs and pipes form sediments under favourable conditions, such as low flow and dead-ends. They generate two problems in water quality. Firstly, they carry bacteria fixed onto their surface, which protect them from disinfection (Ridgway and Olson, 1982; Camper et al., 1986). Secondly, they contribute to the formation of loose deposits in reservoirs and pipes, which are suspended into the water phase when a change occurs in hydraulic regime. Organic matter is suspected to greatly influence the activity of bacteria located inside these loose deposits, even if it is unknown to what extent these bacteria grow on dissolved organic carbon diffusing from the water phase or form accumulated material. After re-suspension of deposits, microbiological and chemical characteristics of water are not controlled (De Rosa, 1993) and thus resulting in degradation of water quality.

Apart from fore mentioned reasons, biofilms plays a prominent role on accelerating chloramine decay. However, the contribution of biofilms on chloramine decay is not well understood. In this context, this research aims to understand and investigate the impact of biofilms on chloramine decay in water distribution system.

Table 2.1: Summary of Chloramine in Water Distribution System

<table>
<tr><th>Merits</th><th>Demerits</th><th>Resulting Effect</th></tr>
<tr><td rowspan="2">Stable disinfectant</td><td>Auto-decomposition</td><td rowspan="4">Chloramine loss</td></tr>
<tr><td>Bulk water reaction
-Microbial decay
-Chemical decay</td></tr>
<tr><td rowspan="3">Less DBPs</td><td>Nitrification
Depending Parameters:
-Chlorine residual
-Chlorine to ammonia-N ration
-Distribution system hydraulics
-Temperature
-pH
-Retention time
-Pipe materials</td></tr>
<tr><td>Sediments (accumulated materials or particles in system)
-Provide good shelter for microbes</td></tr>
<tr><td>Biofilms
-Also create favourable environment for microbes to grow</td></tr>
</table>

2.2 Biofilms in Water Distribution System

Biofilms are basically a complex structure adhering to the surface of reservoir and pipe line that are regularly in contact with water. Normally, in distribution system pipelines they come into existence when microbial cells attach to pipe surfaces (Marshall et al., 1971; Allen et al., 1980). These microbial cells then gradually develop a film or slime layer inside the pipe, eventually developing a biofilms. It consists of colonies of bacteria and other microorganisms that accelerate chloramine

decay. Some of these microorganisms adhere to the pipe surface via appendages that extend from the cell membrane. Other bacteria form a capsular material of extracellular polysaccharides which anchors the bacteria to the pipe surface (Geldreich and Rice, 1987), taking advantage of the macromolecules attached to the pipe surface for protection and nourishment (USEPA, 1992). This shows that surface condition, adhesion of bacteria and slime formation can be considered as prime factors in developing the biofilms in the water distribution system.

Biofilms in distribution system are not a continuous layer on pipe surface. They are formed often in patchy appearance and composed of heterogeneous mixtures and discontinuous structure (Van der et al., 1995; Fleming, 2002; Martiny et al., 2003). It is believed that a mature, fully functioning biofilms is like a living tissue on the pipe surface. It is a complex, metabolically cooperative community made up of different species each living in a customized micro-niche.

It is a microbial activity in the distribution system that causes various water quality related problems such as bacterial regrowth, protection of pathogens from disinfectants, corrosion of iron pipes, nitrification of chloramines and increased disinfectant demand, (Lu et al., 1999). Many researchers have reported that biofilms form a dynamic micro-environment that encompasses the processes such as metabolism, growth, and product formation, and finally detachment, erosion, or sloughing of the biofilms (Characklis, 1981; Characklis and Marshall, 1990).

The rate of biofilms formation depends on the physico-chemical properties of interface, the physical roughness of the surface, and physiological factors of the attached microorganisms (Fletcher and Marshall, 1982). It may grow until the surface layers begin to slough off into water or the pieces of biofilms released into water. This may contribute further to provide protection for the organisms until they can colonize a new section of the distribution system. As a result distribution pipes act as a carrier and become responsible for the deterioration of the drinking water quality and operational problems during transportation to the consumer.

Normally, water distribution systems contain biofilms and sediments which enhance the growth of microbes including nitrifiers, as well as provide protection from disinfectant. It has been estimated that about 95 % of the overall microbial

communities present in distribution systems existed as biofilms, with only 5 % present in water phase (Wingender and Fleming, 2004).

Stewart and Lieu (1997) sampled from a $10.45*10^3$ m^3 reservoir in Southern California and observed no AOB in the water column despite the presence of significant AOB on biofilms surface. The AOB concentrations on biofilms surfaces ranged from a low 11 at the middle level to a high 860 MPN/cm^2 at the bottom level. The sediments sample also had a high level of AOB of 4000 MPN/mg.

Similarly, Ike et al., (1988) reported that higher numbers of AOB per unit area in sediments than biofilms. Baribeau et al., (2002) detected the concentrations of AOB in the bulk water samples, which typically varied between 0 and 10 MPN/mL, whereas in the biofilms from the bottom layer of the reservoir, AOB levels were more than 20-200 MPN/cm^2. Microbes that exist in biofilms are able to resist disinfection more easily than planktonic microbes in water phase. Srinivasan et al., (2008) quantified the relative abundance of bacteria in both biofilms and bulk water in chlorinated pilot scale pipe loops and found that the relative bacterial abundance in bulk water (compared with biofilms) increased with decreased residual.

2.2.1 Steps in Biofilms Development

As mentioned earlier, biofilms begins to form instantly after the clean pipe is filled with water and gradually develop into a complex structure with time. This process could be explained through different steps as explained in the following steps.

Surface Conditioning

The first substances associated with the surface are not bacteria but trace organics (nutrients such as carbon, nitrogen and phosphorous). Almost immediately after the clean pipe surface comes in contact with water, an organic layer starts to deposit on the water solid interface (Mittelman, 1985).These organics are said to form a conditioning layer.

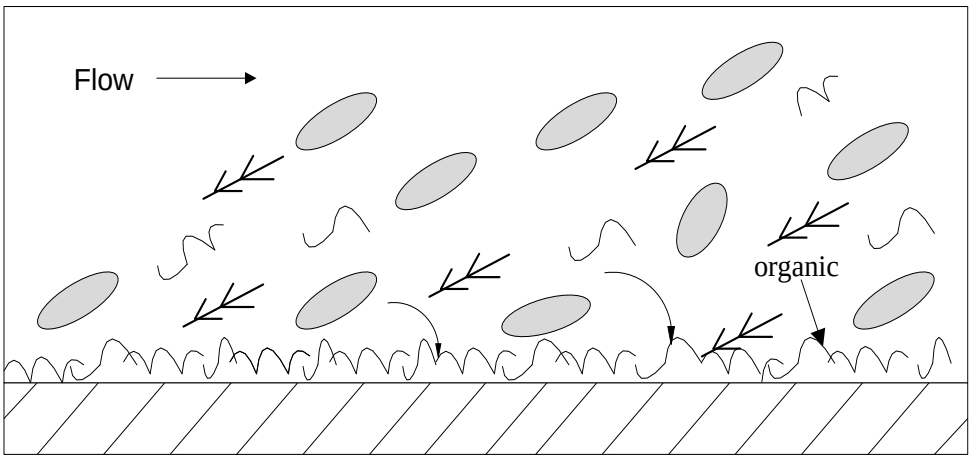

Figure 2.1: Adsorption of organic molecules on a clean surface forms a conditioning film. (Characklis and Marshall, 1990)

Adhesion of Bacteria

In a pipe of flowing water, some of free floating bacteria will approach the pipe wall that has already caught the trace organics and become entrained within the boundary layer, the quiescent zone at the pipe wall that has a null velocity. Some of these cells will strike and adsorb to the surface for some finite time, and then desorbs. This is called reversible adsorption. This initial attachment is based on electrostatic attraction and physical forces, not any chemical attachments. Some of the reversibly adsorbed cells begin to make preparations for a lengthy stay by forming structures which may permanently adheres the cell to the surface resulting in irreversible adsorption (Characklis and Marshall, 1990).

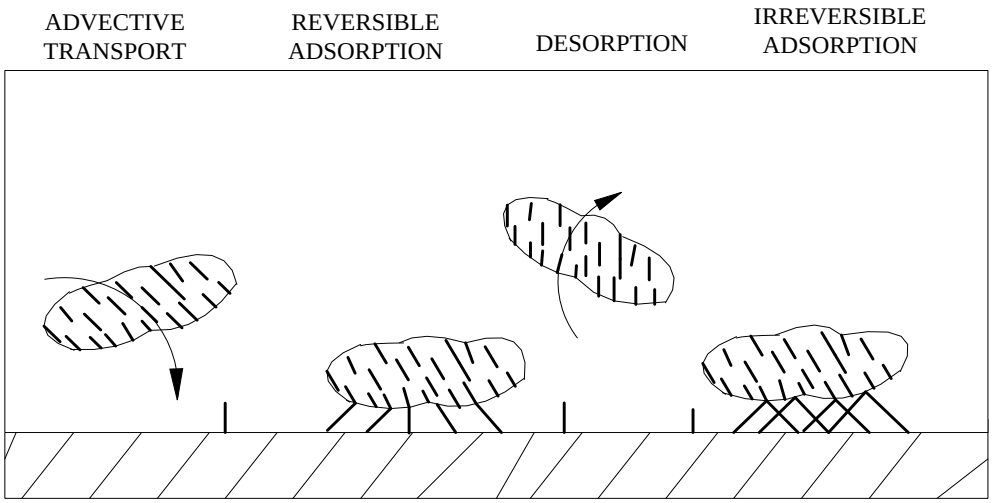

Figure 2.2: Transport of bacteria cells to the conditioned surface, adsorption, desorption and irreversible adsorption (Characklis and Marshall, 1990)

Slime Formation

Biofilms bacteria excrete extracellular polymeric substance, or sticky polymers, which hold the biofilms together and cement it to the pipe wall. In addition, these polymer strands trap scarce nutrients and protect bacteria from biocides.

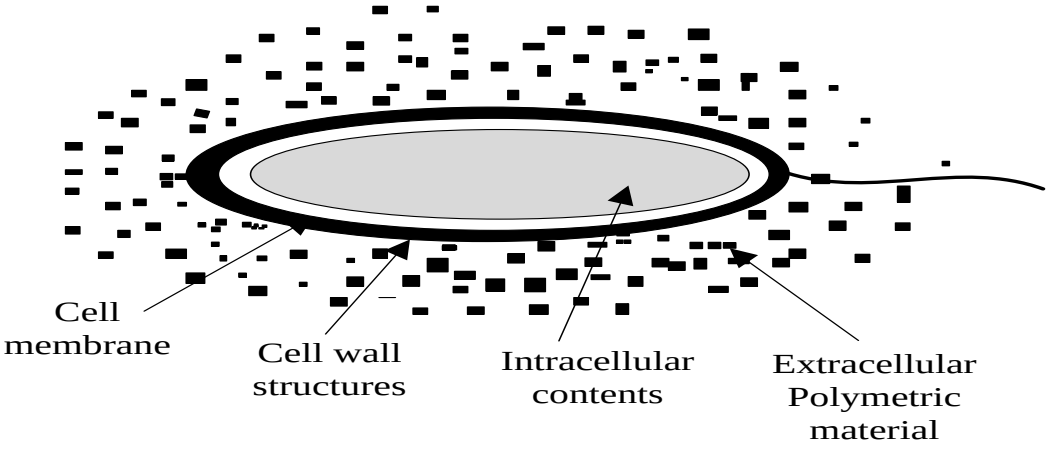

Figure 2.3: Wild bacteria are "bairy" cells with extracellular polymers which stick to surface. (Mittelman, 1985)

2.2.2 Factors Affecting on Biofilms Formation in Water Distribution System

The formation and development of biofilms and subsequent chloramine decay is governed by various factors in both reservoirs and distribution systems. In general, various nutrients such as phosphorous, nitrogen and carbon enhance the microbial activity (Sathasivan and Ohgaki, 1999). The availability of nutrients is a key driver in the formation of biofilms and depends upon the nutrient type, concentration and disinfectant concentration.

Biofilms bacteria predominantly use biodegradable organic carbon as their main nutrient source. It has been suggested that the ratio of 100 carbon: 10 nitrogen: 1 phosphorus is required for bacterial growth (Sathasivan and Ohgaki, 1999). Therefore, principal source of nutrients including carbon, nitrogen and phosphorous provide the favourable environment for the growth of biofilms.

Nitrifying bacteria secrete organic compounds that stimulate the growth of bacteria detected by HPC. They also incorporate organic compounds which help to increase their growth rate and cell yields. They use carbon for the production of new cellular material (assimilation) and an energy source (dissimilation). It is needed mostly for bacterial growth. Studies have shown a positive relationship between the concentration of biodegradable organic matter (BOM) in drinking water and bacterial regrowth in distribution systems (Van der, 1992; Owen et al., 1995).

Similarly, in drinking water derived from surface water source, various conditions such as vegetation decay, runoff containing agricultural fertilizers releases nitrogen encouraging microorganism to build amino acids, genetic material and ammonia. These reduced forms of nitrogen promote the microbial growth in water by generating a friendly environment for microbial growth and biofilms formation both in reservoirs and pipes (Orskov et al., 1984). This may eventually accelerate the chloramine decay in drinking water distribution system.

Apart from this, there are some other sources of nutrients in the distribution system, such as rubber, silicon, polyvinyl chloride (PVC), polyethylene (PE), have been reported to stimulate bacterial growth (Schoenen and Scholer, 1985; Frensch et al.,

1987; Schoenen and Wehse, 1988). These were shown to provide good environment for biofilms formation. Such condition leads to rapid decay of chloramine by bacterial nitrification (Wolfe et al., 1988). Mittelman (1985) have listed some sources of nutrients in water system.

Table 2.2: Sources of Nutrients in Water System.

Nutrient	Sources
Organic Carbon	Humic and fulvic acids (source water)
	Pipe plasticizers and solvents
	Fiberglass - reinforced plastics (FRPs)
	Pump and gage lubricants
	Microbial by-products
	Airborne dust
Nitrogen	Humic and fulvic acids (source water)
	Nitrates and nitrites (source water)
	Microbial by-products
	Airborne dust
Phosphorus	Phosphates (source water)
	Microbial by-products
	Airborne dust
Sulfur	Sulphates (source water)
	Sulphuric acid (RO pre-treatment)
	Membrane surfactants
	Airborne dust
Trace metals and salts	Source water
	Processing piping
	Reinforce plastics (FRPs)
	Stainless steel system components
	RO pre-treatment chemicals
	Airborne dust

Similarly, different temperature condition, lower concentration of disinfection residuals, sediments in the pipes and reservoir, pipe material, corrosion, water hydraulic condition also play a prominent role in the growth of biofilms. (Geldreich and Rice, 1987; Lehtola et al., 2004).

Although, it is difficult to control, temperature also has a large impact on bacterial activity in a distribution system. Dolan and Pipes (1986) have found a close relationship between water temperature and the biofilms development. Directly or indirectly temperature affects all the factors that govern microbial growth. The favourable temperature generally enhances the growth of microbial community. Lund and Ormerod (1995) reported that biofilms formation appeared to be closely related to the fluctuations in water temperature. In their study, they found that the biofilms levels at 18 ˚C are higher than at 6 ˚C and 12 ˚C. Many investigators have observed significant microbial activity in water at temperatures of 15 °C or higher (Fransolet et al., 1985; Dolan and Pipes, 1986; LeChevallier et al., 1990). Similarly, insufficient disinfectant residual also accelerates the bacterial growth in drinking water supplies. (Fleming et al., 2005; Sathasivan et al., 2008).

In one study Dixton et al., (1988) reported that sediments and debris in pipe systems provide a habitat for microbial growth and protection from disinfection. In addition, organic and inorganic sediments can transport microorganism into the distribution system and provide protection from disinfection. So, it generates the good condition for biofilms formation in drinking water distribution system. Those biofilms and accumulated sediments then protect the AOB from bulk water chloramine residual resulting in chloramine decay in distribution system (Cohen et al., 2001).

Drinking water pipes are made of various materials and have their impact on biofilms development either through differences in surface roughness or hydrophobicity. Depending upon their roughness, wettability, and adhesive properties it may act as a source for bacterial growth (Rogers et al., 1994; Bryers, 2000; Kielemoes et al., 2000). Similarly, Corrosion in iron pipes provides a protective surface for microorganisms, slow water flow, and contributes to backflow occurrence. It could be a result of chemical, physical or biological action and could protect HPC and coliform bacteria from the disinfectants (LeChevallier et al., 1987). Therefore,

surface area characteristic is also considered as a primary factor influencing biofilms development.

Further, Victoreen (1980 and 1984) reported that iron may be an important nutrient for microbial growth. He found that substantial coliform growth was stimulated by iron oxides in distribution system tubercles. Under this condition, coliform could rise to 2×10^8 bacteria/100 mL within 90 hours at 20 °C. The bacteria and nutrients may accumulate in tubercles created in corroded iron pipes resulting in the formation of biofilms within the distribution system (Allen and Geldreich, 1977). Therefore, pipe materials are believed to play a major role in the selection of biomass and its organization.

Water hydraulics has also been instrumental in affecting microbial growth in pipe surfaces present in drinking water. It is generally believed that higher the water velocities, greater the flux of nutrients to a pipe surface, greater the transport of disinfectants and greater the shearing of biofilms. Areas of pipe work which have stagnation zones are known to have adverse effect on biofilms in potable water. Particular effect includes a loss in disinfectant residual and accumulation of sediments, debris, and ultimately increases microbial growth (Smith et al., 1989; Opheim and Smith., 1990).

2.2.3 Effects of Biofilms on Water Quality

Numerous studies have shown that bacterial growth within drinking water distribution networks seriously affecting the hygienic and aesthetic quality of drinking water. Biofilms in drinking water distribution system pipes may lead to a number of non-desirable effects on the quality of the distributed water.

1. Bacterial growth may affect the turbidity, taste, odour and colour of water (Servais et al., 1995).
2. Coliform bacteria have been associated with a high abundance of heterotrophic bacteria and biofilms, producing a possible health risk (Goshko et al., 1983).

3. Corrosion of pipe material may be affected by bacterial growth (Lee et al., 1980).

4. Microbes growing in the biofilms degrade the water quality consequently increasing the disinfectant decay in the distribution system (Astier et al., 1995; Lu et al., 1999; Chandy and Angles, 2001).

5. Biofilms in the distribution system also provide a protection to bacteria embedded in matrices of extra cellular polymeric substance and makes disinfectant less effective (Fletcher and Marshall, 1982; LeChevallier et al., 1988).

6. Biofilms have the ability to convert non-reactive organic carbon into reactive species that facilitates reaction with chloramine and results in additional chloramine loss (Exner et al., 1978).

7. The growth of biofilm is significant even in higher chloramine (1.4mg/L) residual in drinking water (Chandy and Angles, 2001).

It is well known that microorganisms colonise any surface in contact with water. Bacterial growth in drinking water distribution system mainly occurs on the internal surface of the pipes. Detachment of bacteria from this biofilms may thus affect the water quality and accelerates the chloramine loss. In addition, biofilms contribute to further pipe corrosion and can deplete the chlorine used to disinfect drinking water and maintain water quality.

2.3 Methods for Quantifying effects of Biofilms in Water Distribution System

Recently, Sathasivan et al., (2010) developed a reservoir acceleration factor (F_{Ra}) method for quantifying factors affecting chloramine decay in full scale service reservoirs. In this method, total chlorine decay within the reservoir is compared with that in the bulk water sample by incubating at the same temperature. This method provides the combined effect of both sediments and biofilms in completely mixed reservoirs. They observed a significant effect of combined biofilms and sediments on

chloramine decay in a smaller reservoir (biofilms surface area to water volume ratio, S/V; 0.31 m^{-1}) compared to two larger reservoirs (S/V ratio; 0.08 and 0.2 m^{-1}). Moreover, they reported a much greater effect of vertical thermal stratification than biofilms and sediments impact on chloramine decay.

The F_{Ra} is defined with respect to base chemical decay rate ($k_{bc,}$) at T °C /hr as shown in Equation 2.7.

$$F_{Ra} = \frac{k_{RT} - k_{bc,T}}{k_{bc,T}} \qquad (2.7)$$

Where, k_{Rt}→ total decay rate coefficient at T °C/hr

Despite such detailed studies, none has separately quantified the impact of biofilms and sediments on chloramine decay at controlled conditions. It is necessary to understand the contribution of sediments and biofilms bacterial activities on chloramine decay for maintaining decent chloramine residuals in water utilities. Therefore, this research aims to understand and investigate the impact of biofilms in drinking water distribution systems.

Literatures review suggests that growth of microbes inside the reservoirs and distribution system eventually favours the formation of biofilms. Previous study found that chloramine decay in the water phase alone indicated the presence of biofilms contributed to disinfectant loss (Angles et al., 1999). Further, as chloramine does not react readily with acetate, the results also show that biofilms are able to convert non-reactive organic carbon species into those able to react readily with chloramine thus facilitates its decay (Exner et al., 1978).

The contribution of biofilms in chloramine can be determined only when the fundamentals of complexity associated with its formation is well understood. Literature reviews give some clues and concepts regarding the fundamentals of biofilms formation and its impact on chloramine decay as listed below.

1. Monochloramine is used as a secondary disinfectant instead of chlorine because of its greater stability.
2. Microbial decay factor (F_m) method can be used to quantify the microbial and chemical chloramine decay in bulk water.
3. Nitrification has a greater role to decay chloramine in chloraminated drinking water.
4. It is a great challenge to maintain the chloramine residual throughout the water distribution system due to nitrification, biofilms, sediments presence, corrosion etc.
5. Biofilms not only protect bacteria from the disinfection, but also provide environment where disinfectant injured cells can repair cellular damage and grow.
6. In addition to bulk water, biofilms and sediments may accelerate chloramine decay in the distribution system.
7. Biofilms may promote the deterioration of metallic pipe surface through a process called microbiologically influenced corrosion or bio-corrosion and it induces a disinfectant demand and consequently increases disinfectant decay in distribution system.
8. Hydraulics affects sediments accumulation in drinking water distribution system, as it can provide habitats for microbial growth.
9. Hydraulic can also affect the formation of biofilms in water distribution system.
10. Biofilms facilitates the chloramine decay by converting the non-reactive organic carbon species to reactive ones that eventually accelerates chloramine.
11. Using F_{Ra} and F_m methods, contribution from biofilms, sediments and bulk water can be separately quantified.

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of the Studied System

The Goldfields and Agricultural Water Supply (Figure 3.1), is perhaps the world's largest water distribution system. The pipeline was commissioned in 1896 and was completed in 1903. This was primarily constructed to deliver water to the communities that had rapidly grown due to a gold rush in Western Australia's "Eastern Goldfields", such as Coolgardie and Kalgoorlie. The pipeline connects Mundaring Weir, near Perth, Western Australia, with the Mount Charlotte Reservoir at Kalgoorlie, 530 km (330 miles) away. It also serves towns further inland via extensions to the north and the south. The Mundaring Weir is fed with water from the Helena River in the Darling Scrap and has also been augmented with treated groundwater in recent years. It continues to operate, supplying water to over 100,000 people and more than six million sheep in 33,000 households, mines, farms and other enterprises.

Major objective of water utilities is to provide safe drinking water to consumer. Therefore, many water utilities are using chloramine as a secondary disinfectant because of its greater stability. However, it has some disadvantages such as auto - decomposition of chloramine, direct chemical reaction with water born constituents, nitrification, promotion and formation of biofilms. Eventually, they accelerate chloramine decay in drinking water distribution system. Drinking water producers try to avoid bacterial growth during water distribution. Bacterial growth in a drinking water distribution system mainly occurs at internal surface of the pipes. Detachment of bacteria from this biofilms may thus affect the water quality and accelerate the chloramine decay. Thus, all the data obtained from lab based experiment is investigated to understand the contribution of biofilms to chloramine decay in drinking water system.

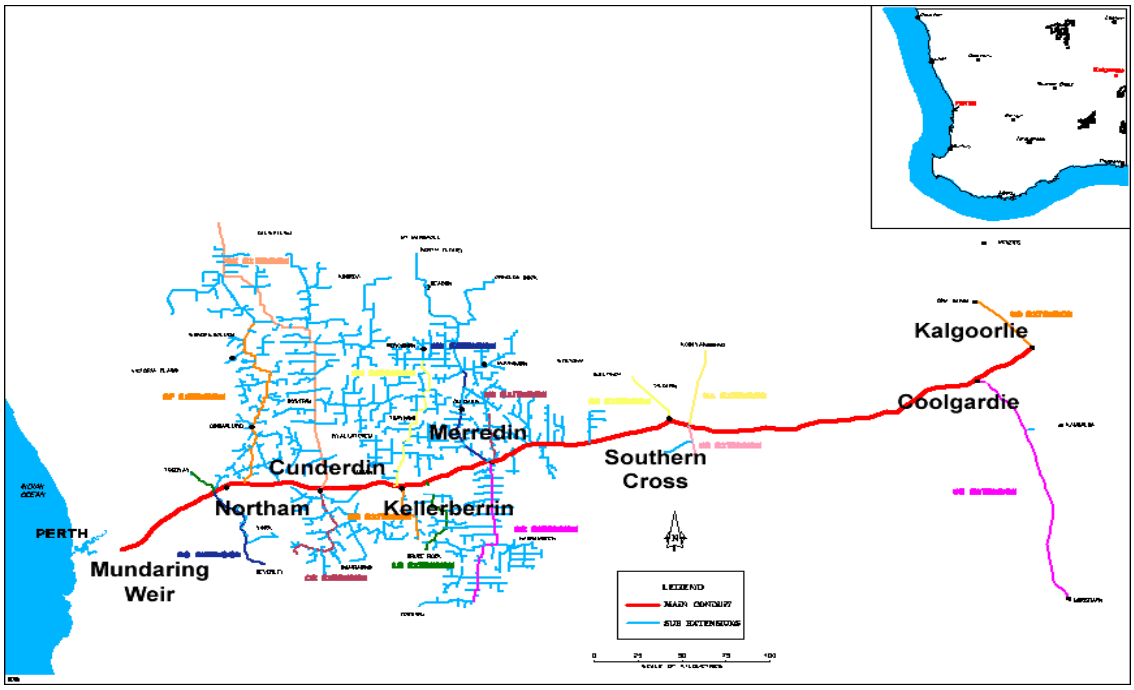

Figure 3.1: Goldfields & Agricultural Water Supply System (Courtesy: Water Corporation, WA)

3.2 Stock Chemical Solutions Preparation

Stock solutions were prepared using analytical grade chemicals in MilliQ water. Monochloramine solution was prepared using stock solutions of ammonium chloride (500 mg-N/L) and sodium hypochlorite (500 mg-TCl/L). Stock solutions of 1 mg-N/L were prepared using ammonium chloride, sodium nitrite and sodium nitrate for TAN, nitrite and NOx (nitrite + nitrate) respectively. The pH was adjusted using 1M hydrochloric acid and 2M sodium hydroxide.

3. 3 Feed Water Sample Collection and Preparation

Sample water was collected from Maundering weir, near Perth, Western Australia. This reservoir is an open reservoir. Rainwater, treated groundwater and river water has been stored. Collected water sample in 1.0 m^3 High Density Poly-ethylene (HDPE) tank was transported to Curtin University and it was stored at room temperature until it was fed into both series of reactors, after dosing chloramine. To

eliminate contamination by indigenous microbes present in feed water, chlorine was added followed by ammonia. About four hours was allowed between chlorine and ammonia dosing. Total chlorine (TCl) to TAN mass ratio of 4.5:1 was maintained. Analytical grade sodium hypochlorite and ammonium chloride were used to maintain desired chlorine and ammonia concentrations. Feed water dissolved organic carbon (DOC) was 2.7±0.4 mg/L during the experimental period. The collected water pH varied from 7.6 to 8.3. Therefore, pH in the feeding tank was adjusted to about 8.0±0.1.

3.4 Reactor Set-up

Two pilot-scale systems set-up were constructed and located at Civil Engineering laboratory in Curtin University. Each system (Figure 3.1) contained five different reactors, named R-1 to R-5, connected in series through HDPE pipes. Each reactor was 25 L in volume and was made of HDPE. Feed water tank was also made of HDPE and was closed by a lid (HDPE). Both systems of reactors were facilitated with automatic water flow and temperature control devices. Water flow rate was controlled using water level sensors and control valves between feeding tank and R-1. However, gravity flow was created from R-1 to R-5 by adjusting outlet and inlet pipe level. All the reactors were closed and stirred once a day by an HDPE rod placed inside each tank. Water temperature was raised using heating plates installed at the bottom of each reactor and temperature was controlled using stainless steel sensors inserted inside the reactors and programmed logical control units. Water samples for analysis were collected from the outlet fixed at the bottom of each reactor.

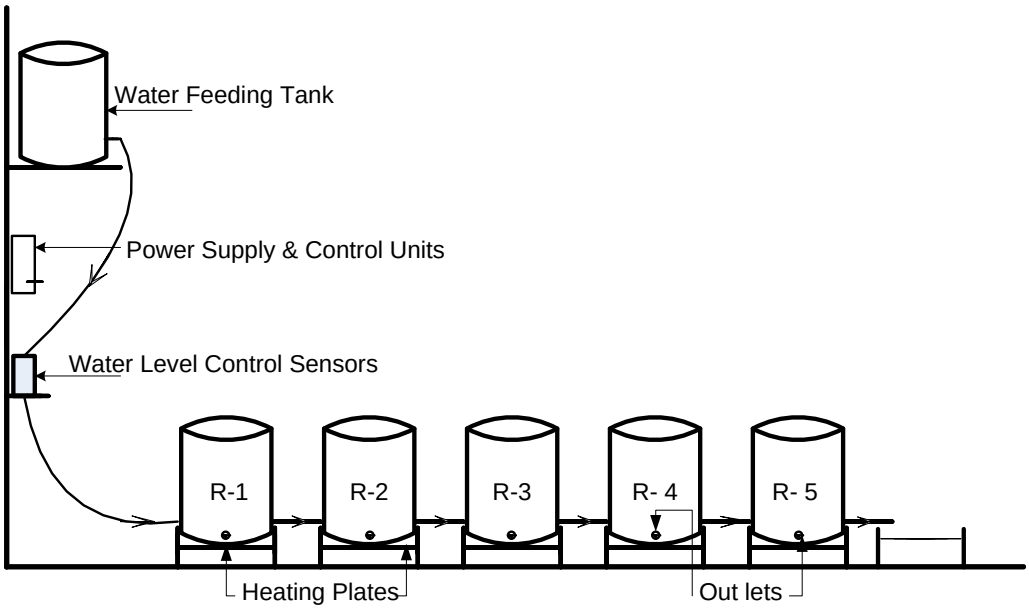

Figure 3.2: Schematic diagram of reactors

3.4.1 Start up of the Reactors

At the start up period, chloramine concentration of about 1.0 mg/L (TCl:TAN ratio, 4.5:1) and pH 8.0±0.1 was maintained in each system. About 20 L water was fed continuously per day and water volume was maintained constant to gain retention time about 20±2 hrs in each reactor. Water temperature was maintained at 20.0±2.0 ˚C in the first three reactors (R-1, R-2 and R-3) and 23.0±2.0 ˚C in the last two reactors (R-4 and R-5) to obtain higher microbial activity. To expedite nitrification and to obtain distribution system specific inoculums, chloraminated water samples collected from G&AWSS, Western Australia, had been placed as seed microorganisms in each reactor, except in R-1. G&AWSS distributes chloraminated water through a 630 km long above ground pipeline and through networks of pipes running in either side. Water in the pipeline undergoes annual temperature fluctuation from 12-50 ˚C. About 10 L nitrified water sample was collected into HDPE containers after initial flushing for 5 minutes. Before collecting the samples, containers were rinsed thoroughly with water containing chlorine concentration of 10-15 mg/L, and then rinsed by distribution system water 5 to 10 times to make sure

the containers were free from contamination of other bacterial species. Collected samples were transported to the laboratory roughly around 20-23 °C temperature.

Gradually, chloramine concentration was increased up to 2.5 mg/L in the feeding tank maintaining TCl:TAN ratio and pH as at the start-up period. The varying nitrifying (none to severe) conditions, which generally occur in real distribution systems, were created along the reactors by varying hydraulic retention time, temperature and chloramine concentration.

3.5 Preparation of Biofilms

PVC and HDPE pipes having dimensions of 2 cm length and 9 mm outer and 6 mm inner diameter pieces were sterilized in 10% sodium hypochlorite solution for 24 hrs. Afterwards, they were cleaned with deionised water for 5 times until they were free from chlorine. The biofilms was then cultivated by placing them inside the reactors under a continuous flow condition. Both pipe coupons were kept in R-2 to R-4 to grow biofilms at different chloramine residuals and nitrification environment. Biofilms surface area (S) to water volume (V) ratio was 2 m^{-1} for each sample.

3.6 Sample Bottle Preparation

Sample bottles (500 ml PET) and all glassware used in this study were soaked into 10 % sodium hypochlorite solution for 24 hrs and rinsed with copious amount of deionised water until they were free from chlorine. All sample collecting glassware, filtration unit and filter papers were autoclaved.

3.7 Analytical Procedures

Total chlorine, TAN, nitrite, nitrate and DOC were measured immediately after collecting the samples. The Aquakem 200, a high precision wet chemistry automated analyzer, was employed to measure TAN, nitrite and NOx residuals. It is a fully automated instrument which provides convenient automated photometric analysis of

water sample. It performs analysis on optical multi-cell cuvette that provides true discrete analysis.

TAN is defined as the summation of ammonia (NH_3), ammonium (NH^{4+}) and ammonia associated with NH_2Cl. The reaction between NH_2Cl and salicylate ions at around pH 12.6 in the presence of sodium nitroprusside produced a blue compound that was measured spectrophotometrically at a wavelength of 660 nm (EPA, 1981a).

Nitrite was measured by the sulphanilamide method (4500-NO_2- B) (Standard Methods, 1998). Before measuring NOx, chloramine residual was reduced stoichiometrically using 0.5 % sodium thiosulphate stock solution. After that it was measured by catalytically reducing to nitrite by nitrate reductive enzyme in the presence of reduced nicotinaminde dinucleotide (Campbell et al., 2006a). The strong azo dye that was produced by the reaction with nitrite was measured spectrophotometrically at 540 nm or 520 nm. Then, nitrate was calculated by deducting nitrite from NOx. The analyzer has a high detection limit for TAN, nitrite and NOx level of 0.002 mg-N/L. Standard curves for TAN, nitrite and NOx were calibrated in the range from 0.0 to 1.0 mg-N/L using a standard solution, prepared from ammonium chloride, sodium nitrite and sodium nitrate, respectively. The experimental errors were 1.5 % (95 % confidence level) for TAN and nitrite measurement whereas NOx measurement was 2.0 % (95 % confidence level).

Total chlorine residuals were measured by DPD colorimetric method using a HACH pocket colorimeter. More than 99% of chloramine is present in the form of monochloramine at pH above 7.5 and TCl:TAN mass ratio about 4.5 (Valentine, 2007). Hence, total chlorine represents predominantly monochloramine residual at pH 8. Total chlorine measurement had an experimental error of ±0.03 mg/L.

DOC was measured using Sievers 5310C Laboratory TOC analyser and experimental error for DOC was ±5 %. Portable pH meter (HACH 40d) was used to measure pH values and the measurement error was ±0.1. Suspended solid was measured as mentioned in Standard Methods (1998).

3.8 Experimental Design

3.8.1 Microbial Decay Factor (F_m) Method

Figure 3.3 shows an experimental protocol to determine the microbiologically assisted monochloramine decay. Determining chemical decay (k_c) and microbial decay (k_m) involved four steps: sample preparation, incubation, monitoring chloramine decay, and estimating decay rate from the resulting data. Sample preparation involved splitting the sample into two sub-samples. The first sub-sample was not processed whereas the second subsample was processed (filtered through 0.2 µm polycarbonate membrane filter) to remove microbial activities. Minimum chloramine residual 1.0 mg/L was adjusted in the bulk water samples which had chloramine residuals less than 1.0 mg/L whereas for bulk water sample which had chloramine residuals more than 1.0 mg/L, chloramine residual was not topped up. Samples were then incubated in a dark water bath maintaining the same temperature measured as in reactors. For each sub-sample, total chlorine was monitored over time and the decay coefficients k_c and total decay coefficient (k_t) were estimated using exponential regression The k_m was calculated from the difference between k_c and k_t. Finally, the F_m is obtained from the ratio of k_m to k_c. The detailed method is given in Sathasivan et al., (2005).

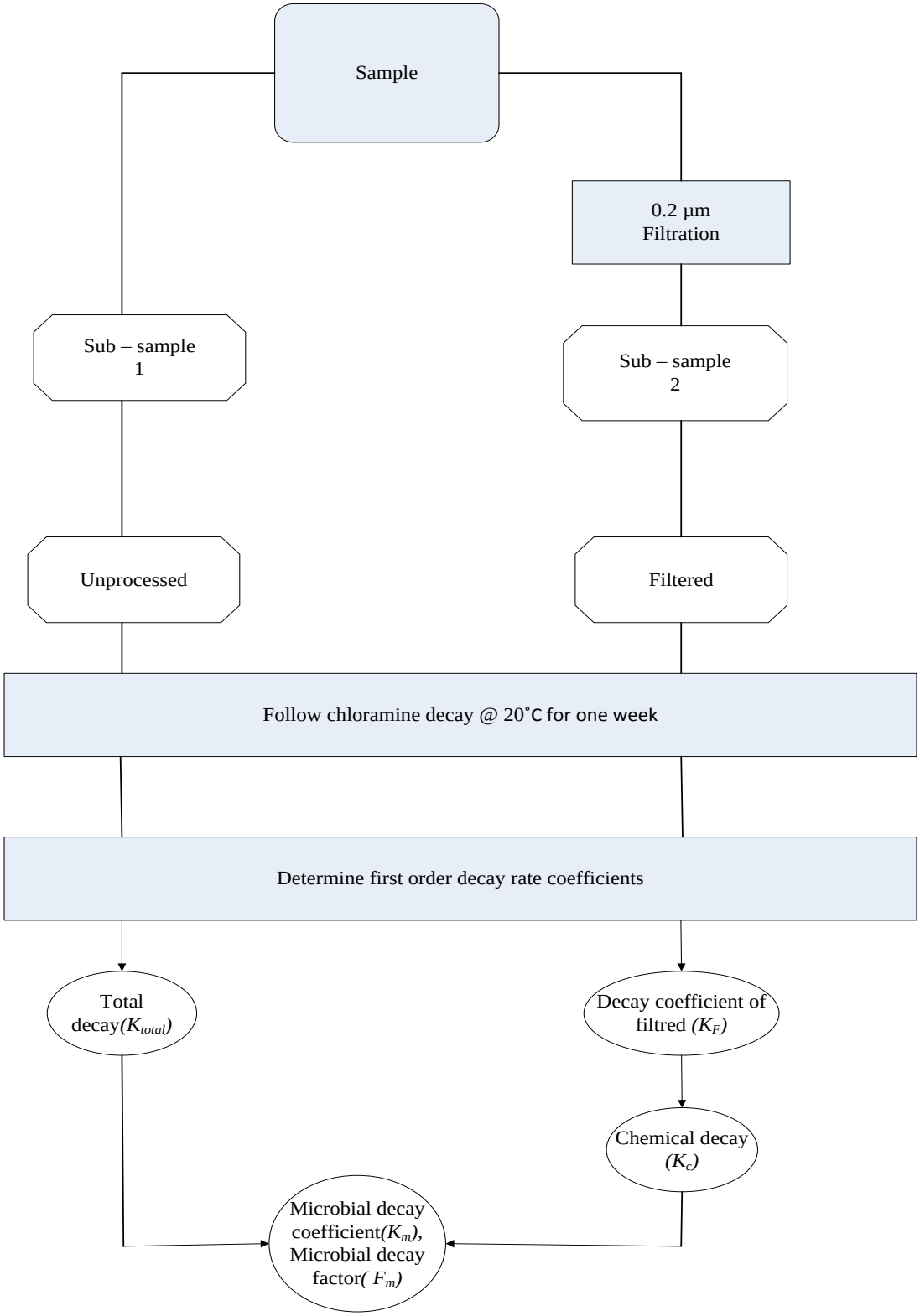

Figure 3.3: Flow chart for determining microbial decay factor

3.8.2 Evaluating the Impact of Biofilms on Chloramine Decay using Batch Tests

Different parameters mainly disinfection doses, age of biofilms, temperature and various nitrification conditions were studied in the reactor system that replicate long water distribution system. All the details are described below.

Sample bottles were prepared as mentioned in Section 3.6. Sample water from Maundering weir, Western Australia was used in this experiment to examine the impact of biofilms on chloramine decay in water distribution systems. Sample water for batch test was prepared by adding chlorine followed by ammonia to avoid contamination by indigenous microbes present in sample water. Time difference was about 24 hrs between chlorination and ammoniation. The mass ratio of TCL:TAN was maintained to 4.5:1 and pH to 8.0 for all samples. Afterwards, biofilms (grown at R-2 to R-4) were collected and kept in 500 ml PET bottles.

In each sample bottle chloramine concentration from 2.0 mg/L, 3.0 mg/L, 4.0 mg/L, 5.0 mg/L, 8.0 mg/L and 16.0 mg/L was maintained by dosing respective stock solution of ammonium chloride and sodium hypochlorite. Similar analysis was also undertaken with PVC and HDPE coupons (without biofilms) material and only with bulk water (Mundaring water) in order to examine their effect on chloramine decay. All sample bottles were incubated in a dark water bath to maintain a constant temperature (20 ˚C) unless effect of biofilms was studied for various temperature effects.

Several experiments were conducted to understand the impact of biofilms on chloramine decay in water distribution system. They are:

1. Impact of biofilms grown under different nitrification environment having different chloramine residual on chloramine decay was investigated. For this experiment, 115 days old biofilms (R-2 to R-4) were considered.

2. Effect of biofilms was studied under various total chlorine concentration (2.0 mg/L to 16.0 mg/L). 115 days old biofilms from R-3 was considered for this experiment.

3. 15, 30, 45, 75 and 105 days old biofilms were studied in order to understand the effect of different ages of biofilms on chloramine decay in water distribution systems.

4. Different temperatures 20 and 25 ˚C were maintained to investigate the impact of biofilms. Thirty days old biofilms from R-3 was considered for this experiment.

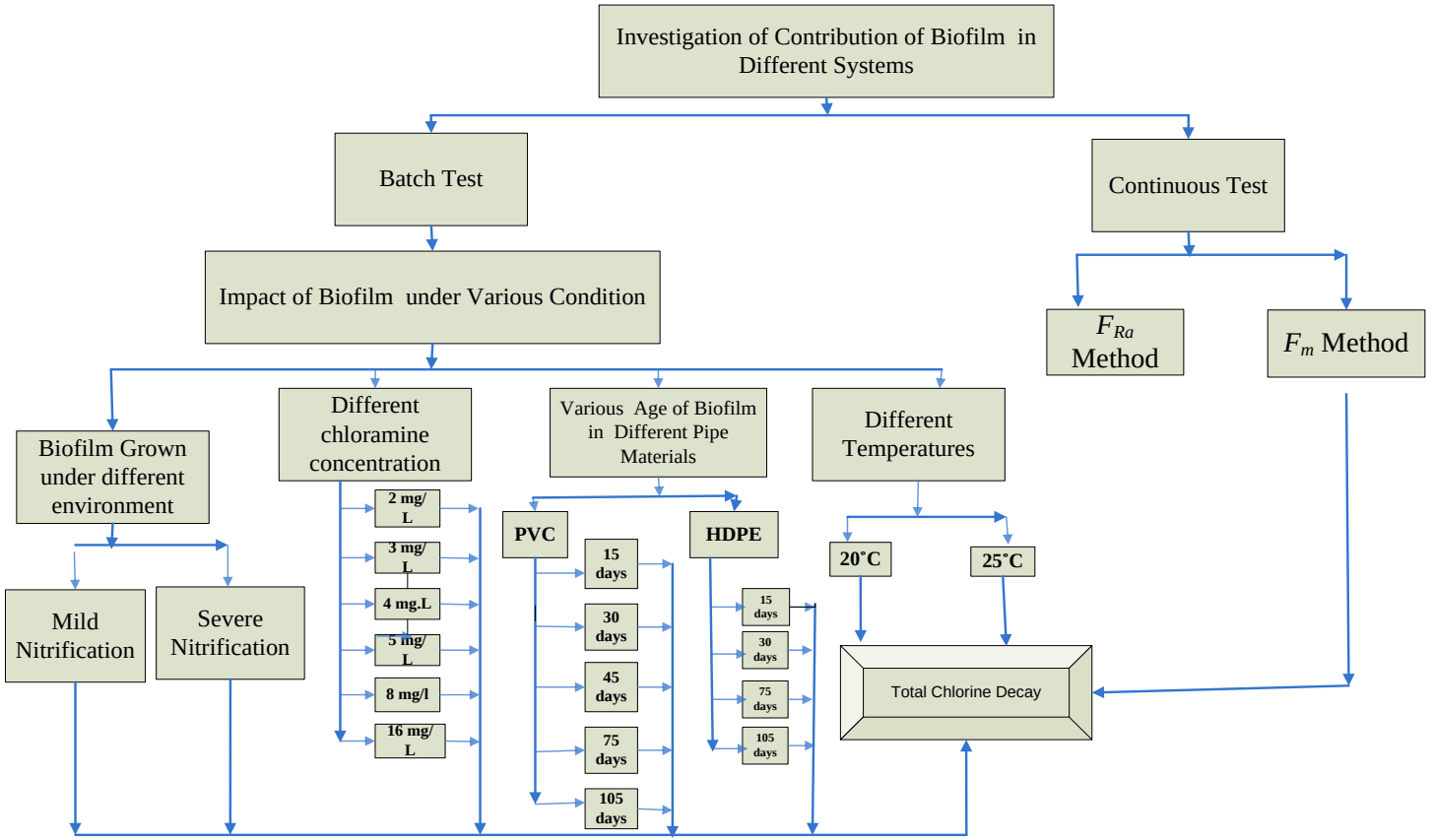

Figure 3.4: Flow chart of impact of biofilms on chloramine decay

CHAPTER 4

Result and Discussion

4.1 Investigation of Biofilms Contribution to Chloramine decay

4.1.1 General

Many drinking water distribution systems are using chloramine as a secondary disinfectant instead of chlorine as it primarily offers reduced DBPs and greater stability. However, the use of chloramine as a disinfectant has some drawbacks such as auto-decomposition of chloramine, direct chemical reaction with water born constituents, nitrification, promotion and formation of biofilms. They eventually accelerate chloramine decay in drinking water distribution system.

In long distribution systems, such as G&AWSS, water spends a greater proportion of time in pipes. So, their role is critical to water quality. But in other systems, such as Sydney Water systems, water spends most time in reservoirs. In reservoirs various factors such as bulk water bacterial activity, biofilms bacterial activity, sediments presence and corrosion degrade the water quality. Thus, it is generally believed that biofilms and sediments provide safe shelter for microbes resulting in acceleration of chloramine decay in chloraminated system. Therefore, in order to understand the impact of biofilms on chloramine decay, several experiments were undertaken. The investigation was focused on how biofilms affect the chloramine decay under various conditions such as:

1. Different nitrifying environment in which the biofilms are grown;
2. The base (pipe) material (PVC and HDPE) on which the biofilms were grown;
3. The age of biofilms, grown on PVC and HDPE coupons in continuous systems;
4. Temperature in batch tests; and
5. The initial concentration of chloramine in batch tests;

In the following sections, results from the experiments are discussed in detail.

4.1.2 Effect of Biofilms Grown Under Various Nitrification Conditions

4.1.2.1 Nitrogenous Species and Chloramine Decay along the Reactors

Figure 4.1(a) reveals the information on total chlorine and nitrogenous species (TAN, nitrite and nitrate) profile along the reactors (R-1 to R-4). In addition, the detail reactors condition during the biofilm growth and investigation is provided in Figure 4.1(b – e). Results for R-5 are not presented as it does not have chloramine residual to understand the role of microbes in accelerating chloramine decay. TAN slowly decreased from R-1 to R-2, but afterwards a sharp drop was observed in R-3 and R-4 (Figure 4.1). Such TAN loss along the reactors (R-2 to R-4) was resulting from TAN oxidation to nitrite by AOB leading to increased nitrite level along the reactors (Figure 4.1(a)). Moreover, there is no other possible pathway for nitrite production by chemical reaction other than microbial oxidation of TAN. Sharp increase in nitrite and decrease in TAN and total chlorine levels in R-3 and R-4 can be defined as severely nitrified conditions (Sathasivan et al., 2008). Nitrate levels were significantly higher in R-3 and R-4 as compared to the first two reactors (R-1 and R-2). There are mainly two pathways of nitrate formation, one is a microbial conversion of nitrite to nitrate and another is chemical oxidation of nitrite in the presence of chloramine. Latter one is possible only when chloramine and nitrite are present, especially in R-2 and R-3.

As discussed in Chapter 2, nitrification plays a prominent role on chloramine loss. Similar results were observed in laboratory scale reactors (R-1 to R-4). Chloramine residual, maintained around 2.5 mg/L in the feeding tank, dropped to 2.1 mg/L in R-1. This was followed by rapid chlorine loss in R-1 to R-4 with almost no residual chlorine in R-4. This is a result of nitrification which is proven by TAN loss, nitrite and nitrate production across the reactors.

However, chloramine loss is accelerated once the onset of nitrification occurs. It is unknown whether such acceleration is due to microbial activities in bulk water or other chemical reactions due to dissolved compounds in bulk water. Therefore, it is necessary to carry out the F_m test along the reactors.

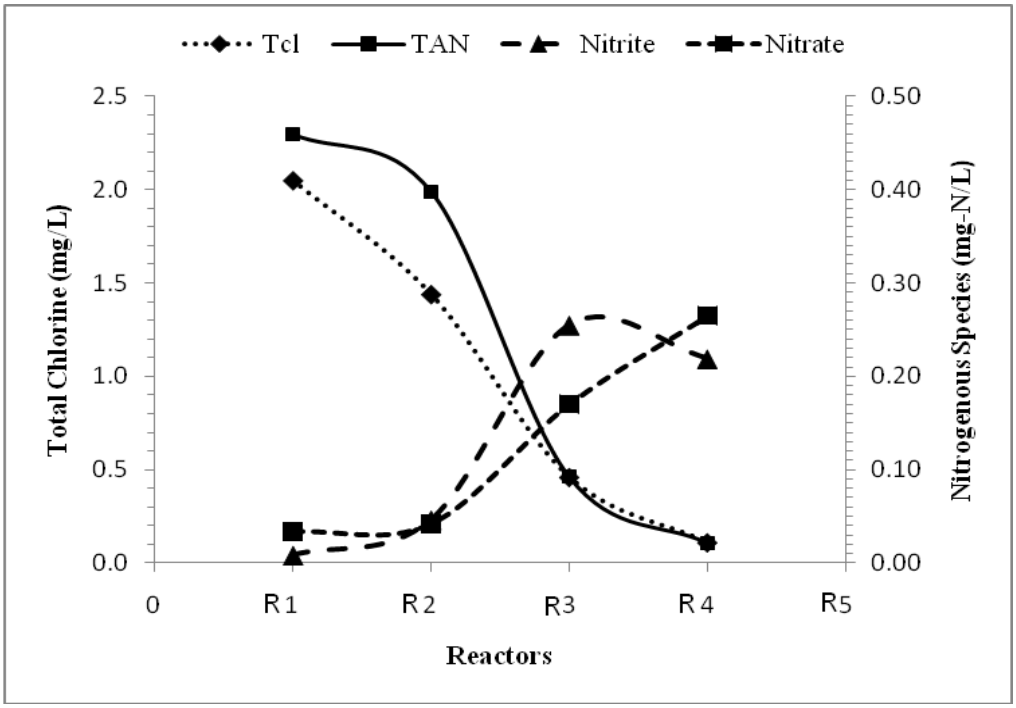

Figure 4.1(a): Total chlorine residuals and nitrogenous species along the reactors (R-1 to R-4) and the age of biofilms on surface of reactor was nearly 2 years

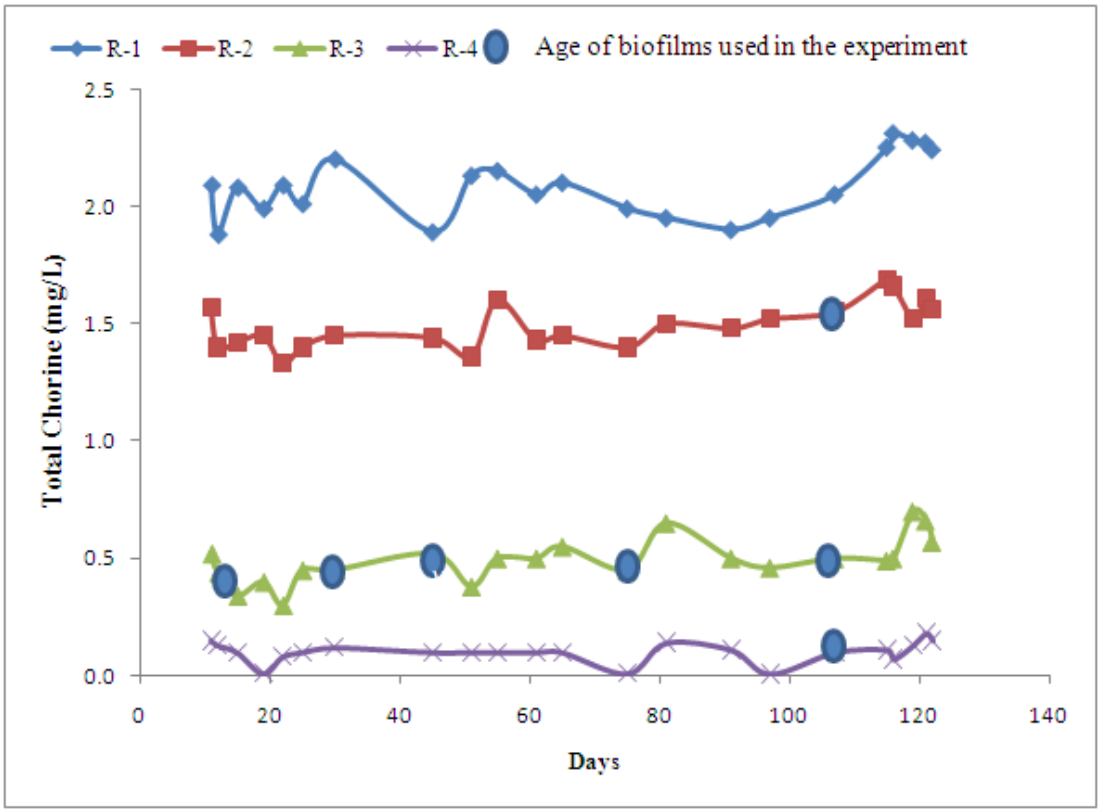

Figure 4.1(b): Total chlorine residuals along the reactors (R-1 to R-4) during the experiments periods.

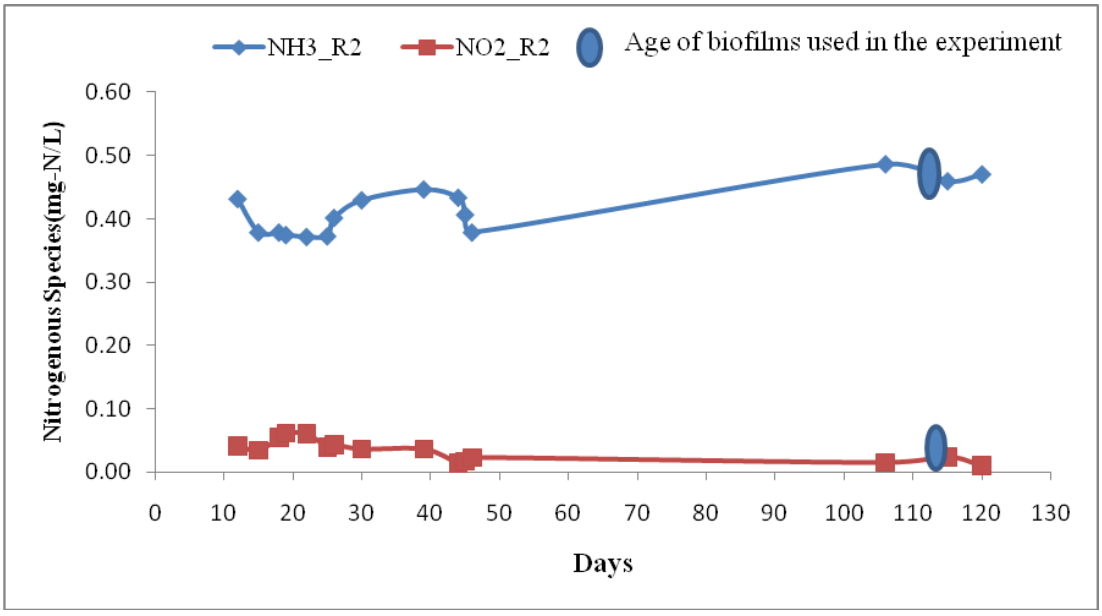

Figure 4.1(c): Nitrogenous species in R-2 during the investigation periods.

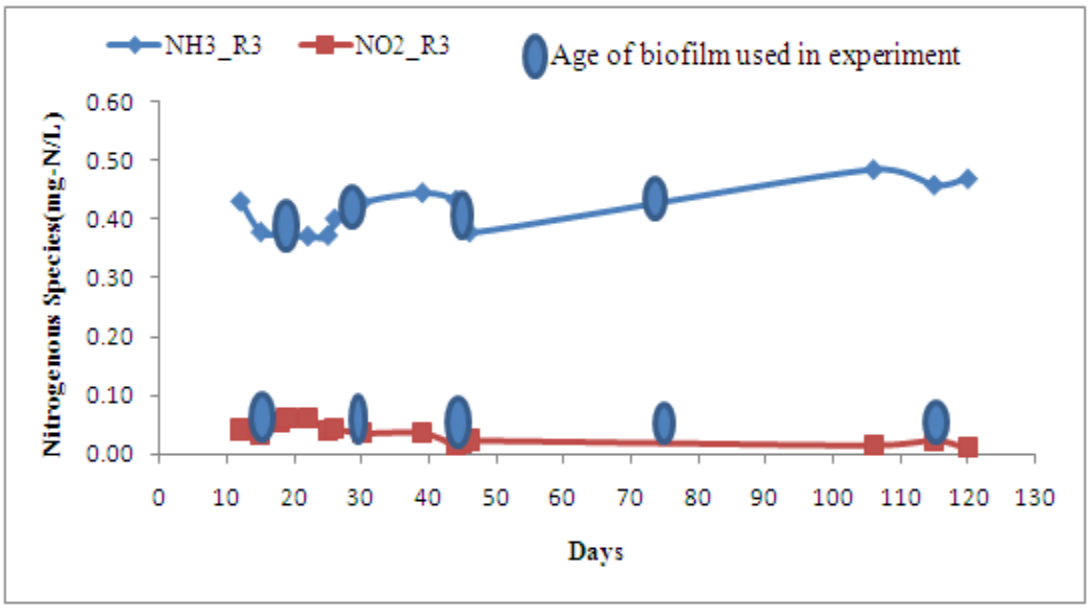

Figure 4.1(d): Nitrogenous species in R-3 during the investigation periods.

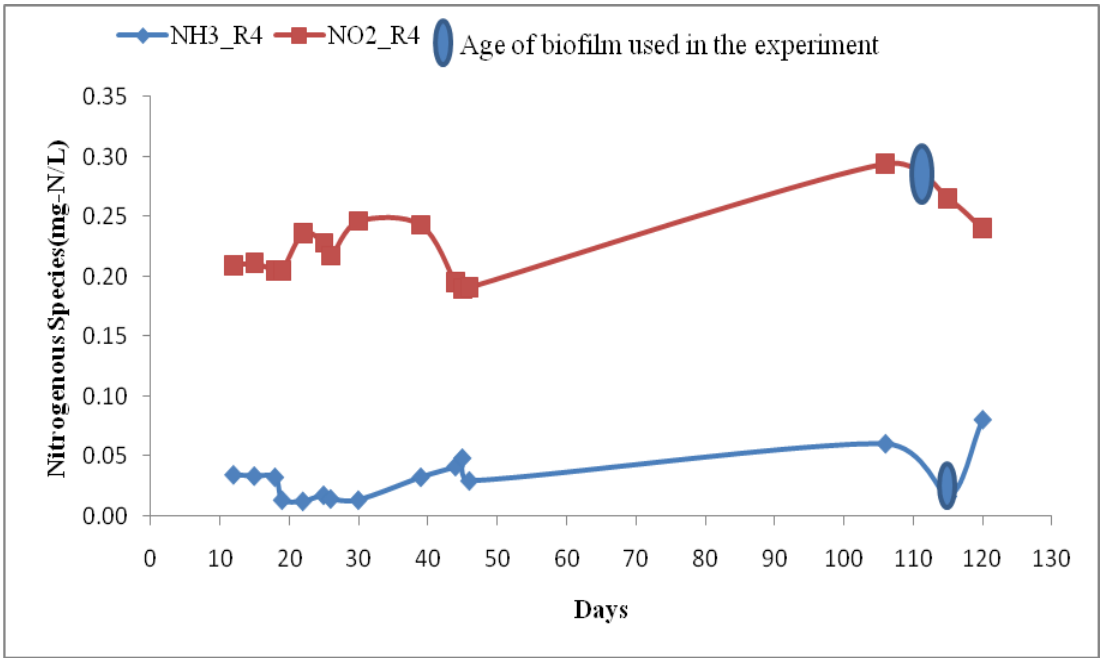

Figure 4.1(e): Nitrogenous species in R-4 during the investigation periods.

4.1.2.2 Microbial Decay Factor (F_m) along the Reactors: Chemical (k_c) and Microbial Chloramine Decay Coefficients (k_m)

Chloramine decay resulted from microbial activities and chemical reactions were analysed using F_m method. Determined F_m related parameters are presented in Table 1. No microbial activities (k_m value zero) were observed in R-1 and chemical reactions were the major cause of chloramine loss. Increased k_m from R-2 to R-4 demonstrated the increase in microbial activities with chloramine loss. Considerably high k_m was measured in R-4; it was probably a result of severe nitrification (Table 4.1). Similar to k_m, k_c also increased along the reactors. Increased nitrite levels (Figure 4.1(a)), decreased pH level (pH in R-3 and R-4 was decreased to 7.8 and 7.7, respectively from 7.95 in R-1 and 7.9 in R-2) and high temperatures could be the main reason of accelerated chloramine loss in R-3 and R-4. However, very high k_c was observed in R-3 and R-4 (Bal Krishna and Sathasivan, 2010). They further reported that high decay coefficients were resulting from soluble microbial products or dissolved organic compounds. However, the reason is not of concern for current work. Therefore, further experiments were conducted to understand how those biofilms grown under contrasting environment impact chloramine decay.

Table 4.1: Microbial Decay Factor, F_m related Parameters

Reactors	Total decay coefficient (k_{tb})	Chemical decay coefficient (k_c)	Microbial decay coefficient (k_m)	Microbial decay factor (F_m)
	hr^{-1}			
R-1	0.002±0.0004	0.002±0.0002	0.000	0.00
R-2	0.0052±0.0002	0.0038±0.0006	0.0014	0.37
R-3	0.0497±0.007	0.0399±0.007	0.0098	0.24*
R-4	0.080±0.010	0.0556±0.009	0.0244	0.44*

* Although k_m increased many folds in R-3 and R-4, due to nature of definition, lower F_m results were obtained.

4.1.2.3 Effect on Chloramine Decay by Biofilms Grown under Various Nitrification Conditions

In this study, biofilms was allowed to grow on PVC coupon in different reactors (R-2 to R-4). Each reactor (R-2 to R-4) represents a different nitrifying condition (mild to severe nitrification) which have various chloramine residual (1.5 mg/L to nearly 0 mg/L) as mentioned in Figure 4.1(a). In this experiment, coupons were placed in sample bottles having chloraminated water and chloramine decay was monitored in order to understand the effect of biofilms grown under different nitrifying environment. Sample (115 days old biofilms) was taken from R-2, R-3 and R-4 respectively and 2 mg/L of chloramine was adjusted in all samples in the beginning. Then , ratio of TCL:TAN to 4.5:1 at pH 8 was maintained for all these samples. The details of reactor's condition during the growth of biofilms is presented in Figure 4.1(b to e).

As presented in Figure. 4.2(a), 1.47, 1.65 and 1.74 mg/L chloramine was consumed within the 42.5 hrs in R-2, R-3 and R-4 respectively indicating rate of chloramine consumption is increased with nitrification condition (mild to severe). Therefore, biofilms grown under severly nitrifyinig condition has higher impact on chloramie decay in R-4 rathaer than R-2 and R-3. However, it should be noted that there is insufficient data to calculate the decay coefficient. Hence futher experiments are needed to conclude.

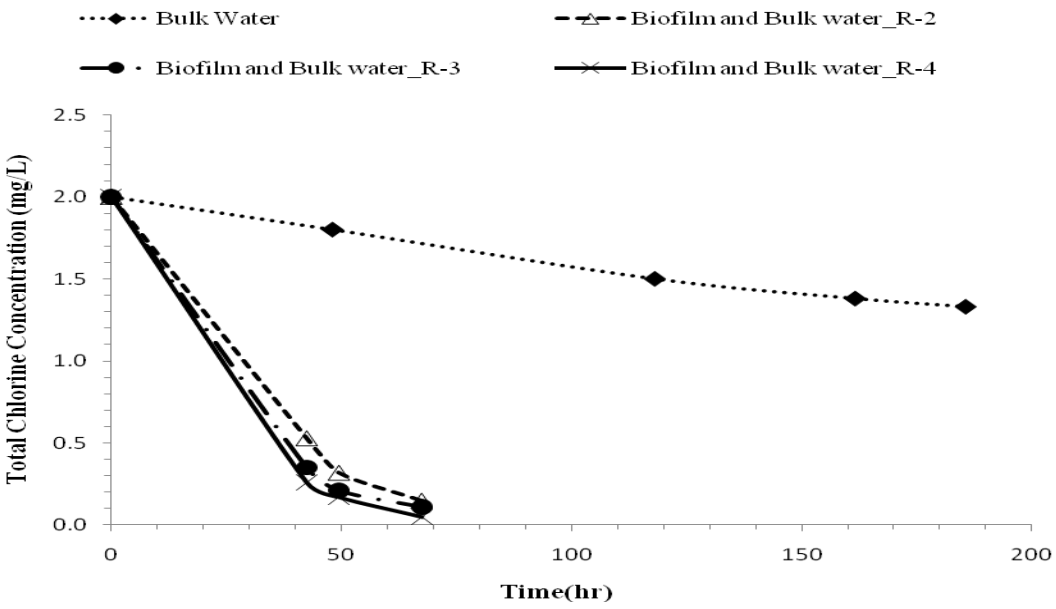

Figure 4.2(a): Impact of 115 days old biofilms grown under various nitrification conditions at 2.0 mg/L

Similarly, as presented in Figure 4.2(b) the rate of chloramine consumption in R2 and R3 samples were same, but in R4 it was very high (as noted from the residual at 42.5 hrs. Table 4.2 illustrates the details of drop in chloramine residual when initial chloramine concentration was topped up to 2.0 and 4.0 mg/L. The experiment was also undertaken with 15 days old biofilms that was grown under similar condition. The chloramine decay was measured in regular interval. These results also indicate higher impact of biofilms on chloramine in severe nitrigfying condtion rather than mildly nitrifiying condition (Figure 4.2(c)).

At the same time, chlorine residual along the reactor was also changing as the nitrification progressed from mild to severe condition. Residual of chloramine was nearly 1.5 mg/L in mild condition (R-2) and dropped close to 0 mg/L when it reached severe nitrification condition (R-4) as clearly mentioned in section 4.1.2.1. This result interestingly indicates that impact of biofilms on severely nitrifying (R-4) condition is higher than mildly nitrifying (R-2) condtion in terms of chloramine decay. However, further investigation is needed prior to reach to this conslusion.

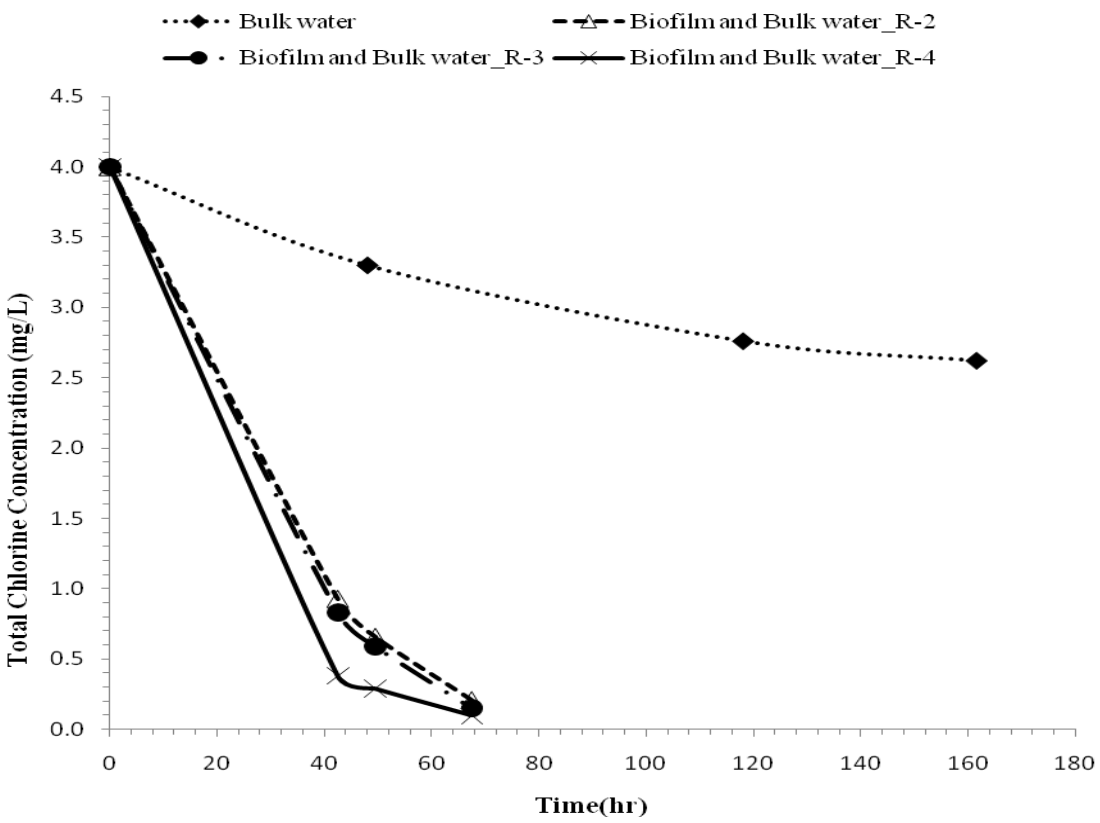

Figure 4.2(b): Impact of 115 days old biofilms grown under various nitrification conditions at 4.0 mg/L

Table 4.2: Drop in Chloramine Residual when Initial Chloramine Concentration was Topped up to 2.0 mg/L and 4.0 mg/L

Time	Origins of Samples					
	R-2		R-3		R-4	
	Drop in residual (mg/L)		Drop in residual (mg/L)		Drop in residual (mg/L)	
0	2.00	4.00	2.00	4.00	2.00	4.00
42.5	1.47	3.07	1.65	3.17	1.74	3.62
49.5	1.68	3.34	1.79	3.41	1.83	3.71
67.5	1.85	3.79	0.11	3.85	1.95	3.9

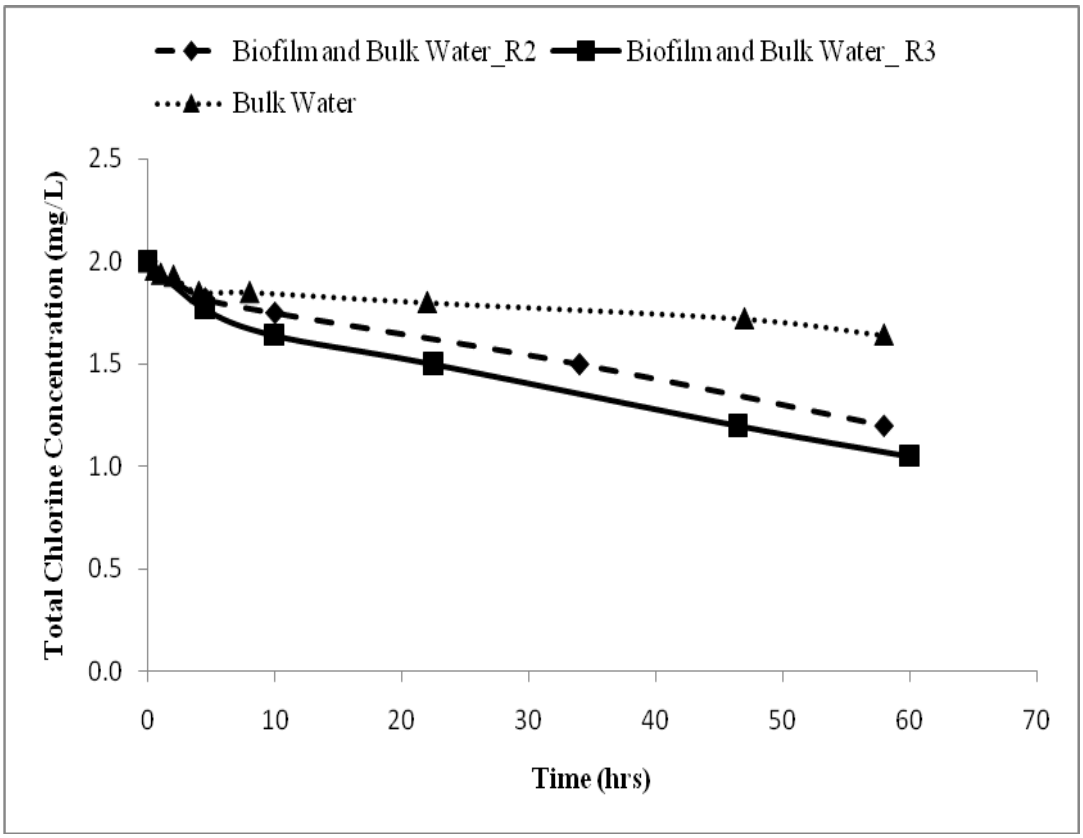

Figure 4.2(c): Impact of 15 days old biofilms grown under various nitrification conditions at 2.0 mg/L

Figure 4.3 illustrates the chloramine decay profiles only for base material (PVC and HDPE coupons) and bulk water. Same chloramine decay obtained in bulk water and base material clearly indicated that pipe material does not demand additional chloramine. This concludes that any chloramine demands obtained in the biofilms grown samples are result of biofilms grown in PVC and HDPE materials, not the materials itself.

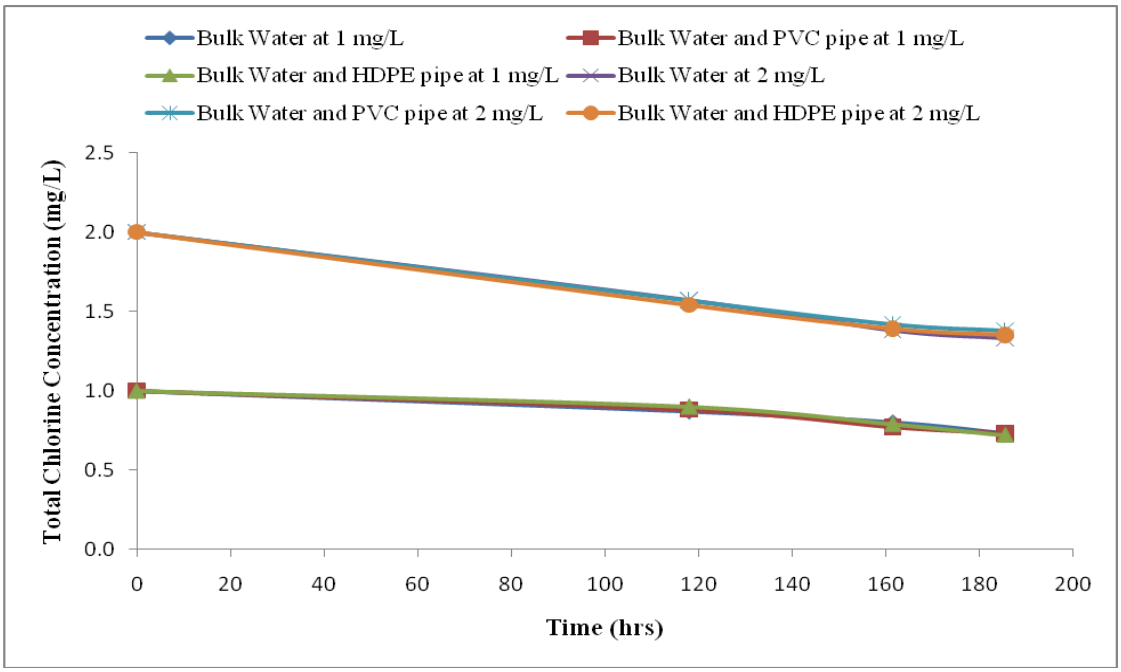

Figure 4.3: Impact of PVC and HDPE pipe material on chloramine decay in chloramine residual 1.0 mg/L and 2.0 mg/L

4.1.3 Conclusions

From this experiment following conclusions can be made.

1. Impact of biofilms grown under severely nitrifying condition is higher than mildly nitrifying condition.
2. There is no contribution from base material (PVC and HDPE) of pipe on chloramine decay.

4.2 Impact of Different age of Biofilms Grown at PVC and HDPE Coupons on Chloramine Decay

The effect of biofilms grown on various materials was investigated using PVC and HDPE coupons with different ages of biofilms. This was basically done in order to understand if there is an additional special effect of various materials on the formation of biofilms and their respective impact. At the same time, these two pipe materials were investigated with various ages of biofilms as well.

4.2.1 Biofilms on PVC Coupons

As presented in Figure. 4.4(a), five different ages of biofilms grown on PVC coupons (e.g. 15, 30, 45, 75 and 105 days) were taken in this experiment to understand the impact of biofilms on chloramine decay in chloraminated system (Refer Figure 4.1(b – e) for detail conditions of reactor during this period). Results illustrated that effect of biofilms on chloramine decay depends on their age. Higher impact of biofilms was observed when they were 30 and 105 days old. On the other hand, the lowest effect was noticed when it was 75 days old. Results indicate that after a certain time of growth, impact of biofilms on chloramine reaches the maximum. Afterwards, it sloughs from the surface. This is in agreement with previous studies that biofilms are dynamic microenvironments, encompassing processes such as metabolism, growth, and product formation, and finally goes through detachment, erosion, or sloughing of the biofilms from the surface (Characklis and Marshall, 1990).

In order to understand this dynamic process of biofilms growth, it was investigated in regular interval as presented in Figure 4.4(b). It clearly illustrates a regular cycle of biofilms formation and its impact on chloramine decay. The impact of biofilms increased with age until 30 days. Then, it again decreased gradually until 75 days. The higher impact again re-appeared in a 105 days old biofilms. This clearly indicates that formation of biofilms goes through a cycle which could be with a fixed or varied period. This is in agreement with the previous studies that found highest impact in chloramine decay at 45 days (Chandy and Angles, 2001). However, further

experiments are needed in regular interval for the given condition of same nutrient level and other affecting parameters.

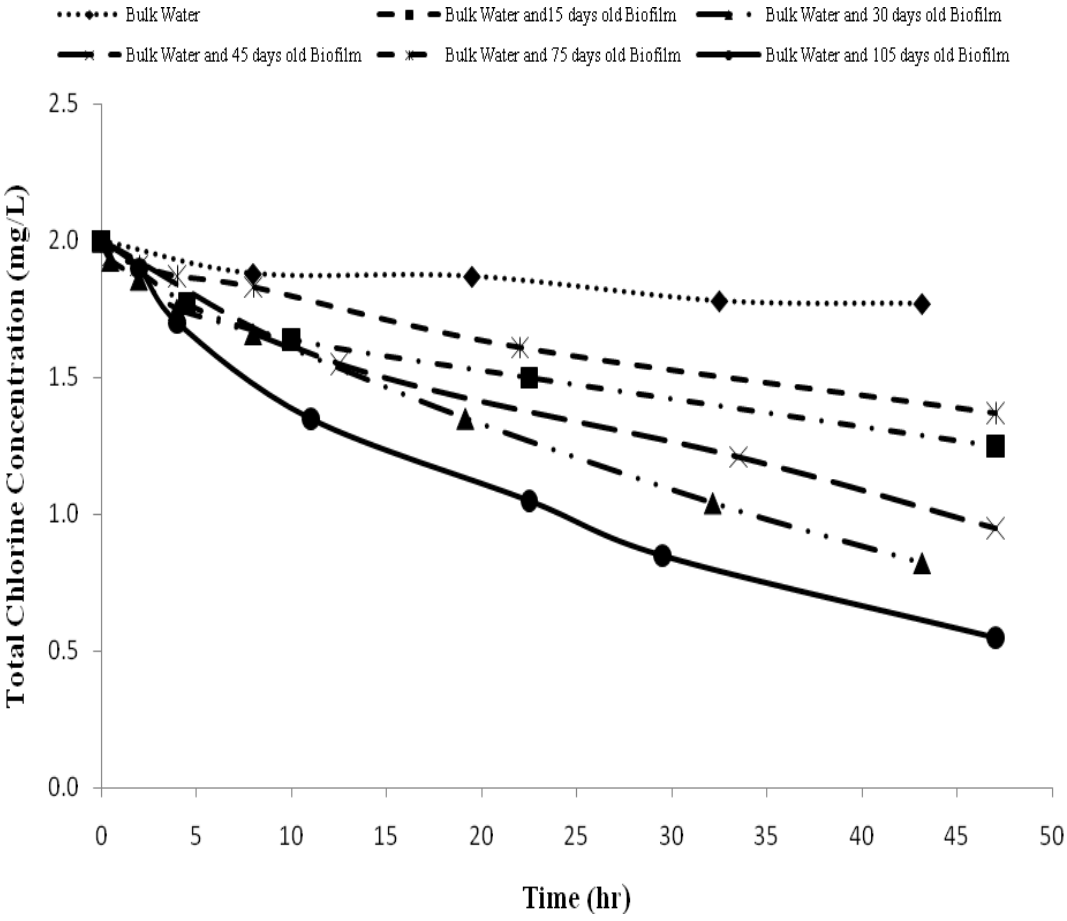

Figure 4.4(a): Impact of different age of biofilms grown on PVC coupons on chloramine decay when an initial chloramine residual 2.0 mg/L was used in batch test

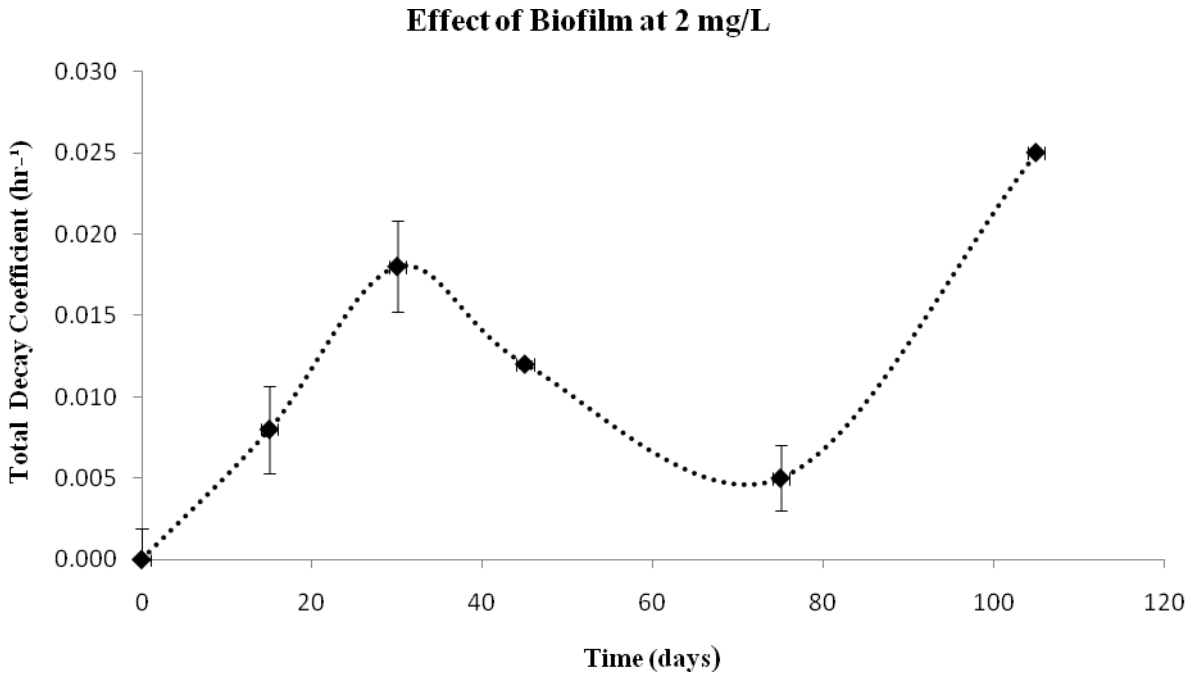

Figure 4.4(b): Impact of different age of biofilms for chloramine decay rate by biofilms grown on PVC coupons on chloramine decay when an initial chloramine residual 2.0 mg/L was used in batch test

4.2.2 Biofilms on HDPE Coupons

Similarly, Figures 4.5(a) and 4.5(b) show the impact of biofilms grown on HDPE coupons on chloramine decay. Total decay coefficient, nearly 0.006±0.003 hr^{-1}, was noticed from a 15 days old biofilms. This was again investigated with the 30 and 75 days old biofilms, but the chloramine decay rate remained roughly constant at 0.005± 0.001 hr^{-1} and 0.004±0.001 hr^{-1} respectively. However, the impact increased significantly for a 105 days old biofilms. Total decay coefficient for 105 days old biofilms was observed 0.019±0.001 hr^{-1} which is nearly 3 times more than both 30 and 75 days old biofilm. This could be the result of biofilms cycle on HDPE coupons as presented in Figure 4.5(b). Its impact on chloramine decay increased gradually up to 75 days and substantially at 105 days. This feature is similar as obtained in PVC coupons although it took longer time to develop biofilms in HDPE.

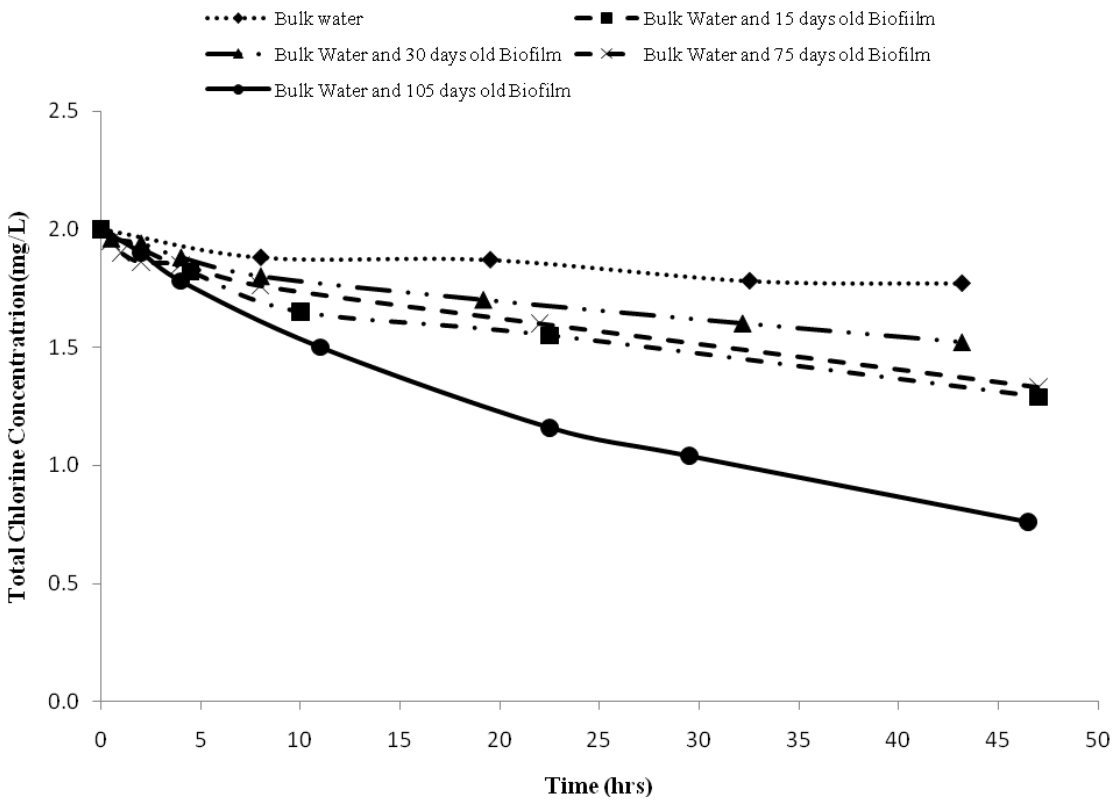

Figure 4.5(a): Impact of age of biofilms grown on HDPE coupons on chloramine decay when an initial chloramine residual 2.0 mg/L was used in batch test

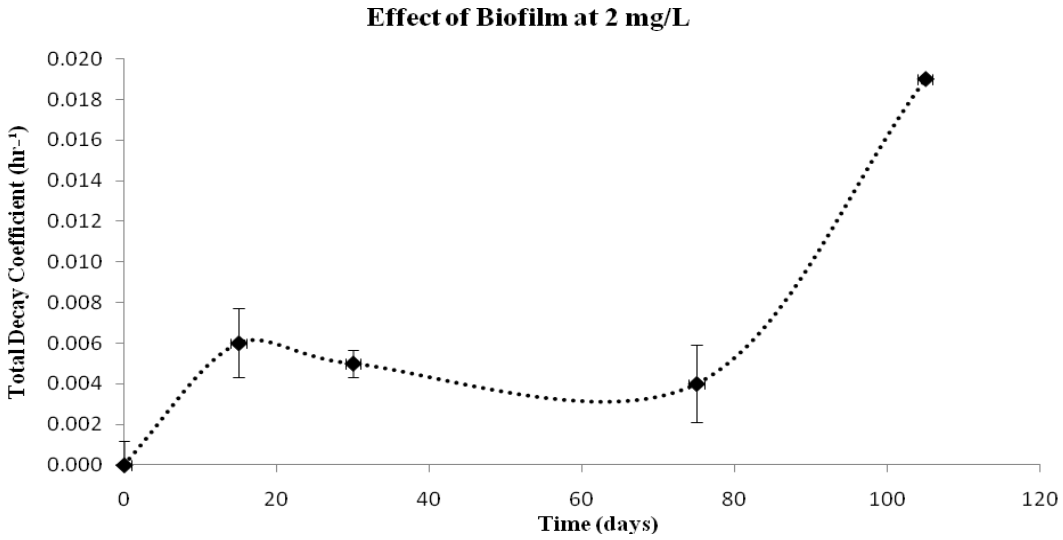

Figure 4.5(b): Effect of the different age (15, 30, 75 and 115 days) of biofilms for chloramine decay rate by biofilms grown on HDPE coupons when an initial chloramine residual 2.0 mg/L was used in batch test

4.2.3 Comparison of impact of biofilms between PVC and HDPE pipe coupons

In Figure 4.6, 15 days old biofilms grown on PVC coupon decay rate was 0.009±0.0019 hr^{-1}. Similarly, for a biofilms grown on HDPE coupon with the same age, decay rate was 0.006±0.001 hr^{-1} with a marginal impact of biofilms on chloramine decay. However, the impact was significantly higher in PVC biofilms (0.019±0.001 hr^{-1}) compared to HDPE biofilms (0.005±0.001 hr^{-1}) for 30 days old biofilms. This is in agreement with the previous studies that higher biofilms potential was observed in PVC pipes with greater affinity than in glass and cement pipes (Hallam et al., 2001). Such affinity towards certain pipe material finally leads to different length of cycle for biofilms formation and sloughing. This could be one of the main reasons that, the PVC coupons biofilms impact peaked at 30 days but in HDPE coupons it was observed only after 75 days. Thus, the result indicates that biofilms grown on PVC coupons has significantly a shorter cycle than HDPE.

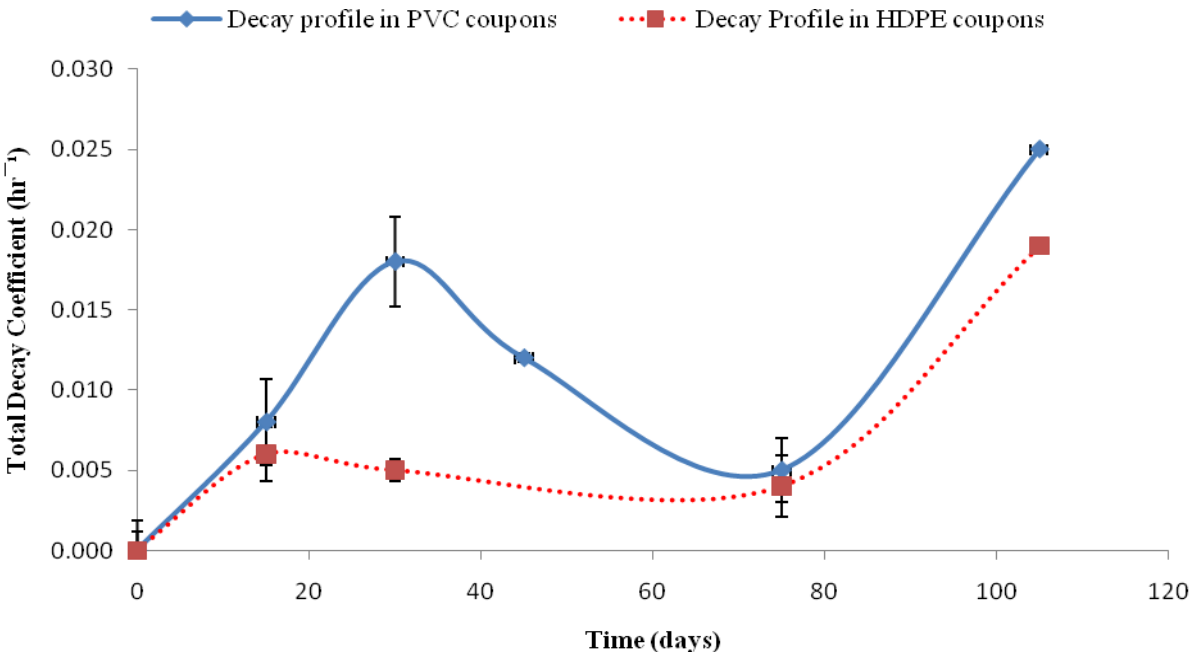

Figure 4.6: Total decay rates for biofilms grown on two different coupons (PVC and HDPE) and when initial chloramine residual in the batch test was 2.0 mg/L (See Appendix 7 for chloramine residual in R-3 during the biofilms formation).

4.2.4 Conclusions

From this study, the following conclusion can be made

1. Impact of biofilms on chloramine decay depends on the age of the biofilms.
2. Growth of biofilms on PVC is faster than HDPE coupons.

4.3 Impact of Biofilms on Chloramine Decay at various Temperatures

Previous experiments illustrated no significant impact of various ages of biofilms on chloramine decay. It was driven to examine the effect of biofilms on chloramine decay at different temperature. Thus, this experiment was investigated under 20 ˚C and 25 ˚C. The same age of biofilms (30 days old) from R-3 with the same topped up chlorine residual (2.0 mg/L), TCl:TAN ratio 4.5:1 and pH 8 was maintained in order to understand the effect of temperature alone. The source water itself (Mundaring water) was used in this experiment.

Figure 4.7 and 4.8 demonstrate that impact of biofilms on chloramine decay under various temperatures. Initially, investigation was undertaken at 20 ˚C. The initial 2.0 mg/L dose of chloramines reduced to half within 50 hrs with total decay coefficient of 0.016±0.004 hr^{-1}. Samples were then investigated at 25 ˚C. It was observed almost same 0.029±0.005 hr^{-1} for 25 ˚C. Biofilms has a less impact on chloramines decay at 20 ˚C as compared to 25 ˚C.

Similarly, Figure 4.9 shows the temperature dependence of microbial decay for sample. In this study, the E/R values for microbiological decay 10,303 k^{-1} was observed. This is very close to calculated E/R value for microbial decay 6,924 ± 1700 k^{-1} (Sathasivan et al., 2009). Therefore, this result indicates that impact of biofilms increases with temperature. However, further investigation with different temperature is recommended to understand its effect.

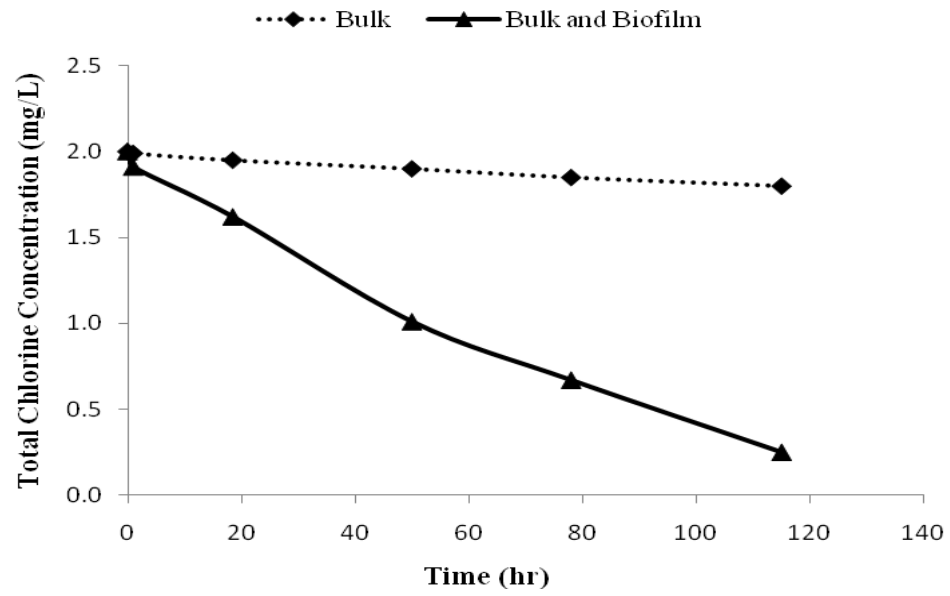

Figure 4.7: Impact of 30 days old biofilms grown on PVC coupons on chloramine decay at temperature 20 ˚C when an initial chloramine residual 2.0 mg/L was used in batch test

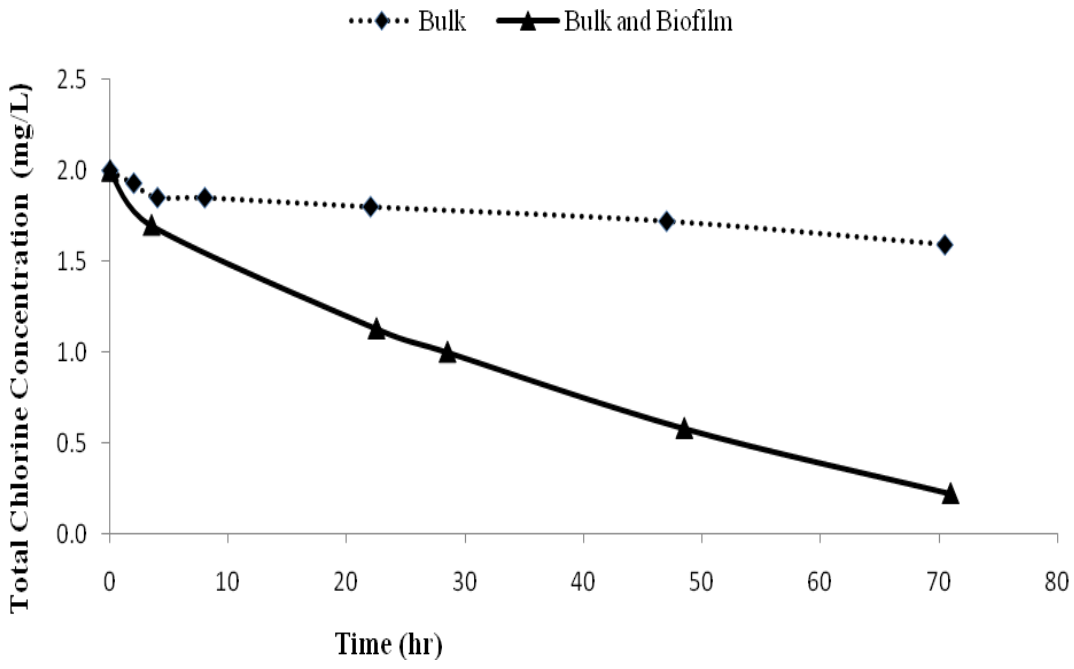

Figure 4.8: Impact of 30 days old biofilms grown on PVC coupons on chloramine decay at temperature 25 ˚C when an initial chloramine residual 2.0 mg/L was used in batch test

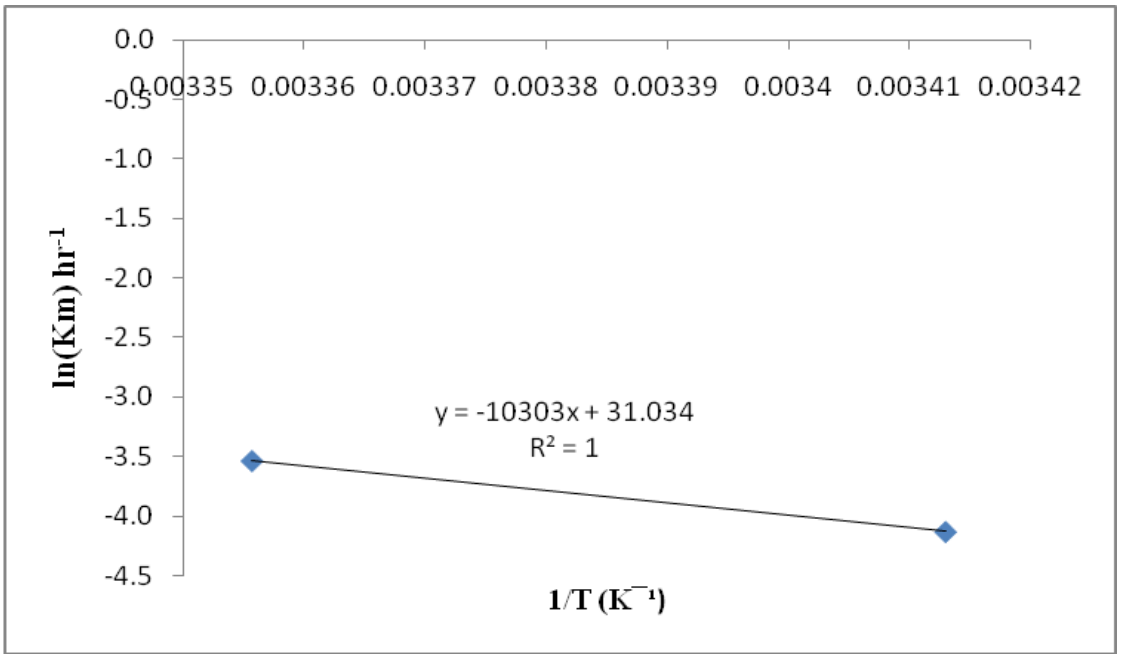

Figure 4.9: Plot of ln km vs 1/T for microbiological decay coefficient

4.3.1 Conclusion

This study helps to draw the conclusion that the impact of biofilms on chloramine decay is directly proportional to the temperature within the tested range.

4.4 Behaviour of Biofilms at Different Initial Chloramine Concentrations used in Batch Test

4.4.1 Biofilms at Different Initial Chloramine Concentration used in Batch Test

The impact of various chloramine concentrations on biofilms was also investigated in terms of chloramine decay. The investigation was undertaken with the biofilms that was grown on PVC coupon. This experiment was undertaken in a biofilms grown in R-3 for a wide range of initial chloramine (2.0 – 16.0 mg/L). Mundaring water was used for this experiment as bulk water and TCl:TAN ratio 4.5:1 and pH 8 was maintained.

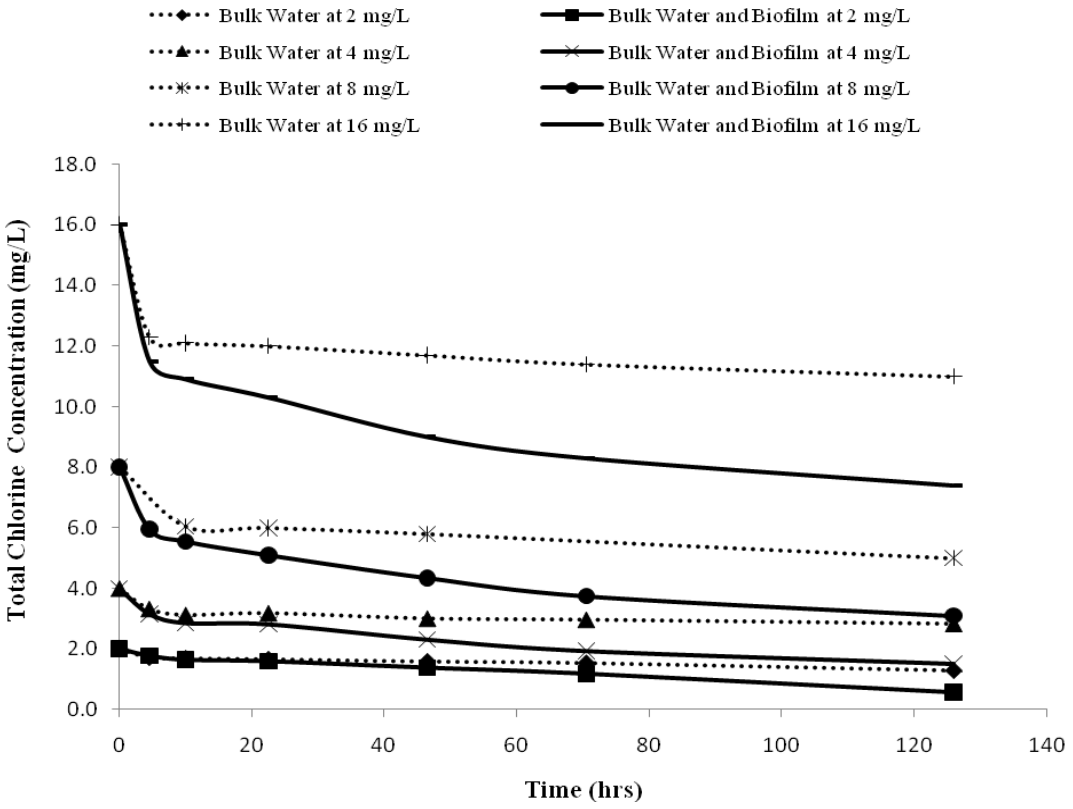

Figure 4.10(a): Impact of various chloramine residual (2.0 mg/L to 16.0 mg/L) on 15 days biofilms grown on PVC coupons under less than 0.6 mg/L chloramine residual on chloramine decay and the temperature of batch test was 20 ˚C

Initially, the experiment was undertaken with a 15 days old biofilms for fore mentioned initial chloramine concentrations. As presented in Figure 4.10(a), the total decay coefficient for the lowest dose (2.0 mg/L) was found to be 0.009±0.001 hr^{-1}. This decay rate remained nearly constant at 0.008±0.003 hr^{-1} despite increase in chloramine dose by 8 times. Result showed that there is only marginal difference in total decay. This clearly indicates that effect of biofilms on chloramine decay does not depend on the topped up chloramine concentration. Again here it should be noted that frequency of chlorine measurements are not sufficient to conclude.

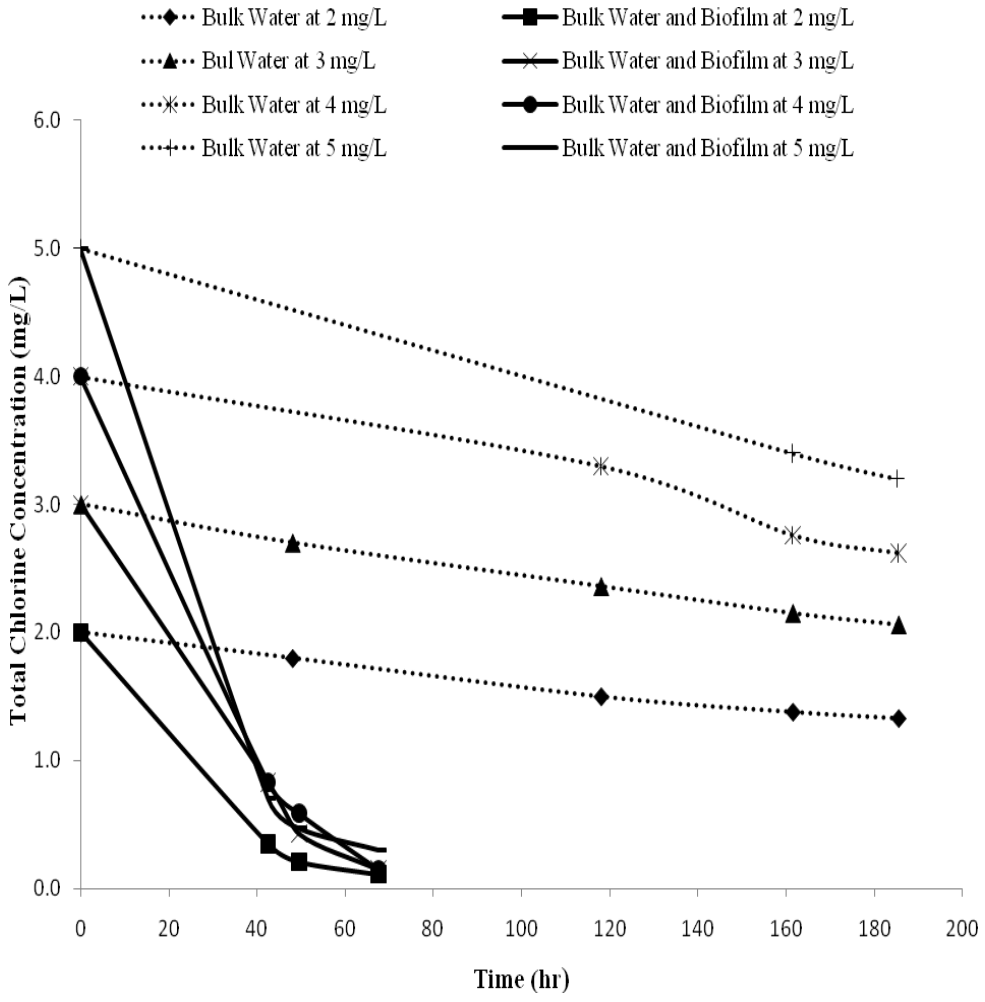

Figure 4.10(b): Impact of various chloramine residual (2.0 mg/L to 5.0 mg/L) on 115 days biofilms grown on PVC coupons under less than 0.6 mg/L chloramine residual on chloramine decay and the temperature of batch test was 20 ˚C

To ensure this, further experiment was undertaken with biofilms of much older age (115 days old). This was investigated within a narrow range (2.0 – 5.0 mg/L) of initial chloramine residual. Concentration of topped up chloramine was increased gradually to understand the impact of biofilms on chloramine decay. Initially, 2.0 mg/L was selected and followed by 3.0 mg/L up to 5.0 mg/L. As presented in Figure 4.10(b), within 70 hrs of chloramination, chlorine residuals reduced to nearly 0 mg/L in all samples. This clearly indicates that chloramine demand is directly proportional to the initial chloramine concentration. Furthermore, similar effect of biofilms was observed on chloramine decay in all different concentration (2.0 to 5.0 mg/L). Total decay rate of 0.043± 0.009 hr^{-1} was obtained in both 2.0 and 4.0 mg/L. Similarly, the same decay rate was observed in rest of them. If experimental errors are considered, there is no significant difference on chloramine decay if the concentration of chloramine was 2.0 or 5.0 mg/L.

Chloramine residual in R-3 was less than 0.6 mg/L since December 2009 and biofilms were grown under this condition. If the microbes only were responsible for accelerating the chloramine, they could have been killed or inactivated with shock dosing of higher chloramine concentrations. These experimental results suggest a similar impact for accelerating chloramine irrespective of various disinfectant levels. This could be a result of biofilms with the same characteristics (in terms of chloramine demanding agents) independent of their chlorine residual in distribution system or also could be the result of severely nitrifying condition.

4.4.2 Conclusions

This experiment indicates that contribution of biofilms on chloramine decay could not be changed significantly by increasing the chloramine residual in bulk water.

CHAPTER 5

Quantification of The Role of Bulk Water Reactions, Sediments and Biofilms on Chloramine Loss

5.1 Continuously Flowing System (Reactors or Reservoirs)

Bulk water reactions, bacterial activities in biofilms, sediments and stratification are the major niches contributing to chloramine loss in service reservoirs or in reactors (Sathasivan et al., 2010). However, the possibility of thermal stratification was minimal in laboratory scale distribution systems due to several reasons. These were less water depth (about 18 to 24 cm), manual mixing of water as detailed in earlier section, outlet and inlet geometry (out let at the top and inlet at the bottom) and placement of heater at the bottom. Therefore, total chloramine decay coefficients due to all reactor contents (bulk water reactions, bacterial activities from sediments and biofilms) was calculated using Equation 5.1 as defined by Sathasivan et al., (2010).

$$TCl_{out} = \frac{TCl_{in}}{(1 + k_{R_t}\theta)} \qquad (5.1)$$

Where, TCl_{out} and TCl_{in} are outlet and inlet chloramine residual (as total chlorine) respectively, k_{Rt} is the total chloramine decay coefficient of all reactor contents and θ is the retention time in the reservoir.

5.1.1 Batch System

Chloramine decay resulting from bulk water reactions was determined by collecting the grab bulk water samples from inside the reactors and carrying out the chlorine decay test (without changing the chloramine residuals) by maintaining the same temperature as in the reactor. Bulk water decay coefficient was determined using Equation 5.2.

$$TCl_{(b)} = TCl_{o(b)}.\exp^{-k_{tb}*t} \quad (5.2)$$

Where, $TCl_{(b)}$ is the total chlorine residuals measured at each consecutive time, t and $TCl_{o(b)}$ is the initial total chlorine residual, k_{tb} is the total chlorine decay coefficient due to bulk water reactions. Subscript '*b*' refers to the bulk water.

In the literature, there was no specific method proposed to determine the direct impact of sediments in the service reservoir or in laboratory scale reactors on chloramine loss. To quantify the impact of sediments duplicate PET sample bottles were filled with bulk water (500 ml). Sediments collected from the bottom of the same reactor from where bulk water had been collected were considered as one sample whereas another sample was taken without sediment. For example, sediments collected from R-2 were kept in sample bottles containing bulk water from R-2. If the chloramine residual was less than 1.0 mg/L in bulk water, then it was topped up to 1.0 mg/L as total chlorine. All samples were incubated in a dark water bath, maintaining the same temperature as in the reactor. Impact of combined bulk water and sediment $(k_{t(se+b)})$ and only bulk water (k_{tb}) were determined using Equation 5.2. Subscript '*se*' refers to the sediments. Differences between $k_{t(se+b)}$ and k_{tb} gives the impact of sediments as presented in Equation 5.3.

$$k_{tse} = k_{t(se+b)} - k_{tb} \quad (5.3)$$

Moreover, the impact of sediments depends on sediments concentration. It was measured as suspended solid in both batch test and in reactors.

5.2 Conversion from Batch System to Continuous Flow System (Reactors or Reservoirs)

Chloramine decay coefficient, $kt_{(w+se)R}$ due to combined effect of sediments and biofilms in reactors (continuous flow system) was quantified using k_{Rt} and k_{tb}

determined using Equations 5.1 and 5.2 as presented in Equation 5.4. Subscript '*R*' refers to the reactor.

$$k_{t(se+w)R} = k_{Rt} - k_{tb} \qquad (5.4)$$

Standardising the same sediments concentration in both batch tests and in reactors from where sediments and bulk water sample collected, chloramine decay due to sediments presence in the reactor was determined as presented in Equation 5.5.

$$k_{t(se)R} = k_{t(se)*} M_R / M \qquad (5.5)$$

Where, $k_{t(se)R}$ is the decay coefficient due to the sediments in the respective reactor, M_R is the sediments concentration in the respective reservoir or reactor, M is sediments concentration in batch test conducted for the same reactor or reservoir. Additional decay in the reactor, beside bulk water reactions and sediments bacterial activities, is due to biofilms bacterial activities. Therefore, biofilms impact on chloramine loss in the reactors was determined using Equation 5.6.

$$k_{t(w)R} = k_{t(w+se)R} - k_{t(se)R} \qquad (5.6)$$

Where, $k_{t(w)R}$ is chloramine decay coefficient due to biofilms in reservoir. The same principle and equations can be applied in completely mixed full scale service reservoirs.

Converting the surface area of biofilms to water volume ratio of batch test same as the reactor from where biofilms and bulk water sample collected, chloramine decay due to biofilms presence in the reactor can be determined as presented in Equation 5.7.

$$k_{t(w)R} = k_{tw} * \frac{(S/V)}{(a/v)} \qquad (5.7)$$

Where, S is the biofilms area (summation of water contact area in reservoir or reactor), V is the volume of water. Similarly, s is the biofilms area in batch test, v is water volume in batch test.

5.3 Result and Discussion

5.3.1 Quantifying Effect of Biofilms and Sediments on Chloramine loss in Tanks of Laboratory Scale System

Each reactor (R-1 to R-4) represents a different nitrifying condition (none to severe nitrification) while having various chloramine residual (2.1 mg/L to nearly 0 mg/L) as mentioned in Section 4.2.1. In chloraminated systems, disinfectant can be impacted by microbial activities and chemical reactions in bulk water, sediments and biofilms. It is generally believed that biofilms and sediments provide safe shelter for microbes, but their impact on chloramine loss has not been quantified. Thus, this experiment investigates to understand the impact of sediments and biofilms on chloramine decay.

5.3.2 Combined Role of Biofilms and Sediments on Chloramine Loss

Beside bulk water reactions (chemical reactions and microbial activities), there could be significant effect of combined sediments and biofilms in the reactors because the possibility of thermal stratification was minimal. To maintain decent chloramine residual and to target critical niches, their roles have to be separated. As mentioned in the section 5.2, Equations 5.1, 5.2 and 5.4 were used to determine the role of reactor contents (total decay), bulk water reactions and combined sediments and

biofilms impact on chloramine loss in reactors, respectively. Results are demonstrated in Figure 5.1.

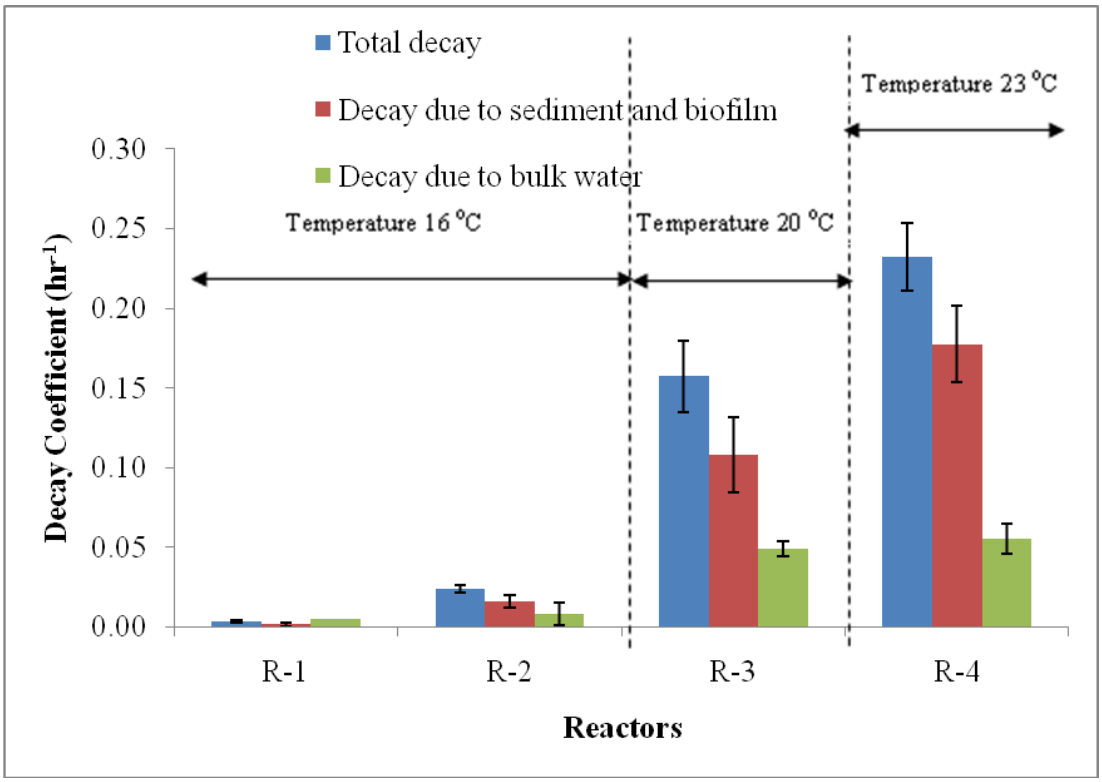

Figure 5.1: Chlorine decay coefficients due to reactor contents (total decay), bulk water reactions and combined effects of sediments and biofilms (calculated as the difference between the former two)

Total chlorine decay within the reactor (k_{Rt}), decay resulting from combined biofilms and sediments ($k_{t(w+se)R}$) and bulk water (k_{tb}) along the reactors increased with increase in bacterial activities or decrease in chloramine(Figures 4.1a and 5.1). Bulk water contribution was minimal in R-1 and R-2. Because of onset of severe nitrification in R-3 and R-4, significant impact of bulk water reaction was observed (Figure 5.1). Similarly, results demonstrated the minimal combined role of biofilms and sediments in R-1. It could be the result of less microbial population present in both biofilms and sediments, or the growth of biofilms was minimal due to higher chloramine residuals (2.1 mg/L) and low temperature maintained in R-1 than that in other reactors. In R-3 and R-4, significant contribution of sediments and biofilms

(about two to three times higher than bulk waters) was observed (Figure 5.1). Less chloramine residual presence may provide a favourable environment for biofilms formation in the surface of reactors in contact with water, as reported by Hallam et al., (2001). Moreover, sloughing of biofilms after its thickness reached maximum level could accumulate as sediments at the bottom, indicating possible presence of microbes in the sediments. In reactors (R-2 to R-4), compared with bulk water, both sediments and biofilms contributed more to accelerate chloramine decay. These results further reinforce the fact that it is very important to first understand the individual role of each niche before a proper strategy is designed.

5.3.3 Separation of Role of Biofilms and Sediments on Chloramine loss

There was a minimal combined effect of biofilms and sediments in R-1 (Figure 5.1). Therefore, further experiments were not conducted for R-1. However, it was higher in R-3 and R-4, although it was not clear if the biofilms or sediments play a dominant role. In order to quantify the role of sediments on acceleration of chloramine, batch tests were conducted in bulk water samples and sediments from reactors (R-2 to R-4), as described in Materials and Methods (Equations 5.2 and 5.3). For this sediments were collected from the bottom of each reactor, concentrated using centrifugation and reconstituted to a known concentration of suspended solids. Then, the decay rates were converted to the suspended solids concentration in the reactor for separation of the role of individual niches.

The experimental results showed the minimum effect of sediments in R-2 (k_{se}; 0.007±0.0013 hr^{-1}/90 mg/L). The effect of sediments was then analysed by converting the batch decay coefficient into continuous system using Eq.5. In the batch test suspended solid was 90 mg/L but in the reactor it was 18 mg/L. Assuming impact of sediments per mg/L on both batch and continuous systems is same; they were calculated for reactors R-2 to R-4 using Equation 5.5. In R-2, it was converted to be 0.0014±0.0013 hr^{-1} (Table 5.1). The results are presented in Table 5.1. Similar conversion was carried out for R-3 and R-4. Interestingly both R-3 and R-4 showed

similar impact after conversion to continuous systems, although they had different sediments concentration.

Table 5.1: Decay Coefficient due to Sediments in Continuous and Batch Systems

Reactors	Batch System		Continuous system	
	Decay coefficient (hr^{-1})	Suspended solids(mg/L)	Decay coefficient (hr^{-1})	Suspended solids(mg/L)
R-2	0.007±0.0013	90	0.0014±0.0013	18
R-3	0.052±0.001	100	0.0104±0.001	20
R-4	0.028±0.003	110	0.0091±0.003	36

Similar to batch test, chloramine decay due to sediments increased along the reactors from R-2 to R-4 (Figure 5.2). Although increased suspended solids could explain the increase in decay rates in R-3 and R-4, it does not explain the behaviour in R-2 because microbes present in R-2 were exposed to high chloramine residual (1.43 mg/L) compared to R-3 and R-4 (Figure 4.1). This might have prevented the growth or survival of microbes in this environment consequently lowering the impact of sediments.

Additional chloramine loss resulting from biofilms along the reactors was calculated using Equation 5.6. Prominent role of biofilms was observed in R-3 and R-4 as presented in Figure 5.2, whereas minimal effect was observed in R-2. Microbes in the biofilms of R-2 were exposed to significantly high chloramine residual compared with that in R-3 and R-4 (Figure 4.1). This might have prevented the growth or survival of microbes in biofilms, hence a lower acceleration. In contrast, low chloramine residuals in R-3 and R-4 provide a favourable environment for biofilms growth; therefore the biofilms impact was significantly higher in R-3 and R-4.

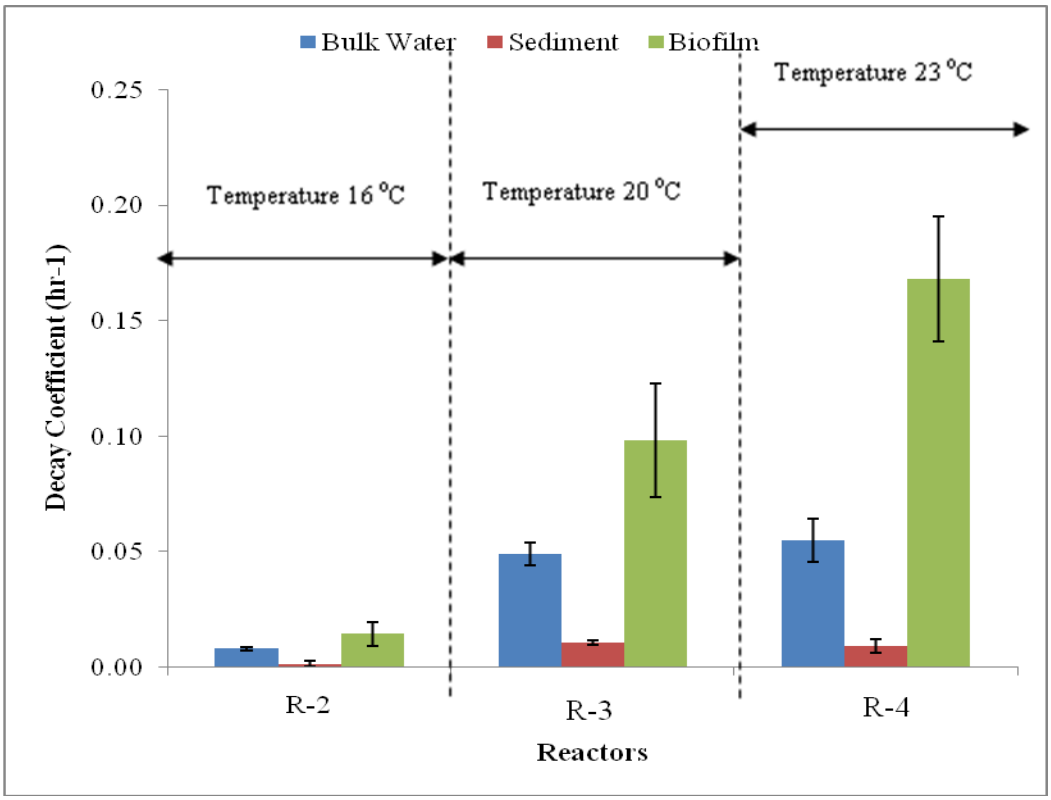

Figure 5.2: Details of chlorine decay coefficients due to bulk water reactions, biofilms and sediments along the reactors (R-2 to R-4)

In conclusion, R-1, which contained higher chloramine residual (2.1 mg/L) didn't show any effect other than bulk water reactions. However, as water travelled from R-2 to R-4, chloramine residuals dropped and nitrification took place. Afterwards, impact of all reactors parameters (bulk water reactions and sediments and biofilms) contribution increased with retention time or with decreased chloramine residual.

It is generally believed that biofilms is the major cause of degrading disinfection in water distribution systems. However, experimental result showed no substantial difference in the effect of bulk water and biofilms in R-2, which could be a result of high chloramine residual present in it. Result showed the impact of biofilms is 2 times higher in R-3 and 3 times higher in R-4 than in bulk water. It indicates that impact of biofilms along the reactors increased with decrease in chloramine residual.

5.3.4 Practical Implications of Impact of Suspended Solids on Chloramine Decay

The results are presented in Table 5.1. The decay rate greatly varied across each reactor. It can be seen that not only the decay rate but also the sediments concentration in each batch samples were different. In order to compare between different reactors it is important to quantify the contribution of the sediments per 100 mg ss/L. Result in Figure 5.3 showed that effect of sediments is minimal in mild nitrification (R-2) as compared to severe nitrification (R-3 and R-4). This could possibly be a result of high chloramine residual in R-2 that may have suppressed the bacterial growth in sediments. On contrary, higher impact of sediments was obtained in R-3 than in R-4. The chloramine residual in R-4 was close to zero (Figure 4.1(a)), but R-3 was experiencing onset of severe nitrification where nutrient levels, including free ammonia (Figure 4.1(a)), can be expected to be higher, as this is the first time in the reactor the microbes are allowed to grow freely. In the R-4, however, it has substantially decreased due to heavy nitrification in R-3. In addition, R-4 bacteria in sediments are exposed to lower (zero) residual. During the batch test, a 1 mg/L residual chloramine was dosed, probably leading to minor shock on microbes on sediments.

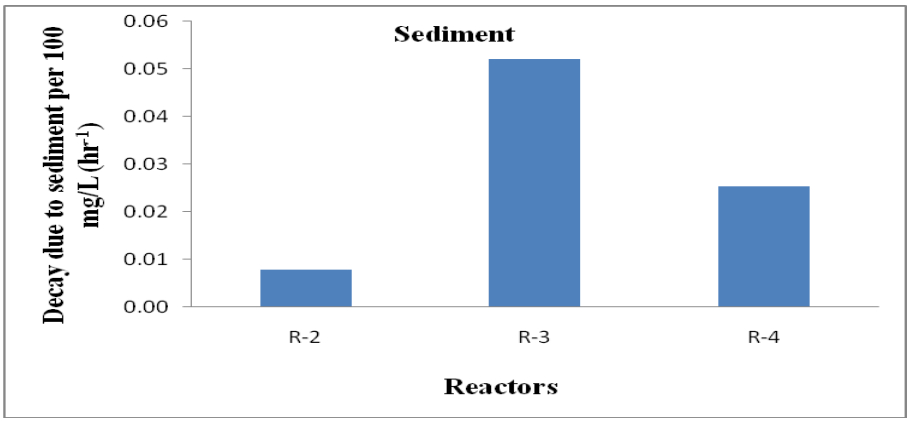

Figure 5.3: Impact of sediments in different nitrification condition

These results therefore imply that sediments in mildly nitrifying stages do not harbour microbes/compounds responsible for heavy acceleration of chloramine decay. In contrast, it is possible sediments can play a crucial role once nitrification is

onset or severe nitrification takes place. If sediments concentrations are known, water utility can take appropriate action in each stage.

5.3.5 Comparison of Biofilms Impact between Batch System and Continuous System

The analysis in continuous system showed that the effect of biofilms on chloramine decay is increased with advancement in nitrification from mild to severe condition. It also increased with decreased chloramine residual. However, in a batch test various nitrification condition and chloramine residual did not affect the chloramines decay (Figure 4.2).

In order to understand this phenomenon, impact of biofilms on chloramine decay was compared between the batch and continuous system. As batch system experiment showed highest decay rate (0.019 ± 0.0019 hr^{-1}) in R-3 with 105 days old biofilms (HDPE pipe), it was considered for the analysis. While, in a continuous system, Equation 5.1 and 5.2 were used to calculate the effect of biofilms in R-3.

The maximum total decay coefficient in batch system was obtained as 0.019 ± 0.0019 hr^{-1}. The effect was then analysed by converting the batch decay coefficient into continuous system using Equation 5.7. However, it showed a larger decay coefficient 0.1730 ± 0.0019 hr^{-1}. On the other hand, total decay coefficient from the biofilms in R-3 was obtained to be 0.0801 ± 0.0212 hr^{-1}, which is 2.1 times lower than the decay resulting from the maximum biofilms impact obtained for the batch system. This result indicates that biofilms has a higher impact on chloramine decay in batch test rather than in continuous system.

This is possibly a result of various reasons such as age of biofilms, pipe material and different level of bacterial activities in batch and continuous system. In our previous study, it is found that effect of biofilms on chloramine decay varies with its age (Fig.4.6). The age of biofilms used for batch test was roughly up to 3 months old but the biofilms in the continuous system (reactor) was much older (2 years) compare to batch system and it was there since the set-up of reactor. This could be a one of the

main reasons in obtaining high difference between decay coefficient obtained in continuous system and the one derived from batch system.

As continuous and batch systems were used for the investigation, different bacterial activities could play crucial role on chloramine decay within the system. In continuous system, there is continuous change of microbial environment along one reactor to another. Further, biofilms might not be distributed homogeneously throughout the reactors walls and their heterogeneous nature may result different impact on the different part of the surface of the reactor. When averaging the impact of biofilms it is possible that only a fraction of observed maximum occurs for the whole surface. But in batch system, situation is completely different in terms of bacterial activities, as they are grown on a smaller surface and their growth is tracked. This possibly could be another reason to have high impact of biofilms on batch test rather than continuous one. This analytical tool could be useful to calculate the impact of biofilms and sediments on chloramine decay independently.

Table 5.2: Total Decay Coefficient due to Biofilms in Continuous and Batch Systems

Reactor	Systems	Total Decay Coefficient (hr^{-1})
3	Continuous*[1]	0.0801±0.0212
	Batch Test*[2]	0.019±0.0019
	Batch to Continuous*[3]	0.1730±0.0019

*[1] Impact of biofilms in Continuous system

*[2] Impact of biofilms in batch system

*[3] Impact of biofilms in continuous system after conversion from batch to continuous

5.4 Practical Implications of Findings

Figure 5.4 (a) compares the contribution of biofilms, sediments and bulk water under severe nitrification. R-3 represents severe nitrification as it contained lower chloramine residual (0.43 mg/L), TAN (0.10 mg-N/L), nitrite (0.285 mg-N/L) and nitrate (0.139 mg-N/L) (Figure 4.1).

As presented in Figure 5.4(a) contribution of biofilms on chloramine decay increased with decrease in pipe diameter in distribution system. However, impact of sediments and bulk water in the distribution systems is increased with increased pipe diameter. Results indicate that biofilms and bulk water play a crucial role on chloramine decay in the distribution system rather than sediment. The detail explanation for this calculation is shown in Appendix (8a and 8b).

In reservoir, contribution of bulk water and sediment remains nearly constant for S/V ratio (0.1, 0.2 and 0.3 m^{-1}). However, the bulk water has substantial impact on chloramine decay rather than sediment and biofilms. Similarly, impact of biofilm is increased with increased S/V ratio (Fig 5.4a). Further, reservoir S/V ratio is much smaller (58 to 225 times) compared to lab scale reactor ($18m^{-1}$) used in this experiment. As a result contribution of biofilms on chloramine decay remained less in reservoir compared to bulk water.

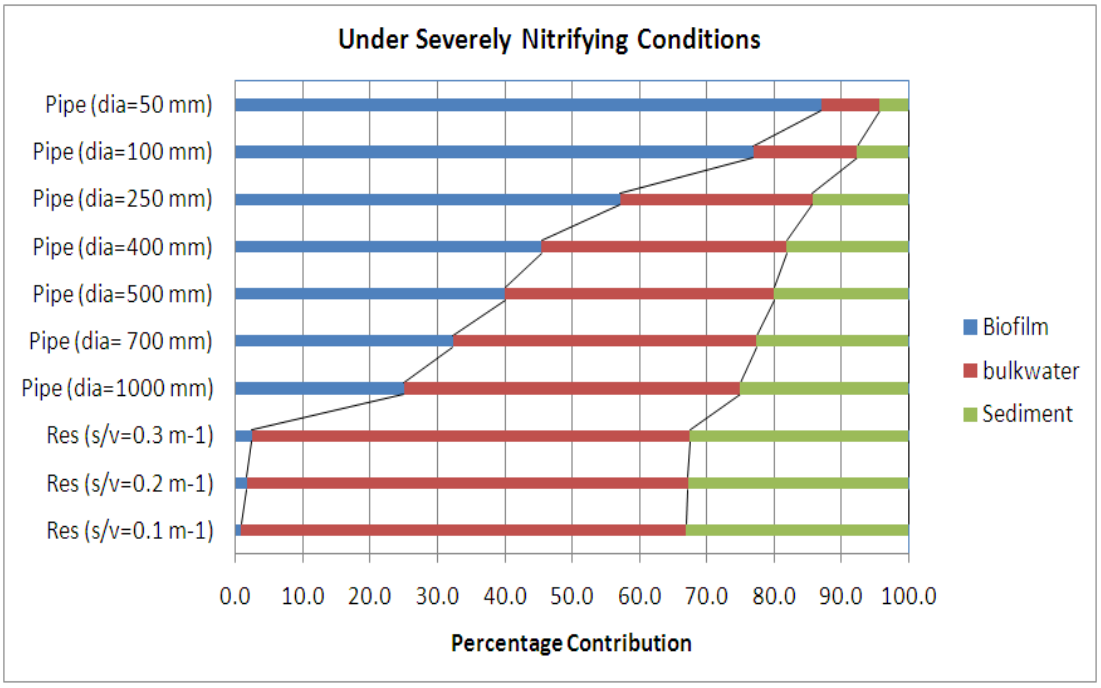

Figure 5.4(a): Relation between surface area to bulk water ratio (S/V) and total decay coefficient (hr^{-1}) for R-3, where TCl (0.43 mg/L), TAN (0.10 mg-N/L), nitrite (0.285 mg-N/L), nitrate (0.139 mg-N/L) and suspended solids (20.0 mg/L)

Similarly, Figure 5.4(b) presents the relation between total decay coefficient resulting from biofilms, bulk water and sediments under mild nitrification condition. R-2 represents mild nitrification having higher chloramine residual (1.43 mg/L), TAN (0.41 mg-N/L), nitrite (0.047 mg-N/L), and nitrate (0.038 mg-N /L). The relation of chloramine decay against the biofilms, sediments and bulk water was observed to be similar as in the severe nitrified condition although the contribution of biofilms nearly 2 or 3 %) remained less (Figure 5.4) in mild nitrified condition.

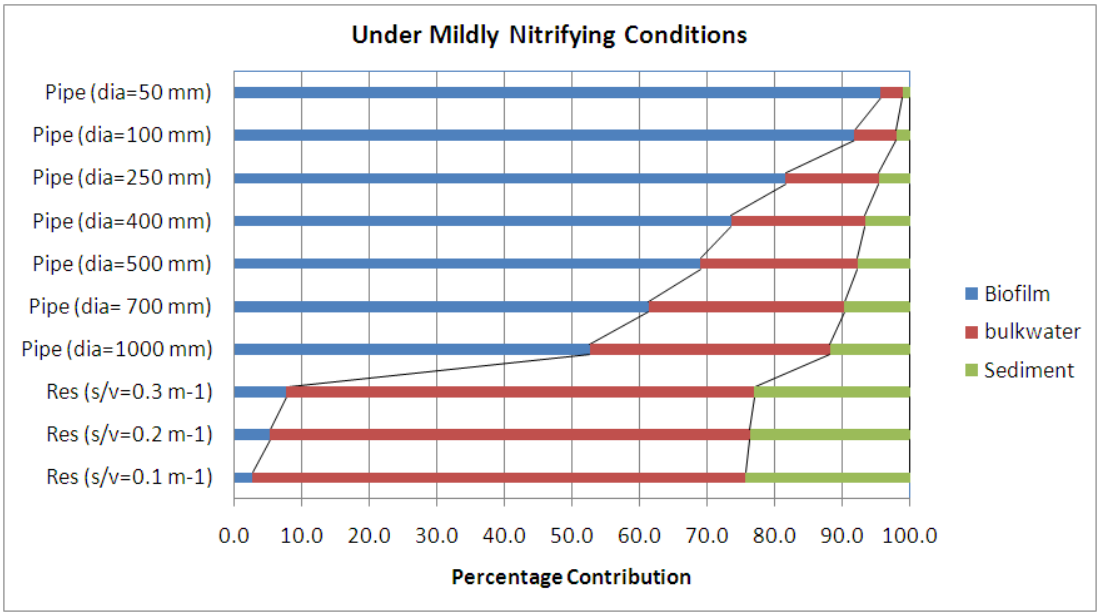

Figure 5.4(b): Relation between surface area to bulk water ratio (S/V) and total decay coefficient (hr^{-1}) for R-2. It contained TCl (1.43 mg/L), TAN (0.41 mg-N/L), nitrate (0.038 mg-N/L), nitrite (0.047 mg-N/L), suspended solids (18.0 mg/L)

These results show the prominent role of bulk water on chloramine decay in reservoir. However, Chloramine decay due to biofilms depends on several parameters such as number of bacteria, temperature and materials. Thus, even small S/V ratio sometimes contributes for higher chloramine decay as it could have more number of microbes resulting from favourable temperature and other conditions. While in a pipe line, biofilms have more contribution on chloramine decay rather than bulk water and sediments.

The experimental result and analysis showed that the impact of biofilms, sediments and bulk water offers less impact under high chloramine residual. Thus, high chloramine residual in full scale distribution system possibly could be an alternative approach to minimize the effect of biofilms, sediments and bulk water.

5.5 Conclusions

This experiment quantified the impact of biofilms, bulk water and sediments on chloramine decay. Impact of all these niches increased with decrease in chloramine residual. Biofilms contributed about 50-60 % of total decay, when surface area to volume ratio was 18 m^{-1}. It is in contrast to what is generally believed. These niches are therefore equally important in pipe lines, but in service reservoirs the bulk water and sediments play crucial roles. Further, there is minimal impact from all these niches if chloramine residual was maintained at 2.1 mg/L.

CHAPTER 6

SUMMARY DISCUSSION AND RECOMMENDATIONS

6.1 Summary and Discussion

There are numerous factors responsible for loss of chloramine both in reservoir and water distribution pipelines. This research mainly focused on the impact of biofilm on chloramine decay. Several experiments were conducted in both batch and continuous systems in order to understand the contribution of biofilms on chloramine decay. Batch test was studied under different condition by varying physical and microbial parameters while continuous system was studied by using F_{Ra} and F_m method. These two modes of experiment lead to the following conclusion by providing better understanding on the effect of biofilm in chloramine decay.

1. There is higher impact of biofilm grown under severely nitrifying condition rather than mildly nitrified condition on chloramine decay exerted by biofilms when a similar age biofilms with smaller area was obtained from various nitrification conditions, especially when the chloramine residual was less than 2.0 mg/L.

2. Impact of biofilms on chloramine decay grown on a smaller area depends on the age of biofilms.

3. Growth of biofilms on PVC coupon is much faster than HDPE one.

4. Impact of biofilms on chloramine decay is increased with increase in temperature.

5. Contribution of biofilms on chloramine decay could not be changed significantly by increasing the topped up chloramine residual in bulk water samples, as it ends up with roughly same decay rate.

6. Biofilms has a significant impact on chloramine decay when grown on a smaller size coupon rather than in a larger area and in a continuous system.

Table 6.1: Impact by various Factors on Chloramine Decay

Various Factors	Age of biofilm(days)								Temperature (˚C)		Nitrification condition		
	Biofilm grown at PVC coupons					Biofilm grown at HDPE coupons							
	15	30	45	75	115	15	45	115	20	25	no	Mild	Sever
Impact on chloramine decay	Low	High	Medium	Low	High	Low	Low	High	Low	High	-	High	High

6.2 Recommendations for Future Studies

The experiments conducted during this research gave some promising results with respect to effect of biofilms on chloramine decay, generating plenty of pathways for further investigations. The main objective of this research is to understand the impact of biofilms on chloramine decay in drinking water systems which could be helpful to maintain the adequate chloramine residual in drinking water while it runs in the distribution pipes. During this research, only one parameter (chlorine decay) was considered to understand the impact of biofilms on chloramine decay in chloraminated drinking water. Thus, it needs detail research to optimize it, the following further studies are recommended:

1. Further laboratory experiments need to be undertaken to quantify the amount of Total protein and carbohydrate present in biofilms.

2. It needs further study to quantify the bacteria numbers and its type present on biofilms.

3. Instead of monitoring the chloramine decay only, some laboratory analysis regarding the mechanism of chloramine decay would be investigated.

4. To minimize the effect of biofilms on chloramine decay in chloraminated drinking water, it is better to understand the controlling measures of biofilms or its impact.

REFERENCES:

Allen, M . J., Geldreich, E . E., 1997. Distribution line sediments and bacterial regrowth. Proceeding of Water Quality Technology Conference., Kansas City, MO.

Allen, M . J., Taylor, R . H., Geldreich, E . E., 1980. The occurance of microorganisms in water main encrustation. J . AWWA. 72(11), 614 -625.

Angles, M . L., Chandy, J., Kastl., G., Jegatheesan, V., Cox, P., Fisher, I., 1999. Biofilms in drinking water: Influence of organic carbon and disinfection. Water 26(4), 30-33.

Astier, R., Paquin, J . L., Mathieu, L., Morlot, M., Hartemann, P., 1995. Study of the development of the musty taste in water according to its ageing process in pilot plant. Journal of Environmental Technology 16, 955-965.

Bal Krishna KC., Sathasivan, A., 2010. Does unknown mechanism accelerate chemical chloramine decay in nitrifying waters? J. AWWA 102 (10), 82-90.

Baribeau, H., Prevost, R., Lafrace, P., Gates, D . J., 2002. Chlorite and chlorate ion variability in distribution system. J. AWWA 94 (7), 96-105.

Brodtmann, N . V., Russo, P . J., 1979. The use of chloramines for reduction of trihalomethanes and disinfection of drinking water. J . AWWA. 71 (1), 40-42.

Bryers, J . D., 2000. Biofilm formation and persistence. In: Bryers, J . D., (Ed) Biofilms II, process analysis and applications, Wiley Liss, New York, USA, 45-48.

Campbell, W . H, Song, P, Barbier, G . G., 2006a. Nitrate Reductase for Nitrate Analysis in Water. Environmental Chemistry Letters 4:69-73.

Camper, A . K; LeChevallier, M . W., Broadaway, S . C., McFeters, G . A., 1986. Bacteria associated with granular activated carbon particles in drinking water. Applied and Environmental Microbiology. 52 (3), 434-438.

Chandy, J . P., Angles, M . L., 2001. Determination of nutrients limiting biofilm formation and the subsequent impact on disinfectant decay. Water Research. 35(11), 2677-2682.

Characklis, W . G., Marshall, K . C., 1990. Biofilms, John Wiley and Sons, Inc., New York.

Characklis, W . G., 1981. Fouling biofilm development: A process analysis. Biotechnology and Bioengineering. 23, 1923-1960.

Cohen, Y. K., Smith, C., Baribeau, H., 2001. Nitrification: Causes, Prevention and Control. AWWA Water Quality in the Distribution System Committee. OPF 27 (12) 6-7, 10, 12.

Cotruvo, J . A., 1981. THMs in drinking water. Environmental Science and Technology. 15 (3), 298 – 274.

De Rosa, S., 1993. Loose deposite in water mains, Report DoE 3118-/2. Department of the Environment, London, pp.119.

Dixton, K . L., Lee, R . G., Moser, R . H., 1988. Residual aluminium in drinking water. Report to the American Water Works Service Company, Voorhees, NJ.

Dolan, R . M., Pipes, W . O., 1986. Pipewall biofilm in potable water mains,14th Annual AWWA Water Quality Technology Conference, Portland.

Exner, J . H., Fox, R . D., Parmele, C . S., Moyer, J . R., 1978. Chlorination effects on typical organic chemical plant effluents. In Water Chlorination, Environmental Impact and Health Effects, Vol. 2, eds R . L Jolley, H. Gorchev and D . H Hamilton Jr., pp. 791-804. Ann Arbour Science Publishers Inc., Ann Arbour, MI.

Fleming, H . C., 2002. Biofouling in water systems – cases, causes and countermeasures. Applied Microbiology and Biotechnology. 59 (6), 629-640.

Fleming, K . K., Harrington, G . W., Noguera, R . D., 2005. Nitrification potential curves: a new strategy for nitrification prevention. J. AWWA. 97(8), 90-99.

Fletcher, M., Marshall, K . C., 1982. Bubble contact angle method for evaluating substratum interfacial characteristics and its relevance to bacterial attachment. Applied and Environmental Microbiology. 44 (1), 184-192.

Fransolet, G., Villers, G., Masschelein, W . J., 1985. Influence of temperature on bacterial development in waters. J. International Ozone Association. 7 (3), 205-207.

Frensch, K., Hahn, J . U., Lesven, K., Nieben, J., Scholer, H . F., Schoenen, D., 1987. Increase in the colony count in drinking water tanks caused by solvents from the coating material. Zentralbl Bakteriol Mikrobiol Hyg 184 (6), 556-559.

Geldreich, E . E., Rice, E . W., 1987. Occurance, significance, and detection of Klevsiella in water systems. J. AWWA. 79 (5), 74-80.

Goshko, M . A., Minnigh, H . A., Pipes, W . O., Christian, R . R., 1983. Relationships between standard plate counts and other parameters in water distribution systems. J. AWWA. 75 (11), 568-571.

Gregory, J . K., 2004. Optimizing chloramine treatement. Ammerican Water Works Association Research Foundation – Nature.

Hallam, N . B., West, J . R., Forster, C . F., Simms, J., 2001. The potential of Biofilm Growth in Water Distribution Systems. Water Research. 35 (17) 4063-4071.

Ike, N . R., Wolfe, R . L., Means, E . G., 1988. Nitrifying bacteria in a chloraminated drinking-water system. Water Science and Technology. 20 (11–12), 441–444.

Kielemoes, J., Hammes, F., Verstrate, W., 2000. Measurement of microbial colonisation of two types of stainless steel. Environmental Technology. 21 (7), 831-843.

LeChevallier, M . W., Lowry, C . D., Lee, R . G., 1990. Disinfecting biofilm in a model distribution system. J. AWWA. 82 (7), 87-99.

LeChevallier, M . W., Babcock, T . M., Lee, R . G., 1987. Examination and characterzation of distribution system biofilms. Applied and Environmental Microbiology. 53, 2714-2724.

LeChevallier, M . W., Cawthon, C . D., Lee, R . G., 1988. Factors promoting survial of bacteria in chlorinated water supplies. Applied and Environmental Microbiology. 54 (3), 649-654.

Lee, S . H., O'Connor, J . T., Banerji., S . K., 1980. Biologically mediated corrosion and its effects on water quality in distribution systems. J. AWWA. 72 (11), 636-644.

Lehtola, M . J., Juhna, T., Miettinen, I . T., Vartiainen, T., Martikainen, P . J., 2004. Formation of biofilms in drinking water distribution networks, a case study in two cities in Finland and Latvia. J. Industrial Microbial Biotechnology. 31 (11), 489-494.

Lieu, N . I., Wolfe, R . L., Means, E . G. 1993. Optimizing chloramine disinfection for the control of nitrification. J. AWWA, 85 (2), 84–90.

Lipponen, M . T . T., Suutari, M . H., Martikainen, P . J., 2002. Occurrence of nitrifying bacteria and nitrification in Finish drinking water distribution system. Water Research. 36 (17), 4319-4329.

Lu, W., Kiene, L., Levi, Y., 1999. Chlorine demand of biofilms in water distribution systems.Water Research. 33 (3) 827-835.

Lund, V., Ormerod, K., 1995. The influence of Disinfection processes on biofilms formation in water distribution systems. Water Research. 29 (4), 1013-1021.

Margerum, D . W., Schurter, L. M., Hobson, J., Moore, E . E., 1994. Water chlorination chemistry: Non metal redox kinetics of chloramine and nitrite Ion. Environmental Science and Technology. 28 (2), 331-337.

Marshall, K . C., Stout, R., Mitchell, R., 1971. Mechanisms of the intitial events in the sorption of marine bacterial to surfaces. J . General Microbiology. 68 (3), 337-348.

Martiny, A . C., Jorgensen, T . M., Albrechtsen, H . J., Arvin, E., Molin, S., 2003. Long – term succession of structure and diversity of a biofilm formed in a model drinking water distribution system. Applied and Environmental Microbiology. 69 (11), 6899-6907.

Mittelman, M . W., 1985. Biological Fouling of purified- water systems. Part 1, Bacterial growth and replication, Microcontanination. 3 (10), 51-55.

Mittelman, M . W., 1985. Biological Fouling of purified- water systems. Part 2, Detection and Enumeration, Microcontanination. 3 (11), 42-58.

Momba, M . N . B., Kfir, R., Venter, S . N., CLoete, T . E., 2000. An overview of biofilm formation in distribution systems and its impact on the deterioration of water quality. J. Water SA. 26 (1), 59-66.

Norton, C . D., LeChevallier, M . W., 1997. Chloramination: Its effect on distribution system water quality. J. AWWA. 89 (7), 66-77.

Odell, L . H., Kirmeyer, G . J., Wilczak, A., Jacangelo, J . G., Marcinko, J . P., Wolfe, R . L., 1996. Controlling nitrification in chloraminated systems. J. AWWA. 88 (7), 86-98.

Opheim, D., Smith, D., 1990. The relationship of Enterobacter cloacae, AOC and ADOC in raw and treated water to confirm episodes, in Proc. AWWA Water Quality Technol. Conf., San Diego, CA, American Water Works Association Research Foundation, Denver, CO,1237.

Orskov., Klebsiella, In., Krieg, N . R., Holt , J . G., (Ed)., 1984., Bergy's manual of systematic bacteriology, Vol.1. Williams and Wilkens, Baltimore, MD, 491-495.

Owen, D. M., Amy, G . L., Chowdhury, Z . K., Paode, R., Mccoy, G., Viscosil, K., 1995. NOM characterization and treatability. J. AWWA. 87 (1), 46-63.

Ridgway, H . F., Olsan, B . H., 1982. Chlorine resistance patterns of bacteria from two drinking water distribution systems. Applied and Environmental Microbiology. 44 (4), 972-987.

Rogers, J., Dowsett, A . B., Dennis, P . J., Lee, J . V., Keevil, C . V., 1994. Influence of plumbling materials on biofilm formation and growth of Legionella pneumophilla in potable water systems. Applied and Environmental Microbiology. 60 (6), 1842-1851.

Sathasivan, A., Chiang, J., Nolan, P., 2009. Effect of temperature on microbiological and chemical chloramine decay. Water Science and Technology. 9 (5), 493-499.

Sathasivan, A., Fisher, I., Kastl, G., 2005. Simple method for quantifying microbiologically assisted chloramine decay in drinking water. J. Environmental Science and Technology. 39 (14), 5407-5413.

Sathasivan, A., Fisher, I., Tam, T., 2008. Onset of severe nitrification in mildly nitrifying chloraminated bulk waters and its relation to biostability. Water Research. 42 (14), 3623-3632F.

Sathasivan, A., Krishna, B., Fisher, I., 2010. Development and application of a method for quantifying factors affecting chloramine decay in service reservoirs. Water Research. 44 (15), 4463-4472.

Sathasivan, A., Ohgaki, S., 1999. Development and application of bacterial regrwoth potential method: Clear evidence of phosphorus limitation. Water Research. 33 (1), 137-144.

Schoenen, D., Scholer, H . F., 1985. Drinking water materials: field observations and methods of investigation, John Wiley and Sons, New York, P 195.

Schoenen, D., Wehse, A., 1988. Microbial colonization of water by the materials of pipes and hoses: changes in colony counts, Zentralbl. Bakt. Hyg. B, 189, 108-117.

Servais, P., Laurent, P., Randon, G., 1995. Comparison of the bacterial dynamics in various French distribution systems. J. Water Supply: Research and Technology (AQUA). 44 (1), 10-17.

Skadsen, J., 1993. Nitrification in a Distribution System. J. AWWA, 85 (7), 95-103.

Smith, D. B., Hess, A . F., Opheim, D., 1989. Control of distribution system coliform regrowth, Proc. AWWA Water Quality Technol. Conf., Philadelphia, PA, American Water Works Association, Denver, CO.

Srinivasan, S., Harrington W . H., Xagoraraki, I., Goeld, R., 2008. Factors affecting bulk to total bacteria ratio in drinking water distribution systems. Water Research. 42 (13), 3393-3404.

Stewart M . H., Lieu, N . I., 1997. Nitrification in chloraminated drinking water and its association with biofilms. In Proceedings of the AWWA Water Quality Technology Conference Denver, Colorado.

United States Environmental Protection Agency (USEPA)., 1992. Seminar publication: Control of biofilm growth in drinking water distribution system. EPA/925/R-92/001, Office of Research and Development, Washington,D.C.

Valentine, R . L., 1985. Disappearance of monochloramine in the presence of nitrite. In water chlorination: Chemistry, Environmental Impact and Health Effects., Jolley, R. L., Eds. Chelsea, Mich. Lewis Publishers. Vol. 5.

Valentine, R . L., 2007. Opinion as to Probable Chloramine Speciation in San Francisco Public Utilities Drinking Water Distribution System. http://www.acwd.org/dms_docs/60ff41c236e702a3d971d1eac7ffaa17.pdf (accessed 17.11.11).

Valentine, R . L., Wilber, G . G., 1987. Water chlorination . Chemistry, Environmental Impact, and Health Effects. Jolley, R. L., Condie, L. W., Johnson, J. D., Katz, S., Minear, R. A., Mattice, J. S., Jacobs, V . A., Eds; Lewis Publishers: Chelsea, MI, Vol. 9, 819-832.

Van der K . D., 1992. Assimilable organic carbon as an indicator of bacterial regrowth. J. AWWA. 84 (2), 57-65.

Van der K . D., Veenendaal, H . R., Baars – Lorist, C., Vander Klift D . W., Dorst, Y . C., 1995. Biofilm formation on surface of glass and teflon exposed to treated water. Water Research. 29 (7), 1655-1662.

Watson, S . W., Bock, E., Harms, H., Koops, H . P., Hooper, A . B., 1989. Nitrifying bacteria; In Bergy's manual of systematic bacteriology, Vol.3; Stately, J.T., Bryant, M.P., Pfenning, N; Holt, J.G., Eds; Williams and Wilkins: Blatimore. 1808-1834.

Victoreen, H . T., 1980. The stimulation of coliform growth by hard and soft water ain deposits. Proceeding of AWWA Water Quality Technology Conference, Miami Beach, FL.

Victoreen, H . T., 1984. The role of rust in coliform regrowth. Proceeding of AWWA Water Quality Technology Conference, Denver, CO.

Vikesland, J . P., Ozekin, K., Valentine, L . R., 2001. Monochloramine decay in model and distribution system Waters. Water Research. 35 (7), 1799-1779.

Vikesland, J . P., Valentine, R., Ozekin, K., 1996. Application of product studies in the eludication of chloramine reaction pathways. Water Disinfection and Natural Organic Matter. Miner, R; Amy, G; Eds; American Chemical Society, Washington DC, 115-125.

Wilczak, A., Jacanelo, J . G., Marcinko, J . P., Odell, L . H., Kirmeyer, G . J., Wolfe, R .L., 1996. Occurance of nitrification in chloraminated distribution systems. J. AWWA. 88 (7), 74-85.

Wingender, J., Flemming, H . C., 2004. Contamination potential of drinking water distribution network biofilms. Water Science and Technology. 49 (11), 277-286.

Wolfe, R . L ., Lieu, N . I., 2001. Nitrifying bacteria in drinking water. Encyclopedia of Environmental Microbiology. Wiley & Sons, New York.

Wolfe, R . L., Lieu, N . I., Izaguirre, G ., Means, E . G., 1990. Ammonia – oxidizing bacteria in a chloraminated distribution system: Seasonal occurrence, distribution and disinfection resisdence. Applied and Environmental Microbiology. 59 (2), 451-492.

Wolfe, R . L., Means, E . G., Davis, M . K., Barrent. S., 1988. Biological nitrification in covered reservoirs containing chloraminated water. J. AWWA. 80 (9), 109-114.

Wolfe, R . L., Ward, N . R., Olsan, B . H., 1984. Inorgarnic chloramines as drinking water disinfectants: a review. J. AWWA. 76 (5), 74-88.

Zhang, Y., Love, N., Edwards, M., 2009. Nitrification in drinking water systems. Critical Reviews in Environmental Science and Technology. 39 (3), 153-208.

Table of Contents

List of Table

List of Figures

List of Appendices

Appendix 8: Calculation of Contribution of Biofilms , Sediments and Bulk Water with respect to S/V for R-3

Pipe ø.(X)	S/V of Reservior and Pipe(Y)	Impact of biofilm =(decay coff of biofilms/SV of reactor*(Y)) (A)	Impact of Bulk water(B)	Impact of sediment(D)		Contribution of Biofilm (%)=((A)/(A+B+D))*100)	Contribution of bulkwater(%)= ((B)/(A+B+D))*100)	Contribution of Sediment(%) = ((D)/(A+B+D))*100)
	0.1	0.0005	0.04	0.02	Res (s/v=0.1 m^{-1})	0.8	66.1	33.1
	0.2	0.001	0.04	0.02	Res (s/v=0.2 m^{-1})	1.6	65.6	32.8
	0.3	0.0015	0.04	0.02	Res (s/v=0.3 m^{-1})	2.4	65.0	32.5
1.0	4.0	0.02	0.04	0.02	Pipe (dia=1000 mm)	25.0	50.0	25.0
0.7	5.7	0.03	0.04	0.02	Pipe (dia= 700 mm)	32.3	45.2	22.6
0.5	8	0.04	0.04	0.02	Pipe (dia=500 mm)	40.0	40.0	20.0
0.4	10	0.05	0.04	0.02	Pipe (dia=400 mm)	45.5	36.4	18.2
0.25	16	0.08	0.04	0.02	Pipe (dia=250 mm)	57.1	28.6	14.3
0.1	40	0.2	0.04	0.02	Pipe (dia=100 mm)	76.9	15.4	7.7
0.05	80	0.4	0.04	0.02	Pipe (dia=50 mm)	87.0	8.7	4.3

Decay Coefficients used for above calculation

Reactors	Bulk water	Sediment	Biofilms
	Decay Coefficient(hr-1)		
R-3	0.04	0.02	0.09