School of Civil and Mechanical Engineering
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Modelling Inhibition of Microbes Responsible for Acceleration of Chloramine Decay in Water Supply System

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This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University

February 2012

Declaration

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

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

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DEDICATION

To my eldest brother **Dwijendra Nath Sarker**
without whom I wouldn’t be here.

ABSTRACT

Chloramine as a secondary disinfectant is widely used instead of free chlorine in USA, Canada and Australia. Free-ammonia, either introduced during chloramination or that released from decay of chloramine, serves as an energy source for ammonia-oxidizing bacteria (AOB) to cause nitrification. Nitrification is thought to be the reason for acceleration of microbiologically assisted chloramine decay. Owing to nitrification, the water utilities face difficulty in maintaining sufficient chloramine residual, especially at the ends of distribution systems.

Usually, traditional preventive measures (breakpoint chlorination, reduction of available free ammonia, increased chloramine concentration, removal of natural organic compounds, flushing of distribution system etc.) are not effective to control nitrification. A novel approach of using copper as an inhibitor for nitrification control has been investigated by several researchers (but not particularly in chloraminated system). Through understanding of fundamental of chloramine decay mechanisms and nitrification in distribution systems, this study focuses on residual management by inhibiting/inactivating the microbes (especially nitrifying bacteria) by using copper as an inhibitor in the distribution system.

For nitrification control and thus to maintain required residual, copper dosing concentrations, dosing pattern, dosing frequency in different reactor systems under different nitrification conditions of samples were studied. The environmental factors that exert influence on chloramine decay/nitrification were also considered. To achieve this target, several aspects were considered in this study.

Most importantly the study uniquely took into account of four different phases of chloramines decay/nitrification. When chloraminated water is introduced into the system, chloramine undergoes only chemical interaction (no nitrification) until microbial decay starts to occur. During the second phase with microbial decay (mild nitrification) very little nitrite production is noticed along with very stable chloramine levels. When the chloramine drops to sufficient level, nitrite production starts to increase heavily (onset of nitrification). Following onset, nitrification was

noted to happen in two ways: with significantly accelerated decay of chloramine (severe nitrification) and the other without significant acceleration (nitrification). The reactors were designed to achieve these different conditions. Due to contrasting conditions experienced by each reactor/conditions the study attempted various non-traditional/traditional control measures (pH or temperature) to influence the most critical phases, although the major target was to control the chloramine decay/nitrification by dosing copper. In doing this, the thesis also recognizes that the onset of nitrification or the point of biostability as an important parameter, because it is the point beyond which most of the problems experienced by utilities start. The point of biostability is defined as the point at which growth rate of AOB balances the killing rate by chloramines. If the residual defines this point it is referred to as biostable residual concentration (BRC).

As utilities usually experience reduced pH levels (~7.5) after the onset of nitrification, pH was attempted as one of the control measures in severely nitrifying samples. Results indicated that maintaining higher pH (8.5) could help to maintain the chloramine residual and suppress the nitrifying bacterial activity, while lower pH (pH 7.5) showed the reverse behavior. Therefore an important conclusion was drawn that the simple adjustment of pH from traditionally observed 7.5 under nitrifying conditions could help in suppressing nitrification and improving chloramine residuals.

For evaluation of the impact of temperature on the BRC, a model defining the temperature effects on maximum growth rate (μ_m) and inactivation rate (k_d) of bacteria similar to AOB was proposed. The model was validated with the batch and continuous flow test experimental data obtained from the pilot-scale and two full scale systems. According to the model, the μ_m/k_d values heavily changed with temperature, defying the literature reported constant value (2.0 mg-Cl_2/L). Also, nitrification triggering time was found to change for the modified growth and inactivation conditions. The model revealed that temperature variations greatly impact on BRC especially at temperatures above 27^{o}C and below 15^{o}C. Under 15^{o}C, as usually observed by many researchers, even smaller concentrations of chloramine residual were sufficient to prevent nitrification. Thus, the model has the potential to aid water utility in residual management throughout the year.

Conducting batch test by applying different concentrations of copper (used as copper sulfate) with 2.0 mg-Cl_2/L initial chloramine in severely nitrified bulk waters showed that nitrification started within 40 hrs for lower copper (<0.20 mg-Cu/L) dosed samples whereas, no sign of nitrification was seen during the experimental period of 500 hrs and 1500 hrs for 0.25 and 0.40 mg-Cu/L dosed samples, respectively. Therefore, nitrification inhibition depends on copper concentration and 0.25 mg-Cu/L was the minimum concentration to inhibit/inactivate the bacteria present in severely nitrified chloraminated bulk waters, especially when chloramine is dosed at 2 mg Cl2/L. These experimental results further revealed that stopping nitrite or NOx-N (nitrite + nitrate) production using copper was not enough to improve chloramine residual. On tracking the onset of nitrification at BRC in unprocessed and copper inhibited samples, a model was proposed to evaluate the combined effect of copper and chloramine using biostability concept. In the model, the disinfection efficacy was considered in three ways; due to the effects of chloramine alone, due to effects of copper only, and due to synergistic effects of chloramine and copper. According to the model, it could be said that copper concentration up to 0.25 mg-Cu/L with chloramine (initial concentration 2.0 mg-Cl_2/L) or 0.40 mg-Cu/L alone play an important role in controlling nitrification.

Chemical effects of copper on chloramine and nutrients profiles were conducted in another experiment. Different doses (0.25, 0.50 and 1.00 mg-Cu/L) of copper in severely nitrified filtered bulk waters were added. Results showed that copper had no chemical effects on chloramine decay and nutrients concentrations. Even pH variation (7.5, 8.0 and 8.5) did not affect chloramine decay and nutrient's levels when copper was added to filtered severely nitrified bulk waters. The use of stoichiometry in severely nitrified filtered samples confirmed that chloramine loss could be explained by auto-decomposition and nitrite oxidation.

The effectiveness of copper inhibition in different nitrification conditions under different reactor flow conditions depends on copper concentrations, chloramine residuals. In semi-continuous flow condition, copper at lower concentration (0.25 mg-Cu/L) could control nitrification but could not improve the chloramine residual even at high chloramine concentration (2.0 mg-Cl_2/L) for severely nitrified bulk waters. Higher amount of copper (1.00 mg-Cu/L) was required to improve the

chloramine residual. For continuous flow conditions, the efficiency depends on both copper concentration and nitrification status of the samples during copper dosing time. Continuous copper dosing for (0.10 mg-Cu/L for 20 days and 0.20 mg-Cu/L for 12 days) 32 days in severely nitrified bulk waters in the pilot-scale reactor could control nitrification but could not improve chloramine residual. On the other hand, by continuous copper (0.10 mg-Cu/L) dosing in the reactor undergoing onset of nitrification stage, it was possible to improve chloramine residual with nitrification minimization. Chloramine decay test results showed that copper was effective for minimising chloramine loss attributed by sediment presence as well in the severely nitrified bulk waters. Furthermore, it was found that there was a stable level of chloramine concentration and no sign of nitrifying bacterial activity in the copper dosed and succeeding reactors even after stopping copper dosing for 50 days. Thus, proper selection of copper dosing point in regards of nitrification stages was the crucial for controlling nitrification and residual management. It also shed a new light that proper monitoring and dosing copper at the time when onset of nitrification conditions are noticed may avert severe nitrification from happening in the distribution systems, especially service reservoirs. As the reactor run in this experiment had surface to volume ratio of 18 m^{-1}, the condition closely resembled pipe of a 350 mm diameter and hence the outcome is equally applicable for nitrification control in pipelines.

ACKNOWLEDGEMENTS

I am grateful to all those who have helped me in different capacities in the attainment of my academic pursuit. Nevertheless, the following are some of those whom I shall always admire for their valuable contributions.

I am sincerely grateful to the chairman, Prof. Hamid Nikraz for his interest and inspiring guidance which proved to be essential for the completion of this work. I feel great pleasure to extend my heartfelt thanks and gratitude to my supervisor Associate Prof. Arumugham Sathasivan for his relentless guidance, wise and sharp comments, and encouragement throughout the study even after he has left Curtin University. This research would not be a mission without his contribution. I would like to acknowledge Dr. Ranjan Sarukkalige for serving as an associate supervisor of my study. I would like to acknowledge Prof. Nikraz and Dr. Andrew White for acceptance to serve as the supervisor and Chairman in the last two weeks of thesis, especially when my supervisor, A/Prof. Sathasivan has left to join another university.

My sincere gratitude goes to Mr. Bal Krishna KC, with whom I worked in a group in an organized manner. I would like to acknowledge him for sharing his ideas and concepts with me especially the results of microbiological analysis presented in chapter 8 in this study. I take this opportunity to express my gratitude to my friends Weixi Zhan and others in water engineering research group for their kind assistance and moral supports. I am also thankful to all the staff of Civil Engineering Laboratory for their valuable help during the laboratory work and sample collection.

I am especially indebted to Australian Research Council and Water Corporation of Western Australia for granting full scholarship for my doctoral study and research grants, without which this study would not be possible. I would like to acknowledge Sydney Water and George Kastl for allowing me to use the pilot-scale reactors in this study.

I would like to thank Gargee, my loving wife and Shreya & Srija, my sweet daughters for their unconditional support. Their inspiration is the ingredient, this

work could not run without and my heartfelt love goes to them. My beloved father, sister, brothers and whoever provided moral support and encouragement to this endeavor are sincerely obliged and deeply appreciated.

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LIST OF ACRONYMS

AOB	:	Ammonia Oxidizing Bacteria
BRC	:	Biostable Residual Concentration
CTR	:	Critical Threshold Residual
DBP	:	Disinfection By-Products
D/DBP	:	Disinfection/ Disinfection By-Products
DO	:	Dissolved Oxygen
DOC	:	Dissolved Organic Carbon
Fm	:	Microbial Decay Factor
HAA	:	Halo-acetic Acids
HPC	:	Heterotrophic Plate Count
HRT	:	Hydraulic Retention Time
k_c	:	Chemical Decay Coefficient
k_m	:	Microbial Decay Coefficient
k_t	:	Total Decay Coefficient
NOB	:	Nitrite Oxidizing Bacteria
NOM	:	Natural Organic Matter
NO_2-N	:	Nitrite Nitrogen
NO_3-N	:	Nitrate Nitrogen
PCR	:	Polymerase Chain Reaction
qPCR	:	quantitative Polymerase Chain Reaction
THM	:	Trihalomethanes
SMP	:	Soluble Microbial Product
TAN	:	Total Ammoniacal Nitrogen
TCl	:	Total Chlorine
TSS	:	Total Suspended Solids

PUBLICATIONS AND PRESENTATIONS AS THE OUTCOME FROM THIS STUDY

A. Journals

1. **Sarker, D. C**. and Sathasivan, A. Modelling the Temperature Effects on Ammonia Oxidising Bacterial Biostability in Chloraminated systems (Prepared for submission in the Journal of "Chemical Engineering").

2. **Sarker, D. C**. and Sathasivan, A. A Novel Approach to Understand the Combined Effect of Copper and Chloramine in Nitrifying Bulk Waters (Prepared for submission in the Journal of "Chemical Engineering").

3. **Sarker, D. C**. and Sathasivan, A. Effect of Copper Addition on Chloramine decay and Nutrient Profiles of Severely Nitrified Bulk Waters (Prepared for submission in the Journal of "Chemosphere").

4. **Sarker, D. C**.; Sathasivan, A. and Bal Krishna, KC. Effects of Copper in Controlling Chloramine Decay in Severely Nitrified Bulk Waters (Semi-Continuous Flow) [Drafted for submission in the Journal of "Science of The Total Environment"].

5. **Sarker, D. C**. and Sathasivan, A. Evaluating the Impact of Copper Inhibition on Nitrification and Chloramine Residuals in a Pilot-Scale Chloraminated System (Continuous Flow Condition) [Drafted for submission in the Journal of "Water Research"].

6. **Sarker, D. C**. and Sathasivan, A. (2011). Nitrification Control by Adjusting pH in Severely Nitrified Bulk Waters. Journal of Water Science and Technology (In press).

7. **Sarker, D.C**. and Sathasivan, A. (2011). Effect of Temperature on Onset of Nitrification in Chloraminated Distribution System. Journal of Desalination and Water Treatment, Vol. 32, p 95-99.

B. Conferences

8. Sathasivan, A., Kastl, G., KC, Bal Krishna and **Sarker, D.C.,** (2011). Role of Nitrification in Accelerating Chloramine Decay through Application of Microbial Decay Factor Method. Water Quality Technology Conference 13-17 November, American Water Works Association, Phoenix, Arizona, USA.

9. **Sarker, D. C**. and Sathasivan, A. (2011). Role of pH on Onset of Severe Nitrification in Chloraminated Distribution System. International Conference on Integrated Water Management. 2–5 February. Murdock University, Perth, Western Australia.

10. Sathasivan, A. and **Sarker, D.C**. (2010). Nitrification Control in Chloraminated System: Role of Temperature on Onset of Severe Nitrification. Challenges in Environmental Science & Engineering, CESE-2010. 26 September to 1 October, The Sebel, Cairns, Australia.

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Why Chloramine is Superior to Chlorine?

Now-a-days, the use of chloramine as a secondary disinfectant is becoming popular, especially under challenging conditions such as; when water has to be carried over long pipelines, under the extremely higher temperature, or when it is difficult to treat the water to remove natural organic matter (NOM). Chloramination has been implemented not only in the United States and Australia by many utilities, but also in countries such as Canada, Scotland and Singapore. The major reason for adaptation of chloramine was that it is less reactive than free chlorine (Jegatheesan et al., 2000) and hence produces less disinfection by-products (DBPs), such as trihalomethanes (THM) and haloacetic acids (HAA) (Taylor et al., 2001).

1.1.2 Challenges of Using Chloramine in the Distribution System

Despite its advantages, chloramine is also known for its problems associated with occasional instability. The instability is mostly thought to be due to proliferation of ammonia oxidizing bacteria (AOB) or the occurrence of nitrification episode in the chloraminated distribution systems (Odell et al., 1996). Nitrification results in nitrite accumulation and is thought to be the reason for accelerated chloramine decay which results in low chloramine residuals.

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1.1.3 Traditional Controlling Measures for Chloramine Decay

Traditional chloramines decay control strategies are aimed at controlling nitrification. A most important strategy is **breakpoint chlorination**, while this is effective in short term, it cannot be adopted as a long term strategy (Odell et al., 1996). The **reduction of available free ammonia** has been reported to control

nitrification with limited success (Wilczak et al., 1996). In some cases, **increased chloramine dose** was found to be effective but if nitrification were already present, this strategy was not found to be effective (Odell et al., 1996). The **removal of organic compounds** at the treatment plant was not found to be effective as a long term strategy (Odell et al., 1996)**. Flushing of distribution system** is another control measure of nitrification but its effectiveness depends on conditions of individual system (Odell et al., 1996). Finally **reduction of system retention time** combined with other controlling measures such as rechlorination or periodic breakpoint chlorination was found to be effective in nitrification control for a long-term strategy. Traditional controlling measures are completely effective for controlling neither nitrification nor chloramine decay. Therefore, the limitations in the above mentioned approaches demand necessity to find an efficient way for chloramine residual management.

There are two approaches to maintain appropriate chloramine residual. Firstly, understanding the fundamental of chloramine decay mechanisms and nitrification occurrences, and secondly, inhibition/inactivation of microbes by using inhibitor in the distribution system. With the primary aim of investigating copper as an effective inhibitor to control nitrification and chloramine in the larger project, the project was done by four students. The project took the view that fundamental understanding is a pre-requisite for better control of chloramine and hence the project was well integrated into other students' work which focused on fundamental understanding in copper free systems whereas the work in the thesis concentrated on understanding how chloramine decay nitrification could be controlled.

1.1.4 Results of Fundamental Studies

Different nitrifying conditions are defined by Sathasivan et al., (2008). They importantly defined three different nitrifying conditions. First condition is referred to as mildly nitrifying conditions wherein chloramine decay is very slow and steady, nitrite levels (less than 0.01 mg-N/L) are low, and ammonia loss is mild. Second condition is referred to as severe nitrification wherein chloramine decays heavily (decay rate is about an order higher than in mildly nitrifying condition) with excessive nitrite production (more than 0.1 mg-N/L) and ammonia drop. Condition

occurring in between the two is referred to as onset of nitrification. Later studies (Siew Teng, 2009) noted that there can be excessive nitrite production without a significant drop in residual. This particular condition is referred to as nitrification. These conditions are likely to host different microbes with varying chloramine concentration and hence behave differently.

1.1.5 Copper Inhibition Strategy in Controlling Chloramine Decay in the Distribution System

Such strategy was also warranted as field and laboratory copper dosing trial in the Goldfield and Agricultural Water Supply System (G&AWSS) conducted by Water Corporation, Western Australia. Their findings indicated loss of copper in the pipe lines which is the predominant part of the G&AWSS. As copper dose can impact the point of onset of nitrification, the biostable residual concept provided by previous researchers (Woolschlager et al., 2001; Harrington et al., 2002) can be utilised. At the biostable residual concentration (BRC), growth rate due to the presence of nutrient and disinfection rate due to the presence of chloramine balances. The BRC can be expected to be changed by addition of copper, change in pH, temperature etc.

1.2 Research Objectives and Scope

The main objective of the study was to examine the effectiveness of copper inhibition to improve chloramine residual in the chloraminated drinking water distribution system. Specifically, this research focused on finding the optimum dose of inhibitor (copper) needed for controlling nitrification and thus to maintain residual at nitrified chloraminated bulk waters systems. For doing this, some factors influencing chloramine decay was taken into account and mathematical models were proposed as needed. To achieve the main aim, several specific objectives were set up as follows:

- To investigate the effects of pH on severely nitrified chloraminated bulk waters as controlling option of nitrification and chloramine decay.

- To develop a model considering temperature effects on bacterial growth rate and inactivation rate incorporating into biostability equation in chloraminated bulk waters.
- To investigate the synergistic impact of copper and chloramines on nitrifying bacteria present in chloraminated severely nitrifying bulk waters.
- To understand the role of AOB on accelerating chloramines decay along with understanding of the chemical effects of copper on chloramine decay and nutrients level in chloraminated bulk waters.
- To investigate the impact of copper when copper is dosed at different nitrifying conditions in the chloraminated distribution system, especially by operating a pilot-scale system.

1.3 Research Significance

Changing climate, increasing populations and drying environment have resulted in scarcity of water in Australia. Though the water used for potable purposes satisfied the health guidelines, maintaining adequate disinfectant levels in distribution systems has become more challenging with decreasing source water quality. Therefore, it is crucial to maintain effective disinfection to minimise exposure to potential waterborne pathogens. Chloramine is the appropriate as a secondary disinfectant but maintaining its decent level throughout the distribution system particularly in summer season is a big challenge for many water utilities. Microbial activities mainly that of nitrifiers, are believed to expedite chloramines decay well beyond the chemically known decay rates. Many corrective strategies have been used once the system experienced nitrification. Among several strategies break point chlorination was found to be effective, however there is a need to compromise the advantages offered by chloramination. In order to find better solution for improving chloramine residuals, a novel approach “the use of copper” as an inhibitor was investigated in this study and the significance of the findings are;

- Detailed study of microbial inhibition using copper conducted in a various systems (batch, semi-continuous and continuous systems) under mildly to severely nitrifying conditions revealed the need of different copper dose to

improve the chloramine residuals. The findings of this study will be particularly helpful for water utilities to determine the proper dose of copper with respect to nitrification conditions. For example if the system has already experienced severe nitrification then need to dose high amount of copper whereas if the system experience mildly nitrifying condition then less amount of copper will be sufficient.

- Result of this study will lead to save the huge amount of resource of water utilities by selecting the proper dose of copper and dosing places (depending on nitrification condition).
- The model developed considering various combinations of copper and chloramine residuals at severe nitrification conditions (the worst condition) will guide to select the right amount of chloramine and copper residues in order to improve chloramine residuals.
- Furthermore outcomes of this research will be directly useful for Water Corporation, Western Australia to establish when and where to apply copper because Water Corporation has been conducting field trial by dosing copper sulphate in chloraminated pipe lines (G&AWSS) to inhibit nitrifiers.
- Beside use of copper, changing pH will help to minimize the nitrifiers activities in the system which had already experienced nitrification. Moreover temperature dependent model developed in this study can easily predict the onset of nitrification at different seasons. Use of this model provide the sufficient time for water utilities operator to take corrective action in advance.
- Finally and most interestingly, the experiments in the thesis proved that it is not just nitrification that controls microbial chloramine decay and hence the utilities have to consider and measure microbial decay in addition to traditionally monitored parameters.

1.4 Research Approach

The concerning bodies of water utilities experienced a lot of problem to maintain the chloramine residual in chloraminated drinking water distribution systems. The major problem is nitrification due to the presence of nitrifying bacteria, occurs in reservoirs as well as pipe lines of the distribution system. The challenging approach of this

research was triggered by using copper as an inhibitor for inhibiting or inactivating the nitrifying bacteria in the chloraminated drinking water distribution system in order to improve chloramine residual.

For doing that, reactors were designed by creating the same conditions that usually happens in the real distribution systems. Three types of reactors e.g. batch, semi-continuous flow and continuous flow reactors were used. In batch and semi-continuous flow reactors, the effect of copper was investigated on chloraminated severely nitrified bulk waters, where copper was dosed initially and daily respectively. For continuous flow experiments, a pilot-scale reactor consisting of two sets (each set contained five reactors connected in series) of reactors had been set up in the laboratory; one set for control and another for copper dosing. Before dosing copper, different stages of nitrification were created in both set of the reactors and the necessary parameters were monitored to ensure similar conditions in both reactor systems containing five reactors (named R1 ro R5 in the direction of water flow). As water flows through each reactor, conditions gradually changed from mildly nitrifying to onset and finally to severe nitrification. The effect of copper was examined in severely nitrified bulk water and at onset of nitrification by continuous copper dosing. In every experiment, the effectiveness of copper inhibition on nitrification control was evaluated by monitoring the surrogate parameters (ammonia, nitrite and NOx-N), whereas the residual improvement was determined by calculating the decay rate using observed total chlorine residual. The schematic diagram of the research approach is presented in Figure 1.1. The obtained results are presented in this thesis that contains ten chapters.

Chapter 1 presents the introduction of the topic that includes the advantages and disadvantages of choosing chloramine as a disinfectant. The strategy of inhibiting microbes including nitrifying bacteria using copper is introduced. The objectives, significance and approach of this research are included in this chapter.

Chapter 2 reviews the valuable information from previous research related to the theme of the topic and current research efforts for nitrification control and chloramine residual improvement.

Chapter 3 presents the major analytical techniques. It consists of water sample collection, preparation of bulk water samples, reactor designs and operational set up, general methods about water analysis, reagents preparation involved in every experiment.

Chapter 4 focuses on the effects of pH on chloramine decay and nitrification control in severely nitrified chloraminated bulk waters. One paper entitled **"Role of pH on Onset of Severe Nitrification in Chloraminated Distribution System"** has been published in International Conference on Integrated Water Management. 2–5 February, 2011, Murdock University, Perth, Western Australia and another one entitled **"Nitrification Control by Adjusting pH in Severely Nitrified Bulk Waters"** has submitted (revised manuscript) for publication in the Journal of 'Water Science and Technology: Water Supply'.

Chapter 5 focuses on the incorporation of temperature effects on nitrification and BRC in chloraminated distribution systems. Two papers have been published and one is drafted. The first one entitled **"Nitrification Control in Chloraminated System: Role of Temperature on Onset of Severe Nitrification"** has been published in the conference of Challenges in Environmental Science & Engineering (CESE-2010), 26 September to 1 October, The Sebel, Cairns, Australia. The second one entitled **"Effect of Temperature on Onset of Nitrification in Chloraminated Distribution System"** has been published in the Journal of 'Desalination and Water Treatment', vol. 32, 2011. The drafted one entitled **"Modelling the Temperature Effects on Ammonia Oxidising Bacterial Biostability in Chloraminated Systems"** is prepared for submission to Journal of 'Chemical Engineering'.

Chapter 6 to Chapter 9 is the main part of the thesis that deals with comprehensive experiments for improving chloramine residual by using copper as an inhibitor. Copper was dosed in chloraminated bulk waters subjected to different nitrification conditions. Results and conclusions are given in each experiment.

Chapter 6 investigated to determine the optimum copper concentration for nitrification inhibition in chloraminated bulk waters in a batch flow experiment and presents a model for co-inhibitory effects of copper and chloramine on nitrification. A paper entitled **"A Novel Approach to Understand the Combined Effect of**

Copper and Chloramine on Nitrification in Bulk Waters" is prepared for submission in the Journal of 'Chemical Engineering'.

In Chapter 7, chemical effects of copper on chloramine decay and nutrient profiles of severely nitrified bulk waters containing dissolved compounds and soluble microbial products were discussed. Behaviour of filtered severely nitrified bulk waters due to pH variation and chloramine decay mechanisms were also discussed in this chapter. A paper entitled **"Effect of Copper Addition on Chloramine Decay and Nutrient Profiles of Severely Nitrified Bulk Waters"** is prepared for submission in the Journal of 'Chemosphere'.

Chapter 8 focuses on the assessment of copper inhibition in semi-continuous flow system reactor. In this chapter, the experiments were designed to recover chloramine residual by dosing copper in severely nitrified chloraminated bulk waters. This chapter studied to find out the optimum dose of copper required to re-establish the chloramine residual in the chloraminated bulk waters by controlling nitrification. A paper entitled **"Effects of Copper in Controlling Chloramine Decay in Severely Nitrified Bulk Waters (Semi-Continuous Flow)"** is drafted for submission in the Journal of 'Science of The Total Environment'.

Chapter 9 focuses on the assessment of copper inhibition in continuous flow system with series of reactors which was similar to real distribution system. In this chapter, the experiments were designed to maintain chloramine residual by dosing copper in two different nitrification conditions. This chapter studied to find out the suitable points of copper application and minimum copper dose in the chloraminated bulk waters for nitrification control and proper residual management in continuous flow system. A paper entitled **"Evaluating the Impact of Copper Inhibition on Nitrification and Chloramine Residuals in a Pilot-Scale Chloraminated System (Continuous Flow Condition)"** is drafted for submission in the Journal of 'Water Research'.

Chapter 10 summarizes the achievements from this research and the recommendations for the future works.

CONCEPTUAL FRAMEWORK

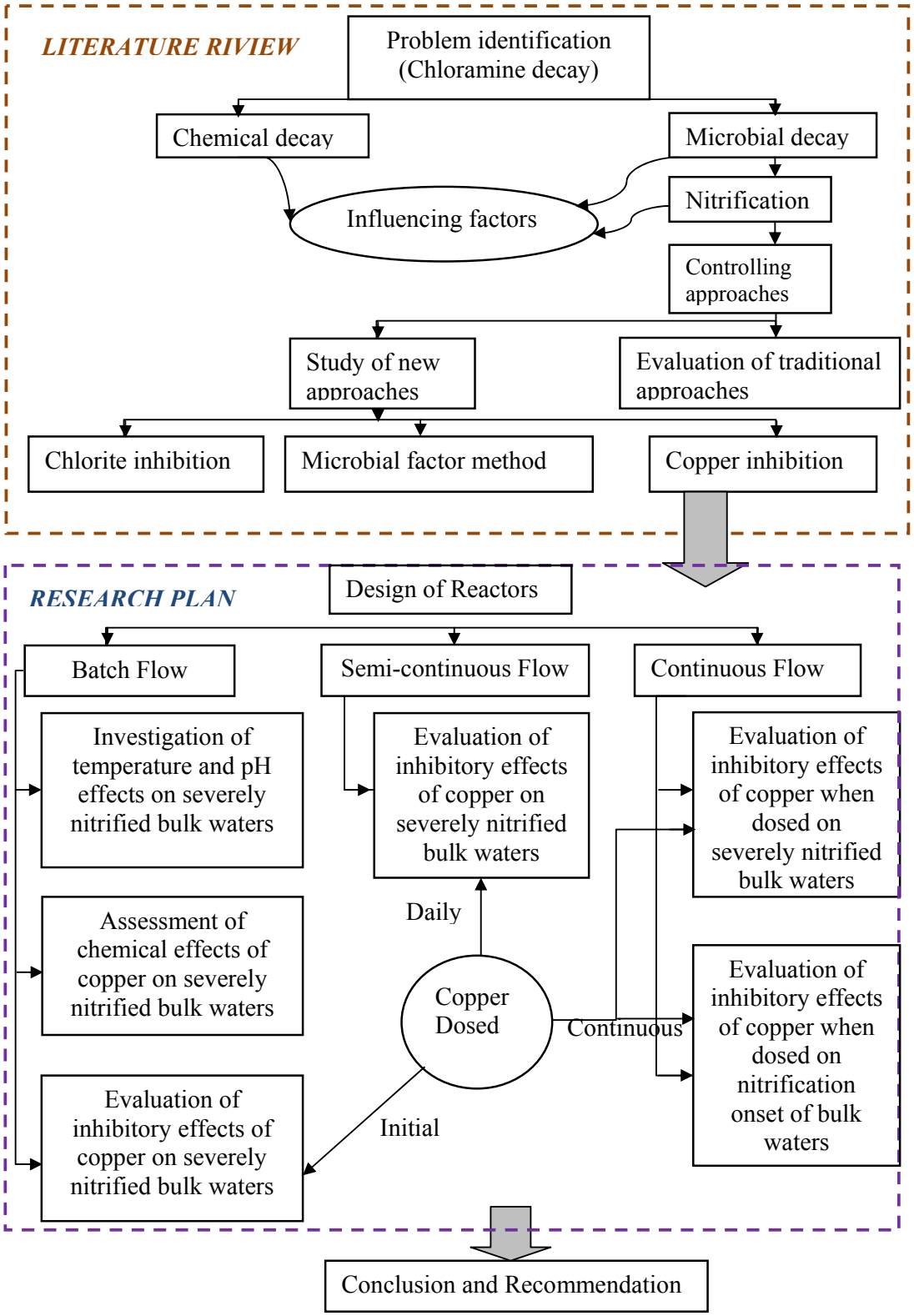

Figure 1.1: Schematic diagram of research approaches of the study

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Many water utilities are switching to chloramine from free chlorine as a disinfectant to reduce the production of regulated disinfection by-products (DBPs), such as trichlormethane (THM) and haloacetic acids (Goslan et al., 2009), produce less chlorinous and chloro-phenolic tastes and odor (Feben, 1935), obtain persistent disinfectant than free chlorine. Although chloramine offers many advantages, utilities experience several ominous consequences while using chloramine. The concerning discouraging aspects of using chloramine are water quality deterioration along the pipe lines (or with travel time) especially due to microbial process and nitrification, the extreme relevance of the study (Seidel et al., 2005).

Nitrification is a microbiological conversion of ammonia to nitrite and then nitrite to nitrate. It is an important challenge to understand and control nitrification in chloraminated water systems because nitrification events concur with reduced disinfectant residual and enhanced heterotrophic bacterial growth (Wolfe et al., 1988; Cunliffe, 1991). In order to colloquial off nitrification, the water utilities practiced several control strategies, including breakpoint chlorination, increased chloramine residual, optimizing chlorine to ammonia ratio, removal of organic compounds, distribution system cleaning, and decreasing detention time. Conversely, these methods are not accepted universally as their effectiveness sometimes depends on specific water utilities or sometimes depends on other control strategies. Although, some utilities used sodium chlorite for nitrification control, little is known about the success of nitrification control by metal inhibition.

Nowadays, metal (such as copper, nickel, zinc etc.) inhibition is used in the drinking water distribution systems as a novel approach for effective nitrification control. Out of these, copper inhibition has the least number of studies committed to expose its effectiveness in controlling nitrification in the drinking water distribution systems.

The following literature review is intended to summarize chloramine-base disinfection practices and its decay associated with nitrification, nitrification response to environmental stressors and the nitrification control approaches emphases on copper inhibition strategy.

2.2 Chloramine as a Disinfectant

Chloramine was introduced in potable water system in 1917 to prevent bacteriological 're-growth' problems and it was first used at a water treatment facility in Ottawa, Canada in 1918 (American Water Works Association (AWWA) Manual M56). As chloramine was more stable than free chlorine and produced less taste and odor problems compared to free chlorine, it was popular between 1920 and 1938. Unfortunately, chloramine lost its favors due to shortage of ammonia in World War II in 1940 (White, 1999). The United States Environmental Protection Agency (USEPA) accepted chloramine as a secondary disinfectant in 1978 to limit the concentration of THM, which was mainly responsible for switching to chloramine at many utilities (Wilczak et al., 1996) and it was accepted as a primary disinfectant by USEPA in 1983 (White, 1999). However, recent concerns over the production of DBPs while using free chlorine as a disinfectant and the explosion of the disinfection and disinfection by-products (D/DBP) rule have revived interest in using chloramine as a secondary disinfectant. Furthermore, it is often used when it is very difficult to maintain free chlorine residuals or when they lead to excessive disinfection (Vikesland et al., 2001 and Duirk et al., 2005) or the level of natural organic matter (NOM) is high (Jafvert and Valentine, 1992). On the other hand, chloramine as a disinfectant has some disadvantages: (i) chloramine is weaker than free chlorine (Haas, 2000), hence it takes longer contact time for pathogen inactivation when used as a primary disinfectant, (ii) Sometimes, chloramine addition in the system leads to increase concentration of by-products such as cyanogen chlorides and dichloropanones (Krasner et al., 1989).

2.2.1 Chloramine Chemistry

Chloramine is derivatives of ammonia by substitution of one, two or three hydrogen atoms with chlorine atoms and is formed by a chemical reaction between chlorine and ammonia. Chlorine and ammonia are added to the water either as a gas, liquid or solid form either sequentially or simultaneously. There are three forms of inorganic chloramine; monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and trichloramine (NCl_3). The generalized formation reactions of these three forms of chloramine are shown in the following (Haas, 1999) Equations 2.1 to 2.3:

Monochloramine formation

$$NH_4^+ + HOCl \rightarrow NH_2Cl + H_20 + H^+ \qquad \textbf{Equation 2.1}$$

Dichloramine formation

$$NH_2Cl + HOCl \rightarrow NHCl_2 + H_20 \qquad \textbf{Equation 2.2}$$

Trichloramine formation

$$NHCl_2 + HOCl \rightarrow NCl_3 + H_20 \qquad \textbf{Equation 2.3}$$

Free chlorine to ammonia ratio and solution pH dictate the formation of these three species of chloramine in the distribution system. Monochloramine is likely to form in drinking water treatment at pH>8.0 and is formed at a chlorine to ammonia ratio is approximately 5:1 (by weight). Monochloramine is preferable than other species (dichloramine and trichloramine) in the distribution system practices because of less susceptible to cause significant taste and odor problems (Kirmeyer et al., 2004).

2.2.2 Chloramine Decay

In the distribution system, chloramine reacts with organic and inorganic compounds associated with distribution system materials, biofilms and the bulk waters. These NOMs may exert an oxidant demand and the interference of micro-organisms results in chloramine decay. Thus, it is very difficult to achieve target residuals at the ends of long distribution systems due to chloramine decay. Chloramine decays in two

ways, one is chemical decay and another is microbiologically assisted decay. The chemical decay of chloramine in drinking water is due to auto-decomposition and reactions with organic and inorganic constituents. Jafvert and Valentine (1987) reported that auto-decomposition of chloramine is radically affected by general acid-catalysis and the generalized form of this reaction is presented in Equation 2.4. During the auto-decomposition reaction, ammonia in chloramine is oxidized to nitrogen gas with production of smaller quantities of nitrate (NO_3^-) [Equation 2.5] (Valentine and Wilber, 1987). Nitrate conversion is so small that it is hard to measure in normal experiments. The direct reaction between monochloramine (NH_2Cl) and nitrite (NO_2^-) or the reaction between nitrite (NO_2^-) and hypochlorous acid (HOCl) produced by NH_2Cl hydrolysis are described by Equations 2.6 and 2.7.

$$3NH_2Cl \rightarrow N_2 + NH_3 + 3Cl^- + 3H^+ \quad \textbf{Equation 2.4}$$

$$4NH_2Cl + 3H_2O \rightarrow 3NH_3 + NO_3^- + 4Cl^- + 5H^+ \quad \textbf{Equation 2.5}$$

$$NH_2Cl + NO_2^- + H_2O \rightarrow NO_3^- + NH_4^+ + Cl^- \quad \textbf{Equation 2.6}$$

$$HOCl + NO_2^- \rightarrow NO_3^- + H^+ + Cl^- \quad \textbf{Equation 2.7}$$

On the other hand, microbiologically assisted chloramine decay occurs due to microbial activities in the distribution systems. The significant concern associated with this decay is nitrification that occurs due to presence of the nitrifying bacteria in the distribution systems. Feben, (1935) found nitrification problems in drinking water systems and their link with chloramine in early 1930s. The ammonia released during chloramine decay can trigger nitrification phenomena and produce nitrite. The first indication of nitrification is often a difficulty in maintaining a stable chloramine concentration (Wolfe et al., 1988; Cunliffe, 1991; Skadsen, 1993; Odell et al., 1996; Wilczak et al., 1996). The two possible explanations for the accelerated chloramine decay: nitrite can accelerate chloramine decay, and chloramine is hydrolysed as free ammonia due to ammonia oxidation (Cunliffe, 1991).

In order to quantify the chemical decay and microbiological decay, and their respective contribution on chloramine decay, Sathasivan et al., (2005) developed a simple tool known as microbial decay factor (F_m). F_m is the ratio between the microbiological decay coefficient and the chemical decay coefficient. The F_m method is more quantitative, sensitive and general than the traditional indicators (e.g. nitrite,

ammonia, total chlorine), in quantifying the microbial contribution to chloramine decay. Later, Sathasivan et al., (2010) proposed the reservoir acceleration factor (F_{Ra}) that defines the degree of acceleration present in the reservoir over and above the chemical decay in the bulk water assuming the reservoir is well mixed. F_{Ra} can be determined using temperature, retention time, inlet and outlet chloramine residuals, that can be easily obtained by an operator. Moreover, because of the advantages over traditional indicators, F_{Ra} and F_m related parameters can be effective indicators to control rapid chloramine loss progressively, especially in summer.

Furthermore, presence of sediment and biofilm take a major part in chloramine decay. Many researchers reported that higher numbers of ammonia oxidizing bacteria (AOB) present in biofilm and sediments than bulk water. Biofilms and sediments provide protection from disinfectant and thus enhance the growth of AOB. The abiotic particle that accumulated or deposited in reservoirs and pipes, form sediment under favorable conditions especially in low flow condition and at dead ends. They affect the water quality in two ways; firstly, they can carry bacteria attached with their surfaces, which protect them from disinfection (Ridgway and Olson, 1982, Camper et al., 1986). Secondly, they act as the suspended organic matter while forming loose deposits in reservoirs and pipes water, when a change occurs in hydraulic regime. It is well-known that the organic matter plays an important role on chloramine decay. After resuspension of deposits, microbiological and chemical characteristics of water are not controlled (De Rosa, 1993) and thus results in water quality deterioration.

Besides, biological activity in distribution system is associated with biofilm formation (Camper et al., 1996). In a reservoir's sample in California, Stewart and Lieu, (1997) couldn't detect AOB in the water column whereas; significant levels of AOB were present in the wall biofilm layer. A recent study quantified the relative abundance of bacteria in both biofilm and bulk water in chlorinated pilot scale pipe loops (Srinivasan and Harrington, 2007). Sathasivan et al., (2010) have noticed a significant contribution of biofilm and sediment on chloramine decay in a small reservoir, although impact was minimal in large reservoirs. Therefore, it can be said that both sediments and biofilm can accelerate the chloramine decay and possibly enhance the growth of AOB, thus impacting on nitrification phenomena.

2.3 Nitrification and Nitrification Controlling Strategies in Chloraminated Drinking Water Distribution Systems

The presence and persistence of nitrifying bacteria in chloraminated water utilities has been well reported by various authors (Wolfe et al., 1988, 1990; Cunliffe, 1991). Conducting a telephone survey, Wilczak et al., (1996) reported that two-thirds of water utilities in US that use chloramine experience nitrification to some extent. Cunliffe, (1991) detected nitrification in 64% of samples from five chloraminated water supplies in South Australia. Lipponen et al., (2002) detected AOB and nitrite oxidizing bacteria (NOB) at the end of distribution systems in at least eleven out of fifteen in Finland.

2.3.1 Nitrification

Nitrification is a two-step microbiological processes by which reduced nitrogen (primarily ammonia) is sequentially oxidized to nitrite and nitrate.
In the first step, ammonia is oxidized to nitrite by AOB according to the Equation 2.8.

$$NH_3+O_2 \rightarrow NO_2^- +3H^+ + 2e^- \qquad \textbf{Equation 2.8}$$

In the second step, nitrite is oxidized to nitrate by NOB according to the Equation 2.9.

$$NO_2^-+H_2O \rightarrow NO_3^- +2H^+ + 2e^- \qquad \textbf{Equation 2.9}$$

Nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources (ammonia or nitrite). The most thoroughly studied ammonia oxidizer is *Nitrosomonas europaea*. Regan et al., (2003) found *Nitrosomonas oligotropha* to predominate in chloraminated distribution systems. Bollmann et al., (2002, 2005) reported that *Nitrosomonas oligotropha* has the high affinity for ammonia and can survive under low-ammonia environments. The first genus of NOB is *Nitrobacter*, which was identified as the predominant NOB in many natural and engineered environments (Painter, 1977). *Nitrospira* are the other common genus found in both pilot (Regan et al., 2002) and full-scale (Regan et al., 2003) drinking

water distribution systems. The water utilities have experienced a lot of problem caused by nitrification.

In Sydney water distribution system, Sathasivan et al., (2008) observed two distinct behaviours in the chloraminated bulk water samples and they defined as mild and severe nitrification conditions. The samples having the nitrite level less than 0.01 mg-N/L and reasonably stable chloramine decay rate was termed as mild nitrification condition. On the other hand, accelerated chloramine decay, higher nitrite production and depletion of ammonia levels were observed when the chloramine residual was below about 0.7 mg/L. This condition was referred as severely nitrifying condition. The same behaviour i.e, the accelerated chloramine loss was observed in severely nitrified water samples in Goldfield & Agriculture Water Supply System in Western Australia (G&AWSS), Western Australia (Sathasivan et al., 2009). Bal Krishna and Sathasivan, (2010) also found the accelerated chloramine decay in the severely nitrified water even after separating the microbial agents by filtration with 0.2 μm filter paper.

2.3.2 Impacts of Nitrification

Basically, nitrification results in the conversion of ammonia to nitrite and nitrate. In this phenomenon, inorganic carbon and dissolved oxygen are consumed and growing nitrifying microorganisms. Nitrification will result in disinfectant residual reduction and will often lead to raised heterotrophic plate counts (HPCs) in chloraminated drinking water systems. In some situations, nitrification may impact corrosion in the distribution system. The consequences of nitrification are not likely to be a direct risk to public health; rather, they may lead to operational or regulatory-compliance challenges. The possible changes of the monitoring parameters and their possible effects on water quality are shown in Table 2.1.

Table 2.1: Effects of Nitrification on Water Quality

Parameters change due to nitrification	Possible effect on water quality
Rapid chloramine decay	Maintaining residual at furthest points from distribution system is merely impossible
Nitrite production	Loss of disinfectant, increased lead from brass
Lower pH and alkalinity	Colour, taste and odour problems
Higher HPC	Loss of disinfectant and concern over pathogen regrowth
Decreased DO	Low redox in iron pipe associated with more red water

Source: Larsen et al., (1956), Pugh et al., (1966) and Guo et al., (2002)

2.3.3 Factors Affecting Nitrification in Drinking Water Systems

Certain physical factors and chemical substrates are required to grow nitrifying bacteria in the distribution systems. Wolfe and Lieu, (2001) found that these factors are likely to affect the growth of nitrifying bacteria in drinking water distribution systems. Some major factors that affect the growth of nitrifying bacteria are described below:

Presence of Free-ammonia: As nitrifying bacteria has limited ability to utilize organic compounds, they use ammonia and nitrite as the only exogenous energy source. In drinking water distribution systems, ammonia can be present from the untreated drinking water and released by chloramine decay. Fleming et al., (2005) reported that the initial chlorine to ammonia ratio used to form chloramine affects the free ammonia levels at the start of the distribution system. Excess free ammonia present at lower chloramine to ammonia ratio (<4:1 mass ratio) tend to encourage nitrification (Skadsen, 1993; Karim and LeChevalier, 2006;). Moreover, Woolschlager et al., (2001) reported that nitrifiers can uptake ammonia directly from monochloramine.

In an aqueous solution, ammonia nitrogen exists as either ammonium ion or ammonia and the equilibrium reaction can be defined as below:

$NH_4^+ \leftrightarrow NH_3\ (aq) + H^+$ **Equation 2.10**

Therefore, the speciation of ammonia nitrogen generally depends on the pH value, the equilibrium being displaced to the left in alkaline water. On the other hand, ammonium ion is largely predominant at neutral or slightly basic pH. Generally, the ratio of the ammonium to the ammonia concentration is equal to 100:1 at 20°C and at a pH of 7.

Dissolved Oxygen: Nitrifying bacteria are obligate aerobes. Drinking water distribution systems have sufficient oxygen for nitrifier growth in the bulk water. However, dead ends, stagnation and biofilms, and corrosion may create micro-anaerobic environments where oxygen could be limiting. Ammonia oxidizers may survive in oxygen limiting environments (Kowalchuk and Stephen, 2001) and they can use nitrite and nitrate as an alternative electron acceptor in low oxygen circumstances (Alleman and Preston, 2005).

Cell Attachment: Nitrifiers can exist as free living cells or attach to a surface as a biofilm. Wilczak et al., (1996) reported that nitrifiers in biofilms can explain the persistence of nitrifiers in drinking water systems. Dead-ends of distribution systems and water storage reservoirs are the persistent and highly active sites of nitrifiers (Cunliffe, 1991; Skarden, 1993). Furthermore, sediment areas (Ike et al., 1988) and plumbing systems in premises (Edwards et al., 2005) can support more nitrifier growth due to low disinfectant residuals and more surface area for attachment. (Odell et al., 1996) reported that sediment and tubercles in distribution pipes may also exert a chlorine demand and further facilitate nitrifier growth.

Temperature: Nitrification is highly influenced by water temperature in drinking water distribution systems. Nitrification incidence was higher in summer or when temperatures were more than 15ºC. Elevated temperatures increase the rate of chloramine decay and therefore increase the availability of free ammonia for nitrifier growth (Nowlin et al., 2001). Temperature greatly affects the onset of nitrification (Sarker and Sathasivan, 2011b). The number of AOB was 100 to 1000 times higher in summer than in winter (Wolfe et al., 1988, 1990). On the contrary, elevated

temperature sometimes helps in controlling nitrfiers by increasing disinfection efficiency of chloramine. The nitrifiers can grow up in distribution systems with temperature ranging from 10-34ºC (Cunliffe, 1991) whereas, the optimum temperature for nitrification and nitrifier growth is 25-30ºC but sometimes nitrification was observed in temperature below 10ºC (Wilczak et al., 1996).

Light: Chloramine degrades when exposed to the atmosphere at varying rates depending on the intensity of sunlight (Alleman et al., 1987), wind and temperature. Nitrifiers are very sensitive to visible and ultraviolet irradiation and even fluorescent lighting (Wolfe and Lieu, 2001).Though Ammonia oxidation by *Nitrosomonas europaea* is inhibited by sunlight and UV light (Hooper and Terry, 1974), they have the capability to recover from light inhibition.

pH: Harrington, (2002) reported that altering bulk water pH is an economical control strategy for reducing nitrification occurrence. In drinking water systems, pH can influence nitrification by affecting the growth of nitrifiers, ammonia release from chloramine decay and chloramine inactivation rate on nitrifiers (Harrington, 2002; Oldenburg et al., 2002). In some utilities, it was noticed that an increase in pH (greater than 9) can be used to reduce the occurrence of nitrification (Skadsen et al., 1996; Sarker and Sathasivan, 2011a). Wilczak et al., (2001) reported that pH is the most important factor controlling the rate of chloramine autodecomposition.

2.3.4 Traditional Control Approaches of Nitrification

Several strategies have been applied in chloraminated distribution system for controlling nitrification. These are: breakpoint chlorination (Wolfe et al., 1988; Odell et al., 1996), maintaining disinfectant residual (Skadsen, 1993; Harrington et al., 2002), optimization of the chlorine-to-ammonia ratio (Wolfe et al., 1988; Odell et al., 1996), removal of natural organic matters (Odell et al., 1996), distribution system flushing (Odell et al., 1996), decrease of distribution system retention time (Odell et al., 1996; Harrington et al., 2002). The brief description of these traditional control methods for nitrification is described below;

Breakpoint Chlorination: Breakpoint chlorination is thought to be a most effective control approach to control nitrification after commencing nitrification (Odell et al., 1996). Many utilities implement breakpoint chlorination for a period of one week to one month once or twice a week. However occasional breakpoint chlorination is not effective for long-term nitrification control strategy and some utilities experienced high percentages of coliform-positive samples during free chlorine periods (Odell et al., 1996). Prolonged uses of free chlorine increase DBPs concentration and unaccepted chlorinous taste (Harms and Owen, 2004; Ferguson et al., 2005). Moreover, breakpoint chlorination process requires extensive labour involvement and huge amount of chlorine at the time of free chlorine addition.

Increase Chloramine Residual: Several studies reported that a minimum of 2-3 mg/L chloramine residual should be maintained to prevent nitrification (Wolfe et al., 1990; Lieu et al., 1993; Kirmeyer et al., 1995; Odell et al., 1996; Harrington et al., 2002). Increasing chloramine residual by adding free chlorine may be an effective nitrification control measure for long term approach but it may not be effective for nitrification control once nitrification starts in the distribution system (Odell et al., 1996). Even, chloramine concentration of 8 mg/L was not effective in controlling nitrification in Ann Arbor, Michigan distribution system (Skadsen, 1993). In South Australia, Cunliffe, (1991) found nitrifiers in samples having more than 5 mg/L of monochloramine. Zhang et at., (2009) reported that nitrification didn't decrease in most pipe materials until the chloramine level was increased to 4 mg/L and maintained at that level for several weeks. The probable reason may be nitrite can degrade chloramine residuals before inactivated by chloramine (Wolfe et al., 1988; Kirmeyer et al., 1995; Odell et al., 1996). Another protective mechanism may be the relative ratio of growth versus inactivation by disinfectant. If the AOB growth rate driven by ammonia concentration is higher than the inactivation rate by chloramine, then theoretically AOB can grow in presence of chloramine (Harrington et al., 2003).

Increasing the ratio of Chlorine to Ammonia: Reduction of available ammonia by increasing the ratio of chlorine to ammonia is probably an effective control measure although evidence suggests that nitrification can take place even when only small amounts of ammonia are available. Moreover, Woolschlager et al., (2001) reported that nitrifiers can uptake ammonia from monochloramine directly. Seidal et al.,

(2005) reported that optimizing the chlorine to ammonia ratio is the most common nitrification control technique. Nitrification was not controlled when chloramine was applied at chlorine to ammonia ratio of 3:1 (McGuire et al., 2004; Karim and LeChevallier, 2006) but it was effective when chloramine was applied at chlorine to ammonia ratio of 5:1 (Wolfe et al., 1988; Karim and LeChevallier, 2006). In an industry survey, Wilczak et al., (1996) did not find any trend between the ratio of chlorine to ammonia and nitrification incidence, and maintaining chlorine to ammonia ratio of 4.75:1 was unsuccessful due to poor ammonia control (Skadsen, 1993). Moreover, there is a chance of dichloramine formation while maintaining chloramine at higher chlorine to ammonia ratio of 5:1, results taste/odour problems and higher DBPs formation (Skadsen and Cohen, 2006).

Removal of Organic Compounds at the Treatment Plant: It is proved that NOM can accelerate chloramine decay (Tomas, 1987; Margerum et al., 1994; Song et al., 1999). Removing NOM at the treatment plant has the potential to be an effective nitrification control measure for long-term strategy and is commonly practised in Europe. It is not accepted as a universal approach due to lack of specific study to establish as a bonfire nitrification control measure (Odell et al., 1996). In a pilot-scale study, Harrington et al., (2002) reported that nitrification could be avoided or prolonged the onset time by maintaining total chlorine residual above 2.2 mg/L and the chlorine to free-ammonia ratio greater than 1.9. In the same study, they also noticed that onset of nitrification was delayed in higher TOC removal samples during a four day retention time and further no nitrification events were observed when the retention time was reduced to one day.

Flushing of Distribution System: Flushing is the second most common practice for nitrification control (Seidel et al., 2005). Flushing can also remove tubercles and sediments, thus disinfectant can penetrate into biofilms containing nitrifiers (Harms and Owens, 2004). Flushing the distribution system with high velocity is a fairly effective for controlling and preventing nitrification but its efficiency depends on individual system's condition (Odell et al., 1996).

Decreasing Detention Time: Reducing system retention time coupled with other controlling measures such as rechlorination or periodic breakpoint chlorination can

be an effective episode control as a long-term improvement measure for nitrification (Odell et al., 1996).

A brief summary of the aforementioned control measures of nitrification is shown in Table 2.2.

Table 2.2: Control Measures of Nitrification-Effectiveness and Limitations

Control Measures	Effectiveness	Limitations
Breakpoint chlorination	Effective for short-term control	Potential taste and odour complains Required intensive labour
Increase chloramine residual	Effective before nitrification occurs	Effective at preventing onset of nitrification but not effective once nitrification occurs
Increasing the ratio of chlorine to ammonia	Effective at chlorine to ammonia ratio of 5:1	Through ammonia concentration reduces, nitrifiers could grow even with small amount of ammonia
Removal of organic compounds at the treatment plant	Effective for long-term control strategy	Not applicable in every country
Distribution system cleaning	Provides long-term improvement for reducing nitrification	Nitrifiers could re-establish between flushing intervals Labour and operation intensive
Decreasing detention time	Effective for long-term control strategy	Effectiveness depends when coupled with other control measures

Source: adapted from Odell et al., 1996; AWWA, 2006.

Traditional controlling measures are not completely effective for controlling neither nitrification nor chloramine decay. Therefore, the limitations in the above mentioned approaches demand necessity to find an efficient way for chloramine residual

management. In this context, using appropriate tools, or addition of some chemicals or metals in the distribution system may give some positive feedback for nitrification control.

2.3.5 Chloramine Residual Management by F_m Method

An adaptive management strategy based on F_m values was developed to help in maintaining chloramine residual in service reservoir (Sathasivan et al., 2005). This method was claimed to provide the most accurate assessment of chloramine stability and showed the early warning of nitrification onset (Fisher et al., 2009; Sathasivan et al., 2009) by calculating the decay rate coefficients (chemical and microbial) with monitoring chloramine residual only rather than observation of traditional parameters. Sathasivan et al., (2010) reported that F_m method was shown to be helpful in maintaining an adequate chloramine residual by minimizing microbial acceleration of chloramine decay in chloraminated water reservoir of Sydney Water Distribution System, Australia. However, this method is useful in predicting the onset of nitrification only and its efficiency depends on others control strategy to maintain the chloramine residual. Moreover, information on nitrification occurring in pipe lines of the distribution system was not reported.

2.3.6 Controlling Nitrification by Using Sodium Chlorite

The Gulf Coast (Texas) Water Authority (GCWA) uses chlorine dioxide as the primary disinfectant while using chloramine as the final disinfectant, has not observed nitrification due to formation a significant amount (0.25 to 0.35 mg/L) of chlorite in their distribution system. It was hypothesized that chlorite can reduced or eliminated the potential for nitrification (McGuire et al., 1999). Further, McGuire et al., (2004) reported that short term application of chlorite ion (0.2 mg/L) is effective in preventing nitrification in full-scale distribution system. In pure cultures, Hynes and Knowles (1983) reported that AOB (*Nitrosomonas europea*) and NOB (*Nitrosomonas winogradski*) were inhibited by chlorite.

On the other hand, the potential impact of using chlorite as a nitrification inhibitor should be considered. Gates, (1989) reported that chlorite could be transformed to

chlorine dioxide in an acidic environment that could create by oxidation of ammonia during nitrification by biofilm on the surfaces. Gagnon et al., (2005) reported that chlorite at 0.1-0.25 mg/L was ineffective in inactivating heterotrophic bacteria. McGuire et al., (1999) reported that chlorite was not effective in preventing nitrification in one system, probably due to presence of higher ammonia (1.4 mg-N/L). Karim and LeChevallier (2006) also found the ineffectiveness of nitrification using chlorite. Rahman et al., (2011) reported that chlorite was effective for nitrification inhibition at higher dose (20 mg/L) whereas permissible limit of chlorite is 0.8 mg/L according to USEPA Stage 1 D/DBP Rule.

2.3.7 Nitrification Control by Various Nutrients Including Metal Inhibition

The additions of toxic substances such as metals exert a significant inhibitory impact on the nitrifying bacteria. Metal inhibition can take place by blocking the enzyme function of the nitrifying bacteria (Martin and Richard, 1982). Nitrification can be controlled by silver addition (Sathasivan et al., 2005; Fisher et al., 2009) and by silver nanoparticles (Choi et al., 2008). Yong-Woo et al., (1997) reported that nitrifying bacteria was found to be inhibited to copper and nickel. Skinner and Walker (1961) reported that nickel ion (Ni^{2+}) at a concentration of 0.25 mg/L reduces the growth of *Nitrosomonas*. Sato et al., (1988) reported that the amine compound of copper and nickel have inhibitory effects on the growth of *Nitrosomonas europaea*. The nitrification inhibition ranges of various metals for pure cultured nitrifiers are shown in Table 2.3. However, it could be significantly difference in mixed culture environment.

Table 2.3: Inhibition Range of Various Nutrients Including Metal

Metal	Specific inhibition range (mg/L)	Culture purity	References
Cu (II)	>0.10	*Nitrosomonas europaea*	Skinner and Walker, (1961)
	0.05-0.56	*Nitrosomonas europaea*	Loveless and Painter, (1968)
	4.0 had 75% inhibition	*Nitrosomonas europaea*	Tomlinson, (1966)
Ni (II)	>0.25	*Nitrosomonas europaea*	Skinner and Walker, (1961)
	11.8 (complete inhibition)	*Pure Nitrosomonas*	Meikleohn, (1954)
	>0.10	Not specified	Martin and Richard, (1982)
Ca (II)	8000	*Pure Nitrosomonas*	Meikleohn, (1954)
Mg (II)	>50	*Nitrosomonas europaea*	Loveless and Painter, (1968)
	12000	*Pure Nitrosomonas*	Meikleohn, (1954)
K (I)	19500	*Pure Nitrosomonas*	Meikleohn, (1954)
Cr (III)	>0.25	*Nitrosomonas europaea*	Skinner and Walker, (1961)
Zn (II)	0.08-0.5	*Nitrosomonas europaea*	Skinner and Walker, (1961)
Na (I)	7000	*Nitrosomonas europaea*	Loveless and Painter, (1968)
Fe	560	*Pure Nitrosomonas*	Meikleohn, (1954)
Cd (II)	0.25	*Nitrosomonas europaea*	Skinner and Walker, (1961)

2.3.7.1 Copper toxicity and nitrification inhibition

Copper is bacteriostatic or toxic to bacteria, viruses or cysts. The use of copper as the control measure for nuisance algae in surface waters has been practiced for a long time and sustains as the most effective algicidal treatment for lakes, reservoirs and other managed bodies (Elder and Horne, 1978; Whitaker et al., 1978; Mcknight et al., 1983; Haughey et al., 2000). Copper can inhibit ammonia oxidizing bacterial activity (Laszlo, 2008; Zhang et al., 2009). Conversely, copper influences the growth of nitrifying bacteria depending upon the concentration. Copper in dissolved form acts as a micronutrient (required for optimal growth) and as a toxicant (impeding growth) to phytoplankton and other microorganisms in natural waters (Manahan and Smith, 1973; Brand et al., 1986; Peers et al., 2005). The growth of ammonia oxidizer *Nitrosomonas* were enhanced by copper at a higher dose of 0.1–0.5 mg-Cu/L (Skinner and Walker, 1961). Copper enhanced the growth of ammonia oxidizer *Nitrosomonas* at a lower dose of 0.005–0.03 mg-Cu/L and inhibited ammonia oxidizer *Nitrosomonas* were at copper concentration of 0.1 – 0.5 mg-Cu/L (Loveless and Painter, 1968). In a pure *Nitrosomonas* culture, Tomlinson, (1966) found about 75% inhibition produced by copper at a concentration of 4 mg-Cu/L. Copper concentrations in low level (0.20 mg-Cu/L) have been found to be toxic to nitrifying bacteria in waters with low chelating capacity (Waara and Wilander, 1985). The effect of copper on nitrification is dependent on the free copper (Cu^{2+}) concentration (Braam and Klapwijk, 1981). Copper in dissolved form, mainly Cu(II), is very persistent and frequently detected in the drinking water (Boulay and Edwards, 2000; Zhang et al., 2002).

2.3.7.2 Copper effects on chloramine residual in chloraminated drinking water distribution system

Jun et al., (2009a) reported that copper plays an important role in the decomposition of monochloamine upon chloramination due to its catalytic activity, especially when pH is not 8. Elemental copper can react with monochloamine through the following reactions at a temperature of 25ºC (Zhang et al., 2002):

$$NH_2Cl + Cu(s) + OH^- \leftrightarrow CuO(s) + Cl^- + NH_3 \qquad \textbf{Equation 2.11}$$

$NH_2Cl + Cu(s) + H_2O + OH^- \leftrightarrow Cu(OH)_2(s) + Cl^- + NH_3$ **Equation 2.12**

$NH_2Cl + Cu(s) + HCO_3^- \leftrightarrow CuCO_3(s) + Cl^- + NH_3$ **Equation 2.13**

The strong ligands for copper ions, include ammonia, chloride, inorganic carbon and NOM weaken the deposit formation by forming dissolved complexes. Jun et al., (2009a) reported that acceleration of monochloramine decay increased when the copper concentration increased from 0.01 to 0.10 mg-Cu/L, but they didn't find any acceleration of chloramine decay when the copper concentration was 1.0 to 5.0 mg-Cu/L. Jun et al., (2009b) reported that monochloramine decay was occurred by dichloramine formation due to addition of Cu(II), where its direct catalysis had the major contribution and pH reduction.

2.3.7.3 Copper complexation with ammonia

pH should be controlled at 8.0 for maintaining the chemical stability of chloramine and maintain the water slightly encrustive (scale forming) in the chloraminated distribution systems. The formation of Cu-Ammonia complex occurs when copper is added in the chloraminated water. Sillen and Martell, (1971) reported a series of equilibrium constants for Cu-Ammonia complex formation. The availability of free ammonia is higher in higher pH (Sarker and Sathasivan, 2011a). Zhan, (2007) confirmed that Cu-NH_3 complex is negligible using Milli-Q water dosed with chlorine and ammonia at the concentrations required in G&AWSS.

2.7.3.4 Impacts of NOM on copper solubility and organo-copper complexation

NOM is a heterogeneous mixture that can bind with metals in aqueous solution. It is believed that the majority of dissolved copper, existing in natural water bodies, is in the form of Cu-NOM complexes, because NOM contains various ligands which can bind with soluble copper, forming soluble or colloidal compounds (Lehman and Mills, 1994). The conditional stability constants for copper–ligand complexes for dissolved organic matter (DOM) steadily increased with pH, indicating that the copper–ligand complexes become more stable at higher pH (Sarathy and Allen, 2005).

At higher pH (8 or more), the stability of Cu-NOM complex is reported to be quite strong and vice-versa (Takacs et al., 1999). Zhan et al., (2009) reported that copper is more soluble in higher in natural water. Therefore, NOM can be enhanced considerably the solubility of copper in bulk waters. Krishna and Sathasivan (2010) found that organic matter in natural waters can be broken down to smaller molecules when they react with chlorine or chloramine. These soluble microbial products can also increase the copper concentration by forming organic copper complexes.

2.3.7.5 Health concern regarding copper application

According to the regulations of World Health Organization, recommended value of copper concentrations in drinking water should be below 1.0 mg-Cu/L with the maximum value of 2.0 mg-Cu/L (WHO, 2008). Following the Australian Drinking Water Quality Guidelines (ADWG, 2004) and authorized by the Department of Public Health, Western Australia, copper dose of 0.25-0.40 mg-Cu/L was implemented in the field.

2.3.7.6 Practical application of copper in G&AWSS

The G&AWSS (Figure 2.1) in Western Australia, is perhaps the world's most extensive water distribution system, consisting a very long pipe line (530 km) from Mundaring Weir, Perth, Western Australia to Kalgoorlie. Mundaring Weir is fed with water from Helena River in the Darling Scarp and has also been augmented with treated groundwater in recent years.

Water Corporation, Western Australia conducted copper dosing in the field to test the feasibility and efficacy of copper inhibition. Copper was dosed in two locations: Merredin reservoir (in 2005) and reticulating pipes at C-K extension (from Cunderdin north toward Minnivale tank). In Merredin reservoir, it was found that copper concentration at the required level was stabilized after two weeks of copper dosing. In the C-K extension, copper (in the form of copper sulphate) had been dosed continuously since April 2006. The dosed copper concentration was gradually increased with the initial dose of 0.25 mg-Cu/L in bulk water. It was found that the growth of nitrifying bacteria was not inhibited due to inadequate copper

concentration at the furthest points of the distribution system pipe lines. As a result, accelerated decay of the disinfectant residuals at furthest points and accumulation of copper in the main in the form of sediments or in the form of adsorbed metals on the pipe walls closer to the dosing were observed.

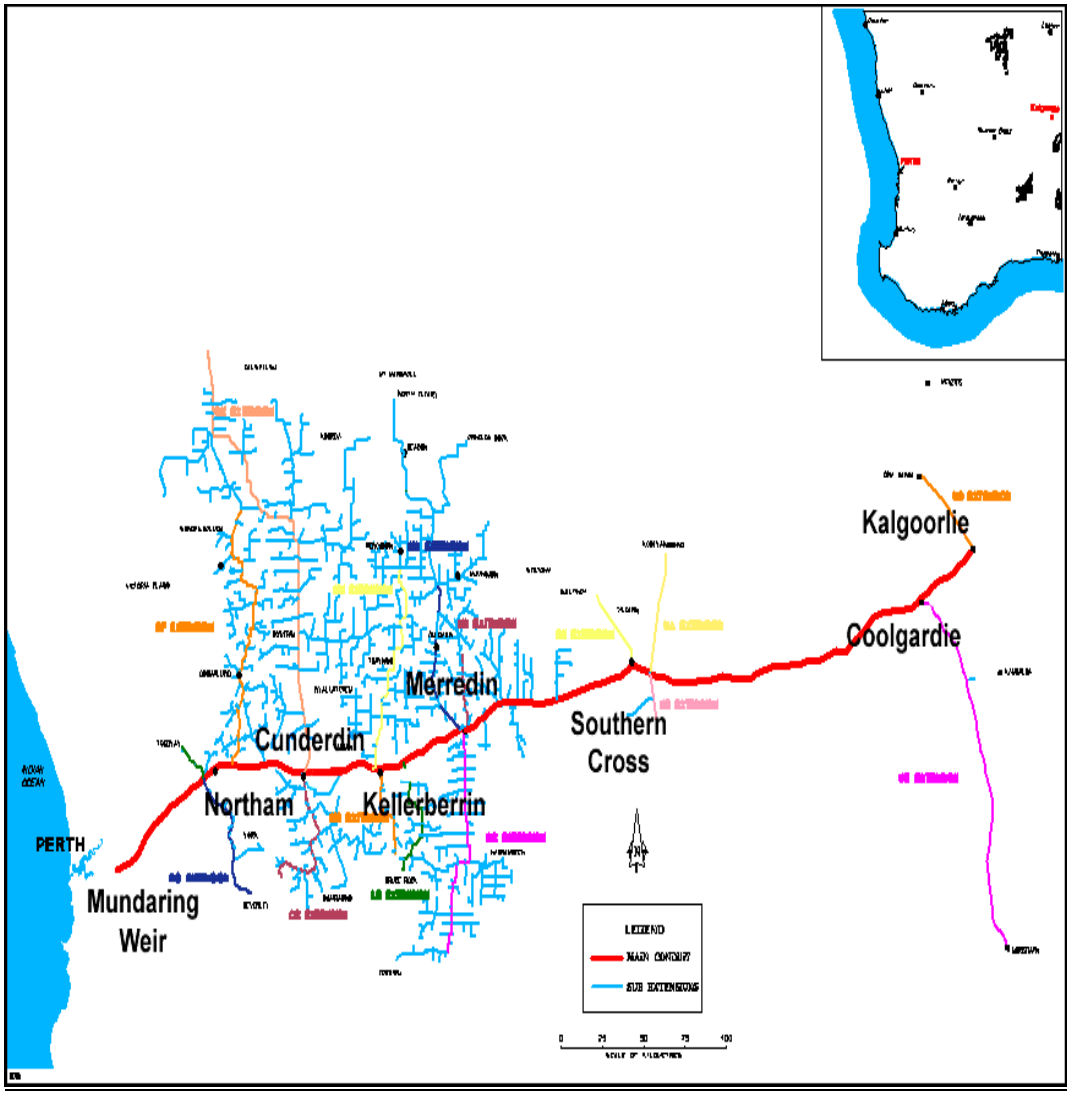

Figure 2.1: Goldfield & agriculture water supply system (Water Corporation, WA)

Based on the past evidences, it can be concluded that nitrification is the main cause of chloramine decay, and biological activity caused by nitrification, associated with

sediments and biofilm formation. Previous studies on nitrification have evaluated the effects of ambient conditions (e.g. temperature) and operational parameters (e.g. pH) on onset of nitrification in the distribution systems (Bone, 1999; Harrington et al., 2003) but their effects on chloramine residual concentration at onset point of nitrification have not been reported. Therefore, mathematical model incorporating the effects of temperature and pH would help in understanding of how nitrification episodes occur and how it can be controlled in chloraminated distribution system subjected to different circumstances.

Controlling nitrification might help in maintaining chloramine residual in the distribution system. There are several methods for nitrification control. The efficacy of the traditional approaches effective only for short term periods and sometimes depends on others strategy. Of all the control strategies discussed, breakpoint chlorination is typically thought of as a last resort when other measures fail to control nitrification. Despite of the benefits, the drawbacks of adopting breakpoint chlorination for nitrification control: the transition periods while switching from free chlorine to chloramine result in a brief period of time where a strong disinfectant residual is absent from the system, which poses a threat to public health. In addition, compliance with D/DBP Rule may be affected when water utilities add chlorine to tie-up free ammonia or to breakpoint chlorination to control nitrification. Furthermore, nitrification control by using chlorite is a relatively new method that is still under investigation. Therefore, controlling nitrification by any means is extreme crucial in order to make the proper use of chloramine in the distribution system. In this context, nitrification control by inhibition will be the feasible solution for long-term strategy. The previous studies focused on the effects of copper on nitrification control when dosed in a particular point. However, copper dosing in different nitrification conditions were not evaluated. Since copper was effective inactivating nitrification in reservoirs, why it was not effective in pipe lines in the distribution system or under severely nitrified bulk waters. Considering nitrification status of the samples during copper dosing time, may bring the fruitful solution for nitrification control in the distribution systems.

CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation of Stock Chemical Solutions

Standard stock solution was prepared by mixing the analytical-grade of chemicals with Milli-Q ultra pure water (18 MΩ/cm, <100 ppb-C/L). Stock solutions of total chlorine (TCl; 1000 mg-Cl_2/L), ammonium-nitrogen (500 mg/L), nitrite-nitrogen (500 mg/L) and nitrate-nitrogen (500 mg/L) were prepared using sodium hypochlorite, ammonium chloride, sodium nitrite and sodium nitrate, respectively. Monochloramine (NH_2Cl) solution was prepared using stock solutions of ammonium chloride (500 mg-N/L) and sodium hypochlorite (1000 mg-Cl_2/L) by maintaining the appropriate proportion. For calibration of standard curves, stock solutions of 1 mg-N/L were prepared using ammonium chloride, sodium nitrite and sodium nitrate for TAN [TAN: total ammoniacal nitrogen- represents sum of ammonia nitrogen (NH_3-N), ammonium nitrogen (NH_4^+-N) , nitrogen associated with chloramine (NH_2Cl-N) and a small portion of organic nitrogen], nitrite and NOx-N (nitrite-nitrogen+nitrate-nitrogen), respectively. Standard copper sulphate solution (1000 mg-Cu/L) was prepared by mixing copper sulphate ($CuSO4 \cdot 5H_2O$) into Milli-Q ultra pure water. The pH of the standard solution of copper sulphate solution was maintained below 6 to preserve copper as cupric ions. The pH was adjusted using 1M hydrochloric acid and 1M sodium hydroxide.

3.2 Details of Water Sample Collection and their Storage for Operating Pilot-Scale Reactors

The water for this study was collected from the outlet of Mundaring weir (from the source of the Goldfields and Agricultural Water Supply System) upstream of chloramination point, where ammonia and chlorine were dosed simultaneously. The water was collected in a container (capacity of 1000L) made of high density poly-ethylene (HDPE). The container was pre-cleaned with sodium hypochlorite (2~3%)

to remove suspected impurities. Milli-Q ultra-pure water (18 MΩ/cm, <100 ppb-C/L) was used to wash the container afterwards. The container was then washed with Mundaring raw water three times prior to collection. The filled container was stored inside the laboratory at room temperature. The quality of Mundaring water is only slightly varying in terms of pH (7.6~8.1), dissolved organic carbon (DOC) of 2.4~3.2 mg/L and UV_{254} absorbance (0.031~0.038 cm^{-1}).

3.3 Description of Pilot-Scale Reactor System

The purpose of operating the pilot-scale system was to produce none to severe nitrification conditions which usually occur in the real distribution system. Therefore, it was possible to conduct several experiments at different nitrification conditions by collecting the bulk water samples from the reactors and into the reactors.

3.3.1 Setting up the Pilot-Scale Reactor System

Pilot-scale system consists two series (five reactors in each series) of reactor, made of HDPE, was set up in the Civil Engineering laboratory at Curtin University, Western Australia as shown in Figure 3.1. Each reactor had one inlet and one outlet valve. Continuous gravity flow of water was maintained along the reactors of R-1 to R-5 through connecting HDPE pipes and flow was controlled according to the reactor's required retention time. However, water flow rate between feed water tank and R-1 was maintained using water level sensors and control valves. The water temperature inside the reactors was controlled through temperature controllers (stainless steel sensor) and a heating pad was attached underneath each reactor.

3.3.2 Preparation of Pilot-Scale Reactor's Feed Water

The reactor's feed water was prepared by maintaining 2.50 mg/L chloramine (chlorine followed by ammonia). Ammonia was added after 20-24 hours of chlorine addition. In the feed water, final TCl toTAN mass ratio of 4.5**:**1 and pH of 8.0±0.1

was maintained. DOC of the feed water was 2.78±0.4 mg/L during the experimental period.

3.3.3 Start-up of Pilot-Scale Reactor System

During the start-up period, chloramine concentration of about 1.0 mg-Cl_2/L was maintained in each reactor. Chloraminated water was fed from feed tank to the reactors continuously. To expedite nitrification and to obtain distribution system specific inoculum, nitrified water collected from G&AWSS had been placed as seed microbes (about 1.0 L water) in R-2 to R-5 in during the start-up period. Chloramine concentration was gradually increased up to 2.5 mg-Cl_2/L at the feeding tank in order to maintain about 2.0 mg-Cl_2/L of chloramine in R-1. Different stages of nitrification (mild to severe) conditions, which generally occur in real distribution systems, were created. The feed water flow rate through each rector was 20 L/day and the quantity of water in each reactor was maintained between 15 and 18L. The hydraulic retention time of reactors (R-1 to R-5) was 22, 21, 20, 19 and 19 hrs respectively, highest retention time was maintained for the first reactor and it dropped gradually to 19 hrs. Temperature in the first three reactors (R-1 to R-3) was maintained at 20±2ºC, but in last two reactors (R-4 and R-5) was 23±2ºC to achieve more microbial activities.

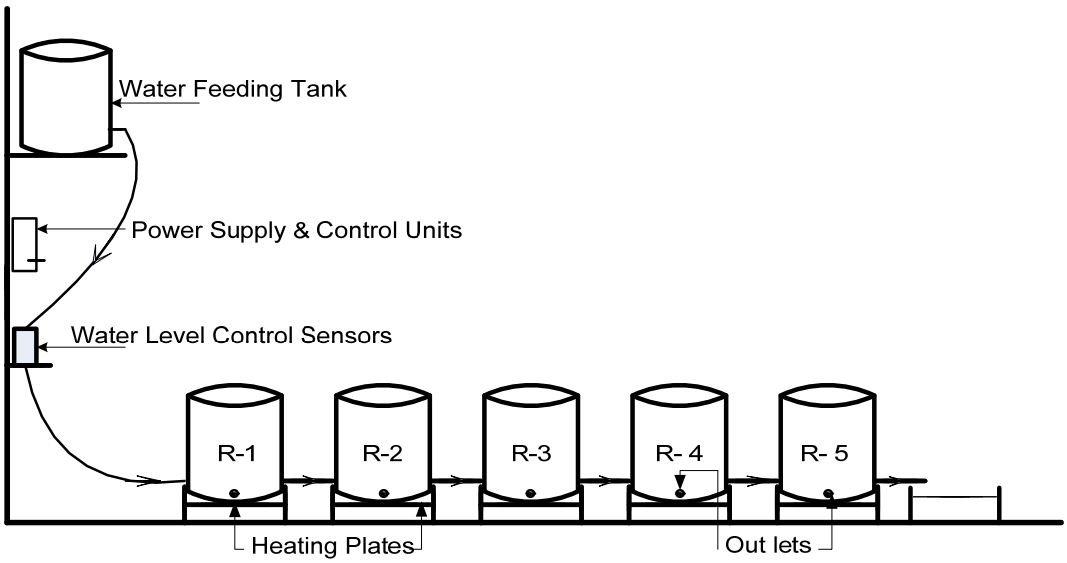

Figure 3.1 A: Pilot-scale reactors set up (Side view)

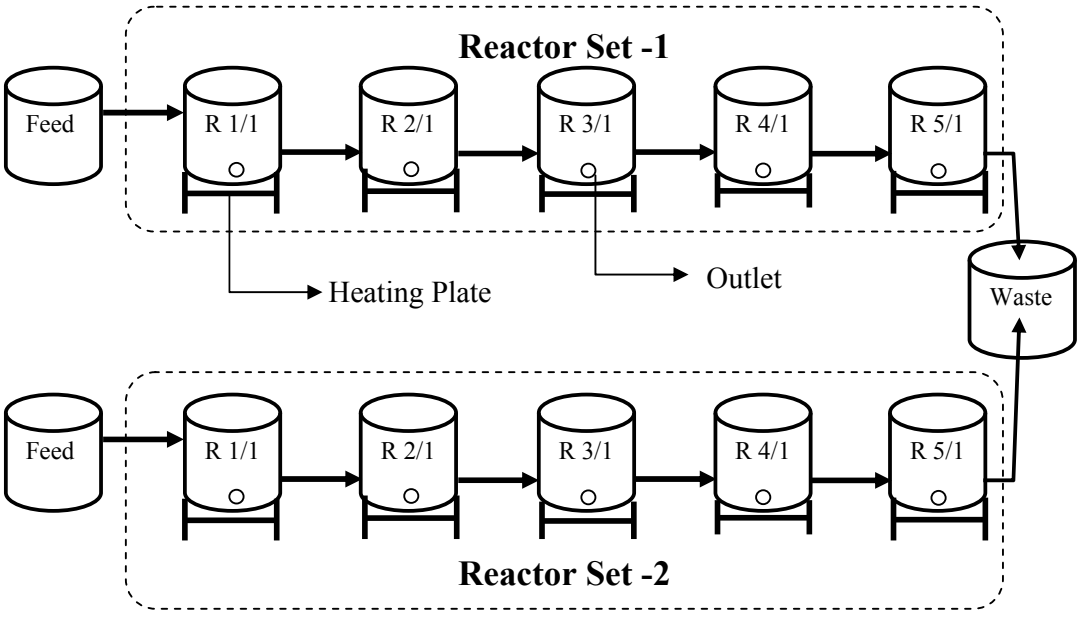

Figure 3.1B: **Pilot-scale reactors set up (Plan view)**

3.4 Preparation of Sample Bottles and Glasswares

Before starting each experiment, sample bottles and glasswares were clean properly. Sample bottles (600ml) were prepared by putting into a 10% sodium hypochlorite solution for 24hrs and rinsed with de-ionized water until sample bottles were free from chlorine. All the sample collection glassware was autoclaved.

3.5 Analytical Procedures

TAN, nitrite (NO_2-N), and NOx-N concentrations were measured using Aquakem-200. TAN present in water sample reacts with hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate and formed monochloramine (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). NO_2-N was measured by the sulphanilamide method (4500-NO2 B) (Standard methods, 1998.) and NOx-N was measured by catalytically reducing nitrate to nitrite by nitrate reductive enzyme in the presence of reduced nicotinamide dinucleotide (Campbell et al., 2006 and Patton et al., 2002). The strong azo dye that was produced by the reaction with nitrite was measured spectrophotometrically at 540nm or 520nm. NH_2Cl residual was reduced to 0 mg-Cl_2/L using 0.5% sodium thiosulphate stock/ascorbic acid solution before measuring NOx-N. This machine has high efficiency with the detection limit of 0.002 mg-N/L for the measurement of TAN, NO_2-N and NOx-N. The experimental error was found to be ±1.5% for TAN and NO_2-N. In case of NOx-N measurement, the experimental error was ±2% due to additional error introduced when reducing TCl using sodium thiosulphate. TCl residuals were measured by the DPD colorimetric method using a HACH pocket colorimeter reagent. TCl measurement had an experimental error of 0.03 mg-Cl_2/L. More than 99% of chloramine is present in the form of NH_2Cl when the pH is above 7.5 and TCl to NH_3-N ratio is less than 4 (Valentine, 2007). Hence, TCl represents mainly NH_2Cl residual at pH 8. The copper concentration was analysed using the spectrophotometer (HACH DR2800, 2005). The bicinchoninate method (Hach method 8506) was used in the laboratory. This method had the measuring range from

0.04 to 5mg-Cu/L and experimental error was 0.02 mg-Cu/L. Samples were digested using nitric acid (1:1) to reduce pH around 4 to 6 for copper measurement. A portable pH meter (HACH 40d) with temperature compensation was used to measure pH values and the measurement error was ±0.10.

3.6 Determination of Chloramine Decay Rate Coefficients

The chemical and total chloramine decay rate coefficients were calculated using exponential regression assuming the decay follows the first order decay kinetics as given in Equation 1

$$C_{TCl,t} = C_{TCl,0} . \exp^{(-k \times t)}$$ **Equation 3.1**

where, $C_{TCl,t}$ = chloramine concentration (mg/L) at time t

$C_{TCl,0}$ = initial chloramine concentration (mg/L)

k = the first order decay coefficient (hr^{-1}), and

t = incubation time in hrs.

3.7 Determination of Reservoir Acceleration Factor (F_{Ra})

For known values of retention time, and inlet and outlet chloramine concentrations, the total chloramine decay coefficient of reservoir water can be estimated from the following Equation, assuming that the reservoir is completely mixed.

$$TCl_{out} = \frac{TCl_{in}}{(1 + k_{Rt} * \theta)}$$ **Equation 3.2**

where, TCl_{out} = outlet chloramine concentration (mg-Cl_2/L)

TCl_{in} = inlet chloramine concentration (mg-Cl_2/L)

k_{Rt} = total chloramine decay coefficient (hr^{-1}) and

θ= retention time in the reservoir (hr)

Average retention time can be estimated by various means, and inlet and outlet residual can be easily measured using either online chlorine analysers or grab sample measurements. Then, the only unknown value, k_{Rt}, can be estimated.

Reservoir status can be standardised against a particular decay coefficient by comparing the k_{Rt} of a reservoir with the "base" chemical decay coefficient (k_{bc}). The k_{bc} was considered as the average chemical decay coefficient and its value was 0.0015±0.0002 hr^{-1} at 20°C (Sathasivan et al., 2008, 2010). If the reservoir temperature is not 20°C, the modified value of k_{bc} has to be converted to reservoir water temperature using Equation 3.3. The k_{bc} is assumed to follow the Arrhenius Equation shown in Equation (Sathasivan et al., 2009).

$$k_{bc,T} = k_{bc,20}.\exp\left[-\frac{E}{R}\left(\frac{1}{T}-\frac{1}{273+20}\right)\right] \qquad \textbf{Equation 3.3}$$

where, T is the temperature and E/R value is $6924K^{-1}$ (Sathasivan et al., 2009).Then, F_{Ra} is defined with respect to k_{bc} can be calculated by the following Equation 3.4

$$F_{Ra} = \frac{k_{Rt} - k_{bc}}{k_{bc}} \qquad \textbf{Equation 3.4}$$

3.8 Determination of Microbial Decay Factor (F_m)

Determining of chemical chloramine decay (k_c) and microbiological chloramine 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 subsamples. The first subsample was not processed whereas the second subsample was processed (filtered through a 0.2 µm membrane filter) to remove microbes. Same chloramine concentration was maintained in both subsamples. Both subsamples were then incubated at a constant temperature (20^oC) in a dark water bath. For each subsample, TCl was monitored regularly over time and the decay rate coefficients (k_c and k_m) were estimated by

exponential regression as presented by Equation 3.1. The decay rate coefficient for filtered sample (k_F) represents k_c. Finally, the F_m value (ratio of k_m and k_c) was calculated using the coefficients (k_c and k_m) at the same time. The detailed method is given in Sathasivan et al., (2005). The schematic diagram of determining F_m is shown in Figure 3.2.

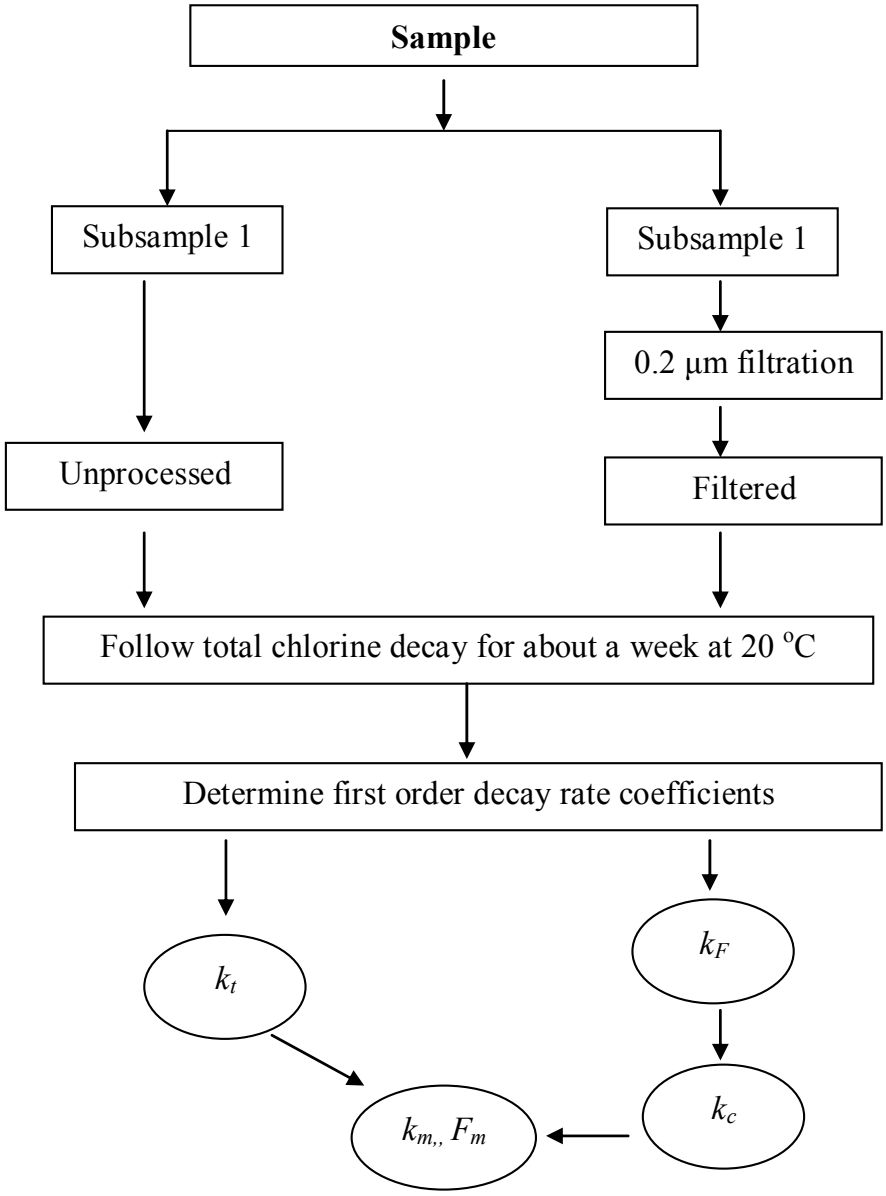

Figure 3.2: Schematic diagram for F_m determination

CHAPTER 4

ROLE OF pH FOR CONTROLLING SEVERE NITRIFICATION IN CHLORAMINATED DISTRIBUTION SYSTEM

Abstract

Nitrification control is complicated and expensive when nitrification has reached severely nitrifying stage. Under this condition, utilities usually apply re-chloramination with limited success. The pH adjustment may benefit utilities. However, it is yet clear whether pH should be moved up or down. The pH adjustment will also adjust chloramine (biocide) decay profile and ammonia (food) concentration. It is important to understand how this behaviour will ultimately impact nitrifying bacterial activity. We collected samples from severely nitrifying bulk waters from a pilot-scale system and adjusted the pH within practical range to know which pH benefits the most. Results showed that even a slight increase in pH can help protecting the chloramine residual and suppressing the nitrifying bacterial activity.

4.1 Introduction

Considering less production of regulated disinfection by-products, such as trihalomethanes and haloacetic acids, many water utilities adopted chloramine over chlorine as a disinfectant in drinking water distribution systems (Goslan et al., 2009). Despite its advantages over chlorine, it has additional challenges in residual management due to microbial acceleration (including nitrifiers) especially in long distribution system (Sathasivan et al., 2005).

Nitrification, a microbiologically mediated process, converts ammonia to nitrite by ammonia oxidizing bacteria (AOB) and nitrite to nitrate by nitrite oxidizing bacteria (NOB). It is the free ammonia in the form of NH_3-N that is reported to be utilised by AOB. In most instances, partial nitrification - mostly AOB activity - is reported in

chloraminated distribution systems (Wolfe et al., 1990). Usually, nitrification occurs over a range of pH of 6.6 to 9.7 (Odell et al., 1996) and above 15^{o}C, but it can also occur at low temperatures (Wilczak et al., 1996). Nitrification is also known to suppress pH to as low as 6.5 (Wilczak et al., 1996).

The threat of nitrification is of great concern to maintain chloramine for water utilities and several control strategies have been attempted. Once nitrification takes place, controlling or overcoming it is very difficult even by increasing chloramine concentration up to 8.0 mg/L (Skadsen, 1993; Cunlife, 1991). Chloramine decays at a much faster rate under nitrified condition (Sathasivan et al., 2008). To improve chloramine residuals and minimize the occurrence of nitrification, different control strategies are implemented. These strategies include: applying break-point chlorination (Odell et al., 1996), reducing concentration of chloramine demanding substances (Harrington et al., 2002), increasing chlorine to ammonia ratio (Lieu et al., 1993), diluting the contents in winter (Sathasivan et al., 2010) and re-chloramination. Realising the importance of ammonia (food) and chloramine (biocide), various authors have used them to define biostability concept to understand the point at which onset of nitrification occurs (Harrington, 2002; Fleming et al., 2005; Sathasivan et al., 2008). Later, Sarker and Sathasivan, (2011b) incorporated temperature effects into the biostability concept. However, previously only the free ammoniacal nitrogen (NH_4^+-N + NH_3-N) was adopted as the controlling nutrient. Chloraminated systems maintain pH at around 8. If water is at pH 8, consideration of free ammoniacal nitrogen or ammonia as the nutrient in the biostability concept will not greatly impact the results. However, when pH is changed consideration of speciation is important to correctly quantify NH_3-N by using Equation 4.1 as substantial changes in NH_3-N concentration can occur even for a small pH variation.

$$[NH_3 - N] = \frac{1}{1+10^{(9.3-pH)}} \cdot \left([NH_3 - N] + [NH_4^+ - N]\right) \qquad \textbf{Equation 4.1}$$

Depending on the value, slight change in pH can suppress/encourage the activity of AOB by three separate mechanisms: first, by modifying the chloramine decay profile and then, by modifying the NH_3-N concentration for given free ammoniacal nitrogen

concentration (Equation 4.1). Finally, pH adjustment can also impact by consuming energy to maintain favourable pH inside the cell and leaving no energy for growth (Bock et al., 1992). How chloramine decays in non-nitrifying water is known (Vikesland et al., 2001), but how it would behave in severely nitrifying bulk waters or impact nitrifying bacterial activity are not known.

The purpose of this study is to show how pH would impact on chloramine decay, ammonia availability and hence finally nitrifying bacterial activity in severely nitrified samples obtained from pilot- scale chloraminated distribution system.

4.2 Materials and Method

The detailed description of stock chemical solutions preparation; water sample collection, preparation and storage; description of pilot-scale reactor setting, operation and feed water preparation; preparation of sample bottles and glasswares and analytical procedures were presented in Chapter 3.

4.2.1 Experimental Design

Three sets of severely nitrifying samples were collected to investigate the pH effects on chloramine decay of severely nitrified bulk waters. These samples were referred to as Sample A, Sample B and Sample C. These samples were dosed with 2.0 mg/L chloramine maintaining TCl to TAN ratio of 3.7:1 by weight. The samples were split into three subsamples and pH was adjusted to 7.5, 8.0 and 8.5. The experiment was carried in a batch mode. All the subsamples were collected in duplicate and incubated in a constant temperature water bath at 20ºC. Chloramine, TAN, NO_2-N and NOx-N levels were monitored regularly.

4.2.2 Rational for Selecting pH Range of 7.5 - 8.5

In selecting the practically viable pH range, many aspects were to be considered: the reported pH range during nitrification in distribution systems, optimal pH range to satisfy the customers' need, pH range that does not seriously affect the chloramine

stability or aid AOB activity. Different authors reported different pH range for nitrification phenomena in drinking water distribution system. Nitrification in drinking water distribution system occurs at a pH range 7.2-9.8 (Odell et al., 1996), 7.9-8.9 (Harrington et al., 2002), 6-10 (Painter, 1977), 4.6-9 (Wolfe and Lieu, 2001) whereas the optimal pH was 7.2-8.5 (Odell et al., 1996), 7-8 (Painter, 1977), 7.5-8.1 (Wolfe and Lieu, 2001). Moreover, in low pH conditions (less than 7.5), there is a possibility for di-chloramine formation. Higher pH may stabilise the chloramine (Vikesland et al., 2001), but customer may complain if the supplied water is too alkaline e.g. higher pH. Furthermore, normal pH in full scale distribution system is around 8.0. Therefore, the selected pH range 7.5-8.5 covers the optimal range that has a significant meaning on residual management and nitrification control.

4.2.3 Rational for Using NOx-N as an Indicator for AOB Activity

In chloraminated systems, AOB activity can be monitored by following the changes in NOx-N concentration. In a chlorminated environment, NO_2-N can be converted to NO_3-N by chloramine or NOB. The change from NO_2-N to NO_3-N will not alter the NOx-N levels. However, there is only one way NH_3-N can be converted to NO_2-N which is by AOB. This conversion from NH_3-N to NO_2-N will increase the pool of NOx-N. Hence, AOB activity can be easily tracked by observing the change in NOx-N levels.

4.2.4 Calculation of NH_3-N Concentration

$$NH_4^+\text{-N} + NH_3\text{-N} = TAN - TCl/5 \qquad \textbf{Equation 4.2}$$

Free ammonia in the form of NH_4^+-N + NH_3-N is the function of TAN and chloramine measured as TCl. In the initial period, there would be a rapid decrease of chloramine as compared to TAN reduction. However with further incubation, the NH_4^+-N + NH_3-N concentration will be reduced to zero due to loss of TAN by nitrification. By substituting the value of NH_4^+-N+NH_3-N in Equation 4.2, the concentration of NH_3-N can be obtained from the following Equation

$$NH_3 - N = \frac{TAN - TCl/5}{1 + 10^{(9.3-pH)}}$$ **Equation 4.3**

Thus, from Equation 4.3, it can be said that NH_3-N depends on pH value.

4.3 Results and Discussion

4.3.1 General Characteristics of Collected Bulk Water Samples

Chemical parameters of the Sample A, Sample B and Sample C at the time of collection from the reactors and the initial conditions maintained for the batch test are presented in Table 4.1. The three sets of samples showed similar results and hence results of Sample A were presented initially and then the results of B and C were summarised. The Sample A contained chloramine and NO_2-N concentrations at 0.46 mg/L and 0.20 mg-N/L respectively. Chloramine decay rate in the sample was found to be 0.0233±0.0042 hr^{-1}, indicating severely nitrifying condition as defined by Sathasivan et al., (2008).

Table 4.1: Water Quality Parameters of Collected Samples

Parameters	Sampling condition	Initial condition for batch test
TCl (mg/L)	0.46-0.82	2.00
TAN (mg /L)	0.16—0.25	0.54
TCl **:** TAN	2.86-3.28	3.70
NO_2-N (mg /L)	0.17-0.20	0.20
pH	7.73-7.80	7.5, 8 & 8.5
Temperature (^oC)	23 ± 1	20 ± 1

4.3.2 Effect of pH on Chloramine Decay and TAN Profiles

In order to investigate the effects of pH on chloramine decay and TAN, an experiment was conducted as defined in the Materials and Methods section. Figure 4.1**(**A) & (B) depict the chloramine decay and TAN profiles of severely nitrifying

samples at different pH conditions. Higher chloramine decay was found within 24 hrs of incubation followed by a slower decay during the remaining period for all pH conditions. Comparatively lower decay was noticed in higher pH (8.5) sample. Moreover, the chloramine decay rate coefficients of the sample were 0.0330±0.0131, 0.0233±0.0042 and 0.0100±0.0036 hr^{-1} at pH 7.5, 8.0 and 8.5 respectively. It should be noted that higher error in estimation of decay coefficients was due to the fact that the curve did not fit the first order decay curve. The TAN profile of higher pH (8.5) was more than that of lower pH (7.5 and 8.0), indicating that TAN loss was lower at high pH (8.5). Based on the chloramine decay profiles, it could be said that higher pH provides better stability of chloramine residual and better protection from nitrification (as more chloramine acts as a biocide) in the distribution system, even after severe nitrification has commenced. Valentine et al., (1998) reported that the rate of chloramine decay decreases with increasing pH in distilled water and in a laboratory experiment, Vikesland et al., (2001) observed much lower chemical chloramine decay at a higher pH (8.34) than pH 7.56 in non-nitrified distribution system waters and in the absence of microbes. Therefore, the finding from current experiments is similar to previous reports.

In the current experiment, however, the chloramine decay profile was also affected by soluble microbial products (SMPs) and microbial activities. SMPs were found to accelerate chloramine decay (Bal Krishna and Sathasivan, 2010). Microbial activity includes NO_2-N production by AOB which could accelerate chloramine decay. NOx-N production (indicative of NO_2-N production in chloraminated environment) is given in Figure 4.1(E). The results indicated the NO_2-N production was lower in the sample with higher pH (8.5) which was another reason for lower chloramine decay at higher pH.

However, the overall results indicate the biocide concentration was higher in the higher pH sample compared to lower pH ones. One would conclude that higher pH should be better if the activity of AOB has to be controlled by biocide concentration.

4.3.3 Effect of pH and Chloramine Decay on Ammonia Concentration (Energy Source for AOB)

Figure 4.1 (C) & (D) show the variation of NH_3-N + NH_4^+-N and NH_3-N for Sample A for different pH conditions, where NH_3-N + NH_4^+-N and NH_3-N concentrations were calculated using the procedure described in Materials and Methods. AOB uses released NH_3-N from chloramine decomposition as the energy source. The activity of nitrifiers could be held back by controlling availability of nutrients (NH_3-N) or chloramine (biocide) concentration. From Figure 4.1D, it is found that higher level of NH_3-N (about 3 times that in pH 8) is observed in high pH sample, whereas lower level of NH_3-N (about half that in pH 8) is found in low pH (7.5) samples. Considering this alone, one could conclude that lower pH would be a better option to control nitrification which is contrast to what was concluded from the chloramine concentration.

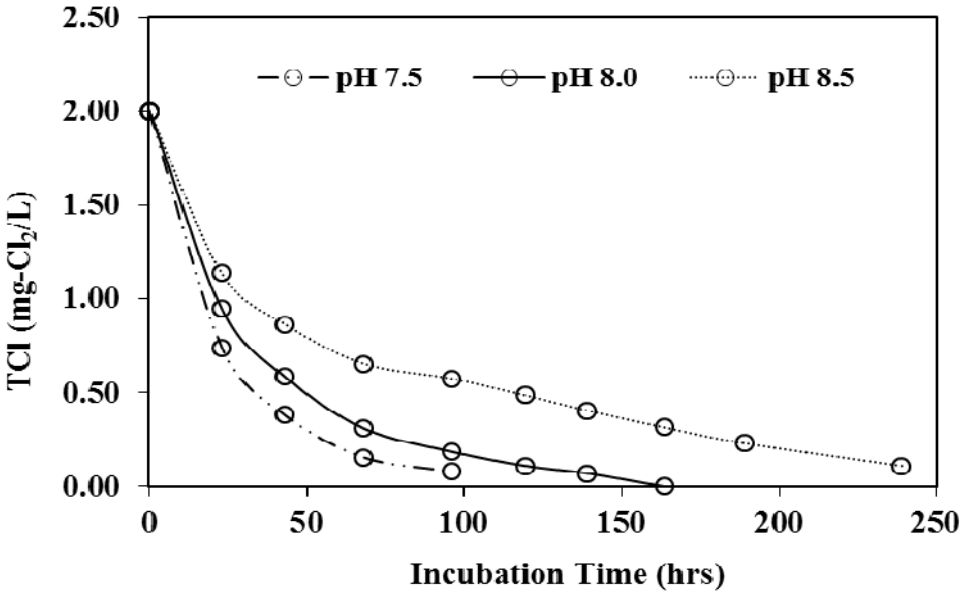

Figure 4.1 A :Effects of pH on TCl profiles at different pH conditions for Sample A

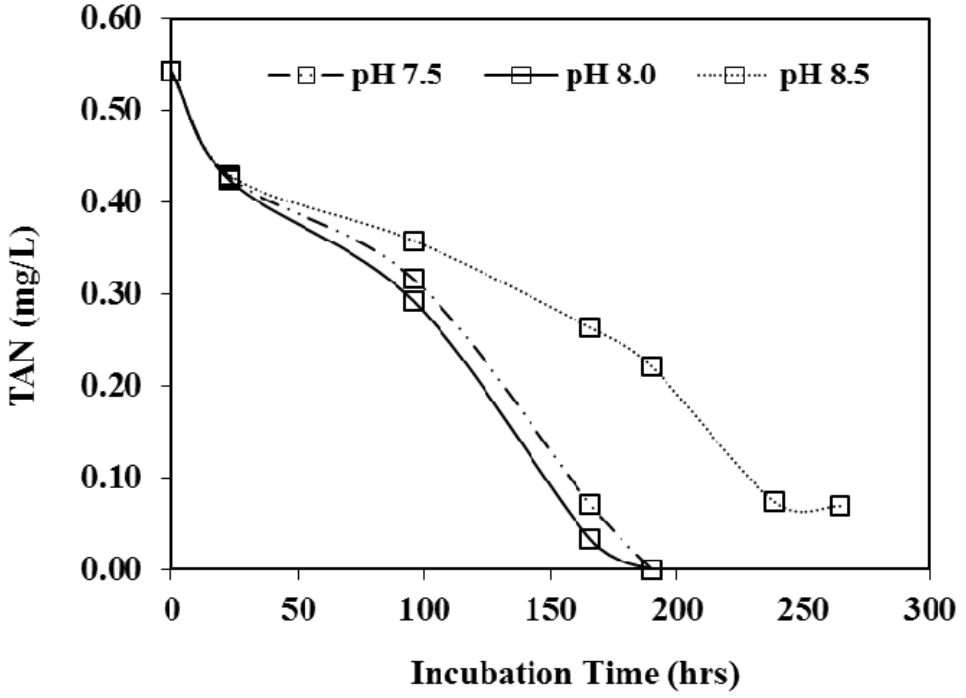

Figure 4.1 B: Effects of pH on TAN profiles at different pH conditions for Sample A

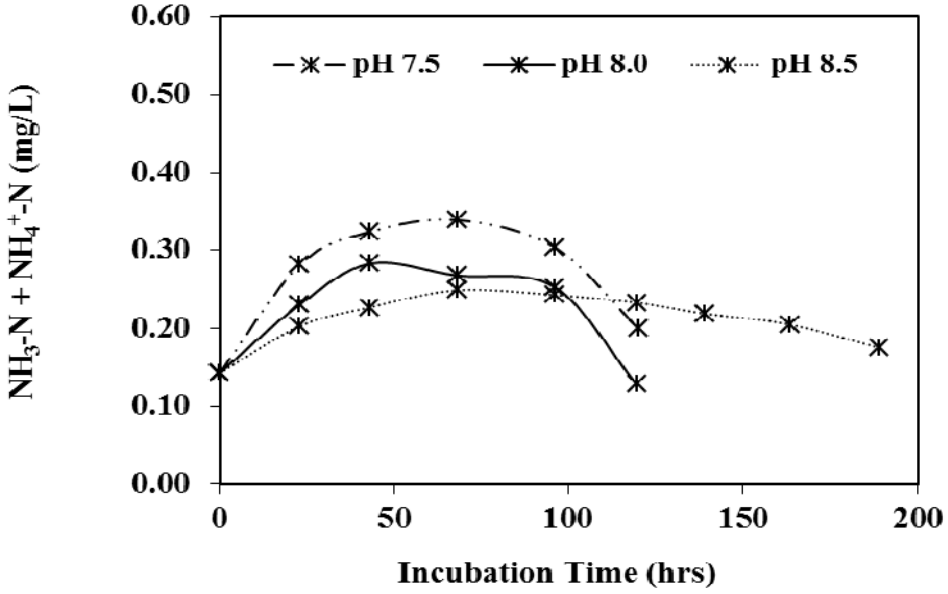

Figure 4.1C: Effects of pH on NH_3-N + NH_4^+-N profiles at different pH conditions for Sample A

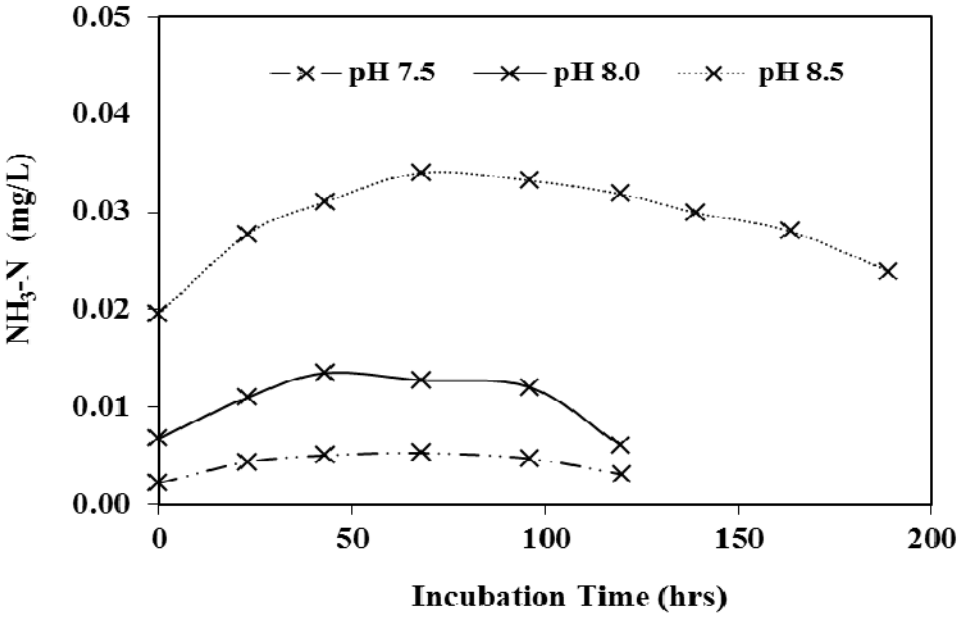

Figure 4.1D: Effects of pH on NH_3-N profiles at different pH conditions for Sample A

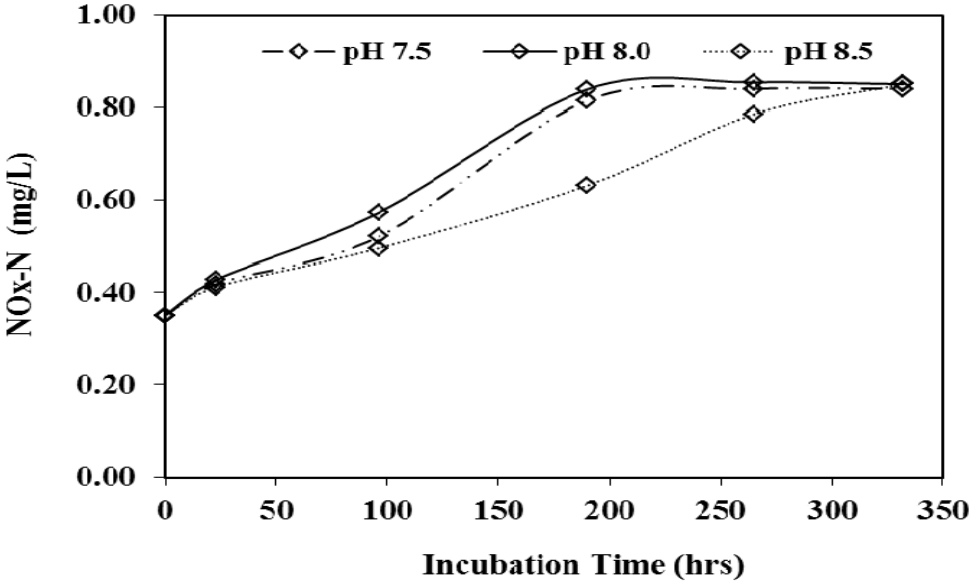

Figure 4.1E: Effects of pH on NOx-N profiles at different pH conditions for Sample A

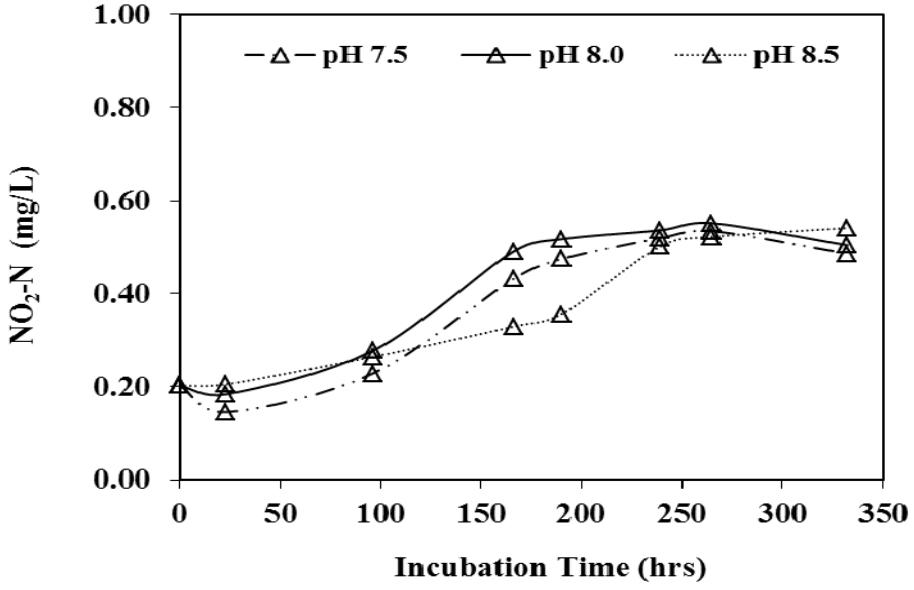

Figure 4.1F: Effects of pH on NO_2-N profiles at different pH conditions for Sample A

4.3.4 Combined Effect of pH, Chloramine, and Ammonia on Activity of AOB

Higher NOx-N production rates in samples indicated the presence of either more number of AOB or AOB were more active. Figure 4.1(E) shows the NOx-N profiles for Sample A when their initial pH was adjusted to different values. It was noticed that NOx-N level increased from the beginning for all the samples as the samples were severely nitrified. Similar behaviour was observed in the other two samples (Table 4.2). From Table 4.2, it is seen that the NOx-N production rate for higher pH (8.5) was lower than that of lower pH (7.5 and 8.0) samples. Meanwhile, the NO_2-N profiles for Sample A at different pH conditions are shown in Figure 4.1(F). From Figure 4.1(F), it is noticed that NO_2-N levels at different pH started to increase after 25 hrs and lower levels were observed at higher pH samples. In the higher pH sample it took about 150-200 hrs to start highly nitrifying. One reason for that could be pH in the higher pH adjusted sample had dropped close to 8. However, the measurement was not made in the current experiment. Finally, however, it could be concluded that higher pH is better for maintaining residual concentration [Figure 4.1(A)] and suppressing nitrification or AOB activity [Figure 4.1 (E) & (F)] in severely nitrifying sample.

Table 4.2: NOx-N Production Rate of Sample A, Sample B and Sample C

Sample	pH value	NOx-N production rate in mg-N/(L.hr) for different incubation time		
		0 to 25 hrs	25 to 100 hrs	100 to 190 hrs
Sample A	7.5	0.0030 ± 0.0017	0.0017 ± 0.0007	0.0029 ± 0.0007
	8.0	0.0033 ± 0.0017	0.0020 ± 0.0007	0.0028 ± 0.0008
	8.5	0.0027 ± 0.0017	0.0011 ± 0.0006	0.0014 ± 0.0006
Sample B	7.5	0.0037 ± 0.0016	0.0021 ± 0.0007	0.0014 ± 0.0007
	8.0	0.0033 ± 0.0016	0.0024 ± 0.0007	0.0010 ± 0.0007
	8.5	0.0021 ± 0.0015	0.0012 ± 0.0006	0.0012 ± 0.0006
Sample C	7.5	0.0025 ± 0.0016	0.0020 ± 0.0007	0.0027 ± 0.0007
	8.0	0.0027 ± 0.0016	0.0018 ± 0.0007	0.0024 ± 0.0008
	8.5	0.0024 ± 0.0016	0.0017 ± 0.0007	0.0014 ± 0.0007

The result was in contrast to what was deduced with NH_3-N profiles. However, higher pH resulted in better stability of chloramine residual. Obviously chloramine decay profile has played a major role in controlling nitrification, rather than the NH_3-N availability. Thus, maintaining higher pH in the sample, gives an advantage in controlling chloramine decay and nitrification.

4.4 Implications for Residual Management

Utilities usually experience nitrification, or severely nitrifying conditions as defined in this study and elsewhere. Operational monitoring usually increases during this period to understand the trend of nitrification and in all instances pH drops, often below 7.5, in severely nitrified samples. Traditionally re-chloramination is practiced without the consideration of pH and it is thought nitrification cannot be controlled and utilities usually resort to break point chlorination. Traditional norm regarding adjustment of pH is; lower pH may be better as minimal NH_3-N would be available for nitrifiers. However, the competing fact is that chloramine decays much faster in lower pH levels. In our previous reports, we have shown that in addition to nitrification, soluble microbial products could accelerate chloramine decay in severely nitrifying samples. Our results with samples that are severely nitrifying conditions, we have shown that chloramine decay can be controlled to an extent.

Although, preventive approach to avoid severe nitrification from triggering is needed for long term control, maintenance of higher pH may eventually help in better control of chloramine in severely nitrified bulk waters rather than simple re-chloramination in bulk waters. In a real distribution system, however, chloramine can be impacted by biofilms and sediments (Sathasivan et al., 2010) and a continuous flow of freshly chloraminated water. While biofilms and sediments will depress the residual freshly chloraminated water will assist in residual improvement efforts. Therefore further experiments in full scale system operating in full continuous flow mode are needed to understand the complications arising from these situations.

4.5 Conclusions

By considering pH effects, the water utilities may get an opportunity to take initiative in chloramine residual management and overcoming nitrification. The finding of the study shows that pH adjustment can benefit nitrification prevention efforts. The major conclusions made from the analysis are presented below:

- Under severely nitrifying conditions, complete prevention of nitrification is impossible as nitrifying organisms are already active and even dosing of 2 mg/L chloramine did not stop nitrification.
- High pH (8.5) is better in controlling chloramine decay and suppressing the activity of nitrifiers. This occurred despite much greater availability of energy source (NH_3-N).
- Low pH (7.5) reduces the energy source (NH_3-N), but is not sufficient to suppress nitrification as chloramine decays much faster.
- Further experiments in full scale/pilot scale systems are needed to understand how pH adjustment would help in improving the chloramine residual.

CHAPTER 5

MODELLING THE TEMPERATURE EFFECTS ON AMMONIA OXIDISING BACTERIAL BIOSTABILITY IN CHLORAMINATED SYSTEMS

Abstract

Biostability concept was successfully used to predict the biostable residual, residual below which onset of nitrification would occur, in chloraminated drinking water distribution systems. At biostable residual, rate of bacterial growth due to substrate (free ammonia) and rate of inactivation due to disinfectant are balanced. Growth rate and inactivation rate greatly vary with temperature, but it is yet to be considered in the biostability equation. Generally, water temperature in distribution systems varies from 6 to 35^{o}C that affects the growth of nitrifiers. Optimum temperature for ammonia oxidising bacteria (AOB) is between 25 and 30^{o}C, which makes the variation of growth rate non-exponential beyond 20^{o}C. In this study, a model is proposed to take into account of the temperature in biostability equation for the full practical temperature range. First the model of growth rate variability is proven for different bacterial species. Then, the model is validated ammonia oxidising bacterial activity using the data collected from pilot-scale and full scale distribution systems. The model has the potential to aid water utility in residual management throughout the year.

5.1 Introduction

Microbial decay including that due to nitrification is a major challenge in chloraminated distribution systems. Nitrification, a microbial conversion of ammonia to nitrite by ammonia oxidising bacteria (AOB) and then nitrate by nitrite oxidising bacteria (NOB), occurs over a pH range of 6.6 to 9.7 (Odell et al., 1996). Nitrification usually occurs at temperatures above 15^{o}C, but it can also occur at low temperatures (Wilczak et al., 1996) in chloraminated distribution systems.

Once nitrification takes place, controlling or overcoming nitrification is very difficult even by increasing the chloramine concentration up to 8.0 mg-Cl_2/L through re-chloramination (Cunliffe, 1991; Skadsen, 1993). Chloramine decays at a much faster rate under nitrified condition (Sathasivan et al., 2008) possibly due to an unknown mechanism (Bal Krishna and Sathasivan, 2010) including nitrification. For overcoming this, different approaches have been taken: applying break-point chlorination (Odell et al., 1996), reducing concentration of chloramine demanding substances (Harrington et al., 2002), increasing chlorine to ammonia ratio (Lieu et al., 1993), diluting the contents in winter (Sathasivan et al., 2010), and adjusting the pH of severely nitrified water to a higher pH (pH 8.5) before re-chloramination (Sarker and Sathasivan, 2011b). All these approaches are either labour intensive, costly or compromise water quality. Therefore, for finding an efficient universal preventive approach, it is vital to understand when the onset of nitrification takes place.

Onset of nitrification is defined differently by different authors. Regan et al., (2003) used molecular microbiological analysis to obtain warning. Pintar et al., (2005) suggested that chloramine drop is a better indicator. Sathasivan et al., (2008) reported that nitrite, chloramine residual or its decay rate could be used.

In chloraminated distribution systems, it is widely accepted that partial nitrification (i.e. microbial conversion of ammonia to nitrite) mostly takes place (Wolfe et al., 1990) and they chose *Nitrosomonas europea* as a representative micro-organism for nitrification phenomena. However, the use of molecular microbiological demonstrated that *Nitrosomonas europea* did not play a major role in controlling the nitrification process in chloraminated systems, especially when low ammonia concentration is present (Regan et al., 2003; Lipponen et al., 2004; Hoefel et al., 2005). In Finland and US distribution systems, *Nitrosomonas oligotropha* was found to be the most abundant ammonia oxidiser. Performing Metagenome studies followed by laboratory isolation using molecular techniques, Prosser and Nicol, (2008) demonstrated that *Mesophilic crenarchaea* does ammonia oxidation in terrestrial, marine and wastewater environment. Recent studies also showed the presence of nitrite oxidising bacterial community in the system (William et al., 2005). Therefore, it is yet imprecise to define the specific micro-organism(s)

responsible for nitrification in drinking water distribution system although the initial step produces the chloramine demanding substance, nitrite. Nitrite is generally used as the indicator of nitrification status (Wolfe et al., 1988). Although other better indicators, such as NOx-N (summation of nitrite and nitrate) production rate or AOB data can be used under well-defined conditions, nitrite is still the best indicator in the absence of appropriate data.

Woolschlager et al., (2001) developed the biostability concept and Harrington et al., (2002) adopted it for the determination of onset of nitrification. Fleming et al., (2005) further developed this concept and applied in laboratory scale system. At this residual, the rate of growth and disinfection are balanced. They used free ammonia [summation of ammonia (NH_3-N) and ammomium (NH_4^+-N)] as substrate for controlling the growth of AOB and dichloramine as the disinfectant, although finally they converted the later to total chlorine. Sathasivan et al., (2008) adopted total chlorine, as there is little dichloramine present under distribution system conditions (Valantine, 2007). The resulting Equation as proposed by Sathasivan et al., (2008) is:

$$BRC = \frac{\mu_m}{k_d}.\left(\frac{free\quad ammonia\_N}{K_s + free\quad ammonia\_N}\right) \qquad \textbf{Equation 5.1}$$

where, μ_m is the maximum specific growth rate of AOB (day^{-1}); free ammonia represents the sum of NH_3-N and NH_4^+-N concentrations (mg-N/L); K_s is the half saturation constant for AOB (mg-N/L); k_d is the rate constant for inactivation of AOB by disinfectant ($L.day^{-1}$ $mg\text{-}Cl_2^{-1}$); BRC is referred to as biostable residual concentration measured as total chlorine concentration (mg-Cl_2/L).

When BRC is drawn as a function of free ammonia, the curve produces two regions: nitrifying (below the curve) and non-nitrifying (above the curve) (Fleming et al., 2005). Using such approach, Fleming et al., (2005) obtained 2.0 mg-Cl_2/L and 0.5 mg-N/L for μ_m/k_d and K_s values respectively for a pilot scale system. Sathasivan et al., (2008) determined a different K_s value (0.18 mg-N/L) for Sydney Water Samples, Australia. In a full scale distribution system, Fleming et al., (2008) reported different values of μ_m/k_d and K_s to pilot scale systems. The reason for the difference was cited

as the presence of different microbiological species in different systems. Before assigning the variation to microbiological diversity, effect of temperature also needs to be considered.

Growth rate of nitrifiers depends on various factors including ammonia concentration, temperature, pH, light and dissolved oxygen concentration (Watson et al., 1986). Gill et al., (1977) reported that temperature is the key factor for controlling the growth rate and no microbial interactions occur until the maximum cell densities are reached under unlimited nutrient availability. Despite few applications of biostability concept in the literature, the impact of temperature was neglected in defining parameters of biostability curves. Investigation of impacts due to temperature variation on bacterial growth and inactivation would help in understanding how onset of nitrification would be affected by temperature.

Traditionally, growth rate is modelled using exponential function (Arrhenius equation). However, Ratkowsky et al., (1982) showed that it may not be valid even for sub-optimal temperature range. Usually, temperature in water distribution systems is between 6–35°C and the optimum temperature for nitrifier growth is 25–30°C (Wolfe et al., 1990; Skadson, 1993; Odel et al., 1996). The maximum temperature experienced by some utilities is about 5°C higher than the optimum temperature. Therefore, a model that reliably predicts the effects of temperature on microbes is of great interest.

Further, it is well known that k_d varies greatly with the temperature. Such temperature variation is usually modelled using exponential relationship (Tchobanoglous et al., 2003), implying that μ_m/k_d value in biostability equation would greatly vary with temperature. Our initial attempt with known literature values has also been shown the same (Sarker and Sathasivan, 2011a). The purpose of the paper is to develop and validate a model that describes the growth rate and thus μ_m/k_d value in the region of interest, i.e. 6-35^{o}C for AOB prevailing in both pilot-scale and full scale distribution systems.

5.2 Model Development for the Effect of Temperature on Biostability

In chemical equilibrium, the temperature effects on rate constant for chemical reactions can be described clearly by Arrhenius Law, or an exponential function. Ratkowsky et al., (1982) reported that the effect of temperature on the growth of bacteria cannot be described completely by Arrhenius law as the growth of bacteria is a complex process that is composed of a variety of substrates and enzymes. To overcome this problem, Ratkowsky et al., (1982) proposed the following relationship for sub-optimal temperatures:

$$\sqrt{\mu_{m,T}} = b(T - \theta_o) \qquad \textbf{Equation 5.2}$$

where, $\mu_{m,T}$ is the maximum growth rate constant (d^{-1}) at temperature T (K), b is the regression coefficient and θ_o is a hypothetical temperature (K) which is an intrinsic property of an organism. They reported that for psychrophilic bacteria, the θ_o value was found to be between 267 and 269K. However, their proposed model is applicable for only sub-optimal temperatures. Therefore, a relationship that describes beyond sub-optimal temperature is also needed. It may be expressed as follows:

$$\mu_{m,T} = A - B(T - T_0)^2 \qquad \textbf{Equation 5.3}$$

where, $\mu_{m,T}$ is the growth rate of bacteria at temperature T (K), T_0 is the optimum temperature (K) of bacterial growth and A & B are constant. The value of A represents the maximum growth rate at optimal temperature. If the growth rate is defined as a percentage of growth rate at optimum temperature, growth rate at optimum temperature will be 100%. Therefore, A is set to 100.

Further, there should be a temperature that divides the region between the relationships defined by Equations 5.2 and 5.3. The temperature T_i where both Equations are valid can be determined by the following Equation:

$$\mu_{m,T_i} = b^2.(T_i - \theta_o)^2 = A - B.(T_i - T_o)^2 \qquad \textbf{Equation 5.4}$$

As Equations 5.3 and 5.4 are common for many microbes, data for several microbes can be used to test this relationship as done in later sections.

The effects of temperature on inactivation rate constant due to disinfection can be modelled using the Arrhenius Equation (Tchobanoglous et al., 2003).

$$k_{d,T} = k_{d,20}.\exp\left[-\frac{E}{R}\left(\frac{1}{T}-\frac{1}{273+20}\right)\right] \qquad \textbf{Equation 5.5}$$

where, $k_{d,T}$ and $k_{d,20}$ are the inactivation rate constant of bacteria at temperature T (K) and 20°C respectively, E is the activation energy (J/mole) and R is the universal gas constant (J/mole.K).

For temperature range ($T<T_i$) where Equation 5.2 is valid, Equations 5.2 and **5.5** can be written:

$$\frac{\mu_{m,T}}{k_{d,T}} = \frac{b^2.(T-\theta_0)^2}{k_{d,20}.\exp\left[-\frac{E}{R}\left(\frac{1}{T}-\frac{1}{273+20}\right)\right]} \qquad \textbf{Equation 5.6}$$

For temperature range ($T>T_i$) where Equation 5.3 is valid, Equations 5.3 and 5.4 can be combined to describe the temperature variation of μ_m/k_d as follows:

$$\frac{\mu_{m,T}}{k_{d,T}} = \frac{A-B(T-T_0)^2}{k_{d,20}.\exp\left[-\frac{E}{R}\left(\frac{1}{T}-\frac{1}{273+20}\right)\right]} \qquad \textbf{Equation 5.7}$$

Using the literature data, it is important to validate the growth rate models (Equations 5.2 and 5.3), and estimate parameters, A, B, θ_o, T_o and b, for several bacteria which live and grow in a wide temperature range, as they would follow the typical growth pattern. It is also better to include a bacterium that grows in a similar range of temperature the distribution water system is experiencing (6-35^{o}C). This will facilitate obtaining a model bacterium to formulate biostability equation for AOB.

Then, the data obtained from the distribution system or pilot-scale could be utilised to fine tune the model for AOB.

5.3 Materials and Methods

5.3.1 Literature Data on Microbial Growth Pattern

For this study, data were collected from Johnson et al., (1974) for a wide temperature range and were used for the calibration of the proposed growth model. Culture containing strains of *Aerobacter aerogenes, Bacillus circulans, Escherichia coli (Barber), Escherichia coli (J. & L.) and Sporotrichum carnis* were incubated at appropriate temperatures and at suitable intervals, and samples were drawn from the culture for bacteriological analyses. Standard plate count method was followed for viable counting, where plates were incubated at appropriate temperature and for a suitable length of time before counting. Full description of the experimental procedure is given in Greene and Jezeski, (1954).

5.3.2 General Description of the Pilot-Scale and Full Scale Systems

The data used for this study was collected from one pilot-scale reactor, two full scale systems and one reservoir inside a full scale distribution system. The full scale systems were 'Sydney Water Distribution System (SWDS)' and 'Goldfields and Agriculture Water Supply System (G&AWSS), Western Australia'. The reservoir was Reservoir C inside SWDS. Brief descriptions of these systems are described below;

The Pilot-Scale Reactor (PSR), set up in Curtin University, Western Australia. The PSR consists of two series of rectors (five in each series) in which different stages of nitrification (none to severe) conditions similar to real distribution systems were created. Water collected from Mundaring weir, Western Australia was fed into the reactors after dosing with chloramine [Total chlorine (TCl) for 24 hr followed by ammonia (NH_3-N) at TCl to NH_3-N ratio of 4.5**:**1 by (weight)] and maintaining temperature from 20 to 22°C in the reactors. The quantity of feed water through each

rector was 20 L/day and the hydraulic retention time of the five reactors was 22, 19, 20, 19 and 19 hrs respectively. The general characteristics of PSR's samples are shown in Table 5.1.

SWDS has the capacity of delivering 1700 ML of water per day to the customers in Sydney metropolitan area and contains about 200 service reservoirs. Chlorine is practiced as the primary disinfectant. As soon as appropriate CxT (concentration x time) values are obtained in the clear water tank, NH_3-N is added. Chloramine is used as a secondary disinfectant. TCl to NH_3-N ratio (by weight) is very close to 4**:**1. Water temperature downstream of the treatment plant is above 12°C in winter and generally rises up to 25°C in summer. General descriptions of SWDS collected samples are presented in Table 5.1 and detailed description of SWDS is given in Sathasivan et al., (2008).

The other full scale distribution system is G&AWSS and probably, it is the longest above ground water distribution system in the world. A 530 km long pipe line connects Mundaring Weir, near Perth, Western Australia with the Mount Charlotte Reservoir at Kalgoorlie but it also branches into more inland area with an estimated total of 7000 km of main pipelines. The day time temperature of this system can be as high as 50°C and can experience a sharp diurnal variation. Chloramine is used as the primary disinfectant and dosed at Mundaring pump station. Chloramine is prepared by addition of chlorine (gas form) followed by direct dosing of ammonia solution (20% w/v) at downstream. Once chloramine residual has dropped to certain level along the system, only chlorine is topped up by maintaining TCl to NH_3-N ratio (by weight) close to 5. The general characteristics of the collected samples are shown in Table 5.1 and detailed description of G&AWSS is given in Sathasivan et al., (2009).

Reservoir C is the fourth reservoir in SWDS. It is a circular tank with 18 and 13 m diameter and height respectively. Full capacity of this reservoir is $3.0x10^3$ m^3. The reservoir has a common inlet/outlet pipe of diameter 0.45 m. Water temperature in this reservoir varies between 12 and 25°C, seasonally. General characteristics of the samples are shown in Table 5.1 and description of Reservoir C is detailed in Sathasivan et al., (2010).

Table 5.1: General Characteristics of the Collected Samples

Parameters	Sampling Sources				
	Batch experiment			Continuous flow experiment	
	SWDS	G&AWSS	PSR	Reservoir C	PSR
Chloramine (as TCl) (mg-Cl_2/L)	0.62-1.54	1.57-1.68	0.13-0.82	0.50-1.22	0.05-2.10
TAN* (mg /L)	0.25-0.35	0.60-0.63	0.05-0.25	0.09-0.34	0.107-0.55
NO_2-N (mg /L)	0.03–0.10	0.03–0.09	0.17-0.38	0.001-0.061	0.01-0.336
pH	8.0±0.2	8.2±0.1	7.8±0.2	8.0±0.2	7.8±0.2
DOC (mg/L)	3.3±0.4	3.0±0.2	3.0±0.3	3.3±0.4	3.0±0.3

5.3.3 Sampling Details

In all cases, sample bottles were cleaned by dipping them into a 10% sodium hypochlorite solution for 24 hrs, followed by rinsing with deionized water until the bottles were completely free of chlorine. All sample collection glassware was autoclaved before used.

In PSR, samples for batch test were collected from the reactors of both conditions (nitrified and non-nitrified) and were analysed for TCl, TAN and NO_2-N. For continuous flow experiments, samples were collected from each reactors of PSR and were analysed for TCl, TAN and NO_2-N immediately. The temperatures of each reactor were also recorded. The detailed description of stock chemical solutions preparation; water sample collection, preparation and storage; description of PSR setting, operation and feed water preparation were presented in Chapter 3.

In SWDS, samples were collected from 21 locations at different temperature. Duplicate samples collected from reservoirs and pipelines were transported to the laboratory under dark conditions at constant temperature, immediately after collection. Temperature of incubation in different samples ranged between 20 and 25°C, but only one temperature was adopted for a given sample. They were

incubated in new 500 mL PET containers (precleaned with 10% NaOCl solution) under dark conditions in duplicate. Decay tests were performed immediately in the laboratory after sample collection. To minimise the measurement error, measurements were made on both duplicate samples and average results were reported. The other collected samples were analysed for TAN and NO_2-N. Details of sample collection of SWDS are presented in Sathasivan et al., (2008).

In G&AWSS, about 10L samples were collected from one of the branches of G&AWSS located about 200km from the first chloramination point and were transported to the laboratory by maintaining 15~17°C temperature. The samples were taken into the clean bottles and necessary batch tests at different temperatures were conducted immediately. The other collected samples were analysed for TAN and NO_2-N. Details of sample collections from G&AWSS are presented in Sathasivan et al., (2009).

In Reservoir C, water samples were collected manually from the inlet/outlet of Reservoir C. Samples were collected from the surface and mid-depth. Temperature and chloramine were measured at the time of sampling. Samples were put on ice for transport to the laboratory. Decay tests were conducted at 20°C, when samples arrived at the laboratory. All other samples were refrigerated until measuring for TAN and NO_2-N were analysed. The more detail of sampling from Reservoir C is described in Sathasivan et al., (2010).

5.3.4 Pilot-Scale and Full Scale Distribution System Data

For this study, two types of results were used: results of batch test and that of in-situ for samples collected from different systems. For batch tests, bulk water samples were collected from PSR, SWDS and G&AWSS, and were incubated at desired temperature after making sure that there was sufficient chloramine residual. Several tests were conducted with different initial chloramine concentration for samples having different NO_2-N levels or different nitrification conditions (nitrified or non-nitrified) at different temperature. For in-situ assessment, samples from PSR and Reservoir C were analysed for the status of nitrification by using various indicators.

5.3.5 Analytical Methods

TCl residuals were measured by DPD colorimetric method using a HACH pocket colorimeter for all samples. TCl measurement has an experimental error of 0.03 mg-Cl_2/L. In SWDS, G&AWSS and Reservoir C, TAN and NO_2-N were measured using Flow Injection Analysis (FIA) as described in APHA et al., (1998). TAN was measured by phenate method and NO_2-N was measured by sulphanilamide method. NO_2-N has a lowest detection limit of 0.002 mg/L and TAN has a measurement error of 10%, a discrepancy of 10% was allowed. In PSR samples, TAN and NO_2-N concentrations were measured spectrophotometrically using the Aquakem 200 according to the methods mentioned in EPA, (1981). TAN was measured by phenate method and NO_2-N was measured by sulphanilamide method. TAN and NO_2-N has an experimental error of 2% and has the detection limit of 0.002 mg/L.

5.3.6 Criteria for Identifying Onset of Nitrification

In Batch Tests, two different criteria were used to identify the onset of nitrification. As initial NO_2-N levels were very low in non-nitrified samples, the TCl and TAN concentrations at which NO_2-N increased to 0.015 mg/L, was used to define the point of onset. In nitrified samples, the point of onset was decided as a point at which NO_2-N concentration started to increase by more than 10% of the value as NO_2-N levels were already higher. This data was utilised in biostability analysis for batch tests.

For in situ samples and continuous flow experiments, NO_2-N is used to understand the onset of nitrification and it was determined when NO_2-N exceeded 0.015 mg-N/L. Samples containing the NO_2-N levels more than 0.015 mg-N/L were assumed as undergoing nitrified and that below this level were assumed as non-nitrified. The respective chloramine (TCl), TAN and temperature were used as data input for determining the parameters.

5.3.7 Calculation of Free Ammonia Concentration

Free ammonia = TAN – TCl/5 **Equation 5.8**

Free ammonia is the function of TAN and chloramine measured as TCl.

5.3.8 Determination of Constants A, B, b, θo , To and Ti

For the sub-optimal temperature range, Equation 5.2 could be utilised. By minimising the squares of errors between estimated μ_m values and actual ones, and by allowing Excel tool "Solver", b and θ_o could be obtained. The data at which square root function cease to be valid, could be obtained by checking the error in estimation. According to the assumption, A is 100%. Values of B and T_o were estimated by Excel tool 'Solver' by minimising the squares of errors with calculated values of μ_m using Equation 5.3 and analysed experimental data of SWDS with the help of Equation 5.1. Temperature (T_i) where both Equation 5.2 and 5.3 were valid could be obtained by using Equation 5.4.

As BRC varies with temperature, free ammonia etc, normal biostability curve could not be utilised to understand the point of nitrification onset. Instead, the difference between measured TCl and BRC (e.g. TCl-BRC) could help in determining the status of the water sample.

If a curve is plotted for TCl-BRC against respective temperature, one can obtain regions of nitrifying and non-nitrifying samples as well as the points at which onset of nitrification occurs. When (TCl-BRC) >0, i.e. the measured chloramine concentration is greater than BRC, it indicates the absence of nitrification. If samples show chloramine concentration values less than BRC [i.e., (TCl-BRC) <*0*], it indicates the presence of nitrification.

5.4 Results and Discussion

5.4.1 Validation of the Proposed Growth Rate Model

The relationship of bacterial growth and temperature for sub-optimal temperature range has already been modelled by Ratkowsky et al., (1982), but that beyond the sub-optimal temperature was proposed (Equation 5.3) in this study. For verification of the model's effectiveness, experimental data for different species of bacteria *(Aerobacter aerogenes, Bacillus circulans, Escherichia coli (Barber), Escherichia*

coli (J. & L.), and Sporotrichum carnis) were collected from literature (Jonson et al., 1974) and used to provide a validation for the proposed models describing temperature effect on growth rates.

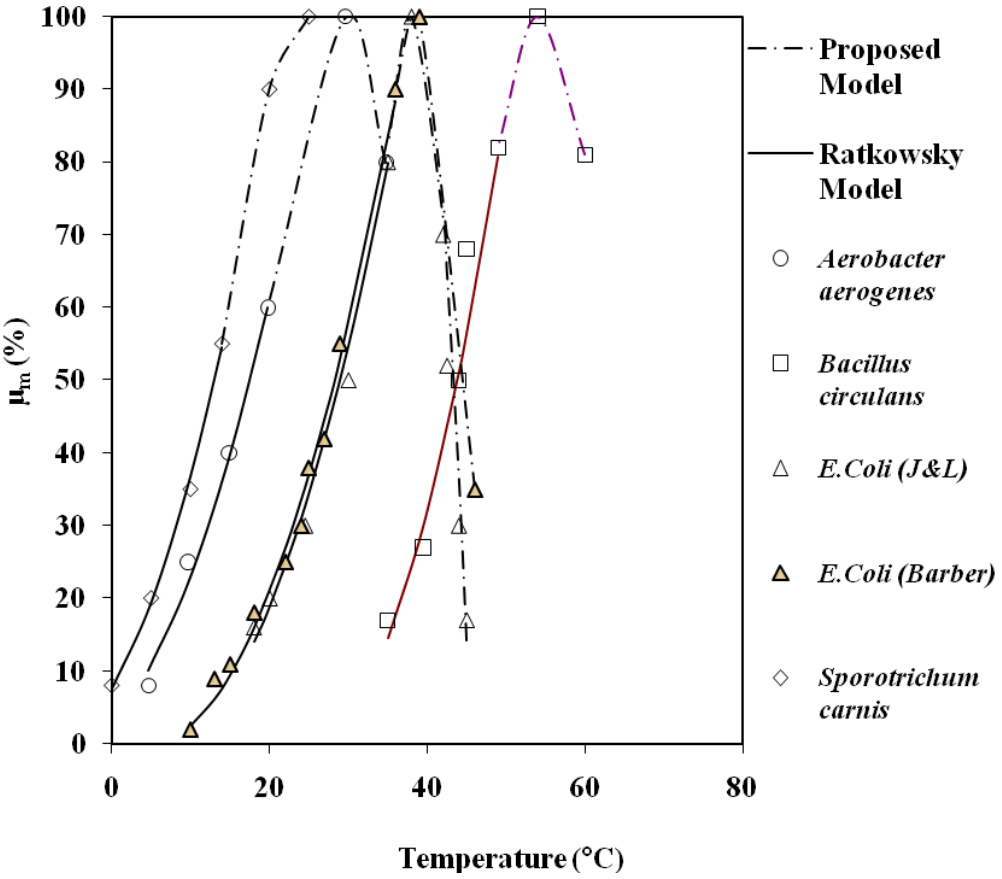

Figure 5.1: Growth rate of various species of bacteria as proposed by the model

The growth rate profiles of *Aerobactor aerogenes, Bacillus circulans, Escherichia coli (Barber), Escherichia coli (J. & L.), and Sporotrichum carnis* as proposed by the model were shown in Figure 5.1. The appropriate fitting was obtained when two models were used to fit the maximum specific growth rate variation of five different bacterial species (Figure 5.1). The observed maximum value of μ_m was taken as the highest value (100%) and other values for various temperatures were calculated relative to the maximum.

Table 5.2: Values of the Proposed Model Constants for Different Bacterial Species

Parameters	Bacterial Species				
	Aerobactor aerogenes	*Bacillus circulans*	*Escherchia coli (Barber)*	*Escherichia coli (J. & L.)*	*Sporotrichum carnis*
A	100	100	100	100	100
B	0.510	0.615	1.26	1.80	0.35
T_0 *(K)*	301.5	327.4	311.8	311.1	298.4
b	0.302	0.369	0.302	0.305	0.33
θ_0 *(K)*	267.03	297.6	277.9	278.68	264.63
Ti (°C)	19.6	48.8	35.5	34.6	14
$\Sigma(error)^2$ *in Equation 2*	14.09	0.00	0.00	245.71	293.92
$\Sigma(error)^2$ *in Equation 3*	1.07	151.71	45.74	38.11	3.76

For illustration, *Aerobactor aerogenes* was selected. It was observed that μ_m was maximum (100%) at the optimum temperature (28.5°C) and then started to decrease with the rising or falling of temperature from the optimum. In the sub-optimal temperature range, growth rate could be described by Equation 5.2. When the squares of errors were minimised as described in Materials and Methods, b and θ_o were estimated to be 0.302 and 267.03 K. It was found that error was high if the data for 28.5°C was utilised in Equation 5.2. Therefore, for estimating parameters the data points starting from the temperature below 28.5°C (19.8°C) to the maximum temperature, were fitted to Equation 5.3. The trial resulted in the following parameters: A = 100; B = 0.51; and T_o = 301.5 K (or 28.5°C). From Table 5.2, it can be seen that sum of error squares ranged from 0 to 293.92 for Equation 5.2 and 1.07 to 151.71 for Equation 5.3 (the proposed model). Since the growth was described as percentage of maximum specific growth rate at optimum temperature, the error is in squares of percentage. This makes the error appears high; otherwise the fitting of each curve is excellent.

The common temperature *(Ti)* which divides the validity was found to be 292.6 K (or 19.6^{o}C) using Equation 5.4. Similarly, values of different constants *(A, B, T_0, b, θ_0)* defining the proposed models for *Bacillus circulans, Escherichia coli (Barber), Escherichia coli (J. & L.), and Sporotrichum carnis* were calculated and are shown in Table 5.2. Although, it was seen that values of constants defining growth rate model for different bacterial species were not the same, they followed the same pattern and their growth rate beyond the sub-optimal temperature could be defined by the quadratic equation (Equation 5.3).

5.4.2 Selection of Appropriate Model Bacteria for AOB Found in Distribution Systems

It is reported that the optimum temperature for nitrifier, especially AOB growth is 25–30°C (Wolfe et al., 1990; Skadson, 1993; Odell et al., 1996), whereas the optimum temperature for *Aerobactor aerogenes* is 28.5^{o}C (301.5 K) as in Table 5.2. *Aerobactor aerogenes* also grow in the temperature between 6 and 35^{o}C, the interested region. Therefore, the behaviour of *Aerobactor aerogenes* due to temperature variations may represent AOB behaviour subjected to different temperatures. As our concern was to assess temperature effects on AOB activity, behaviour of *Aerobactor aerogenes* within the governing temperature of AOB would be considered.

5.4.3 Modelling the Effects of Temperature on µm/kd

It is known that growth rate (μ_m) (Figure 5.1) and k_d (Equation 5.5) vary with temperature. Therefore, μ_m/k_d changes with temperature (Equations 5.6 and 5.7) and could be calculated with the different values of μ_m and k_d for different temperatures. The relative values of μ_m at different temperature could be evaluated from Figure 5.1. To calculate k_d values at different temperatures, E value was obtained from literature (Fair et al., 1948) and the standard value of R was used in Equation 5.5. The universal value of *R* is 8.3144 J/mole.K and the value of E for chloramine at 20°C is 50,250 J/mole for pH 7 and 58,630 J/mole for pH 8.5 (Fair et al., 1948). By interpolation and simplifying, *E/R* value could be considered as 6500 K.

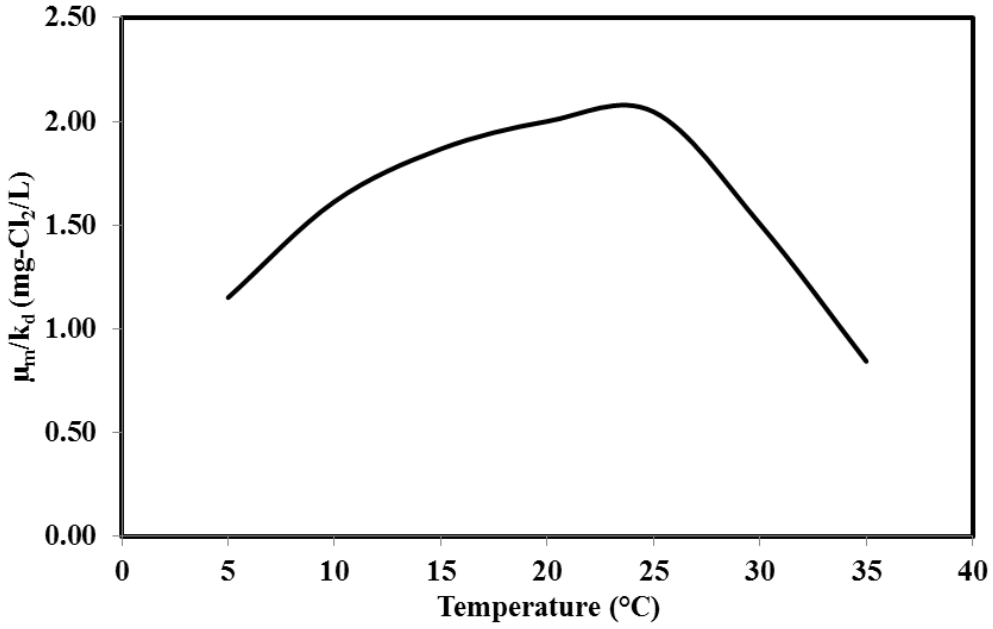

Figure 5.2: Profiles of μ_m/k_d for *Aerobactor aerogenes* as proposed by the model, if the value at 20°C is set to 2 mg-Cl_2/L and E/R value is 6500 K.

Using Equation 5.5, value of k_d at different temperatures could be estimated with the estimated E/R value (6500 K). To determine the variation of μ_m/k_d with temperature, first the value of μ_m/k_d was set at 2.0 mg-Cl_2/L at 20°C as this was used by other researchers (Harrington et al., 2002; Fleming et al., 2005; Sathasivan et al., 2008), then the unknown parameters defining the models were calculated. Figure 5.2 shows the effect of temperature on μ_m/k_d of *Aerobactor aerogenes* where μ_m/k_d values were calculated as specified by the Equations 5.6 and 5.7 based on temperature regime. This model could be used to calculate the μ_m/k_d values for different temperatures. From Figure 5.2, it is noticed that the maximum value (2.07 mg-Cl_2/L) of μ_m/k_d is observed at 23°C, whereas the value of μ_m/k_d at 20°C from the proposed model is 2.00 mg-Cl_2/L which is the same as the literature reported value.

5.4.4 Effect of Temperature on BRC for Bulk Water Samples in Batch Experiment

The experimental results obtained from batch experiments of two full scale distribution systems (SWDS and G&AWSS) and one PSR used for ensuring the variation of μ_m/k_d values proposed in Equations 5.6 and 5.7 were found to be acceptable. BRC was calculated using Equation 5.1, taking K_s as 0.18 mg-N/L and μ_m/k_d values at different temperatures were taken from the proposed model (Figure 5.2). TCl-BRC was calculated for the samples and a graph of TCl-BRC versus temperature was plotted in Figure 5.3.

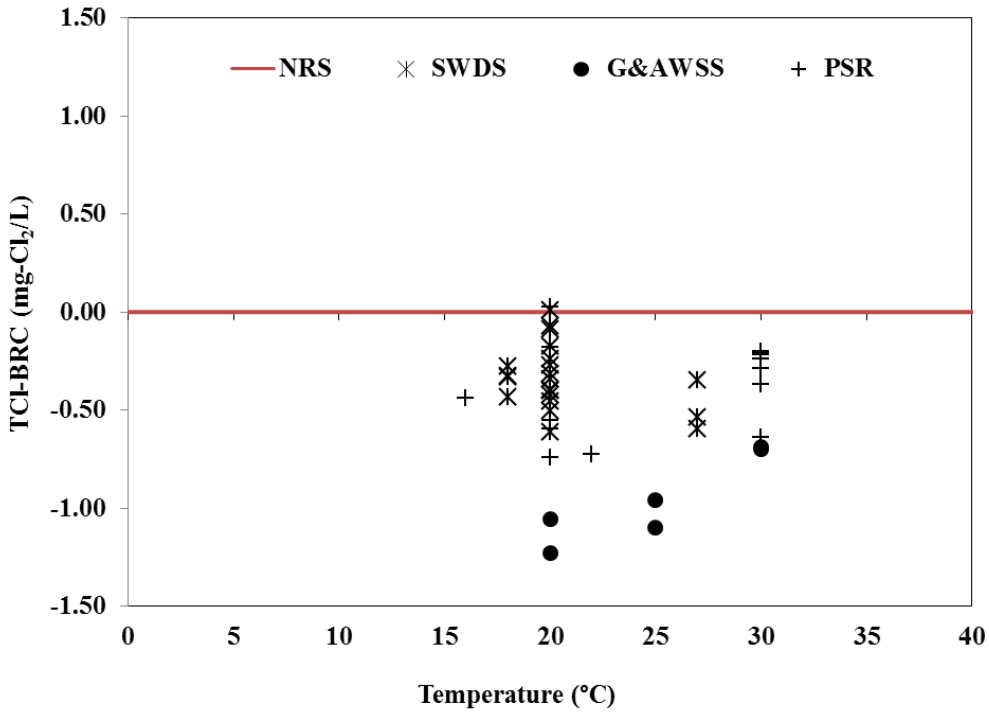

Figure 5.3: Variation of TCl-BRC with temperature of full scale distribution and pilot-scale reactor samples in batch experiment

A straight line (TCl-BRC = 0 at different temperatures) presented the line that divides the nitrified region from non-nitrified one and was referred as 'nitrification region separator (NRS)'. It was observed that all the samples except one of SWDC stayed below the NRS. This one sample also met the criteria while considering the

experimental error. Therefore, all the nitrified samples met the criteria as mentioned in Table 5.1. The results confirmed that the estimation of μ_m/k_d values using Equations 5.6 and 5.7 for different temperatures are reasonable.

5.4.5 Effect of Temperature on BRC for Bulk Water Samples in Continuous Flow Systems

Figure 5.4 shows the variation of TCl-BRC with temperature in Reservoir C and PSR to validate the proposed variation of μ_m/k_d with temperature in full scale distribution system under continuous flow condition. For doing this, both the nitrified and non-nitrified sample's results of Reservoir C and PSR were used. Based on sample's NO_2-N concentration, the samples of the Reservoir C and PSR were divided into two categories: non-nitrified and nitrified. Samples having NO_2-N $\geq$ 0.015 mg/L were considered nitrified. According to the criteria, 6 out of 30 samples from Reservoir C and 23 out of 82 samples of PSR were nitrifying. A graph of TCl-BRC versus temperature was drawn in Figure 5.4 where the determination of BRC and other parameters were same as that explained in section 5.4.4. According to nitrification identification criteria mentioned above, position (below or above the NRS) of each sample decides the presence and absence of nitrification. From Figure 5.4, it is noticed that all the samples met the criteria. Therefore, the proposed parameters and variation of μ_m/k_d with temperature was validated for the data from the full and pilot-scale system under continuous flow condition.

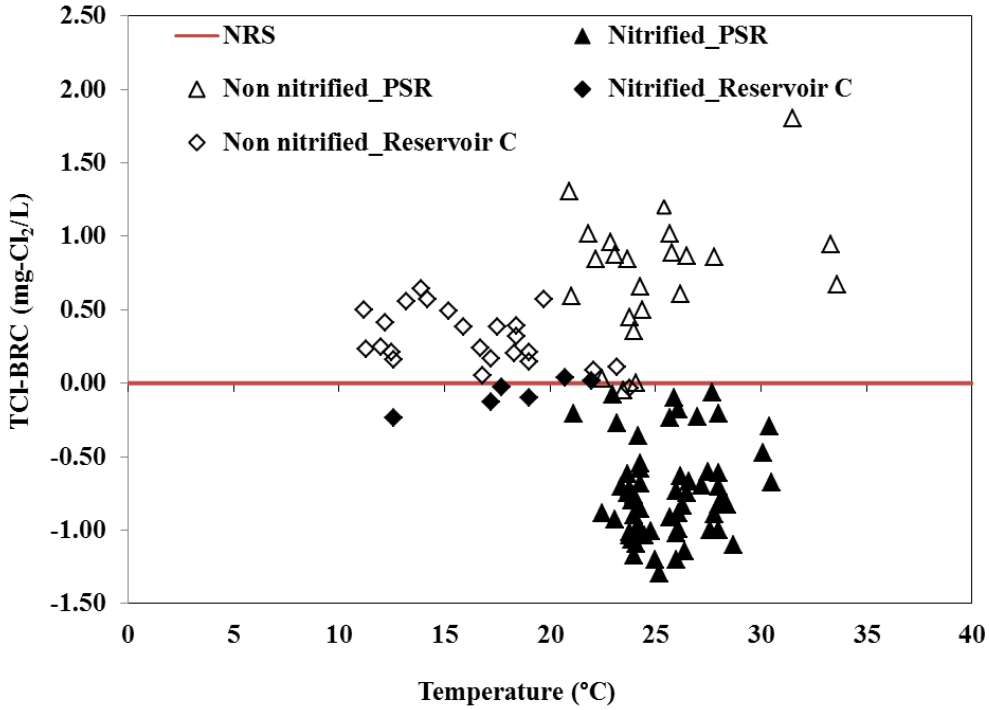

Figure 5.4: Variation of TCl-BRC with temperature of full scale distribution and pilot-scale reactor system samples during continuous flow experiment

5.4.6 Impact of Temperature on BRC Values in Full and Pilot-Scale Distribution Systems

From Figure 5.2, it is noticed that the value of μ_m/k_d at 15°C and 27°C are 1.87 and 1.83 mg-Cl_2/L respectively and that are very close to 2 mg-Cl_2/L (as reported in the literature). The maximum value of μ_m/k_d is 2.07 at 23°C which lies within the range. As mentioned in Equation 5.1, BRC is the function of μ_m/k_d for a given value of free ammonia where, μ_m/k_d always taken at maximum growth conditions. BRC values obtained by using μ_m/k_d from the proposed model and 2.0 mg-Cl_2/L would not be differ within the temperature range. Therefore, the temperature effect on μ_m/k_d and BRC within 15°C to 27°C temperature range can be neglected. Conversely, there are notable differences of μ_m/k_d values from the proposed model and 2.0 mg-Cl_2/L at temperatures above 27°C or below 15°C. Therefore, the variation of μ_m/k_d due to temperature changes will greatly impact on BRC values at temperatures above 27°C or below 15°C.

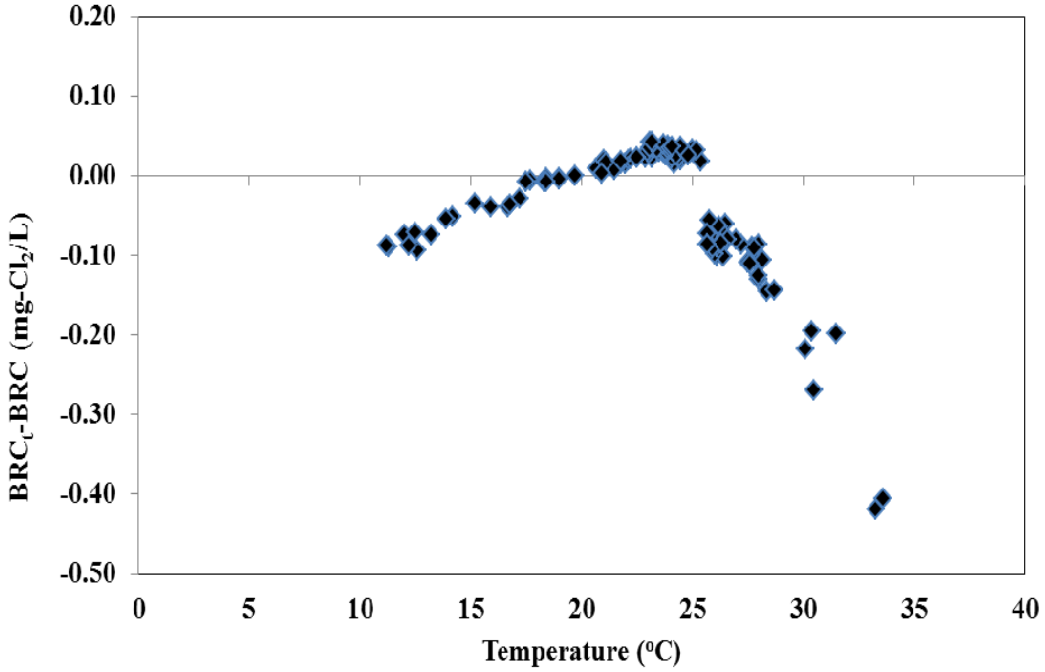

Figure 5.5: The difference between BRC values calculated considering temperature (BRC_t) and not considering temperature (BRC) effect on μ_m/k_d.

To clearly demonstrate, a comparative study between the calculated values of BRC by considering temperature effects and without temperature effects has done for pilot-scale and full scale system bulk water samples as shown in Figure 5.5. As expected, these two calculated values of BRC were same for the temperature range of 15°C to 27°C. On the other hand, a noticeable difference was observed out of the range (15°C to 27°C) due to remarkable difference between the model value (Figure 5.2) and literature value of μ_m/k_d (2.0 mg-Cl_2/L).

In this study, the samples used for the experiments were subjected to the temperature range (15°C to 27°C). Due to acclimatization of bacteria in the samples, the maximum growth of bacteria were occurred within the temperature that prevailed higher μ_m/k_d value than other temperatures. If the bacteria grow in higher (above 27°C) or lower (below 15°C) temperature, there were a possibility of shifting the μ_m/k_d curve (Figure 5.2). In those cases, the constants defining the model need to be adjusted.

5.4.7 Applicability of the Model in Water Distribution Utility

Properly maintaining the water quality standard in the distribution system networks demands the implementation of numerous tools like the proposed mathematical model to enable the choice of the most appropriate monitoring modes, to adjust residual chloramine concentration. Controlling bacterial growth by disinfectant in the distribution system may serve as a better solution. For controlling bacterial proliferations, it is necessary to understand the influence of the factors that might influence on bacterial growth and inactivation. This model makes it possible to develop a relation of μ_m/k_d with temperature in relation to biostability that will help the utility manager to select the chloramine residual for avoiding nitrification under different temperature. Until now, we have limited our modelling to temperature only. For better result it needs to be extended to include pH and other effects.

5.5 Conclusions

Generally most of the water distribution systems have been subjected to different temperature (6-35°C) due to changing weather conditions round the year. Changing temperature with some other environmental factors affect the chloramine residual especially in water utilities with long pipe lines or water age. In this study, we proposed a novel approach for calculating BRC considering varying μ_m/k_d values at different temperatures using literature data obtained for *Aerobactor aerogenes*, which behaves somewhat similar to AOBs found in distribution systems. The μ_m/k_d of the proposed model at 15°C to 27°C temperature ranges is almost same with literature reported value but a big difference is found for other temperatures. Based on above discussion it can be concluded that the proposed model can be more helpful in predicting the BRC at different temperature round the year specially at lower temperature (below 15°C) and higher temperature (above 27°C) in full scale as well as laboratory scale distribution system.

CHAPTER 6

A NOVEL APPROACH TO UNDERSTAND THE COMBINED EFFECT OF COPPER AND CHLORAMINE ON NITRIFICATION IN BULK WATERS

Abstract

Although chloramine has been popular in some countries, its popularity is dampened by microbial acceleration of its decay. Copper is known to inhibit nitrification, but how synergistically it works in the presence of chloramine is not known, as most of the studies evaluated individual effect usually using pure cultures. Indigenous organisms are shown to behave differently. Hence, this study devised a method to evaluate combined effect of copper and chloramine using biostability concept on heterogeneous organisms containing samples. Samples were collected from a pilot-scale system. The samples were collected in such a way that they can show the onset of nitrification at the point of biostable residual, and different concentrations of copper were added. Change in the point of biostable residual was noted and modelled to understand the combine effect of chloramine and copper. The results showed that copper (≥ 0.25 mg-Cu/L) in combination with chloramine can strongly inhibit nitrification and that could be modelled using the biostability concept. Further, stopping activities of nitrifying bacteria could not improve chloramine decay greatly. This result confirmed our previous finding that there is an unknown mechanism accelerating chloramine decay than nitrification. Such novel finding will pave the way for better chloramine decay control in the system.

6.1 Introduction

In order to meet the rigid regulations regarding disinfection by-products (DBPs) formation, use of chloramine as a secondary disinfectant is increasingly becoming popular, particularly in Australia and USA. The reasons behind selecting chloramine as an alternative to chlorine are less reactivity, lower decay rate, and less production of regulated DBPs, such as trichlormethane and haloacetic acids (Goslan et al.,

2009). It is often used when it is difficult to maintain free chlorine residuals or when they lead to excessive disinfection (Vikesland et al., 2001) or the level of natural organic matter (NOM) is high (Jafvert and Valentine, 1992). However, in long distribution systems, it is very difficult to achieve target residuals at the ends of the system due to regrowth of microbes, especially nitrifiers.

Chloramine decays in two ways, one is chemical decay and another is microbiologically assisted decay. The chemical decay of monochloramine in drinking water is due to auto-decomposition and reactions with organic or inorganic constituents. Jafvert and Valentine (1987) reported that auto-decomposition of monochloramine is radically affected by general acid-catalysis and the generalized form of this reaction is presented in Equation 6.1. During the auto-decomposition reaction, ammonia in chloramine is oxidized to nitrogen gas with production of smaller quantities of nitrate [Equation 6.2] (Valentine and Wilber, 1987). Nitrate conversion is so small that it is hard to measure in normal experiments. The direct reaction between monochloramine and nitrite or the reaction between nitrite and hypochlorous acid produced by monochloramine hydrolysis are described by Equations 6.3 and 6.4.

$$3NH_2Cl \rightarrow N_2 + NH_3 + 3Cl^- + 3H^+ \quad \textbf{Equation 6.1}$$

$$4NH_2Cl + 3H_2O \rightarrow 3NH_3 + NO_3^- + 4Cl^- + 5H^+ \quad \textbf{Equation 6.2}$$

$$NH_2Cl + NO_2^- + H_2O \rightarrow NO_3^- + NH_4^+ + Cl^- \quad \textbf{Equation 6.3}$$

$$HOCl + NO_2^- \rightarrow NO_3^- + H^+ + Cl^- \quad \textbf{Equation 6.4}$$

It is commonly believed that nitrification accelerates chloramine decay in distribution systems. Wolfe et al., (1990) noted that the accelerated chloramine decay is related to high levels of nitrification. Different authors (Woolschlager et al., 2001; Fleming et al., 2005; Pintar et al., 2005; Sathasivan et al., 2008) concluded that chloramine residual can play a significant role along with availability of free ammonia on onset of severe nitrification in distribution systems. Woolschlager et al., (2001) and Harrington et al., (2002) proposed a simple formula to determine the point of biostability. By implementing this concept, Fleming et al., (2005) determined the residual below which potential for nitrification occurrence exists. This was done by balancing growth and disinfection. They used ammonia as

substrate for controlling growth of AOB and dichloramine as the disinfectant. The resulting Equation is:

$$\frac{\mu_m \ (free \ \ ammonia \ as \ \ N)}{(K_S \ + \ free \ \ ammonia \ as \ \ N)} = k_d \ \times \ TCl \qquad \textbf{Equation 6.5}$$

where, μ_m is the maximum specific growth rate of AOB (day^{-1}); K_s is the half saturation constant for AOB (mg-N/L); k_d is the rate constant for inactivation of AOB by disinfectant ($L.day^{-1}$ mg-Cl_2^{-1}); *TCl* is the total chlorine concentration (mg-Cl_2/L) referred to as biostable residual concentration (BRC).

Severely nitrifying condition is defined as the conditions at which nitrite levels are higher than 0.10 mg/L, chloramine residual is lower than 0.5 mg-Cl_2/L and chloramine decay is more than two to three times that was observed at mildly nitrifying conditions**.** Sathasivan et al., (2008) developed a method to identify chloramine residual at which mild nitrifying samples becomes nitrifying by showing sudden increase of the decay rate and nitrite level. They proposed a concept of critical threshold residual (CTR), the residual at which signs of severe nitrification were detected for mildly nitrifying samples. In severely nitrifying bulk waters, this approach has to be modified to take into account of the fact that there is already a very fast decay.

As nitrification is thought to be of serious concern, several control measures have been investigated. Additions of metals like copper and silver to control nitrification have been attempted in the literature (Sathasivan et al., 2005; Laszlo, 2008; Fisher et al., 2009). Copper is bacteriostatic or toxic to bacteria, viruses or cysts. Copper can inhibit AOB activity (Laszlo, 2008; Zhang et al., 2009). Copper in dissolved form, mainly Cu(II) is very persistent and frequently detected in the drinking water (Boulay and Edwards, 2000; Zhang et al., 2002). Jun et al., (2009a) reported that copper plays an important role in chloramine decomposition upon chloramination due to its catalytic activity, especially when pH is not 8. Trials were conducted in the Goldfields and Agricultural Water Supply System (G&AWSS) in Western Australia, using copper (as cupric sulphate) in the pipelines to inhibit nitrification positively. According to the regulations of World Health Organization, recommended value of

copper concentrations in drinking water should be below 1.0 mg-Cu/L with the maximum value of 2.0 mg-Cu/L. Following the Australian Drinking Water Quality Guidelines (ADWG, 2004) and authorized by the Department of Public Health, Western Australia, copper dose of 0.25-0.40 mg-Cu/L was implemented in the field.

There was no report concerning the combined effects of copper and chloramine on inhibiting nitrifiers, especially on indigenous bacteria in chloraminated distribution system. In the context of using copper as an inhibitor, it is important to know whether copper with chloramine can overcome chloramine decay by controlling nitrification under severely nitrifying conditions. As chloramine is usually present in waters of concern, it is important to know how copper and chloramine together will show synergistic effect. In our laboratory, different stages of nitrification (from none to severe) were created to mimic various conditions experienced by the utilities. Out of all, severely nitrifying conditions are worst to get rid of. The purpose of this study is to report our finding on how copper inhibition impacts various aspects in severely nitrifying bulk waters, especially when copper and chloramine were present.

6.2 Concept Development: Modelling Co-Inhibitory Effects of Copper and Chloramine

Biostability concept (Equation 6.5) is defined by balancing the growth rate and disinfection rate. It can be assumed that disinfection rate will increase in the presence of copper and chloramine in three different ways: Firstly, it can be expected that there is direct disinfection by chloramine. Effect of chloramine alone can be modelled as described elsewhere. Secondly, it would be due to an individual effect of copper. Effect of copper is assumed to be proportional to copper concentration, although it need not be always the case. Thirdly, it would be due to synergistic effect of copper and chloramine on nitrification, it could also be referred to as co-inhibition. Co-inhibition, in this paper, is assumed to be proportional to the product of copper and chloramine concentration. These three components can be described in the following way:

$$\mu_m\left(\frac{(free\ \ ammonia\ \ as\ \ N)}{(K_S\ +\ free\ \ ammonia\ \ as\ \ N)}\right)=k_d BRC\ +\ a.BRC.Cu\ +\ b.Cu \qquad \textbf{Equation 6.6}$$

In Equation 6.6, the term 'k_d.BRC' describes the killing rate by chloramine. The term '*b.Cu'* refers to the killing/inhibition rate of copper, when copper is alone and *'b'* has a unit of L.day^{-1}/(mg-Cu). The term 'α.*BRC.Cu*' describes the co-killing/inhibition rate of copper and chloramine, where '*a'* is a constant expressed in L^2.day^{-1}/(mg-Cl_2. mg-Cu).Or the same equation can be written as follows, if both sides of Equation 6.6 is divided by k_d and 'a/k_d' and 'β/k_d' are replaced by 'α' and 'β':

$$\frac{\mu_m}{k_d}.\left(\frac{(free\ \ ammonia\ \ as\ \ N)}{(K_S\ +\ free\ \ ammonia\ \ as\ \ N)}\right)=BRC\ +\ \alpha.BRC.Cu\ +\ \beta.Cu \qquad \textbf{Equation 6.7}$$

From this Equation 6.7, the equivalent effect of copper on inhibiting/killing nitrifiers can be estimated. The unit of α and β are L/mg-Cu and mg-Cl_2/mg-Cu respectively.

6.3 Materials and Methods

The detailed description of stock chemical solutions preparation; water sample collection, preparation and storage; description of pilot-scale reactor setting, operation and feed water preparation; preparation of sample bottles and glasswares, analytical procedures and F_m value determination were presented in Chapter 3.

6.3.1 Criteria for Ensuring Nitrification Inhibition

To make sure nitrification continuation or inhibition in severely nitrifying samples, NOx-N (NO_2-N + NO_3-N) production has to be tracked, as there is no other mechanism than AOB activity that can produce NO_2-N and NO_3-N production by Equation 6.2 is very small (Valentine and Wilber, 1987).

6.3.2 Experimental Design

In order to understand how copper inhibits AOB activity and how it eventually affects chloramine decay, two sets of experiment were conducted on samples collected from severely nitrifying reactors (Figure 6.1).

In both sets of experiments, experiments were done following the same experimental procedures. TCl to TAN ratio of 4.1:1 by weight and pH 8.0±0.1 were maintained whenever chloramine was dosed. Samples were collected in duplicate and incubated in water bath at a constant temperature of 20ºC. Chloramine, TAN, NO_2-N and NOx-N levels were monitored regularly for all samples.

In the first set of experiment, severely nitrifying samples were not processed other than dosing chloramine (2.0 mg-Cl_2/L) and was referred to as "unprocessed (Unp)".

In the second set of experiment, collected severely nitrifying samples were dosed with 2.0 mg-Cl_2/L chloramine and inhibited by various copper sulphate concentrations (0.10, 0.20, 0.25 and 0.40 mg-Cu/L) to understand the inhibitory effects of copper on nitrifying bacterial activity present in chloraminated bulk waters and was referred to as "Inhibited (Inh)". Similar experiments were repeated thrice, and all of them showed a consistent pattern.
The schematic diagram of the experimental protocol is shown in Figure 6.1.

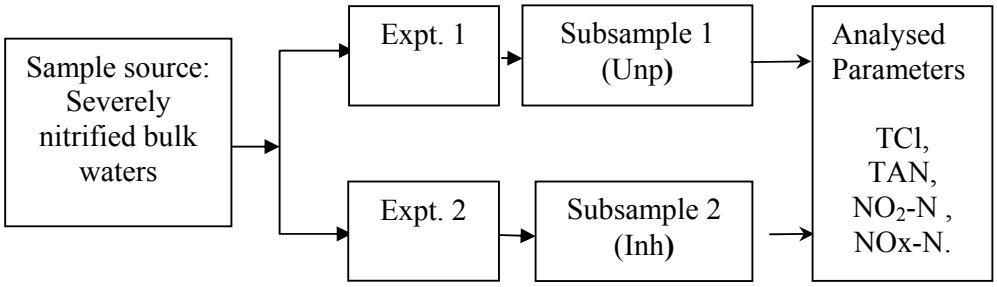

Figure 6.1: Schematic diagram of experimental protocol

For controlling nitrification using copper inhibition with or without chloramine, Equation 6.7 could be utilized. The left hand side (L.H.S) of Equation 6.7 could be calculated (for known μ_m/k_d and K_s) using free ammonia concentration obtained from

experimental results and right hand side (R.H.S) of Equation 6.7 represents proposed relationship between copper and chloramine concentration present in the samples. By minimizing square of errors between L.H.S and R.H.S of the Equation 6.7 by allowing Excel tool "Solver" to select appropriate values, α *and* β could be obtained.

6.4 Results and Discussion

6.4.1 General Characteristics of Pilot-Scale Reactors from where Severely Nitrifying Samples were drawn

The purpose of operating the pilot-scale reactor was to produce none to severe nitrification conditions to allow for collection of severely nitrifying samples – with sufficient nitrifying bacteria to show signs in nitrogenous compounds such as NOx-N when there is an activity/growth.

Results of monitored parameters, measured on a single day, of five different reactors showed the evidence of none (R-1) to severe nitrification (R-5) stages (Figure 6.2). Reactor performance was steady over two months when samples were collected for further experiments. About 2.0 mg-Cl_2/L of TCl was maintained in R-1 and it decayed gradually along the reactors, resulting in residuals below 0.5 mg-Cl_2/L in fourth reactor (R-4) and 0.11 mg-Cl_2/L in fifth reactor (R-5). TAN gradually decreased, but the decrease was same as NOx-N production (Figure 6.2). Changes in NOx-N levels could be used to understand AOB activity, since NO_2-N has to be produced before NO_3-N production and there was no other pathway that significantly produces NO_3-N directly from TAN (Valentine and Wilber, 1987). Therefore, nitrifying bacterial activity gradually increased along the reactors. Total inorganic nitrogen (TIN) did not change more than the experimental error. Moreover, the difference in pH between none and severe nitrification was about 0.3, which was similar to the value reported by Wilczak et al., (1996)**.** These results are similar to what is experienced in a typical distribution system.

First two reactors (R-1 and R-2) showed signs similar to mild nitrification, but the last three (R-3, R-4 and R-5) showed severe nitrification. In the first two reactors,

NO_2-N or NOx-N increased slightly. Although, last three reactors were experiencing nitrification, the most AOB activity was noted in R-4. NOx-N production rate in each reactor could be calculated by dividing NOx-N change by hydraulic retention time. The resulting value was the slope of the curve between each reactor. For example, NOx-N production in R-4 was the difference between NOx-N in R-4 and that in R-3. Therefore, the NOx-N production rate was 0.0093 mg/L/hr, the highest of all reactors. The results clearly indicated that this was the best candidate to conduct further experiments on biostability, as all the nitrifying bacteria were active and sufficient enough to show signs of nitrification through TAN/NO_2-N/NOx-N.

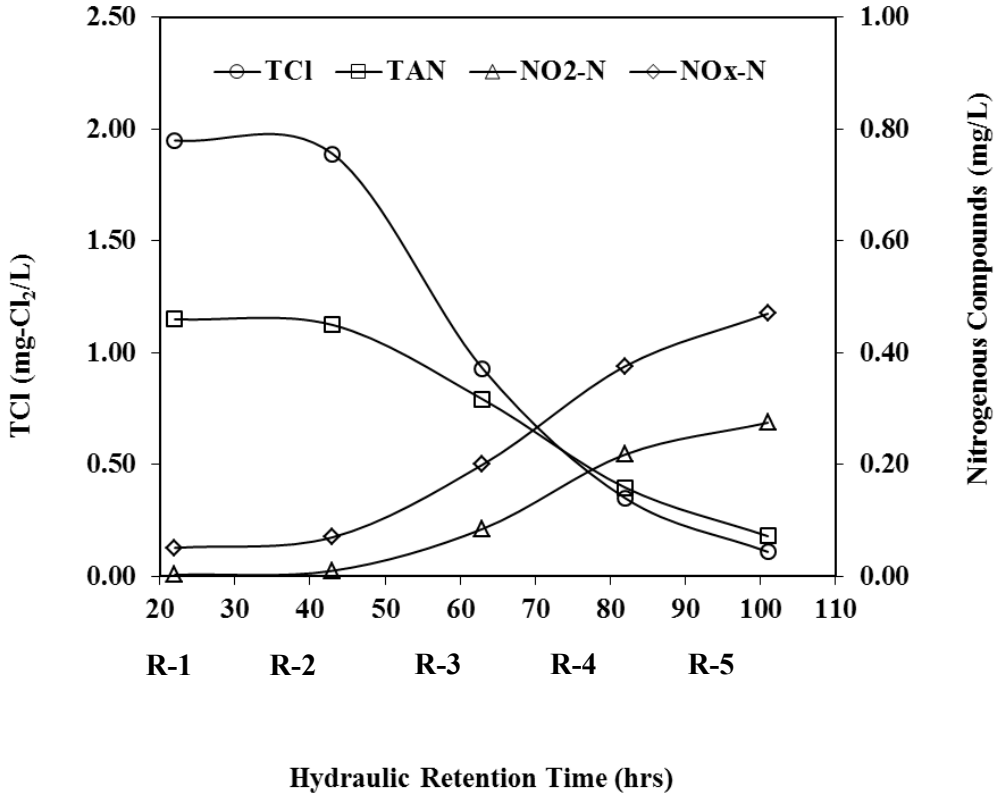

Figure 6.2: Operational parameters of the Pilot-scale reactors.

6.4.2 Profiles of Chloramine and Nitrogenous Compounds Observed in Unprocessed Sample from R- 4

When a sample containing NO_2-N of 0.315 mg/L (collected from R-4) with active AOB was dosed at 2 mg-Cl_2/L chloramine, nitrification was suppressed for the first few hours, but later, signs of ammonia oxidising bacterial activity (changes in NOx-

N) were noted. Several trials were done on samples collected from R-4 following similar experimental procedure described for the first set. The results obtained from one of those trials are shown in Figure 6.3. It was observed that chloramine decayed fast within the first 20hrs of incubation period from 2.0 to 1.2 mg-Cl_2/L. Within the 20hrs, TAN decreased significantly from 0.483±0.007 to 0.324±0.005 mg/L, but NO_2-N decreased slightly from 0.315±0.005 to 0.302±0.004 mg/L. Within the period, TAN reduction was not associated with NOx-N change, indicating that ammonia oxidising bacterial activity was suppressed in the first 20 hrs. The reason for TAN and NO_2-N decrease without increase in NOx-N needs explanation and was given in section 6.4.3, but it could be noted that the behaviour was significantly different from the one observed in the reactors (R-1 to R-5) where the loss in TAN was equal to NOx-N production.

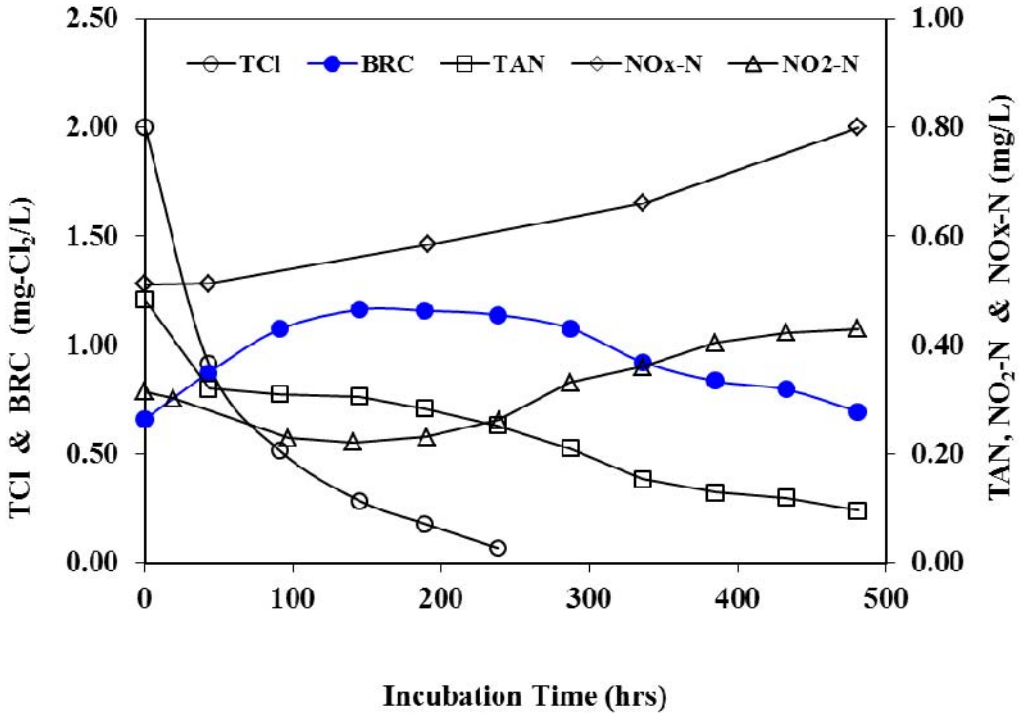

Figure 6.3: Profiles of TCl, BRC, TAN, NO_2-N and NOx-N for unprocessed sample

In contrast to the first 20 hrs behaviour, from Figure 6.3, it was observed that NOx-N has started to increase after the first 20 hrs, indicating that ammonia oxidising

bacterial activity has increased. For example, within this period, NOx-N gradually increased along with incubation time from 0.512±0.010to 0.800±0.016 mg/L. The change of NOx-N was 0.288±0.006 mg/L during this period. Within this period, TAN dropped from 0.324±0.005 to 0.096±0.002 mg/L. It was noticeable that TAN drop (0.228±0.003 mg/L) and NOx-N production (0.288±0.006mg-N/L) were almost same while considering experimental error. Overall these results indicated that AOB activity was present in the unprocessed sample after 20hrs, explaining the TAN loss was mainly due to nitrification. This condition was similar to what was observed in the reactors.

Results of biostabilility analysis showed that the point at which NOx-N started to increase could be predicted using biostable residual concept. At the point of nitrification, TCl, free ammonia, NO_2-N and NOx-N were 0.88 mg-Cl_2/L, 0.15, 0.28 and 0.512 mg/L respectively. If BRC for this condition was calculated, it was 0.90 mg-Cl_2/L. This result was very close to 0.88 mg-Cl_2/L. Before 20 hrs BRC was lower than TCl indicating biostable conditions, but later it was higher indicating conditions suitable for nitrification activity/growth. Therefore the concept of biostability could be utilised to understand the point at which nitrification commenced in these samples.

6.4.3 Behaviour of an Inhibited Severely Nitrifying Bulk Water Sample

In an unprocessed sample, both microbial activities and chemical activities existed together. Now, it was important to understand the impact of copper dosing on chloramine decay and nitrification. Experiments were conducted by dosing various levels of copper into water samples (second set). Tested levels of copper were 0.10, 0.20, 0.25 and0.40 mg-Cu/L. They showed varying degrees of inhibitory effect. Most effect was seen when high concentration of copper (0.40 mg-Cu/L) was dosed into the water sample, although 0.25 mg-Cu/L was sufficient to inhibit ammonia oxidizing bacterial activities. Results of 0.25 mg-Cu/L dosed sample were discussed in detail.

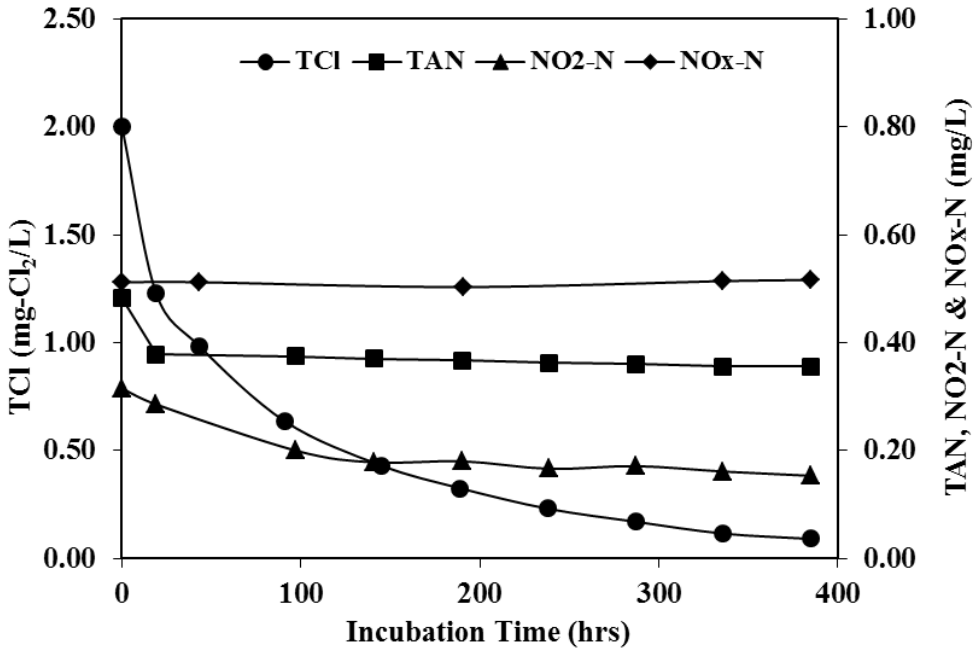

Figure 6.4: Profiles of TCl, TAN, NO_2-N and NOx-N for copper inhibited sample

Figure 6.4 shows that 0.25 mg-Cu/L was sufficient to completely inhibit ammonia oxidizing bacterial activities under the experimental conditions. The initial and final NOx-N concentrations were 0.512±0.010 and 0.516±0.010 mg/L respectively. Hence NOx-N remained constant throughout the experimental period. If AOB were active, NOx-N level would have gradually increased as there was no other mechanism producing NO_2-N or NO_3-N directly from TAN. However, the NOx-N concentration remained same. Results therefore indicated that there was no new production of NOx-N, indicating the absence of AOB activity. Similar conclusion could be drawn if one looked at the NO_2-N level in the absence of chloramine. NO_2-N did not increase when conditions were suitable for nitrification to take place, i.e. chloramine concentration has dropped to 0 mg-Cl_2/L level and free ammonia concentration was high.

Similarly, mass balance of TIN and TAN was carried out. TIN loss was 0.159±0.015 mg/L that was almost same as TAN loss (0.162±0.002 mg/L). Therefore, there was only one pathway of TAN loss which was to convert into nitrogen gas. These results

collectively indicated that only chemical mechanism of chloramine loss was present in the water sample and that 0.25 mg-Cu/L has completely inhibited ammonia oxidising bacterial activity. It could be noted that nitrogen loss happened in the unprocessed sample in the similar way as in inhibited sample.

6.4.4 Inhibition of AOB with Various Copper Concentrations

The second set of experimental results were also used to understand the effective copper concentration that was capable of inhibiting or inactivating AOB present in bulk waters, especially when chloramine was also present. From Figure 6.4, it is found that NOx-N level remained unchanged in inhibited sample and there was no NO_2-N production throughout the experimental period. On the other hand, it was noted that NOx-N started to change after 19 hrs (Figure 6.3) in the unprocessed sample.

Table 6.1: Nitrification Onset Time at Various Copper Concentrations

Copper Dose (mg-Cu/L)	Nitrification onset time (hrs)	Chloramine at nitrification onset (mg-Cl_2/L)
0	19.0	0.84
0.10	24.5	0.32
0.20	40.0	0.14
0.25	>500.0	0.05
0.40	>1500.0	0.00

If the time of beginning of NOx-N increment was referred to as "nitrification onset time", .BRC was calculated at nitrification onset point by using Equation 6.5 taking μ_m/k_d = 2.0 mg-Cl_2/L and Ks = 0.18 mg-N/L and the results could be summarized in Table 6.1 for different copper concentrations. From Table 6.1, it is noticed that nitrification started within 40 hrs for up to 0.20 mg-Cu/L inhibited samples whereas, no sign of nitrification was seen during the experimental period of 500 hrs and 1500 hrs for 0.25 and 0.40 mg-Cu/L dosed samples respectively. Therefore, it could be concluded that 0.25 mg-Cu/L was the minimum concentration to inhibit/inactivate the bacteria present in nitrifying bulk waters if similar chloramine residuals were

present. As both chloramine and copper could inhibit the activity of AOB it was important to understand how well they inhibit and in which relation.

6.4.5 Modelling Synergistic Effect of Copper with Chloramine

The results showed that copper and chloramine together could be an effective way of controlling AOB activities in severely nitrifying bulk waters. At biostable residual, rate of bacterial growth due to substrate (free ammonia) and inactivation due to disinfectant are balanced. When residual reaches concentration below BRC, onset of nitrification and thus increase of NOx-N was observed. Based on experimental results and above discussion, it was possible to show the relationship of copper and chloramine to avoid nitrification by Equation 6.7.

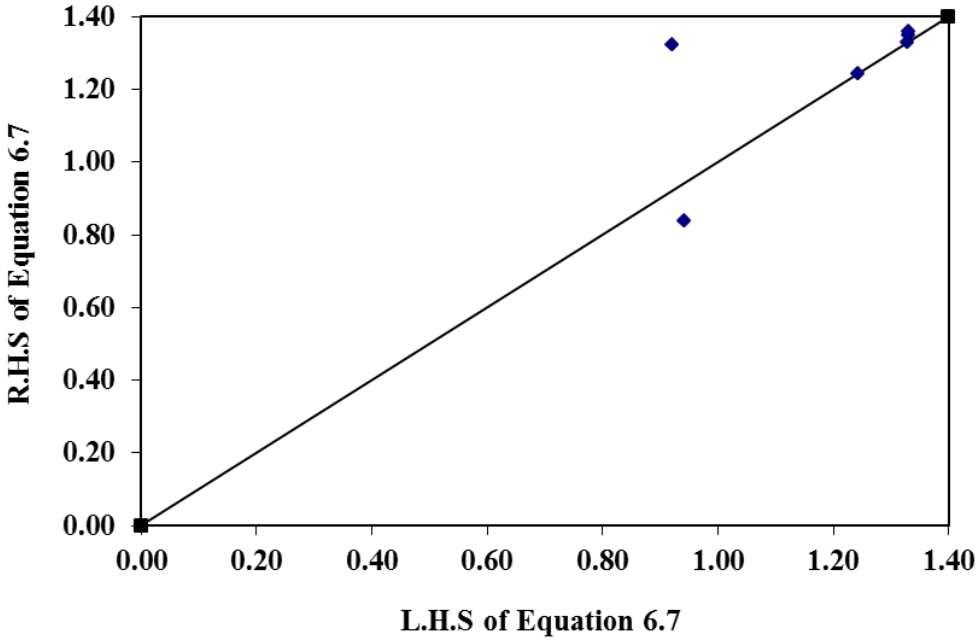

Figure 6.5: Variation of the proposed model with BRC

The calculated BRC based on free ammonia was shown in the left hand side and a relation between copper inhibition and chloramine was proposed on the right side in Equation 6.7. For determination of the constants α and β, the squares of errors were minimized as described in Materials and Methods. The estimated values of α and β were 18.2 and 3.4 respectively. From the model, it could be said that copper concentration up to 0.25 mg-Cu/L with chloramine or 0.40 mg-Cu/L alone might

play an important role in controlling nitrification. A graph was drawn for the proposed relationship against BRC based on free ammonia concentration as shown in Figure 6.5. Therefore, if one is interested in controlling nitrification, copper dosing alone or with chloramine may be sufficient in severely nitrifying bulk waters. Copper inhibition may be helpful as an early intervention to stop nitrifying bacterial activity and to protect chloramine residual.

6.5 Conclusions

For better understanding of how copper would act as an inhibitor, experiments were performed on samples collected from worst, severely nitrifying condition. The chloramine residual as well as nitrogenous compounds were monitored periodically to investigate the effects of copper. The effect was quantified by measuring the time at which onset of nitrification was found. The experimental results showed the following:

- Copper strongly inhibited the ammonia oxidising bacterial activities (nitrite production) in chloraminated severely nitrifying samples at concentrations greater than or equal to 0.25 mg-Cu/L.
- The results confirm the traditional belief that nitrite production was one of the major mechanism by which chloramine decay is accelerated.
- From the proposed model, it can be said that copper alone or with chloramine can prevent nitrification thus can help in maintaining residual in the distribution system.

CHAPTER 7

EFFECT OF COPPER ON CHLORAMINE DECAY AND NUTRIENT PROFILES OF SEVERELY NITRIFIED BULK WATERS

Abstract

The use of chloramine as a secondary disinfectant in drinking water utilities is preferred due to its minimal disinfection by-products formation over chlorine and its vulnerability to nitrification. Under severe conditions, nitrification can greatly accelerate chloramine decay. Using copper as an inhibitor may be feasible to control nitrification in chloraminated drinking water. Copper is used as an inhibitor in Water Corporation of Western Australia, but the chemical effects of copper on chloramine decay and nutrients are not known. In this study, a series of experiments were carried out for severely nitrified samples (the worst conditions) collected from a pilot-scale system under varying conditions of pH and different doses of copper was added in the filtered bulk water samples. Results showed that chloramine decay of filtered samples was not influenced by copper addition, but residual was improved with the increase of sample pH and vice-versa. Moreover, copper has similar effects on ammonia and nitrite profiles, whereas has no effects on NOx-N profiles.

7.1 Introduction

Now-a-days, chloramine has been chosen over chlorine as a disinfectant due to less production of regulated disinfection by-products - such as trihalomethanes and haloacetic acids, less reactivity, lower decay rate, and longer stability in drinking water distribution systems. It is often used when free chlorine residuals are difficult to maintain or when they lead to excessive disinfection (Vikesland et al., 2001) or the level of natural organic matter (NOM) is high (Jafvert and Valentine, 1992). Chloramine decay occurs generally in two ways: One is chemical decay, and another one is microbial decay, caused due to presence of microbes in the system. Chemically chloramine decays due to auto-decomposition and reaction with

chloramine demanding matters present in water distribution system. Jafvert and Valentine (1988) reported that auto-decomposition of chloramine is radically affected by general acid-catalysis and the generalized form of this reaction is presented in Equation 7.1. The direct oxidation of nitrite in the presence of monochloramine is presented in Equation 7.2.

$$3NH_2Cl \rightarrow N_2 + NH_3 + 3Cl^- + 3H^+ \quad \textbf{Equation 7.1}$$

$$NH_2Cl + NO_2^- + H_2O \rightarrow NO_3^- + NH_3 + HCl \quad \textbf{Equation 7.2}$$

The potential barrier of using chloramine is the proliferation of ammonia oxidizing bacteria (AOB) or the occurrence of nitrification episode in the chloraminated distribution systems (Odell et al., 1996; Skadsen, 1993; Wolfe et al, 1990; Cunliffe 1991). Nitrification results in nitrite accumulation and accelerates chloramine decay. Pintar et al., (2005) and Sathasivan et al., (2008) suggested that chloramine concentration could possibly play an important role on onset of severe nitrification. Several nitrification control strategies have been attempted to maintain chloramine residuals in distribution systems. The traditional approaches that have been applied in chloraminated distribution system for controlling nitrification are: breakpoint chlorination (Wolfe et al., 1988; Odell et al., 1996), maintaining high disinfectant residual (Skadsen, 1993; Harrington et al., 2002), optimization of the chlorine-to-ammonia ratio (Wolfe et al., 1988; Odell et al., 1996), removal of natural organic matters (Odell et al., 1996), distribution system flushing (Odell et al., 1996), decrease of distribution system retention time (Odell et al., 1996; Harrington et al., 2002). The effectiveness of these methods is not satisfactory for controlling neither nitrification nor chloramine decay for a long term period. In this context, the novel approach of controlling nitrification is to use inhibitor(s) in the distribution systems. Controlling nitrification by metal (copper, silver) inhibition has been attempted in the literature (Fisher et al., 2009; Laszlo, 2008; Sathasivan et al., 2005). It is well-known that copper is bacteriostatic or toxic to bacteria, viruses or cysts and it can inhibit AOB activity (Zhang et al., 2009). In drinking water, copper in dissolved form, mainly Cu(II) is very persistent (Boulay and Edwards, 2000; Zhang et al., 2002). Wang et al., (2003) and Lin et al., (2004) reported that copper can act as a heterogeneous or homogeneous catalyst in many reactions.

Jun et al., (2009a) reported that copper plays an important role in the decomposition of monochloramine upon chloramination due to its catalytic activity. This catalytic effect increased as the solution pH decreased and become disappeared at pH 8. Elemental copper can react with monochloramine through the following reactions at 25ºC (Zhang et al., 2002):

$NH_2Cl + Cu(s) + OH^- \leftrightarrow CuO(s) + Cl^- + NH_3$, **Equation 7.3**

$NH_2Cl + Cu(s) + H_2O + OH^- \leftrightarrow Cu(OH)_2(s) + Cl^- + NH_3$, **Equation 7.4**

$NH_2Cl + Cu(s) + HCO_3^- \leftrightarrow CuCO_3(s) + Cl^- + NH_3$, **Equation 7.5**

The strong ligands for copper ions - include ammonia, chloride, inorganic carbon and NOM - weaken the deposit formation by forming dissolved complexes. Acting as a bridging substance, the majority of dosed copper can aggregate small organic molecules via intermolecular dicarboxylate chelation which is thought to be a dominating chelation between Cu(II) and organic compounds (Zhan, 2011). He also reported that majority of dosed Cu(II) exist in the forms of Cu(II)-NOM complexes. Holakoo et al., (2006) reported that copper can chelate with soluble microbial products (SMPs) depending on molecular weight.

Jun et al., (2009a) reported that acceleration of monochloramine decay increased when copper concentration increased from 0.01 to 0.10 mg-Cu/L, and this acceleration continually increased up to 1.00 mg-Cu/L copper. They didn't find any acceleration of chloramine decay when copper concentration was increased from 1.0 to 5.0 mg-Cu/L. Jun et al., (2009b) reported that monochloramine decomposition was enhanced by dichloramine formation due to addition of Cu(II), where its direct catalysis had the major contribution along with pH reduction. However, Jun et al., (2009 a, b) conducted the experiments using deionized water with higher chloramine concentrations. This is entirely different from a real water distribution system.

There is a lack of information regarding the chemical effects of copper on chloramine residual in chloraminated nitrifying bulk waters. As chloramine is usually present in the waters of concern, it is important to know whether copper and chloramine have the chemical synergistic effect. In this context, yet the role of copper on chemically accelerated chloramine decay in the water distribution system

is not defined. The purpose of this paper is to understand the chemical effects of copper that may influence on chloramine residual stability as well as nutrients in nitrified bulk waters, especially when copper and chloramine are present together.

7.2 Materials and Methods

The detailed description of stock chemical solutions preparation; water sample collection, preparation and storage; description of pilot-scale reactor setting, operation and feed water preparation; preparation of sample bottles and glasswares, analytical procedures and decay rate coefficients determination were presented in Chapter 3.

7.2.1 Experimental Design

How onset of nitrification was affected by pH, temperature and copper addition were presented in Chapters 4, 5 and 6 respectively. In this chapter, how copper impacts chloramine decay rates and nutrient profiles (nitrogenous compounds: TAN, NO_2-N, NOx-N) are discussed. Since copper would impact both dissolved compounds and particulate matters, it was important to evaluate them separately. Especially, it is of interest to know how copper inhibition of nitrification modifies chloramine decay rates, especially if copper has to be used as an effective strategy to improve chloramines stability. Three sets of experiments were conducted to evaluate the impact of copper on chloramine decay and nitrification for severely nitrified bulk waters. The severely nitrified bulk waters were collected from the pilot-scale reactor (R-4). The target of these experiments were first to evaluate whether copper could control nitrification and chloramine decay in a sample containing high nitrifying bacterial activity, then to understand chemical effects of copper on filtered sample containing dissolved compounds. Lastly, experiments were designed to see chemical effects of copper on chloramine decay under various pH conditions, as pH affects greatly in controlling nitrification and chloramine decay. It is known pH is modified gradually when a sample undergoes nitrification. Hence, the impact of pH was studied for 7.5, 8.0 and 8.5 to evaluate feasible pH levels. These three pH levels are important as 8 is the normal pH of a chloraminated water; about 7.5 is the pH

reached by a nitrified sample; and 8.5 was seen as improving chloramine decay rates of severely nitrified samples in Chapter 3. The flow diagram of the experimental design is shown in Figure 7.1.

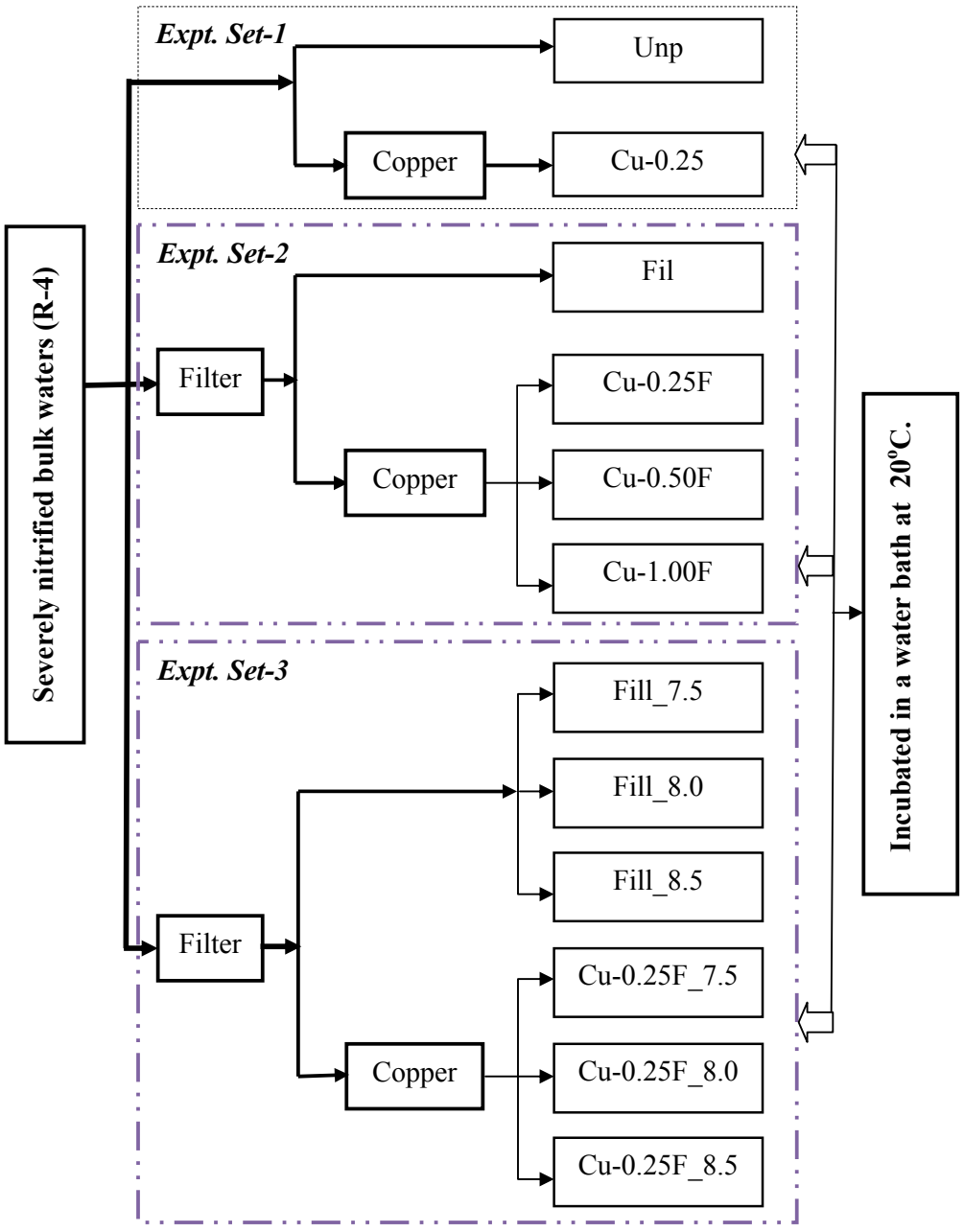

Figure 7.1: Flow diagram of experimental design

First set was conducted to investigate the effects of copper on chloramine decay rate and nutrient profiles in a sample containing highly active nitrifying microbial population. Severely nitrified samples were divided into two sub-samples. The first sub-sample was not processed other than dosing chloramine (2.0 mg-Cl_2/L) and was referred to as an unprocessed (Unp) sample. In the second sub-sample, collected severely nitrified sample was dosed with 2.0 mg-Cl_2/L of chloramine and inhibited by copper sulphate (0.25 mg-Cu/L). This copper dose was selected, because lower concentration did not effectively inhibit the nitrifying microbes in the previous chapter (Chapter 6). The sub-sample was referred to as 'Cu-0.25'. In both sub-samples, TCl to TAN ratio of 4.1:1 by weight and pH 8.0 was maintained, whenever chloramine was dosed.

To investigate the chemical effects of copper or in other words its effect on dissolved compounds present in severely nitrified bulk waters, the second set of experiment was designed, as the tested sample would contain microbes, particulates and dissolved compounds. Severely nitrified bulk water sample was filtered through a 0.2 μm polycarbonate membrane filter and split into four sub-samples. The first sub-sample was not further processed, other than adding appropriate chloramine residual and hence it was named as 'Fil'. In the second, third and fourth sub-samples, 0.25, 0.50 and 1.00 mg-Cu/L was added as copper sulphate and designated as Cu-0.25F, Cu-0.50F and Cu-1.00F, respectively. In all sub-samples, initial chloramine concentration of 2.0 mg-Cl_2/L, TCl to TAN ratio of 4:1 by weight and pH 8.0 were maintained.

The aim of the third set of experiment was to investigate the chemical effects of copper, when pH of the samples was changed. For this, severely nitrified samples were filtered through 0.2 μm polycarbonate membrane filter and split into three categories adjusting pH to 7.5, 8.0 and 8.5. Each category was divided into two sets of sub-samples. One set of sub-sample remained unchanged and was marked as 'Fil_7.5', 'Fil_8.0' and 'Fil_8.5' for adjusted pH of 7.5. 8.0 and 8.5, respectively. In another set of sub-sample, 0.25 mg-Cu/L copper was added and termed as 'Cu-0.25F_7.5', 'Cu-0.25F_8.0' and 'Cu-0.25F_8.5' for adjusted pH of 7.5. 8.0 and 8.5, respectively. In all sub-samples, initial chloramine concentration of 2.0 mg/L, TCl to TAN ratio of 4:1 by weight was maintained.

Sub-samples for all sets of experiments were prepared in duplicate and incubated in water bath at a constant temperature of 20ºC. Chloramine, TAN, NO_2-N and NOx-N levels were monitored regularly for all the samples. The chloramine decay rate coefficient for all samples is estimated using the Equation 3.1.

7.2.2 Calculation of Auto-decomposition and Nitrite Oxidation Mechanism of Chloramine Decay

According to the stoichiometry of Equation 7.1, auto-decomposition of 3 millimoles chloramine (3x71 mg-Cl_2/L) will result in 28 mg/L of nitrogen loss (or converted to nitrogen gas). Therefore, 7.5 mg-Cl_2/L chloramine loss will result when 1 mg/L of nitrogen loss is observed. Using this conversion, the chloramine demand exerted by auto-decomposition could be calculated for each TAN loss. In a nitrifying sample, TAN change cannot be used to track ammoniacal nitrogen loss, but total inorganic nitrogen loss can serve as a good indicator of nitrogen loss from sample.

Other possible mechanism of chloramine decay is by direct oxidation of NO_2-N to NO_3-N. When chloramine is lost by NO_2-N oxidation, 1 millimole of NO_2-N (14 mg-N/L) will result in 1 millimole (71 mg-Cl_2/L) loss of chloramine (Equation 7.2). Therefore, 1 mg/L of NO_2-N oxidation will result in 5 mg-Cl_2/L of chloramine. In a nitrified sample, NO_2-N is oxidized by chloramine and NOB, and hence it is difficult to assign any value. However, if copper can completely stop nitrification and if the behaviors of unprocessed and copper added samples are compared it is easy to assign values NO_2-N oxidation in samples where chemical oxidation and nitrification take place.

7.3 Results and Discussions

7.3.1 General Behavior of Unprocessed Severely Nitrified Bulk Waters

The first set of experiment was conducted for monitoring chloramine decay and nitrogenous compounds for the unprocessed severely nitrified bulk water sample and the profiles are shown in Figure 7.2. It was observed that chloramine decayed quickly within the first 20 hrs of incubation from 2.0 to 1.2 mg-Cl_2/L. The

chloramine decay coefficient for the first 20 hrs was calculated to be 0.0268 ± 0.002 hr^{-1}. Within this 20 hrs, TAN decreased from 0.483±0.007 to 0.324±0.005 mg/L, but NO_2-N slightly reduced from 0.315±0.005 to 0.302±0.005 mg/L. These were associated with some change in NOx-N level (increase of 0.02 mg/L), indicating that there was some nitrification activity in the sample and the chloramine decay within this period should be both by microbiological and chemical. The higher net reduction of total inorganic nitrogen (0.106 mg/L) or TAN reduction (0.159 mg/L) but smaller NOx-N production (0.02 mg/L) indicates a net loss of nitrogen, possibly via nitrogen gas production of 0.139 mg/L or auto-decomposition. This is in alignment with what was observed earlier by Bal Krishna and Sathasivan, (2010) and Sathasivan and Bal Krishna (2012) in a filtered severely nitrifying sample.

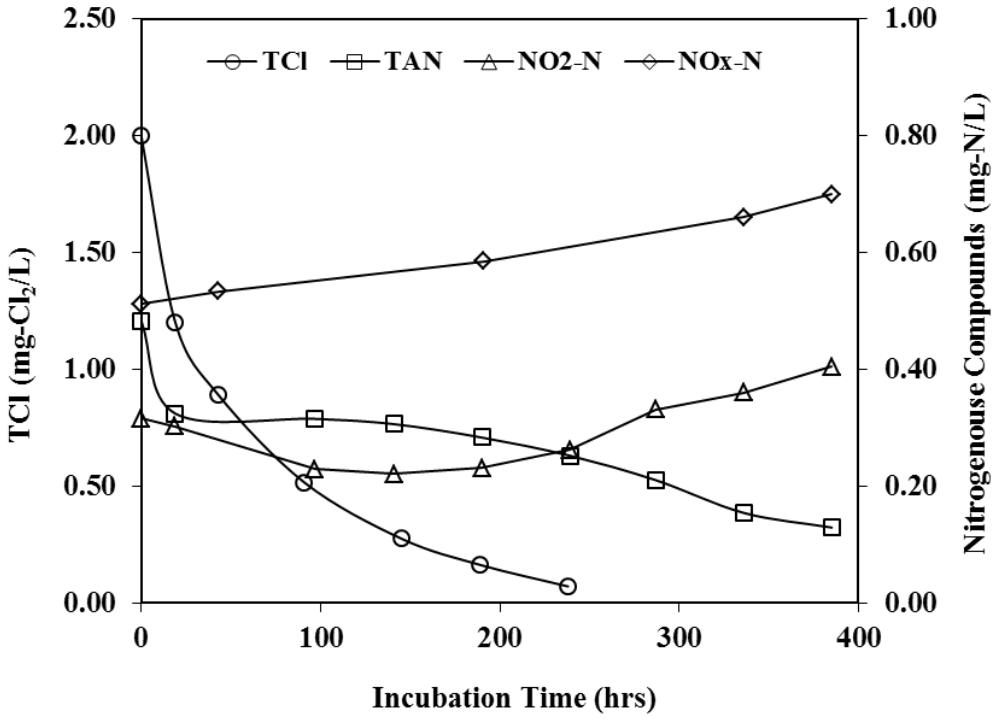

Figure 7.2: Profiles of chloramine decay and nitrogenous compounds for severely nitrified bulk waters

After 20 hrs, TAN gradually dropped to 0.129 mg/L and it was observed that the chloramine decay rate coefficient was 0.0128±0.0042 hr^{-1} on average. Within the same period, NOx-N level gradually increased with incubation time from

0.565±0.010 to 0.700±0.014 mg/L. The change of NOx-N was 0.135±0.024 mg/L. Within this period, TAN dropped from 0.324±0.005 to 0.129±0.002 mg/L. In contrast to the period up to 20 hrs, it was noticeable that TAN drop (0.195±0.007 mg/L) and NOx-N production (0.135±0.024 mg/L) were not same, indicating the presence of both chemical and microbial mechanisms of TAN loss. Overall, these results indicated that AOB activity was present in the unprocessed sample. TAN drop and NOx-N production were equal, explaining the TAN loss was mainly due to nitrification. It was therefore likely that the chloramine decay was also controlled by AOB in addition to other chemical mechanisms.

One of the possible ways to overcoming/controlling nitrification would be the inhibition or inactivation of microbes by adding inhibitors. The behaviour of severely nitrified bulk waters under copper inhibition was discussed in the following section.

7.3.1 Effect of Copper on Severely Nitrified Bulk Water Behavior

The first set of experiment was conducted to understand the inhibitory effects of copper on AOB and the profiles of TCl, TAN, NO_2-N and NOx-N are shown in Figure 7.3. The chloramine concentration decreased rapidly from 2.0 to 1.23 mg-Cl_2/L within the initial 20 hrs of incubation time. The calculated decay coefficient was 0.0256 ± 0.002 hr^{-1} within the 20 hrs followed by a slower decay for the remaining period of the experiment. TAN decreased from 0.483±0.007 to 0.378±0.006 mg/L within the initial 20 hrs and it was found to be stable for the rest of the experimental period. Similarly, NO_2-N quickly decreased from 0.315±0.005 to 0.200±0.003 mg/L within the first 97 hrs and slowly decreased afterwards. However, the NOx-N concentrations remained the same with the initial and final concentrations at 0.512±0.010 and 0.516±0.010 mg/L, respectively. Therefore, there was no AOB activity and copper has effectively inhibited the nitrification in this sample.

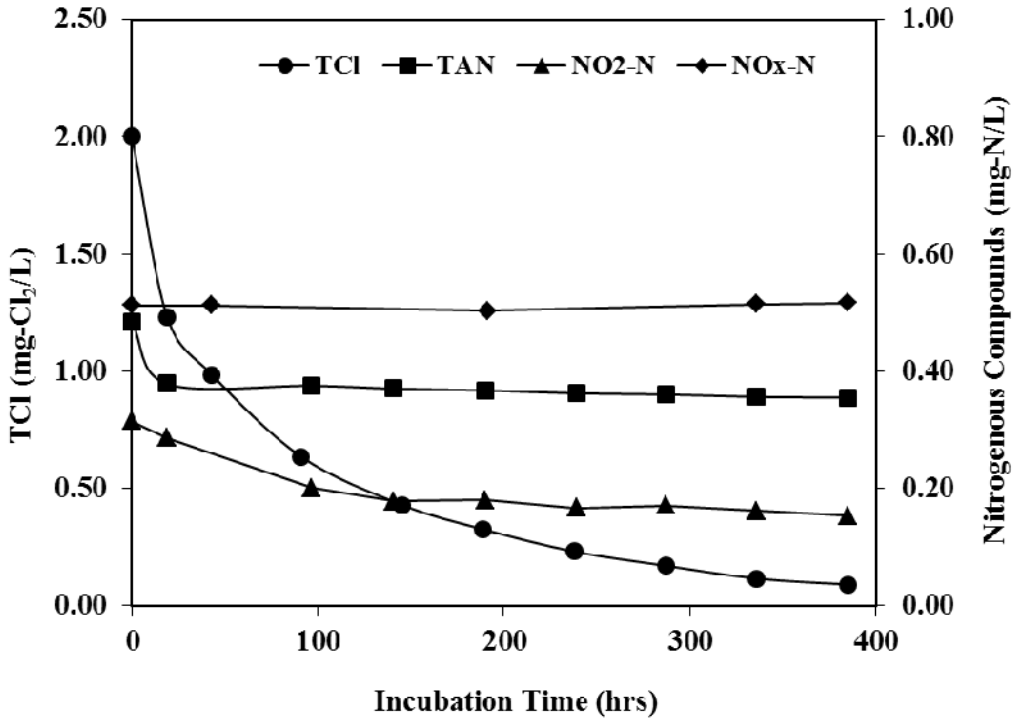

Figure 7.3: Profiles of chloramine decay and nitrogenous compounds for inhibited severely nitrified bulk waters

7.3.2 Effectiveness of Copper Inhibition on Improving Chloramine Decay Rate and Possible Reasons

In comparison to unprocessed samples, copper added samples showed a suppressed ammonia oxidation activity and improved chloramine residuals (Figure 7.4 A and B). In the first 20 hrs of incubation, chloramine decay profiles of both samples followed closely and the decay rate coefficients were same; but a marginally faster decay was observed in unprocessed samples than inhibited samples after 20 hrs (Figure 7.4A). From Figure 7.4B, it was noticed that NO_2-N and NOx-N levels were higher due to ammonia oxidation for unprocessed sample at any point. On the other hand, NO_2-N and NOx-N levels remained lower and stable throughout the incubation time in the copper inhibited sample. A marginal improvement of chloramine residual and stable pattern of NOx-N level was seen probably due to inhibition and/or killing of nitrifying bacteria in these copper inhibited samples. Therefore, the conclusion is significant as it provides the first evidence that controlling nitrification alone in severely nitrified samples would not significantly improve chloramines decay and

supports the earlier study (Bal Krishna and Sathasivan, 2010; Sathasivan et al., 2011) that SMPs play a critical role on accelerating chloramine decay rather than nitrification alone.

However, the decay rate could have been controlled by chemical interaction of SMPs with copper or simply by controlling of nitrite production and thus reduction of chemical chloramine demand. Therefore, it is important to investigate the copper effects on chloramine decay and nutrients content for filtered severely nitrified bulk waters which contain SMPs and nitrite, but not the particulate matters.

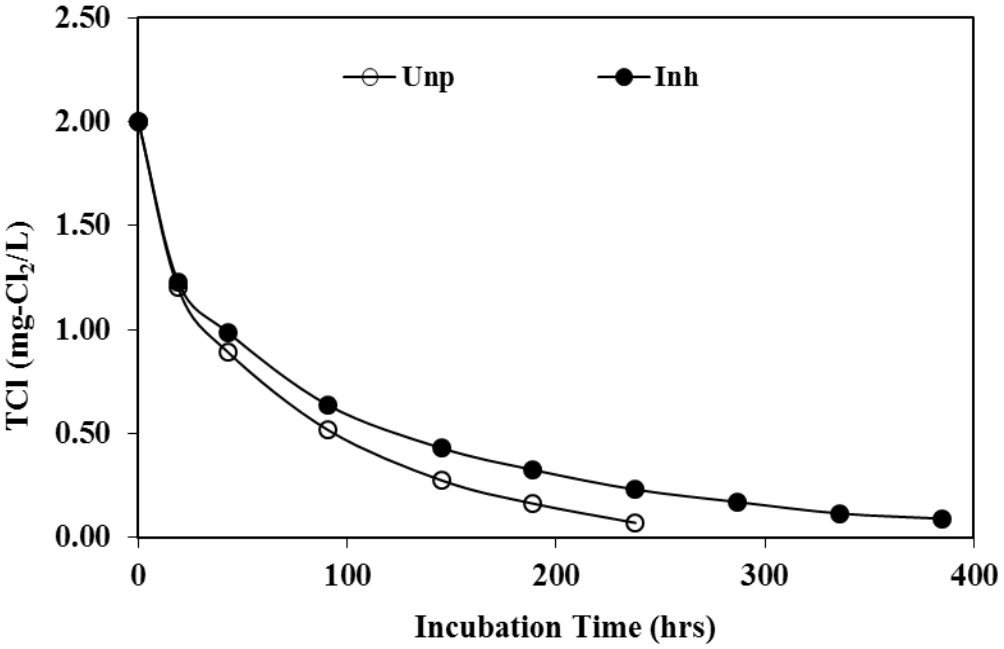

Figure 7.4A: Impact on chloramine decay due to copper inhibition on severely nitrified bulk waters

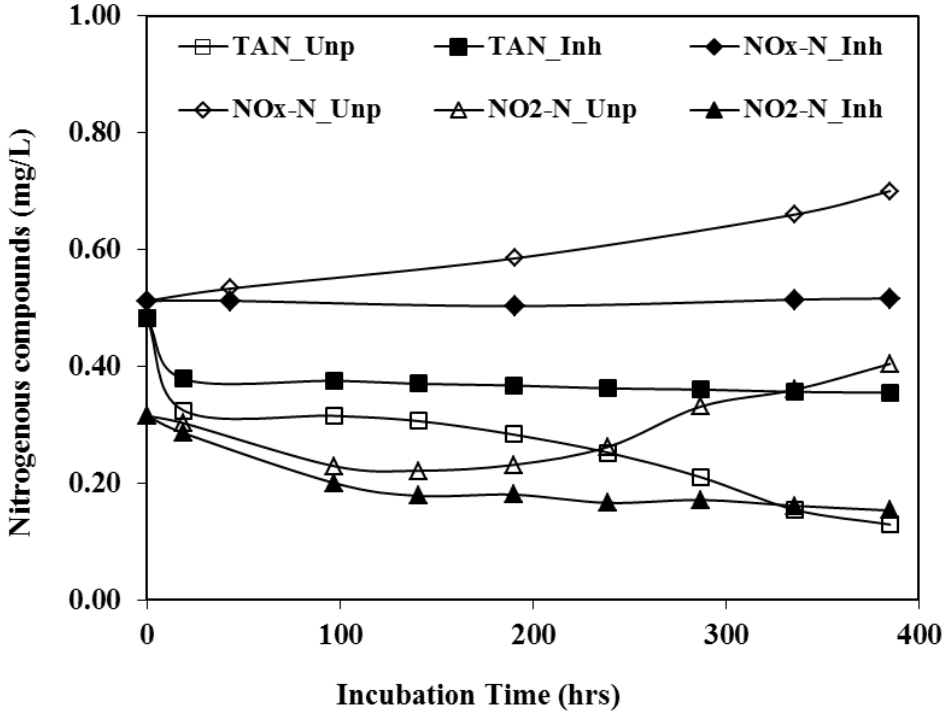

Figure 7.4B: Impact on nitrogenous compounds due to copper inhibition on severely nitrified bulk waters

7.3.3 Chemical Effects of Copper in Severely Nitrified Bulk Waters

The second set of experiment was conducted to find out if there was any effect of copper on chloramine decay by dissolved compounds in the severely nitrified bulk waters. For completeness, different concentrations of copper were dosed in the filtered severely nitrified bulk waters and the profiles are presented in Figures 7.5 A, B, C & D. From the figures, it is observed that TCl, TAN, NO_2-N and NOx-N profiles of filtered severely nitrified samples were not greatly altered by the addition of copper, although there was a slight increase in decay rate for each incremental copper addition. The calculated chloramine decay rate coefficients for Fil, Cu-0.25F, Cu-0.50F and Cu-1.00F samples were 0.0230±0.0022, 0.0252±0.0077, 0.0262±0.0105 and 0.0272±0.0114 hr^{-1}, respectively. After 407 hrs of incubation time, the final concentrations of TAN for Fil, Cu-0.25F, Cu-0.50F and Cu-1.00F samples were 0.381, 0.394, 0.409 and 0.420 mg-N/L respectively. The final concentrations of NO_2-N were 0.243, 0.245, 0.241 and 0.230 mg/L respectively. Similarly, the final concentrations of NOx-N for Fil, Cu-0.25F, Cu-0.50F and Cu-1.00F samples were 0.739, 0.747, 0.721 and 0.734 mg/L respectively. The chloramine decay rates, and changes in TAN, NO_2-N and NOx-N concentrations for Fil, Cu-0.25F, Cu-0.50F and Cu-1.00F samples were same even in higher copper added samples (Cu-1.00 mg/L) while experimental errors were considered. Thus, it could be said that Fil, Cu-0.25F, Cu-0.50F and Cu-1.00F samples behaved identically with time. Therefore, copper had no significant chemical effects on chloramine decay as well as nutrients level. However, it should be noted that the level of some components such as decay rate coefficients, TAN levels constantly changed and hence there could be some chemical effects of copper on these but due to lower chloramine/copper application compared to the literature reported chloramine/copper ratio 3.19-319 (3.19 mg-Cl_2/(0.01-1.0) mg-Cu/L[Jun et al., 2009a) we may not have noticed the difference.

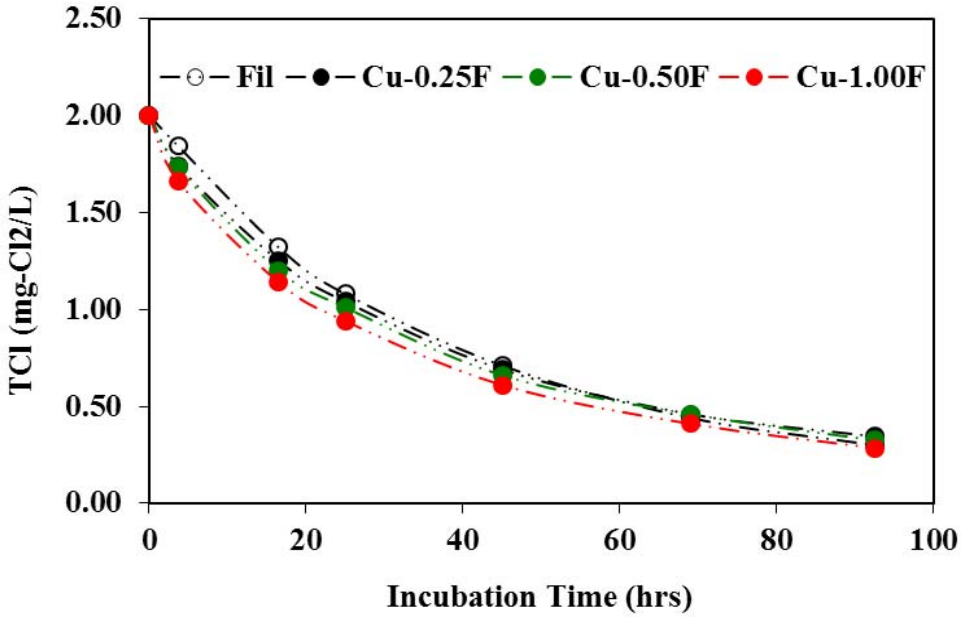

Figure 7.5A: Impact of copper addition on TCl profiles for filtered severely nitrified bulk waters

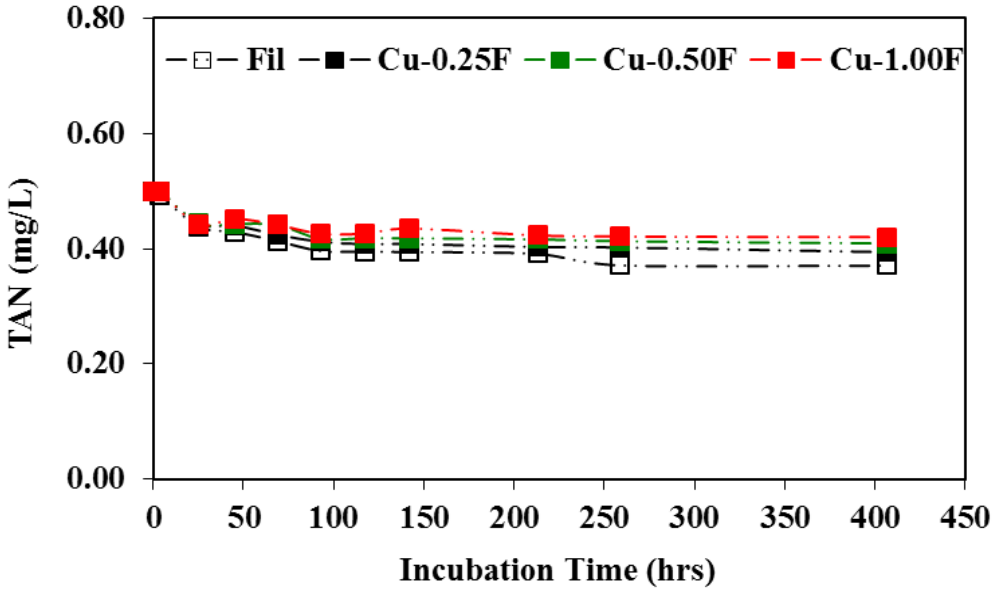

Figure 7.5B: Impact of copper addition on TAN profiles for filtered severely nitrified bulk waters

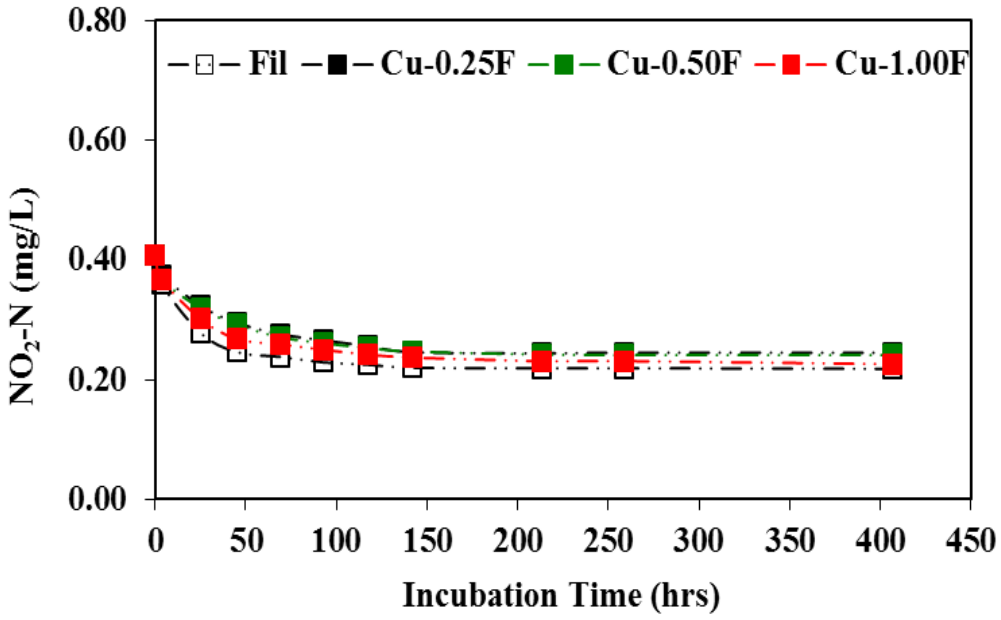

Figure 7.5C: Impact of copper addition on NO_2-N profiles for filtered severely nitrified bulk waters

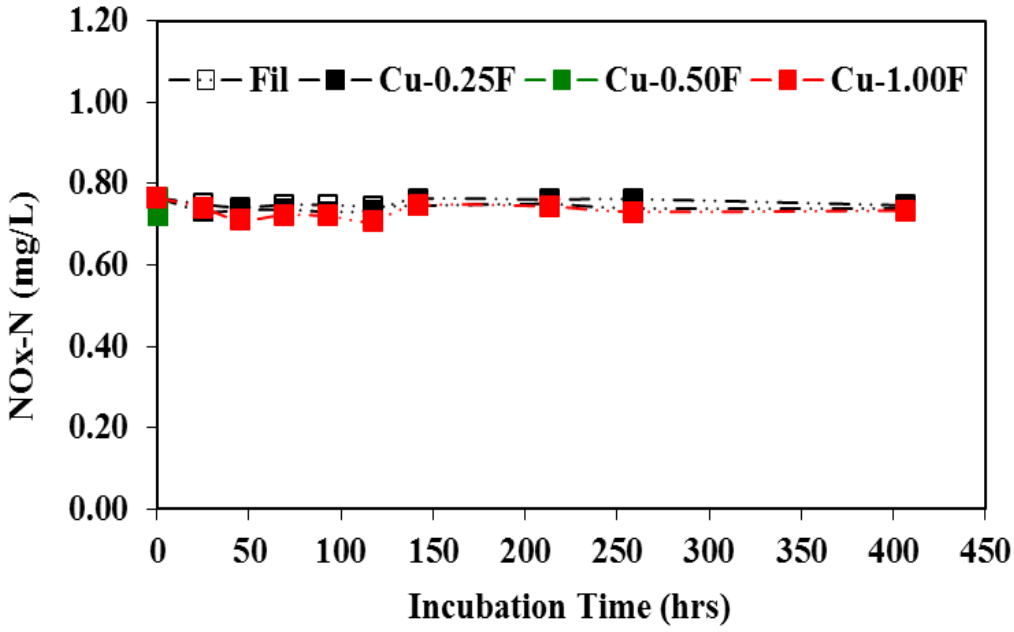

Figure 7.5D: Impact of copper addition on NOx-N profiles for filtered severely nitrified bulk waters

Based on the above discussion, it has been proven that copper didn't play a significant role in accelerating chloramine decay when comparing the profiles for smaller differences of copper dosing such as 0.25 mg-Cu/L, but it did when decay profiles of substantially different doses, for example 1.0 mg-Cu/L were compared with that of zero copper concentration. In these experiments (Set 2), however our concentration of concern was about 0.25 mg-Cu/L where we show different chloramines/nutrient behaviour. Therefore, it could be other factors that contributed to controlling chloramine decay rate and nutrient profiles in severely nitrified bulk waters. In this context, investigation of the influence of the governing factors (like pH, NO_2-N levels, etc.) that might be responsible for affecting chloramine decay in the unprocessed samples should be considered. Therefore, these should be carefully studied (section 7.3.1). Bal Krishna and Sathasivan, (2010) reported that chloramine decay observed in severely nitrified filtered samples were much higher than that observed in mildly nitrified samples, for a given set of conditions (NO_2-N, TCl, TCl:TAN ratio, temperature and pH). Hence, there might be a possibility to alter the chloramine decay behaviours with the change of such environmental factors. In this study, chemical effects of copper addition on chloramine decay were discussed under varying pH conditions.

7.3.4 Effect of pH and Copper on Chloramine Decay and Nutrient Profiles of Filtered Severely Nitrified Samples

The pH was found to be modifying the decay profiles of chloramines, but the addition of copper did not further modify any profiles as also noted previously for pH 8 (Figure 7.6 A, B, C &D). The decay behavior showed that the decay rate decreased with increased pH, within the tested range. Figure 7.6 A, B, C & D compares the chloramine decay and nutrients profiles of severely nitrified samples that had been filtered and pH adjusted (Fil_7.5, Fil_8.0 and Fil_8.5) with that of copper added samples (Cu-0.25F_7.5, Cu-0.25F_8.0 and Cu-0.25F_8.5). These results were obtained by conducting the experiments according to the protocol set out for the third set of experiment. From Figure 7.6A, it could be observed that pH has modified the TCl decay profiles of severely nitrified samples. However, the copper addition (0.25 mg-Cu/L) did not further modify them. This is evident from chloramine decay coefficients for all samples as shown in Table 7.1.

Table 7.1: Chloramine Decay Rate Coefficients at Different pH Conditions

Sample Type	Incubation Time (hrs)		
	0 to 25 hrs	25 to 285 hrs	0 to 285
Fil_7.5	0.0344±0.0048	0.0231±0.0010	0.0263±0.0064
Cu-0.25F_7.5	0.0364±0.0108	0.0231±0.0072	0.0252±0.0077
Fil_8.0	0.0252±0.0068	0.0196±0.0046	0.0203±0.0025
Cu-0.25F_8.0	0.0260±0.0133	0.0149±0.0068	0.0174±0.0052
Fil_8.5	0.0205±0.0059	0.0151±0.0020	0.0159±0.0017
Cu-0.25F_8.5	0.0211±0.0171	0.0141±0.0046	0.0154±0.0033

From Figure 7.6 B, C & D, it was noticed that TAN, NO_2-N and NOx-N profiles show that they are not modified greatly by either adjusting pH or adding copper at 0.25 mg-Cu/L. From Figure 7.6 B, it was noticed that there was an initial rapid reduction of TAN, otherwise were constant for all samples. NO_2-N levels were also constant except there was an initial rapid loss for all samples (Figure 7.6 C). From Figure 7.6 D, it was found that the NOx-N levels for all the samples were constant throughout the experimental period. It could be said that TCl, TAN, NO_2-N and NOx-N concentrations were same while considering experimental errors. Therefore, pH variations did not affect chloramine decay and nutrient's levels in severely nitrified bulk waters although the increase in pH has slightly increased.

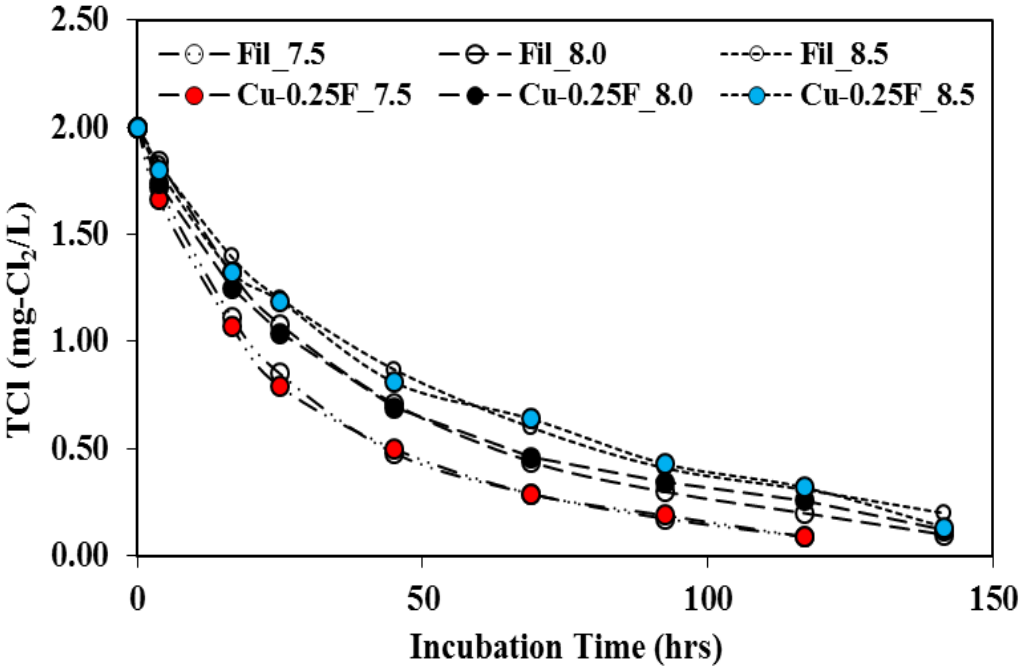

Figure 7.6A: Effects of pH and Copper on chloramine decay profiles

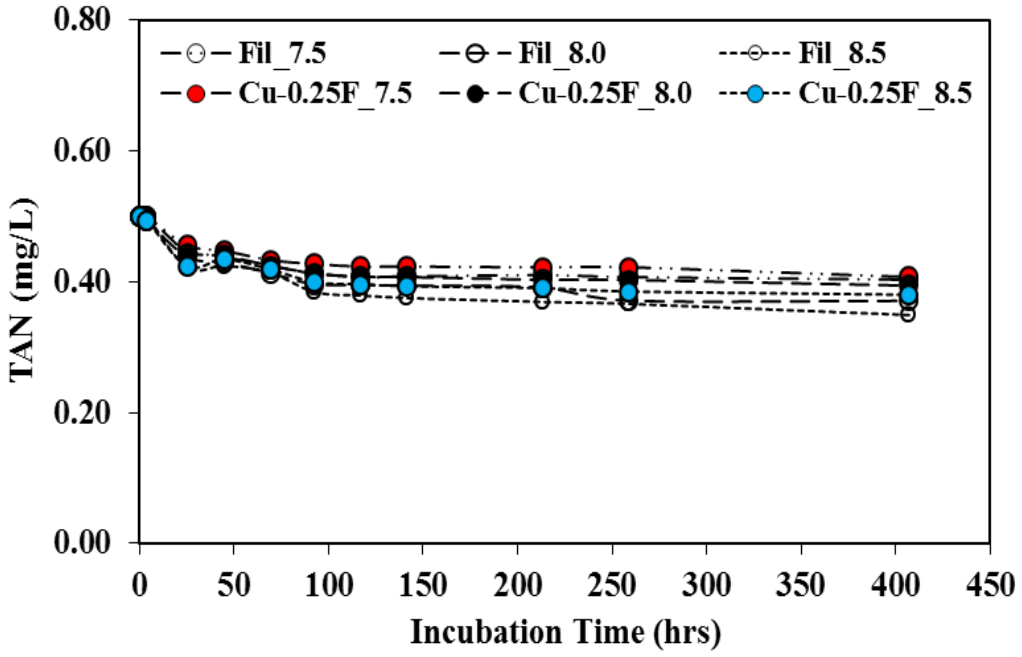

Figure 7.6B: Effects of pH and copper on TAN profiles

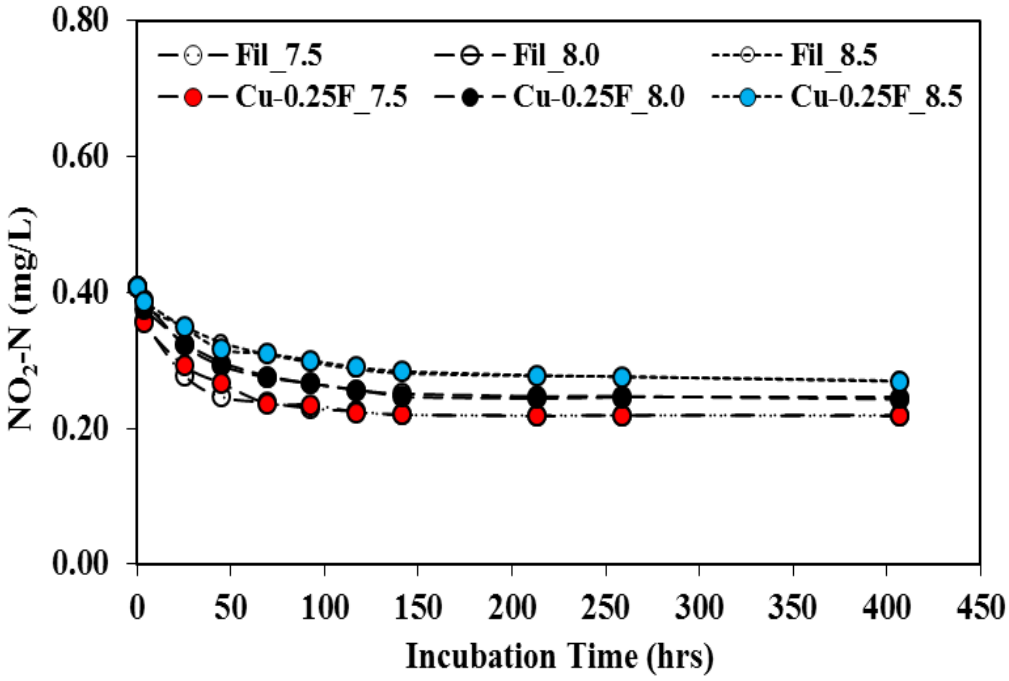

Figure 7.6C: Effects of pH and copper on NO_2-N profiles

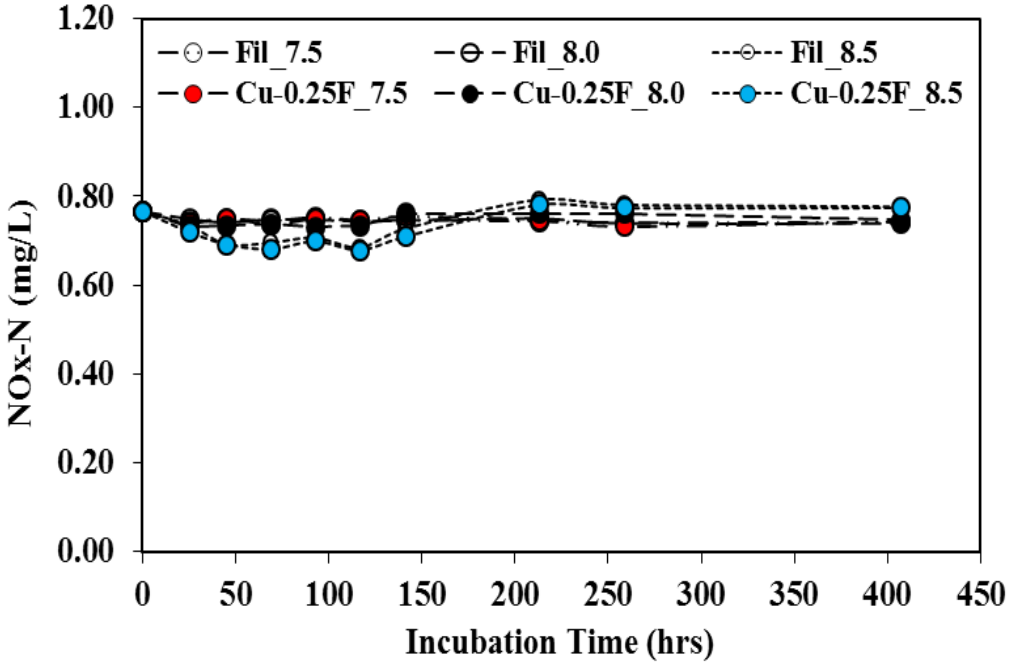

Figure 7.6D: Effects of pH and copper on NOx-N profiles

7.3.5 Chloramine Decay Mechanism in Filtered Severely Nitrified and Copper Inhibited Severely Nitrified Bulk Waters

Sathasivan and Bal Krishna, (2011) reported that about 85-90% of chloramine loss can be explained by auto-decomposition and NO_2-N oxidation in filtered severely nitrified samples. Chloramine decay in these samples generally occurs in two phases with a high initial decay followed by a slower one. The initial 20 hrs was taken for explaining the decay mechanism for both samples. For calculating chloramine decay in severely nitrified filtered sample, TAN loss and NO_2-N loss within 20 hrs were considered. In the first 20 hrs, TAN loss and NO_2-N loss was occurred due to chemical decay of chloramine rather than microbiological. From the experimental results, it was found that loss of TAN concentration was 0.062 ± 0.002 mg/L (Figure 7.5C) and the reduction of NO_2-N was 0.085 ± 0.002 mg/L (Figure 7.5C). According to the auto-decomposition and NO_2-N oxidation calculation (Section 7.2.2), 0.062 mg/L of TAN loss resulted in 0.47 ± 0.015 mg-Cl_2/L of chloramine decay and 0.085 mg/L of NO_2-N oxidation resulted in 0.43 ± 0.010 mg-Cl_2/L of chloramine decay. Therefore, combined chloramine decay due to auto-decomposition and NO_2-N oxidation was (0.47 + 0.43 =) 0.90 mg-Cl_2/L. From Figure 7.5A, it was observed that the chloramine decay within the initial 20 hrs was (2.0-1.10=) 0.90 mg-Cl_2/L. Therefore, the chloramine loss of severely nitrified filtered samples within initial 20 hrs could be defined by auto-decomposition and NO_2-N oxidation.

In case of copper inhibited severely nitrified bulk waters, the chloramine decay could be explained by auto-decomposition only. From Figure 7.3, It is noticed that chloramine loss within the initial 20 hrs was (2.0±0.03 to 1.23±0.03=) 0.77±0.06 mg-Cl_2/L. Meanwhile, TAN loss was 0.105±0.013 mg/L which resulted in 0.78±0.09 mg-Cl_2/L of chloramine decay. Therefore, chloramine loss in copper inhibited samples could be defined by auto-decomposition.

7.3.6 Assessment of Copper Effects in Severely Nitrified Bulk Waters

By filtration, it was possible to remove microbial agents including nitrifying bacteria and particulates that might accelerate chloramine decay from unprocessed sample whereas, the microbes were inhibited/inactivated but particulates were present in

copper inhibited samples. From the experimental results, it was observed that the trend of chloramine decay profiles of filtered and copper added filtered samples were same for a particular pH (8.0) condition (Figure 7.5 A). The decay rate coefficients between filtered and copper added filtered samples were same while considering the experimental error. Jun et al., (2009a) did not find any impact of copper at pH 8, when they used very high copper and chloramine concentrations. The results of the experiment reported in this study also have found the same conclusion. Similar behaviours of filtered and copper inhibited samples indicate that presence of particulates didn't show any impact on chloramine decay and nutrient profiles (Figure 7.4 & 7.5). Jun et al., (2009a) also found that decay rate in copper added sample increased at lower pH and decreased at higher pH but remained unchanged when pH was 8.0. But in severely nitrified case, the findings were different. No additional chloramine decay was found due to addition of copper in severely nitrified bulk water. Furthermore, in Section 7.3.4, it was reported that copper addition could not accelerate chloramine decay at different pH conditions. The probable cause could be the SMPs present in the severely nitrified bulk waters which combined with heavy metal to form complexes and hence prevented it from catalysing the reaction or the catalytic reaction of copper (Cu^{2+}) was dampened by the presence of NOM. In case of Jun et al., (2009a), they used MilliQ or pure water which can contain only inorganic Cu compounds (Cu^{2+} ion and other compounds). Therefore, these results indicated the role of SMP or NOM in chelating the copper and thus dampening the catalytic acceleration by Cu^{2+}.

7.4 Conclusions

The study investigated the chemical effects of copper for controlling chloramine decay as well as nitrification in chloraminated drinking water distribution systems. Experiments were performed on raw and filtered samples collected from severely nitrified bulk waters and chloramine residual as well as nitrogenous compounds were monitored periodically. The experimental results showed the followings:

- Copper effectively inhibited nitrification when 0.25 mg-Cu/L was present in re-chloraminated (2 mg-Cl_2/L) severely nitrifying sample.

- Effective copper inhibition of nitrification did not significantly alter the chloramine decay profile and hence it proves that nitrification process is not the major mechanism accelerating chloramines decay as traditionally believed.
- Copper addition did not alter the chloramine decay when only dissolved compounds were present in severely nitrified bulk waters. The phenomenon remained the same when pH was altered.
- Within the pH range (7.5 to 8.5), chloramine decay rate in severely nitrified samples increased with pH drop.
- Copper has no effect on nutrients profiles of severely nitrified bulk waters when only dissolved compounds were present.
- Attempt to control nitrification in severely nitrified sample with rechlorination will not improve the chloramine decay although pH increase could help in improving the chloramine residual stability.

CHAPTER 8

EFFECTS OF COPPER IN CONTROLLING CHLORAMINE DECAY IN SEVERELY NITRIFIED BULK WATERS (SEMI-CONTINUOUS FLOW)

Abstract

Controlling nitrification is the first preference for maintaining chloramine residual in the distribution system. Traditionally it is believed that nitrification, greatly accelerates chloramine decay, making it a less desirable disinfectant. Traditional nitrification controlling measures are not effective for long-term. Water Corporation of Western Australia has attempted to overcome this issue by dosing copper as an inhibitor. The study evaluated the inhibitory effects of copper in bulk waters undergoing severely nitrified conditions (the worst condition with nitrification and highly accelerated chloramine decay) in a pilot-scale reactor operating in a semi-continuous mode. Various concentrations of copper were applied in the reactor after establishment of severe nitrification (chloramine residual reduced to zero and ammonia was converted to nitrate). Chloramine and copper concentrations were gradually increased from 0.85 to 2.00 mg-Cl_2/L and 0.25 to 1.00 mg-Cu/L, respectively. Nitrification activity was significantly reduced from the beginning when copper (0.25 mg-Cu/L) and chloramine (0.85 mg-Cl_2/L) were dosed together. There was no residual improvement when copper was dosed up to 0.60 mg-Cu/L or even when chloramine concentration in the feed was increased from 0.85 to 2.00 mg-Cl_2/L. Minimal residual improvement was noticed at copper concentration of 0.80 mg-Cu/L with 2.0 mg-Cl_2/L chloramine concentration. Higher dose of copper was required due to presence of soluble microbial products (SMPs), possibly due to reduction in effective copper concentration as copper can form Cu-SMP complexes. Copper at higher concentration (1.00 mg-Cu/L) in combination with 2.00 mg-Cl_2/L chloramine was effective in proper residual management by inhibiting nitrification. This condition is very hard to obtain and hence if water has already undergone severe nitrification conditions, copper dosing is not the effective applicable control strategy.

8.1 Introduction

Chloramine is often used as a secondary disinfectant instead of chlorine to maintain a longer lasting residual and/or a reduction in the formation of chlorinated disinfectant by-products in chloraminated distribution systems (Brodtmann et al., 1979; Cotruvo, 1981). Despite many advantages, chloramine decay accelerated once the systems experienced nitrification. Nitrification is a two-step microbial process, in which ammonia-oxidizing bacteria (AOB) initially convert ammonia to nitrite and then nitrite-oxidizing bacteria (NOB) convert nitrite to nitrate. Nitrification, occuring in the distribution systems, can be categorized into three different conditions (Sathsivan et al., 2008). The first condition is known as mildly nitrifying condition wherein chloramine decay is very slow and steady, with lower nitrite levels (less than 0.01 mg-N/L) and mild ammonia loss. The second condition is referred to as severe nitrification, where rapid chloramine decay with excessive nitrite production (more than 0.1 mg-N/L) and rapid ammonia drop are observed. Condition occurring in between these two or the transition period is referred to as the onset of nitrification.

Controlling nitrification is the pre-requisite for maintaining chloramine residual in the distribution system, but it is very hard to retrieve the residual once the nitrification episode is underway. In the literature, various methods are suggested for controlling nitrification. The traditional approaches for controlling nitrification are: breakpoint chlorination (Wolfe et al., 1988; Odell et al., 1996), maintaining high disinfectant residual (Skadsen, 1993; Harrington et al., 2002), optimization of the chlorine-to-ammonia ratio (Wolfe et al., 1988; Odell et al., 1996), removal of natural organic matters (Odell et al., 1996), distribution system flushing (Odell et al., 1996; Sathasivan et al., 2010), decrease of distribution system retention time (Odell et al., 1996; Harrington et al., 2002) adjusting the pH of severely nitrified water to a higher pH (pH 8.5) before re-chloramination (Sarker and Sathasivan, 2011b).

Out of these methods, breakpoint chlorination is usually effective. However, Odell et al., (1996) reported that breakpoint chlorination led to a rise in total coliform counts which is likely to be due to increased biofilm detachment caused by the aggressive reactivity of free chlorine. Increasing monochloramine concentration was found to be ineffective in overcoming nitrification once it is already established (Skadsen, 1993).

Flushing with fresh (non-nitrifying) water is sometimes used as the control option of ongoing nitrification stages but it is a temporary approach (Skadsen, 1993). In a full-scale system, McGuire et al., (2006) reported that chlorite was effective in controlling nitrification but it is not preferred option as chlorite is a regulated compound. Sathasivan et al., (2010) reported the reservoir management strategy by F_m method to determine when the reservoir was at risk in regards of nitrification. Identifying the time to dilute, the reservoir was serially diluted with freshly chloraminated water.. This method requires careful manipulation of reservoir. The complications of traditional approaches require a simple but an effective solution that is also safe in health perspectives.

In this context, addition of suitable inhibitor(s) in the distribution system can be an effective approach for controlling nitrification. The additions of metals such as silver, copper etc. exert a significant inhibitory impact on the nitrifying bacteria by blocking their enzymatic function (Martin and Richard, 1982). It has already been reported that nitrification can be controlled by silver addition (Fisher et al., 2009 and Sathasivan et al., 2005) and by silver nanoparticles (Choi et al., 2008). Due to toxicity concern, silver may not be adopted as the preferred inhibitor in the distribution system. However, copper can be a safe option as copper is one of the essential nutrient for human body functions with recommended daily dose level. Hence, copper can be used as a nitrification inhibitor in the distribution systems. In the literature, it has been already reported that copper could inhibit ammonia oxidizing bacterial activity (Laszlo, 2008; Zhang et al., 2009; Loveless and Painter, 1968). Further, (Zhang and Edwards, 2005) reported that it could be achieved at concentrations greater than 0.1 mg-Cu/L. Laszlo, (2008) reported that Water Corporation, Western Australia (WCWA) had successfully used copper to inhibit nitrification by dosing copper sulphate into a reservoir and specific pipe lines in Goldfield and Agriculture Water Supply System (G&AWSS).

Furthermore, in the pilot-scale system, experimental results obtained from batch reactor revealed that copper had significantly inhibited nitrifying bacterial activity but little impact on chloramine decay in the severely nitrified chloraminated bulk water. These results were presented in Chapter 6. The effectiveness of copper inhibition may be different under different reactor conditions, and hence they are

likely to host different microbial community. In order to understand how copper controls nitrification and ultimately how it affects chloramine decay, progressively increasing concentration of copper (copper sulphate) was added to a reactor containing severely nitrified bulk waters operated in semi-continuous flow mode. The purpose of this study was to examine the inhibitory effects of copper on severely nitrified bulk waters – water undergoing the worst possible condition - under semi-continuous flow condition.

8.2 Materials and Methods

The detailed description of stock chemical solutions preparation; water sample collection, preparation and storage; preparation of sample bottles and glasswares, analytical procedures of F_m value determination were presented in Chapter 3.

8.2.1 Design of Semi-continuous Flow System

8.2.1.1 Feed Solution Preparation

Feed solution was prepared by nutrients and chloramine in Reverse Osmosis (RO) (Ibis mini model) treated water. DOC and conductivity were <0.1 mg/L and <1 μS/cm, respectively in the RO treated water. The added chemicals and the quantity were: $MgSO_4.7H_2O$, 40 mg/L; KH_2PO_4, 100 mg/L; $CaCl_2$, 15 mg/L; total chlorine (TCl), 0.85 mg-Cl_2/L; total ammoniacal nitrogen (TAN), 0.60 mg-N/L and 1.0 mL/L for the trace elements solution. The trace elements solution was prepared as detailed in Lipponen *et al.,* (2002) except Na_2EDTA (Titriplex III). The chemicals composition were; $FeCl_2.4H_2O$,1988 mg/L; $MnCl_2.4H_2O$, 99 mg/L; $NiCl_2.6H2O$, 24 mg/L; $CoCl_2.6H_2O$,24 mg/L; $CuCl_2.2H_2O$, 17 mg/L; $ZnCl_2$, 68 mg/L; $Na_2MoO_4.2H_2O$, 24 mg/L; H_3BO_3, 62 mg/L. Feed water pH was adjusted to 8.0±0.1 using 1M KH_2PO_4 and 1M NaOH.

8.2.1.2 Operation of Reactor

A container (5L) fitted with a lid made of High Density Poly-ethylene was used as a reactor. It was filled up with feed solution as detailed in Section 8.2.1.1 and TCl, 0.25 mg-Cl_2/L; TAN, 0.18 mg/L and NOx-N, 0.2 mg-N/L were adjusted on the first day of operation. To expedite the microbial activities and to obtain similar microbial inoculums, 100 mL seed water collected from the severely nitrified reactor (fourth reactor (R-4) of pilot-scale system as described in Chapter 3) had been placed as seed microorganisms in the reactor. The chemical compositions of severely nitrified water were: TCl, 0.25 mg/L; TAN, 0.18 mg/L; NOx-N, 0.37 mg/L; DOC, 2.85 mg/L and pH, 7.80. The reactor was placed in a covered water bath maintaining a constant water temperature (25.0± 1.0 ^oC).

From the second day of operation, feed solution as detailed in Section 8.2.1.1 was used. The reactor was operated in a semi-continuous mode; everyday 1.5 L water was drained out and then the same volume of feed solution was fed manually to obtain 3.3 days of retention time. Before draining out and after feeding, the reactor contents were thoroughly mixed by shaking and placed in dark water bath. Nitrification surrogate parameters (TCl, TAN and NOx-N) were continuously monitored. Experiments were conducted when severe nitrification condition was set in the reactor. During copper dosing in the reactor, samples were collected at different time to perform chloramine decay test.

8.2.2 Experimental Design

Three sets of experiments were conducted on the feed solution and bulk waters collected from the reactor. Reactor water and feed water represent severely nitrified and non-nitrified waters, respectively.

The first experiment set was designed to prove the role of dissolved compounds, beyond known parameters (TCl, TCl to TAN ratio, NO_2-N, pH and temperature) that affect chloramine decay, in the RO treated water fed reactor. Samples were collected from the reactor (at the 17^{th} operation day) and feed solution and filtered through 0.2 µm polycarbonate filter paper. The samples were known as filtered reactor water and filtered feed water. The known parameters that affect chloramine decay were

adjusted to similar levels in both samples. The adjusted chemical parameters were TCl= 2.0 mg-Cl_2/L; TAN = 0.45 mg/L; NO_2-N= 0.21 mg/L; and pH = 8.0 ± 0.1. All samples were kept in a water bath (20^oC). TCl, TAN, NO_2-N and NO_3-N were monitored regularly and the chloramine decay coefficients were determined using exponential regression as defined in Equation 3.1.

The second experiment set was designed to confirm the responsible dissolved compounds in the reactor was SMPs. The bulk waters were collected from the reactor and feed solution. The collected samples were filtered through 0.2 µm polycarbonate filter paper. The filtered reactor water was didived into two categories. First one remained unchanged and 0.05 mg-Ag/L silver was added in the other portion. Therefore, the experiment was conducted on three sets of samples; filtered feed water, filtered reactor water and filterted inhibited reactor water with 0.05 mg-Ag/L silver. Theses three samples were reffered as 'FFW', FRW' and 'FRW+Ag'. The adjusted chemical parameters for the samples were TCl= 2.0 mg-Cl_2/L; TAN = 0.45 mg/L; NO_2-N= 0.21 mg/L; and pH = 8.0 ± 0.1. All samples were kept in a water bath (20^oC). TCl readings were monitored regularly.

The third set of experiment was designed to check the inhibitory effects of copper on severely nitrified water when dosed directly in the reactor. In order to understand how copper and chloramine collectively inhibits AOB activity and how they eventually affects chloramine decay, copper was dosed daily during the feeding time in severely nitrified bulk waters in the reactor. The copper addition was done when the reactor condition was in stable condition i.e. there was a stable level of TCl and NOx-N. The copper dose was started at 98th day of reactor operation. At the start of copper dosing, TCl and NOx-N concentrations of the reactor were 0.00 mg-Cl_2/L and 0.419 mg/L. The concentrations of chloramine and copper were increased gradually following the combination mentioned in Table 8.1. TCl, TAN and NOx-N levels were monitored regularly for the samples collected from the reactor.

Table 8.1: Various Phases of Copper Dosing in Different Chloramine Concentrations

Copper dosing time (days)	Chloramine (mg-Cl_2/L) in the feed water		Copper (mg-Cu/L)
	TCl (mg-Cl_2/L)	TAN (mg/L)	
0 to 24	0.85	0.60	0.25
25 to 38	1.50	0.60	0.25
39 to 45	2.00	0.60	0.40
46 to 51	2.00	0.60	0.60
52 to 60	2.00	0.60	0.80
61 to 145	2.00	0.60	1.00

8.2.3 Microbiological Analysis

8.2.3.1 DNA Extraction

Bulk water volume of 1.0 L (after 60 days of operation) was collected from the reactor. The bulk water samples were filtered through a 0.22 μm filter paper (Polycarbonate membrane, Cat No. GTBP02500, Millipore, UK) to concentrate the biomass. Then DNA was extracted from the concentrated biomass using the Fast DNA® spin kit for soil (Cat. #6560-200, MP Biomedicals LLC, France) following the manufacturer's instructions. The extracted DNA was stored at -80 ^{o}C until further use. The DNA was electrophoresed on a 1.0 % (wt/vol) agarose gel to confirm whether DNA is extracted.

8.2.3.2 Quantitative Real-time PCR (qPCR)

The used primer pairs to quantitatively estimate 16S rRNA gene copy numbers of the microorganisms are listed in Table 8.2. The primers were selected based on the study of microbial community diversity in a pilot-scale chloraminated system carried out by Bal Krishna, (2012).

The optimum thermocycler conditions used for all primer pairs included an initial denaturation step at 95°C for 15 min followed by 50 cycles of denaturation at 95°C for 60 sec, annealing at 60°C for 60 sec and an elongation at 72°C for 45 sec. All qPCR reactions were carried out in an iQ5 real-time PCR detection system (Bio-Rad) using IQ SYBR green supermix (Bio-Rad) following manufacturers' instructions. Plasmids carrying the respective cloned genes used as standards for calibration of the assay are also given in Table 8.2. A negative control (1 to 5 base mismatches, Table 8.2) and negative control with no template DNA was also included in each qPCR run. All qPCRs were performed in triplicate. At the end of each qPCR assay, a single band of expected size was observed using agarose gel electrophoresis. Additionally, the specificity of each qPCR reaction was confirmed by comparing melting curve analysis of the sample and its respective reference clone-derived PCR product. Data analysis was carried out using IQ^{TM} software (version 5.2).

Table 8.2: Oligonucleotide Primers Used in This Study

Primers	Specificity	Sequence (5'-3')	Target genes	Base position	Application	Reference
27f	*Bacteria*	GAGTTTGATCCTGGCTCAG	16s RNA	9 to 27	PCR/qPCR	
EUB338r	*Bacteria*	GCTGCCTCCCGTAGGAGT	16s RNA	338-355	qPCR	
Acido1232f	*Candidatus*	ATTGCTGCTTTCTTCTCT	16s RNA	1232-1215	qPCR	
Acido1313r	*Solibacter* genus	TGGATTATCTACCTCTTAGTG	16s RNA	1313-1333	qPCR	
Methylo770f	*Methylobacterium*	GGTGTTCTTGCGAATATCT	16s RNA	770-751	qPCR	
Methylo844r	genus	GGACGCTTGAGTATGGTA	16s RNA	844-861	qPCR	
Sphingo1152f	*Sphingomonas*	GCTACAATGGCAACTACA	16s RNA	1152-1135	qPCR	
Sphingo1226r	genus/*Sphingopyxis* genus	AATCCGAACTGAGACAAC	16s RNA	1226-1243	qPCR	This study
Nsp20f	*Nitrosomonas*	CTTTACACATGCAAGTC	16s RNA	20-4	qPCR	
Nsp141r	genus	TATTAGCACATCTTTCGAT	16s RNA	141-159	qPCR	
Ntspa625f	*Nitrospira* genus	GGATACTTAATGCGTTAG	16s RNA	625-608	qPCR	
Ntspa722r		CAAACAGGATTAGATACC	16s RNA	722-739	qPCR	
Pseu614f	*Pseudomonas*	TGAGCTAGAGTACGGTAG	16s RNA	614-597	qPCR	
Pseu690r	genus	ACTGGTGTTCCTTCCTAT	16s RNA	690-707	qPCR	

8.2.4 Indicator of Nitrification Status

In partial nitrification cases, ammonia (NH_3-N) oxidation by AOB usually occurs in distribution systems and is of usual concern as the product (NO_2-N) is a reducing agent responsible for accelerating chloramine decay. Thus, NO_2-N is generally used as the indicator of partial nitrification status (Wolfe et al., 1988). However, change in NOx-N (summation of NO_2-N and NO_3-N) is the best indicator because, NO_2-N can be oxidized to NO_3-N by chloramine or NOB, but microbial oxidation of NH_3-N is the only mechanism that converts NH_3-N to NO_2-N in a chloraminated environment. Therefore, change in NOx-N level was the best indicator for AOB activity in chloraminated system.

8.2.5 Calculation of NOx-N Production Rate

When samples were in severely nitrified conditions, the NOx-N production rate was calculated by the following Equation;

NOx-N production rate = $[(\text{NOx-N})_{\text{final}} - (\text{NOx-N})_{\text{initial}}]/ \text{HRT}$ **Equation 8.1**

where, $(\text{NOx-N})_{\text{initial}}$ = NOx-N concentration in the reactor just after feeding (mg/L)

$(\text{NOx-N})_{\text{final}}$ = NOx-N concentration in the reactor before feeding after HRT (mg/L)

HRT = Hydraulic retention time (days)

8.2.6 Evaluation of the Inhibitory Effects of Copper

Two criteria were used to check the effectiveness of copper inhibition in severely nitrified bulk waters.

The first criterion was to monitor the concentrations of TCl, TAN and NOx-N in copper dosed reactors. Increasing trend of TCl and TAN levels, and decreasing trend of NOx-N level indicated the effectiveness of copper inhibition.

The second criterion was to compare the calculated decay rate coefficients and F_m at different time during copper dosing. Decreasing trend of decay rate coefficients and F_m could prove copper effectiveness positively.

8.3 Results and Discussion

8.3.1 Initial Operational Results of the Reactor

The severe nitrification occurred in the reactor within a short period of time. TCl residual was reduced to 0 mg-Cl_2/L within a day and remained the same except at the feeding time. At the feeding time, TCl increased to 0.26 mg-Cl_2/L (=0.85*1.5/5), but it quickly reached 0 mg-Cl_2/L. The profiles of TAN and NOx-N during the operational period are shown in Figure 8.1. From Figure 8.1, it was seen that TAN level gradually increased whilst the NOx-N level continuously decreased in the initial 5 days due to minimal microbial activities. After 5 days, nitrification started; resulting in progressive increase of NOx-N and decrease of TAN levels.

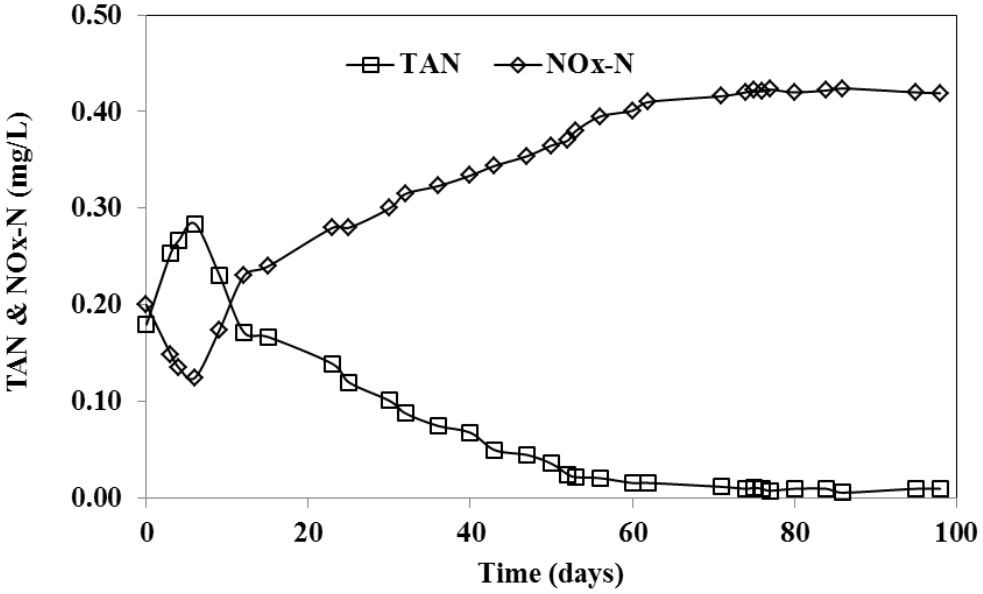

Figure 8.1: TAN and NOx-N profiles in the reactor during the operational period

Nitrification surrogate parameters indicated that severe nitrification had established in the reactor, especially after 10 days. In complete nitrification, TAN fully converted to NO_3-N. It was observed that the complete nitrification occurred in 60 days after which NOx-N production was constant (Figure 8.1). Stable NOx-N level indicated the production of NOx-N. Otherwise NOx-N level would decrease

continuously as the reactor was diluted with 1.5 L feed water (NOx-N=0) daily during the feeding time. The NOx-N production rate was calculated by monitoring the consecutive two NOx-N readings by using Equation 8.1, when the NOx-N readings were stable (after 60 days). During that period, the average NOx-N concentration just after feeding was 0.293 mg/L and after one day (HRT) just before feeding was 0.420 mg/L. Therefore, the average NOx-N production rate in the reactor was [(0.420-0.293)/3.33=] 0.038 mg/L/day.

8.3.2 Microbiological Analysis of the Reactor Contents at the Start of Copper Dosing

Microbial community in the reactor before starting copper dosing was quantified by means of molecular tool, qPCR by another student working in the same project the results are presented here for completeness. Seven set of primers were used to quantify the genus *Methylobacterium* spp., *Pseudomonas* spp., *Sphingomonas* spp. *Candidatus Solibacter* spp, *Nitrosomonas* spp. and *Nitrospira* spp. The samples were collected at 60th day of reactor operation and were used for microbial quantification. The *Sphingomonas* spp., were found to be the highest number in the reactor. The abundance of this genus in the chloraminated environments has been previously reported (Noguera *et al.,* 2009; Regan et al., 2002, 2003; William et al., 2004) and according to Noguera et al., (2009) this genus specifically dominated in chloraminated systems (bench-, pilot- and full-scale) but not in the non-chloraminated systems. Similar to the chemical results the genera *Nitrosomonas* spp. (2% of total bacteria) and *Nitrospira* spp., (4% of total bacteria) belonging to AOBs and NOBs, respectively were detected in the reactor. However, the reactor condition was severely nitrified, relative abundance of AOBs and NOBs were very less (6% of total bacteria). It could be due to the primers which we used in this study may not target all AOBs and NOBs.

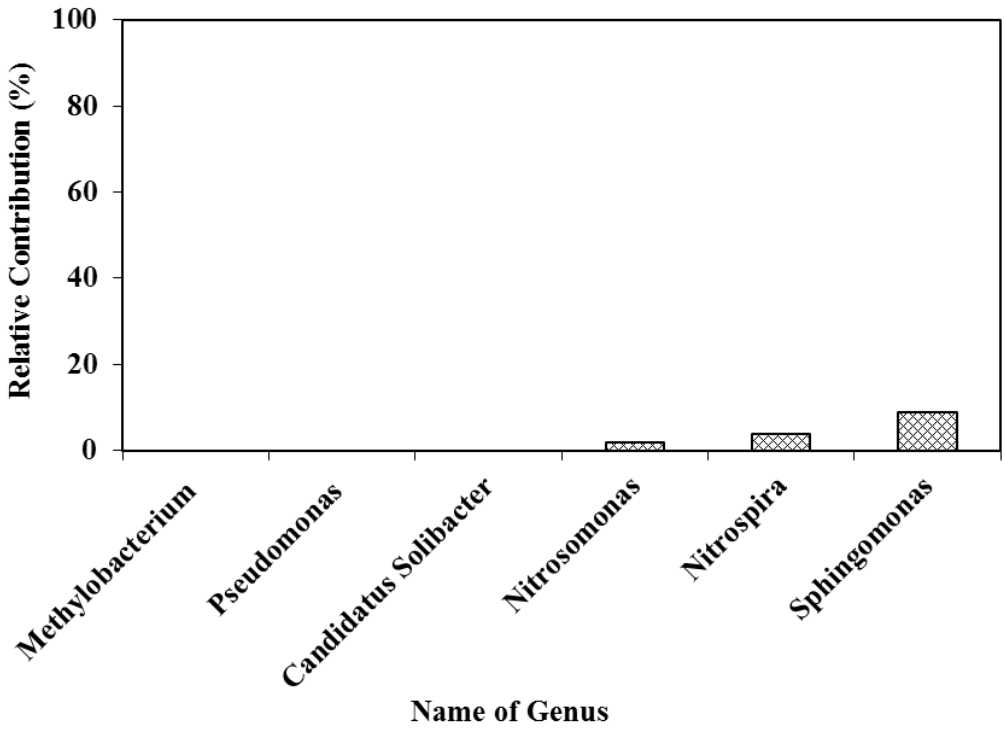

Figure 8.2: Relative quantification of the six different bacterial Genus

8.3.3 Decay Characteristics and Fm values of the Reactor during Operational Period

Table 8.3 shows the total decay coefficients (k_t), chemical decay coefficients (k_c) and microbial decay coefficients (k_m), conducted on the 11th, 25th and 98th days after the reactor operation. It was found that k_t increased with nitrification (TAN reduction and NOx-N increment). The k_c had also increased between the 11th and 25th days, but there was a reduction from the 25th to 98th days (Table 8.3). The k_m value was constant within the 25th day and then started to increase. Initially, the k_c value was increased due to a higher NO_2-N concentration. The k_c value would gradually decrease when there would be more NOB than AOB, e.g., all NO_2-N converted to NO_3-N (after 60 days). Initially, there were both TAN conversions to NO_2-N by AOB and NO_2-N conversion to NO_3-N by NOB, chemical decay of chloramine was more pronounced than microbial decay (within 60 days). The probable reasons were pH and NO_2-N level. Bal Krishna and Sathasivan, (2010) reported that NO_2-N and

pH change was not sufficient to explain the chemical decay of chloramine when the reactor or systems were fed with surface water (DOC of 2.5 to 3.0 mg/L). In this experiment, however, DOC free water was used. Therefore, further experiments were needed to define the significant occurrence of chemical decay in the reactor.

Table 8.3: Decay Rate Coefficients and F_m Values during the Reactor Operation

Operating days	NO_2-N (mg/L)	NOx-N (mg/L)	k_t (hr^{-1})	k_c (hr^{-1})*	k_m (hr^{-1})	F_m
11	0.15±0.002	0.230±0.005	0.053±0.007	0.023±0.003	0.030±0.01	1.30
25	0.25±0.004	0.280±0.006	0.068±0.008	0.038±0.004	0.030±0.01	0.79
98	0.012±0.002	0.419±0.008	0.060±0.011	0.017±0.006	0.043±0.008	2.63

8.3.4 Investigation the Factors Responsible for Chemical Decay Rate in the Reactor

The reason for the higher chemical decay rate in the reactor content could be understood if the decay rates of feed solution and reactor water were compared after removing microbes and adjusting the parameters that are known to be modified by microbial activities and affect chloramine decay. Figure 8.3 A & B illustrates the chloramine decay and nitrogenous species profiles in the filtered reactor water and filtered feed waters (First set of experiment). Accelerated chloramine loss was observed in the reactor sample compared with the feed water. The k_c in the reactor sample was 0.029±0.003 hr^{-1}, but only 0.005±0.001 hr^{-1} in the feed water. According to the model proposed by Vikesland et al., (2001), after adjusting similar chemical parameters (TCl, TCl to TAN ratio, NO_2-N, pH and temperature), the k_c should have been the same in both filtered reactor water and filtered feed water.

Accelerated chemical loss of TCl in filtered reactor water was associated with an accelerated TAN and NO_2-N loss (Figure 8.3A). Within the initial 22 hrs, TAN concentration (0.45±0.007 mg/L) dropped to 0.34±0.005 and 0.43±0.006 mg/L in

filtered reactor water and filtered feed waters, respectively. Afterwards, TAN continuously decreased in the filtered reactor water, whereas TAN loss was very slow in filtered feed water. Similarly, within the 22 hours, NO_2-N residuals dropped from 0.21±0.003 mg/L to 0.099±0.002 and 0.161±0.002 mg-/L in filtered reactor water and filtered feed waters, respectively.

From Figure 8.3 A & B, it is noticed that NO_2-N loss could be defined by NO_3-N gain in both filtered reactor water and filtered feed water. For example, within the first 22 hours, NO_2-N loss in filtered reactor water and filtered feed water were 0.111±0.004 and 0.04±0.004 mg/L, and NO_3-N gain was 0.106±0.008 and 0.045±0.006 mg/L, respectively. After TCl had dropped to 0.0 mg/L, NO_2-N and NO_3-N profiles remained stable (Figure 8.3A & B); demonstrating conversion of NO_2-N to NO_3-N was present only in the presence of TCl. Therefore, NO_2-N loss could be explained by gain in NO_3-N, showing that the most probable pathway of NO_2-N loss was oxidation of NO_2-N to NO_3-N in the presence of TCl. Moreover, total inorganic nitrogen (TIN; summation of TAN and NOx-N) and TAN losses were 0.153±0.018 and 0.147±0.012 mg/L, respectively in filtered reactor water at the end of experiments (after 200 hrs of incubation). Similar TIN and TAN losses were found in the filtered reactor water, therefore, demonstrated the other possible decay mechanism was auto-decomposition.

In summary, much faster TCl, TAN and NO_2-N loss were observed in the filtered reactor water than filtered feed water, after adjusting with similar conditions. This indicated an unknown dissolved compound(s) were present in the filtered reactor water, that was similar to what Bal Krishna and Sathasivan, (2010) reported. In DOC containing bulk water, they reported that soluble compound(s) responsible for acceleration of chloramine decay were modified NOM and/or SMPs. These experimental results conducted with DOC free bulk water. Therefore, the absence of DOC confirmed that the accelerated decay was due to the presence of SMPs.

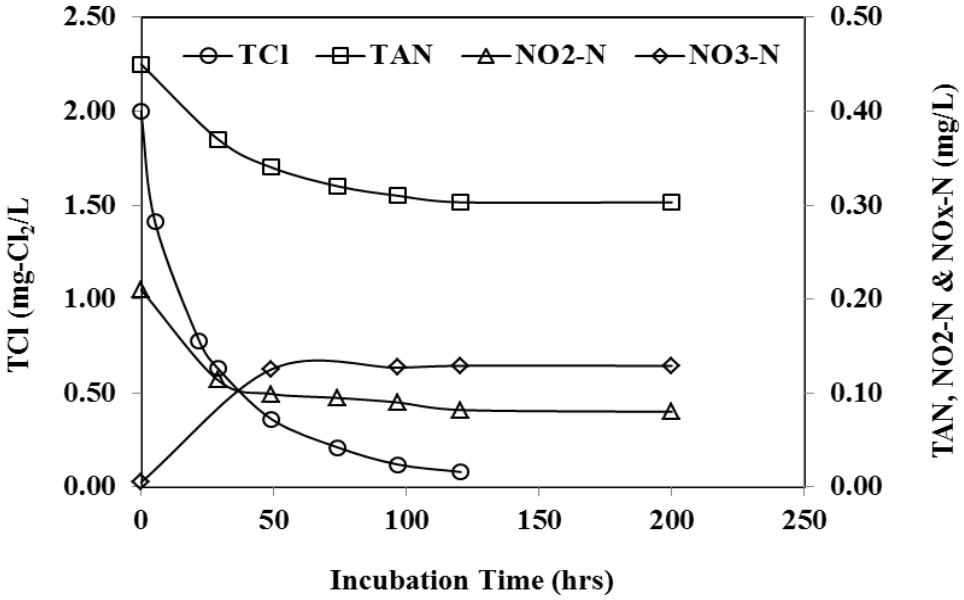

Figure 8.3A: TCl, TAN, NO_2-N and NO_3-N profiles in the filtered bulk water samples of Reactor

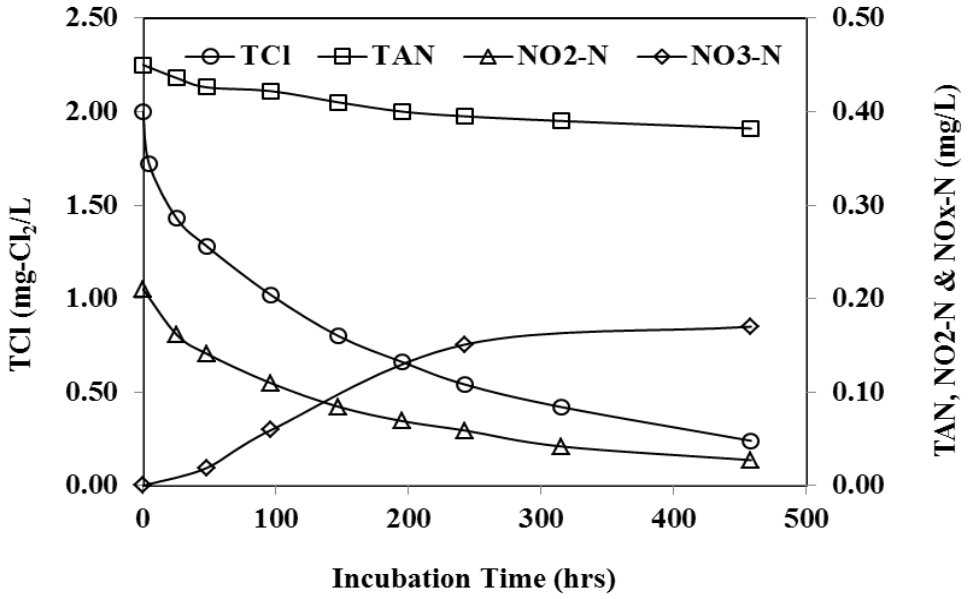

Figure 8.3B: TCl, TAN, NO_2-N and NO_3-N profiles in filtered Feed water Reactor

8.3.5 Confirmation of Catalytic Reaction by SMPs Present in Reactor by Silver Inhibition

SMPs are known as a pool of complex organic matter such as proteins, extracellular enzyme, polysaccharides, humic substances, organic acids, amino acids etc (Tao et al., 2010). In one of our study (Bal Krishna, 2012), we showed that silver can stop the reaction by SMPs. Hence, the conduct of test with addition of silver can prove the existence of SMPs. Therefore, a comparative study was done for the samples of 'FRW' 'FFW' and 'FRW+Ag' (Second set of experiment). From Figure 8.4, it is observed that the chloramine decay profiles of FFW and FRW+Ag followed the same pattern demonstrating the presence of SMPs. Qilin et al., (2008) reported that silver normally combines with active sites of enzymes/proteins, blocking the reaction with other compounds. Figure 8.4 demonstrates that the behavior of reactor water was similar to the feed water, after addition of silver (0.05 mg-Ag/L). The inhibition of silver completely stopped the catalytic reaction of SMPs in the reactor. The experiment proved the existence of SMPs in severely nitrified bulk waters.

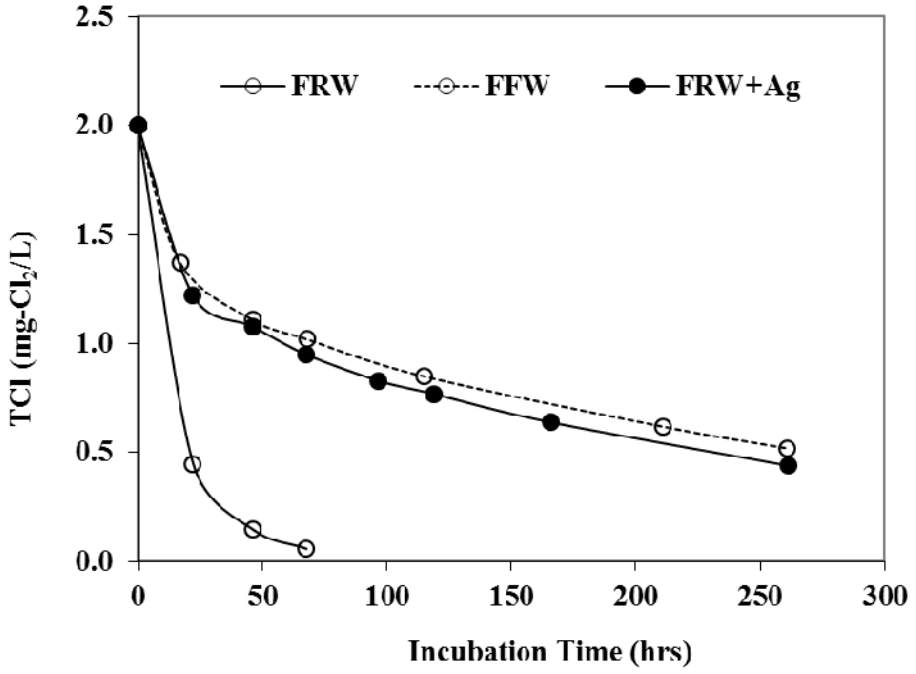

Figure 8.4: TCl profiles of filtered reactor water, filtered feed water and silver inhibited filtered reactor water.

8.3.5 Behavior of Severely Nitrified Bulk Waters after Copper Dosing

8.3.5.1 Copper effects on chloramine decay and nitrogenous species profiles

The profiles of TCl, TAN and NOx-N of severely nitrified bulk waters due to consecutive daily copper doses and chloramine are shown in Figure 8.5A & B. In fact, the Figures 8.5A & B were representing the results of the same experiment, but separated to show the results of different time scale much more elaborately. The Figure 8.5 A & B showed the profiles of TCl, TAN and NOx-N for 0 to 60 days and 60 to 145 days, respectively. From Figure 8.5A, it was noticed that NOx-N gradually declined from the start of copper dosing. On the other hand, there was no improvement of chloramine residual by dosing copper up to 0.60 mg-Cu/L, even the TCl concentrations were increased from 0.85 to 2.0 mg-Cl_2. Simultaneously, there was an increasing trend of TAN concentration and it continued when the copper dose was increased to 0.80 mg-Cu/L but without any improvement of TCl residual. Thus, it could be said that copper inhibition was effective in controlling nitrification but not effective to improve the chloramine residual when copper was dosed up to 0.60 mg-Cu/L. A slight increase of TAN at copper dose of 0.80 mg-Cu/L drew a possible indication for improving chloramine residual and controlling nitrification by copper inhibition at a higher dose. Therefore, copper concentration was increased to 1.0 mg-Cu/L and the profiles of TCl and nitrogenous compounds are presented in Figure 8.5B. From the Figure 8.5B, it was noticed that the profiles of TCl and TAN increased whereas the profile of NOx-N decreased gradually to zero after 140 days. Therefore, from the profiles of TCl, TAN and NOx-N, it could be said that copper was effective in controlling chloramine decay by inhibiting nitrifying bacteria. To examine how copper could control chloramine decay, copper impact on chloramine residual stability, decay characteristics and F_m values were considered and discussed in the following section.

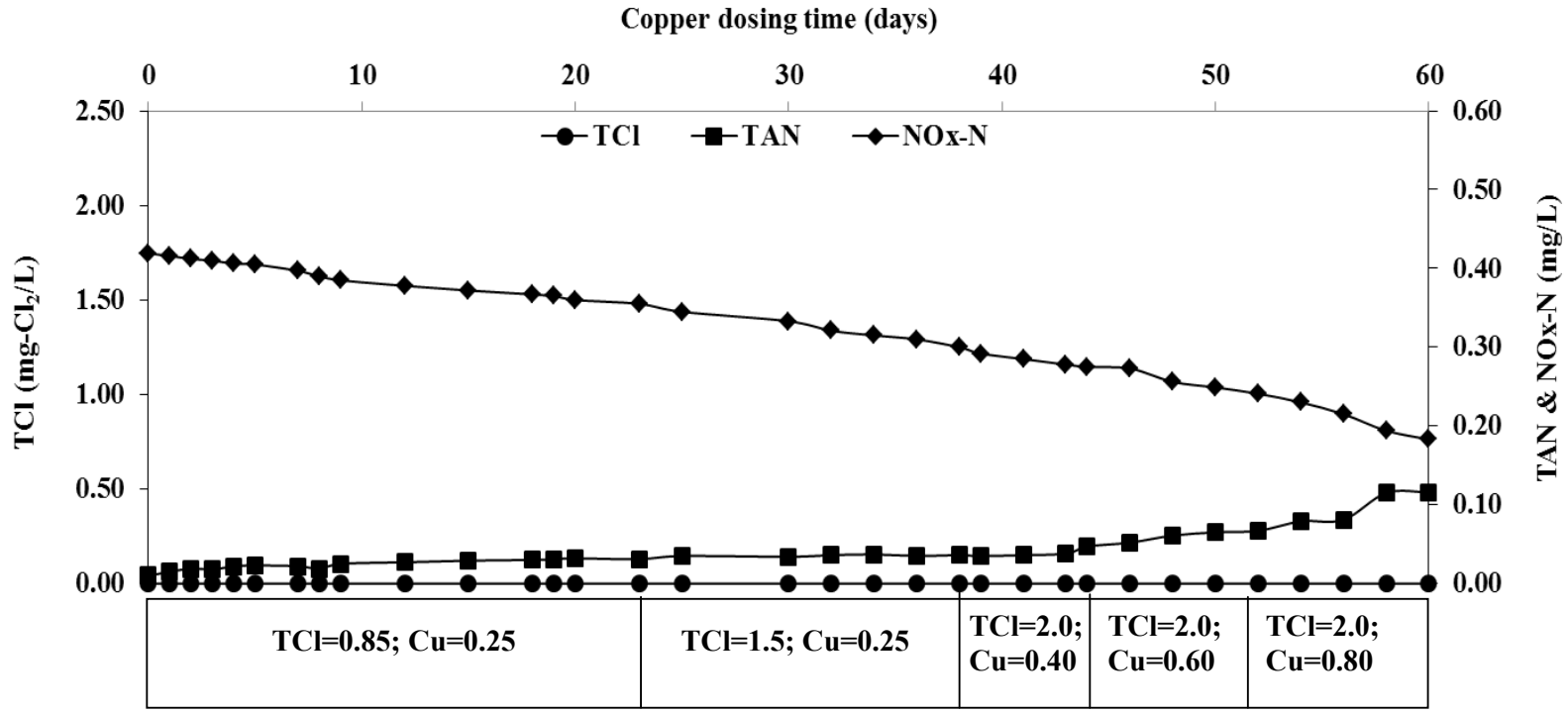

Figure 8.5(A): TCl and nitrogenous profiles of severely nitrified bulk waters inhibited at different copper concentrations. Unit of TCl and copper were in mg/L and mg-Cu/L.

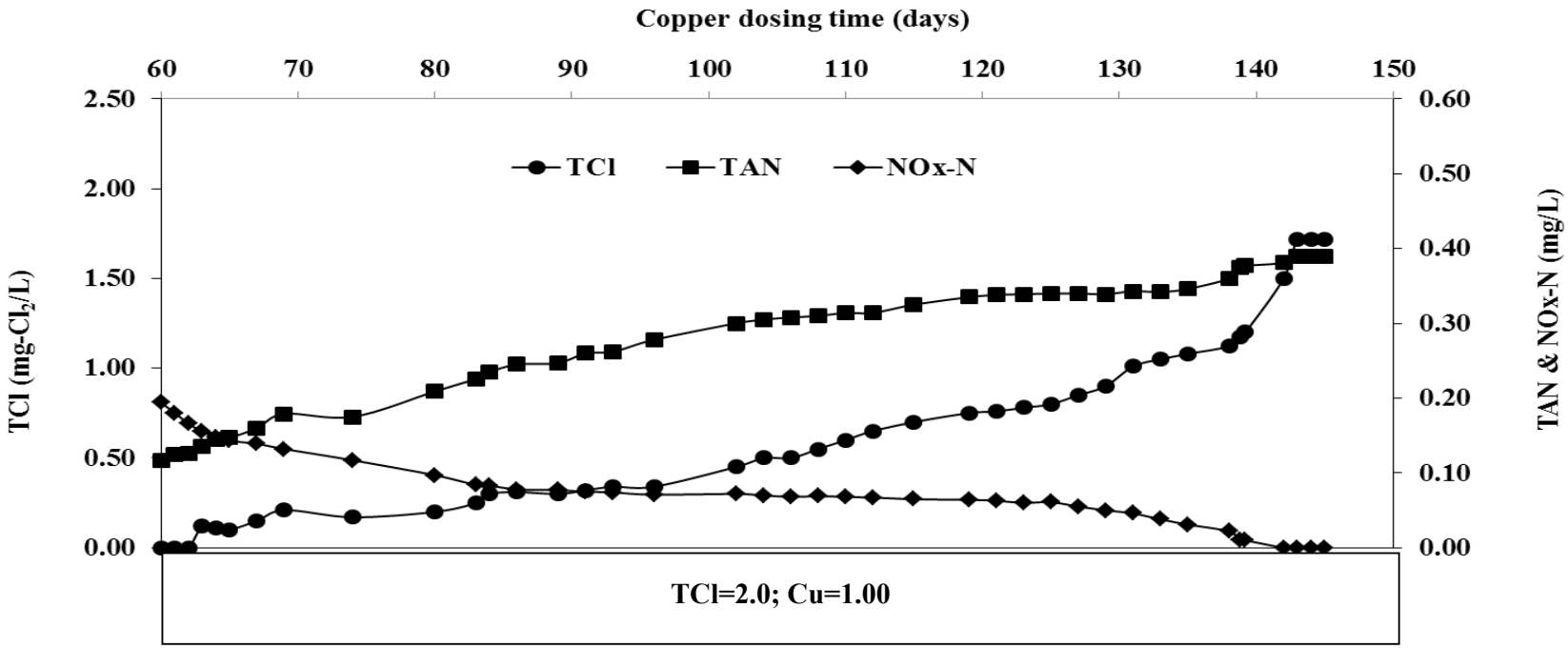

Figure 8.5(B): TCl and nitrogenous profiles of severely nitrified bulk waters inhibited at different copper concentrations. Unit of TCl and copper were in mg/L and mg-Cu/L.

8.3.5.2 Copper Effects on Chloramine Residual Stability

These experiments were conducted by collecting the bulk water samples from the reactor at different times during copper dosing in the reactor as like F_m test. The decay tests were performed by adjusting the initial TCl concentrations maintaining TCl:TAN of 4.1:1. The adjusted initial TCl concentrations for 0, 6, 35 and 135 days were 0.60, 0.70, 0.65 and 1.00 mg-Cl_2/L respectively. The ratio (TCl_{out}/TCl_{in}) of observed TCl (TCl_{out}) concentrations at different incubation times with respect to initial adjusted TCl (TCl_{in}) concentration for each experiment were calculated and presented in Figure 8.6. It was observed that there was no improvement of residual improvement due to copper dosing up to 6 days but a noticeable residual improvement was found after 45 days. This increment was continued until the end of the experiment. Due to copper dosing for 135 days, the ratio of TCl_{out}/TCl_{in} was one for 5 hrs followed by a very slower decreasing trend. However, it could be said that copper at a higher concentration with chloramine was effective to improve the chloramine residual. For more explanation, variation of chloramine decay rate coefficients and F_m values due to copper dosing were presented in the next section.

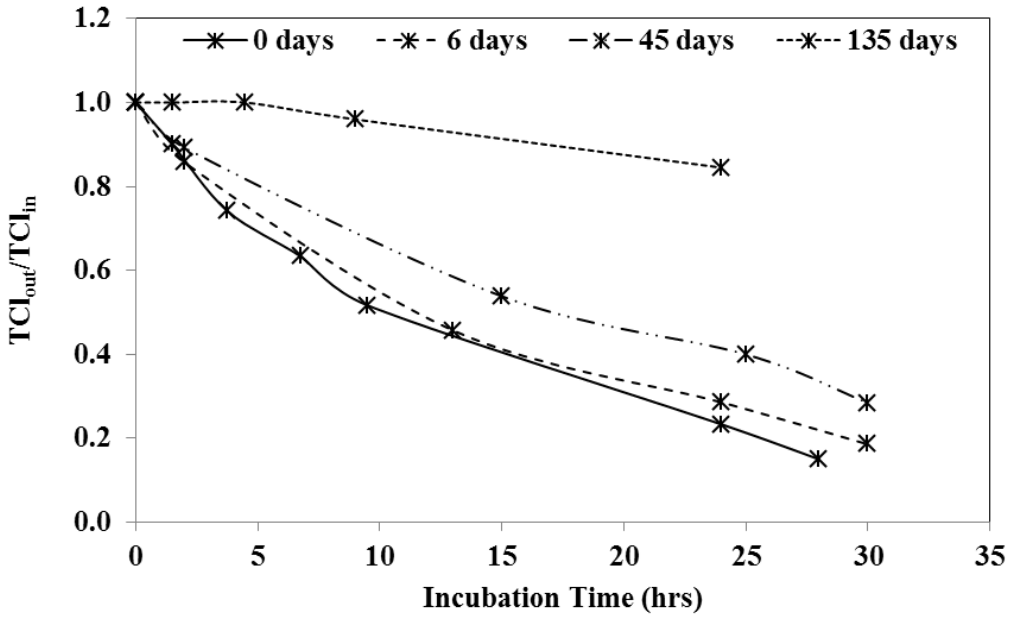

Figure 8.6: Ratio of TCl_{out}/TCl_{in} at different copper dosing periods

8.3.5.3 Copper Effects on Chloramine Decay Rates and Fm Values

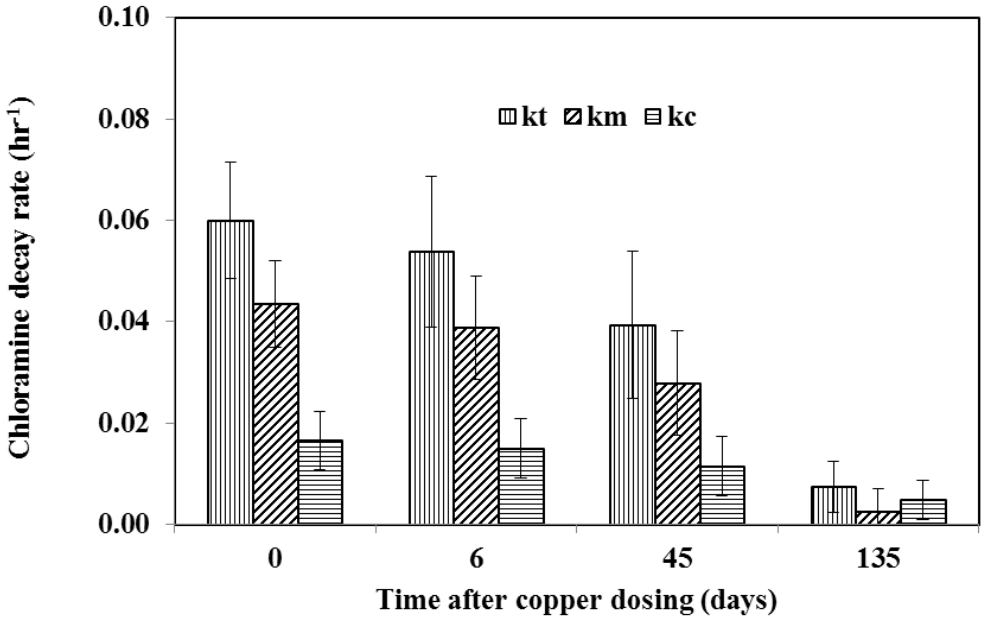

Figure 8.7A: Chloramine decay rate coefficients along the copper dosing time

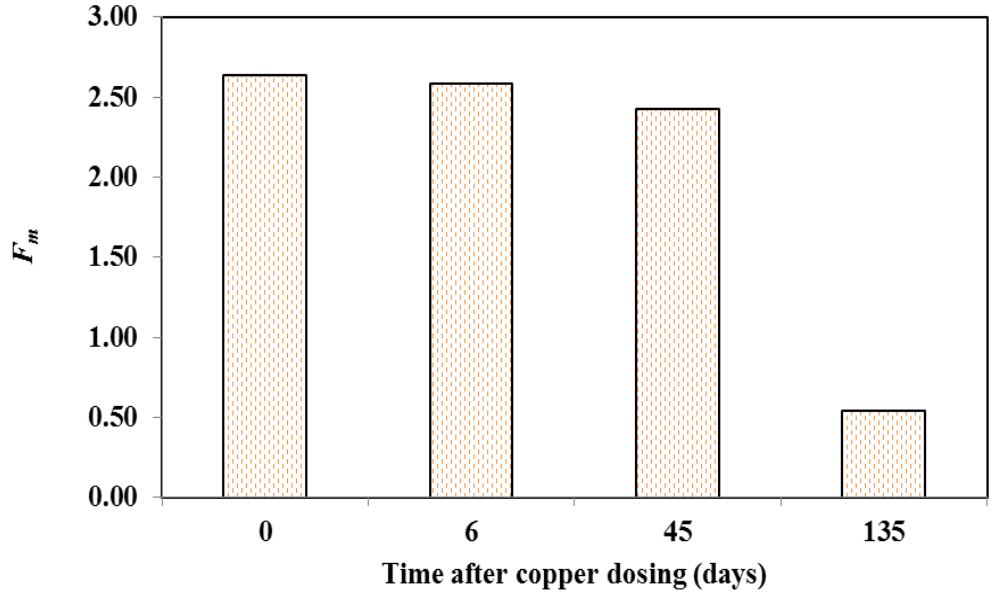

Figure 8.7B: Variation F_m values along the copper dosing time

For better understanding of inhibitory effects of copper on chloramine decay, chloramine decay tests were performed for samples collected from the reactor at different times during the copper dosing. Figure 8.7A shows the variations of k_t, k_m and k_c at different times after copper dosing in the reactor. It was noticed that the decay rate coefficients were reduced significantly with the increasing copper concentrations along with copper dosing time. At high copper concentration (1.0 mg-Cu/L), the decay rates especially microbial decay was significantly reduced. It was observed that k_m values decreased from 0.0435±0.0100 hr^{-1} to 0.0026±0.0100 hr^{-1} indicated that microbial activities were greatly inhibited by copper. On the other hand, k_c values were reduced initially and then remained same while considering experimental errors. The k_c value was 0.0048±0.0039 which is closer to 0.0013-0.0016 hr^{-1}, which was observed in distribution systems, indicative of the absence of SMPs.

The variation of F_m values at different times during copper dosing is shown in Figure 8.7B. It was observed that initially there was a little reduction of F_m value when the copper concentration was low. At higher doses of copper, the F_m value was reduced dramatically. Thus, copper at a higher concentration was effective in overcoming chloramine decay by controlling nitrification.

8.4 Effectiveness of Copper Inhibition for Severely Nitrified Bulk Waters

From Figure 8.1, it was noticed that NOx-N level remained same after 60 days, though the reactor water was diluted with feed water during feeding time, that indicated the NOx-N production. On the contrary, from Figure 8.5A & B, it was observed that the trend of NOx-N was downward. The possible reasons of the reduction of NOx-N were; dilution of the reactor water with fresh water (NOx-N= 0), and reducing NOx-N production. From Figure 8.5A & B, NOx-N was decreased from the beginning of copper dosing without any improvement of chloramine residual, and chloramine residual was improved when copper was dosed at high concentration (0.80-1.00 mg-Cu/L) with 2.0 mg-Cl_2/L chloramine concentration. Thus, copper inhibited the nitrifying bacteria at a lower concentration (0.25 mg-Cu/L) but required higher concentration for residual improvement. In Chapter 6, it

was already proved that copper at a lower concentration (0.25 mg-Cu/L) was effective for controlling nitrification. The efficiency of copper inhibition depends on bacterial number as well as the particulates and dissolved compounds present in the reactor. In this case, the particulates were not considered as the feed solution was prepared with RO treated water. From microbiological analysis (Section 8.3.2), it was found that the majority of the bacteria was *Sphingomonas* spp., whereas the contribution of nitrifying bacteria (*Nitrosomonas* spp. and *Nitrospira* spp.) was less. In addition, it was found that the reactor contained SMPs that were responsible for acceleration of chloramine decay. The probable reasons for required higher copper concentration were due to presence of SMPs and the bacteria other than nitrifiers. Therefore, it could be reported that copper was effective in controlling nitrification at a lower concentration (0.25 mg-Cu/L) and chloramine decay was controlled at copper concentration of 1.00 mg-Cu/L.

8.5 Conclusions

Copper was dosed in severely nitrified bulk waters in semi-continuous flow reactor to control chloramine decay. Different concentrations of copper at different chloramine concentrations were dosed into the reactor. The chloramine residual as well as nitrogenous compounds were monitored regularly to investigate the effects of copper. The experimental results showed the followings:

- Copper was effective for inhibiting nitrification but not effective for controlling chloramine decay at lower concentration of 0.25 mg-Cu/L.
- The acceleration of chloramine decay was not only due to nitrification but also due to presence soluble microbial products in severely nitrified bulk waters.
- At a higher dose (1.00 mg-Cu/L), copper was effective for controlling nitrification as well as chloramine decay.

CHAPTER 9

EVALUATING THE IMPACT OF COPPER INHIBITION ON NITRIFICATION AND CHLORAMINE RESIDUAL IN A PILOT-SCALE CHLORAMINATED SYSTEM (CONTINUOUS FLOW CONDITION)

Abstract

Controlling nitrification is the most important challenge as it is believed to be responsible for accelerated chloramine decay in the distribution system. The effectiveness of nitrification control practised by many researchers is not effective for long-term strategy. Addition of metal is the new approach for residual management by overcoming nitrification. Water Corporation of Western Australia has already used copper as nitrification inhibitor in water distribution systems. They failed to inhibit nitrification at the end of distribution system due to difficulty in maintaining appropriate copper concentration. By selecting proper copper dosing location, pattern, frequency and concentration, it would be possible to do residual management and nitrification control in the distribution systems. In our previous research (Chapter 6 to 8), it was reported that copper could control nitrification and improve chloramine residual at higher concentration. In this study, an attempt has been made to control nitrification and chloramine decay by dosing copper at severely nitrified and onset of nitrification conditions in the reactor operating with continuous flow mode. Copper could inhibit the ammonia oxidising bacterial activities at a lower concentration (0.10 mg-Cu/L) when dosed in both nitrification conditions. In case of copper dosing in severely nitrified condition, no residual improvement was found even at higher concentration (0.20 mg-Cu/L). However, significant improvement of chloramine residual was noticed when copper (0.10 mg-Cu/L) was dosed in the reactor where onset of nitrification was occurring. Furthermore, copper was effective in inhibiting sediment associated chloramine decay. When copper dosing was stopped in the reactor where onset of nitrification was occurring, nitrification did not bounce back in any reactors even when the reactor system was operated for 50 days. Therefore, this study offers a long-term solution to nitrification control and

chloramine residual management by using copper as inhibitor in the distribution system.

9.1 Introduction

Many water utilities use chloramine as a secondary disinfectant due to less production of carcinogenic disinfection by-products (Goslan et al., 2009) and longer residual stability over chlorine. Besides the advantages, unintentionally increased level of free-ammonia due to chloramine decay in the distribution system serves as an energy source for indigenous nitrifying organisms. Chloramine decays in two ways. One is chemical decay, which is due to auto-decomposition and reactions with organic or inorganic constituents. The other is microbial decay, caused due to the presence of microbes including nitrifiers in the distribution system. As a result, nitrification occurs in the distribution system. Nitrification is a microbial process by which ammonia is sequentially oxidized to nitrite and then to nitrate. Many authors (Skadsen, 1993; Odell et al., 1996; Wilczak et al., 1996; Wolfe and Lieu, 2001; Seidel et al., 2005) reported that nitrification is one of the most frequent operational problems encountered by water utilities that use chloramine as a secondary disinfectant. It is commonly believed that nitrification accelerates chloramine decay in distribution systems although the recent finding of Sathasivan et al. (2011) and in Chapter 7 the traditional norm is questioned.

Many researchers attempted to control nitrification in different ways. Based on the common belief, many control measures have been practiced. These are; breakpoint chlorination (Wolfe et al., 1988; Odell et al., 1996), maintaining high disinfectant residual (Skadsen, 1993; Harrington et al., 2002), optimization of the chlorine-to-ammonia ratio (Wolfe et al., 1988; Odell et al., 1996), removal of natural organic matters (Odell et al., 1996), distribution system flushing (Odell et al., 1996), decrease of distribution system retention time (Odell et al., 1996; Harrington et al., 2002). The effectiveness of these measures is not satisfactory for controlling neither nitrification nor chloramine decay over long term.

The effectiveness could be increased, if chloramine control could be achieved by either dosing a suitable inhibitor or increasing the fundamental understanding of the causes of chloramine decay - the major aim. The additions of metals such as silver, copper etc. can significantly inhibit nitrifying bacteria by blocking their enzymatic function (Martin and Richard, 1982). It has been already reported that microbial decay including that due to nitrification can be controlled by addition of silver (Sathasivan et al., 2005 and Fisher et al., 2009), silver nanoparticles (Choi et al., 2008), and copper (Laszlo, 2008; Zhang et al., 2009; Loveless and Painter, 1968). Some authors suggested that copper could inhibit nitrifiers at concentrations greater than 0.1 mg-Cu/L (Zhang and Edwards, 2005).

Water Corporation, Western Australia (WCWA) obtained patent for its application in water distribution system (Laszlo, 2008). WCWA also attempted to dose copper into a reservoir and specific pipe lines in Goldfield and Agriculture Water Supply System (G&AWSS). They found that copper was effective to inhibit nitrification. However, they found it difficult to maintain appropriate copper concentrations in the pipe lines farther to the dosing point although they had a good success in protecting copper concentrations in the reservoirs. As a result, accelerated decay of the disinfectant at the farthest points and accumulation of copper in the form of sediments or adsorbed metals on the pipe walls closer to the dosing point was observed (Zhan, 2011). By investigating further, Zhan, (2011) hypothesised that the major reason for copper loss could be corrosion of cement lined cast iron pipes. Therefore, it is important to understand the point at which copper could be dosed to effectively control nitrification without too much jeopardising the loss of copper that was seen to occur in distribution system pipelines.

From the point water has entered into the water distribution system, microbial and chemical decay undergoes different phases depending on nitrification or chloramine residuals. They happen in a sequential manner although some of the stages could be missing in some systems, following same stages seen in bulk water incubated under batch conditions (Sathasivan et al., 2008). Sathasivan et al., (2008) defined three different conditions. The first condition is known as mildly nitrifying conditions wherein chloramine decay is very slow and steady, nitrite levels are low (less than 0.01 mg-N/L) with mild ammonia loss. The second condition is referred to as severe

nitrification, where rapid chloramine decay with excessive nitrite production (more than 0.1 mg-N/L) and rapid ammonia drop are observed. Condition occurring in between these two is referred to as onset of nitrification. Later studies (Siew Teng, 2009) noted that there can be excessive nitrite production without a significant drop in residual. This particular condition is referred to as nitrification. Samples subjected to these conditions are likely to host different microbes with varying chloramine concentration and hence behave differently. This has been confirmed when a fellow student (Bal Krishna, 2012) working on the same project investigated microbial community in each reactor.

The effectiveness of copper dosing depends on selecting the point where copper could be dosed to maintain an effective nitrification or most importantly protect chloramine residual. There are a variety of copper dosing arrangements with its own advantages and disadvantages: intermittent or continuous, appropriate dosing location (reservoir or pipe line), and suitable dosing points in terms of nitrification stages. The quantity of copper dose that needs to be dosed for a given situation (TCl, free ammonia concentration) can be determined using the model developed earlier in Chapter 6 to control AOB activity.

Sathasivan et al., (2005) proposed microbial decay factor (F_m) method to help in maintaining chloramine residual. This method was claimed to provide the most accurate assessment of chloramine stability and showed the early warning for onset of nitrification (Fisher et al., 2009 and Sathasivan et al., 2009) by calculating the decay rate coefficients (chemical and microbial) with monitoring chloramine residual only rather than observation of traditional parameters. Later, Sathasivan et al., (2010) proposed the reservoir acceleration factor (F_{Ra}) that defines the degree of acceleration present in the reservoir over and above the chemical decay in the bulk water assuming the reservoir is well mixed. F_{Ra} can be determined using temperature, retention time, inlet and outlet chloramine residuals, that can be easily obtained by an operator. Moreover, because of the advantages over traditional indicators, F_{Ra} and F_m related parameters can be effective indicators to control rapid chloramine loss progressively, especially in summer.

Service reservoir is a critical location for nitrification control (Sathasivan et al., 2010) as water spends a majority of travel time in it. Water quality in reservoirs can deteriorate due to chloramine decay and other chemical and microbial processes in bulk water, biofilms and sediments. Sathasivan et al., (2010) also noticed a significant effect of biofilm/sediment on chloramine decay in a small reservoir (water contact surface area to water volume ratio = 0.3 m^{-1}). Therefore, service reservoir can be chosen as the location where any control measures can take place. To understand the overall effect on chloramine decay compared to a clean reservoir with no microbial contribution Sathasivan et al., (2010) used F_{Ra} for a completely mixed reservoir. This could be used to understand the overall impact an inhibition strategy is having on chloramine protection.

The purpose of the study was to examine the copper inhibition effectiveness in residual management by preventing nitrification in continuous flow system. In order to understand how copper controls nitrification and ultimately how it affects chloramine decay, varying copper dose within the acceptable limit, was added in continuous flow system containing five reactors connected in series which behaved similar to real distribution system. In the continuous flow system, the reactors were created to represent all these three nitrification conditions so that copper could be dosed at appropriate condition. Further details of the reactor set up are given in Chapter 3 and Section 3.2.3. Such understanding is the key to control the chloramine decay in distribution systems, or to apply inhibition strategies. The dosing of copper with the consideration of different nitrifying conditions is the uniqueness of the study.

9.2 Materials and Methods

The detailed description of stock chemical solutions preparation; water sample collection, preparation and storage; description of pilot-scale reactor setting, operation and feed water preparation; preparation of sample bottles and glasswares, analytical procedures F_m, and F_{Ra} value determination were presented in Chapter 3.

9.2.1 Experimental Design

Out of two sets of reactors, one set (R 1/1, R 2/1, R 3/1, R 4/1 and R 5/1) was operated without dosing copper, and it was called control reactor and denoted as 'Ctrl'; whereas the other one (R 1/2, R 2/2, R 3/2, R 4/2 and R 5/2) was inhibited with copper, and it was called the copper dosed reactor and was referred as 'Inhi'. To make it clear, the additional schematic diagram of the reactors is shown in Figure 9.1.

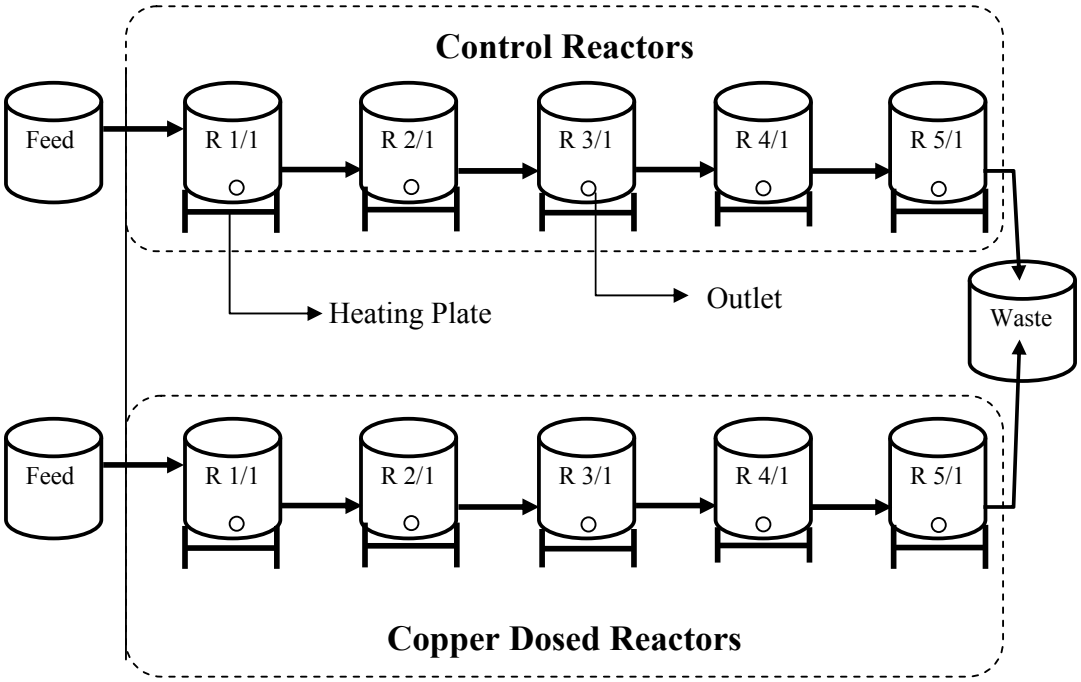

Figure 9.1: Schematic diagram of pilot-scale reactors

Four sets of experiments were conducted and samples were collected from the reactors. The experiment started first by establishing two parallel reactors with similar operating conditions behaving in a reproducible manner, before the copper was dosed in one of the severely nitrified reactor followed by a reactor where a switching to nitrification (onset) was happened. The major aim was to first check to see if it controlled nitrification then to see if it improved chloramine residual. If any of these were not fully achieved copper dosing was increased.

The copper dose was selected based on two principles. First was to consider starting at a concentration of 0.10 mg-Cu/L. to check the copper concentration required to inhibit the nitrifying organisms and the second was using the Equation 6.7 to check the validity of the Equation 6.7 for a continuous flow system.

Experimental Set–1: The first set of experiments was conducted to see the copper effects on severely nitrified reactor (R 4/2) by dosing copper. Initially, 0.10 mg-Cu/L copper was dosed for 20 days and then increased to 0.20 mg-Cu/L for the next 12 days. Samples were collected from the reactors (R 3/1, R 3/2, R 4/1, R 4/2, R 5/1 and R 5/2) three times in a week and analysed for TCl, TAN, NO_2-N, NOx-N and copper concentration immediately.

Experimental Set–2: The second set of experiment was designed to examine the inhibitory effects of copper while dosing at nitrification onset point. 0.10 mg-Cu/L copper was dosing in reactor R 3/2 continually for 40 days. Samples were collected from the reactors (R 2/1, R 2/2, R 3/1, R 3/2, R 4/1, R 4/2, R 5/1 and R 5/2) three times in a week and analysed for TCl, TAN, NO_2-N, NOx-N and copper concentration immediately.

Experimental Set–3: The third set of experiments were conducted to check the inhibitory effects of copper on sediment contribution to nitrification and thus to see the improvement in chloramine residual. During copper (0.10 mg-Cu/L) dosing in nitrification onset point (R 3/2), samples were collected from reactors (R 3/1, R 3/2, R 4/1 and R 4/2) in two ways: from middle after a vigorous mixing for bulkwater with sediment, from top after letting settle down the sediments for bulkwater only. Samples were prepared in two categories, namely bulkwater with sediment, and bulkwater. Bulkwater with sediments sample provided the decay due to bulkwater and sediment *(kt(b+s)),* and bulkwater sample provided the decay coefficient *(ktb)* due to bulkwater. Difference between *kt(b+s)* and *ktb* provided the contribution of sediment. The decay rate coefficients were calculated using Equation 3.1.

Experiment Set–4: The fourth set of experiment was designed to understand how quickly nitrification/acceleration of chloramine decay would bounce back after stopping the copper dosing. The copper dosing reactor and its downstream reactors

were monitored for 50 days to understand the post-effectiveness of copper inhibition. The copper dosing was stopped and samples were collected from the reactors (R 2/2, R 3/2, R 4/2 and R 5/2) regularly for fifty days and were analysed for TCl, TAN, NO_2-N, and NOx-N immediately. The copper dosing in the reactors in different times is shown in Figure 9.2.

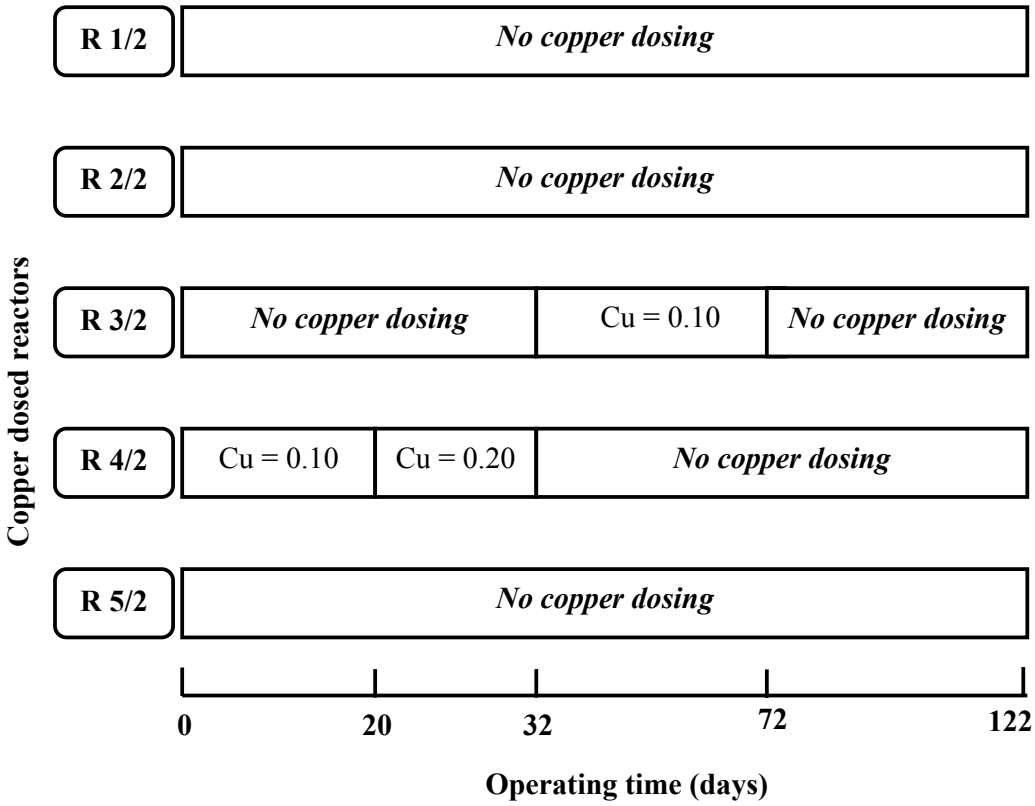

Figure 9.2: Bar chart showing the copper dosing in the reactors along the operating time. Unit of copper is in mg-Cu/L.

9.2.2 Evaluation of Nitrification Inhibition Effectiveness

Three criteria were considered to examine the effectiveness of nitrification inhibition or inactivation.

The first criterion was to monitor the change of parameters that usually produced due to presence of responsible microbes in the copper dosed reactors and by comparing the measured values of parameters between the copper inhibited reactors and control

reactors. Wolfe et al., (1990) reported that mostly partial nitrification (ammonia oxidation) occurs in distribution systems. Thus, NO_2-N is generally used as the indicator of nitrification status (Wolfe et al., 1988). The recent work of Regan et al., (2003) and Hoefel et al., (2005) reported that nitrite oxidising bacteria (NOB) is present in distribution system bulk waters. However, it is wise to select the parameter that can represent the sign of activity of both AOB and NOB. In this context, NOx-N is the best option because in a chloraminated environment as NH_3-N converts NO_2-N by AOB and NO_2-N converts to NO_3-N by NOB. Hence, there is a reduction of TCl and TAN, and an increment of NOx-N as a result of nitrification. Therefore, increment or stable trend of TCl and TAN profiles, and downward trend of NOx-N profile in copper dosed reactors than control reactors indicates the copper inhibition effectiveness on nitrification and residual improvement.

The second criterion was by comparing reactor's condition (effluent value) with the upstream reactor's (influent value) condition. Comparison of the ratio of effluent to influent of the monitored parameters (mentioned in the first criteria) was used to find effectiveness of copper inhibition. If the value of this ratio is equal to one, it represents the same condition of the reactor as the upstream reactor that indicates the expected copper inhibition efficiency. The ratio between effluent and influent concentration of TCl and TAN was used in this study.

The third criterion was to compare F_m, F_{Ra} and NOx-N production rate at different time during copper dosing. Decreasing trend of F_m, F_{Ra} and NOx-N production rate proves copper effectiveness positively.

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9.3 Results and Discussions

9.3.1 General Characteristics of Pilot Scale Reactors Associated with Nitrification Stages

As noted before two identical set of reactors were operated with the same feed and same operational conditions, such as temperature and retention time. If the effectiveness of copper needs to be understood one set should be operated as a

control. Before any copper dosing was practiced, it should be proven that those two sets behave similarly. The characteristics of these two sets of reactors were assessed by monitoring the surrogate parameters (TCl, TAN and NOx-N) for fifteen days, before copper was dosed into the reactors. The profiles of TCl, TAN and NOx-N of R-2 (R-2/1, R-2/2), R-3 (R-3/1, R-3/2), R-4 (R-4/1, R-4/2) and R-5 (R-5/1, R-5/2) for 15 days representing the behaviour of the reactors are presented in Figure 9.3A. From Figure 9.3A, it is noticed that TCl and TAN profiles were higher in R-2 and then started to decrease along the reactors. On the other hand, there was an increasing trend of NOx-N from R-2 towards R-5.

To make a clear understanding of chloramine loss and nitrification status inside the reactors, F_{Ra} and nitrification rates were used. Figure 9.4A and 9.4B shows the F_{Ra} and NOx-N production rate along the reactors. From Figure 9.4A and 9.4B, it is noticed that the chloramine decay acceleration and NOx-N production rate were high in R-3 and low in R-2. These findings indicate that nitrifying bacteria were more abundant or active in R-3 than other reactors. From Figures 9.3 and Figure 9.4, it is observed that the trend of all parameter's profiles of reactors R-2/1, R-3/1, R-4/1 and R-5/1 were similar to R-2/2, R-3/2, R-4/2 and R-5/2 respectively. Therefore, it can be said that the characteristics of both sets of reactors were same. To categorise the reactors into different nitrification conditions, the typical values of the monitoring parameters are summarised in Table 9.1.

It is well known that nitrification, in addition to other microbes, plays an important role on chloramine loss. The same phenomenon occurred in the pilot-scale reactors (R-1 to R-5). From Table 9.1, it is observed that there is a gradual reduction of TCl and TAN, a slight change in NO_2-N concentration (<0.01 mg-N/L) in R-1 and R-2. This condition was defined as mild nitrification according to Sathasivan et al., (2008). Nevertheless, significantly low levels of TCl and TAN with very high NO_2-N and NOx-N were noticed in R-4 to R-5 as compared with R-1 or R-2. This condition was defined as severely nitrified conditions (Sathasivan et al, 2008). From the NO_2-N and NOx-N concentration of the reactors, it might be said that there was a transition in between mild and severe nitrification in R-3 and this stage is referred to as onset of nitrification. However, chloramine loss started accelerating once nitrification has started. This nitrification has to be overwhelmed for proper

chloramine residual stability. Therefore, it is necessary to inhibit the nitrification first by any means. In this study, continuous copper dosing was carried out at stages of severe nitrification and onset of nitrification in order to improve the chloramine residual and inhibiting/controlling nitrification.

Table 9.1: Typical Chemical Parameters Measured Before Addition of Copper in a Pilot-Scale System

Reactors	R-1	R-2	R-3	R-4	R-5
TCl (mg-Cl_2/L)	2.15±0.03	1.96±0.03	0.75±0.03	0.15±0.03	0.00±0.03
TAN (mg/L)	0.520±0.007	0.490±0.007	0.260±0.005	0.115±0.002	0.045±0.002
NO_2-N (mg/L)	0.005±0.002	0.007±0.002	0.180±0.002	0.280±0.002	0.210±0.002
NOx-N(mg/L)	0.090±0.002	0.120±0.002	0.350±0.002	0.450±0.003	0.550±0.004
Temp. (°C)	18±2	20±2	22±2	22±2	22±2
DOC (mg/L)	2.75±0.20	2.76 ±0.02	2.76± 0.02	2.79±0.02	2.60±0.02
pH	7.9±0.1	7.9±0.1	7.8±0.1	7.6± 0.1	7.6±0.1
Nitrification stage	Mild nitrification	Mild nitrification	Onset of nitrification	Severe nitrification	Severe nitrification

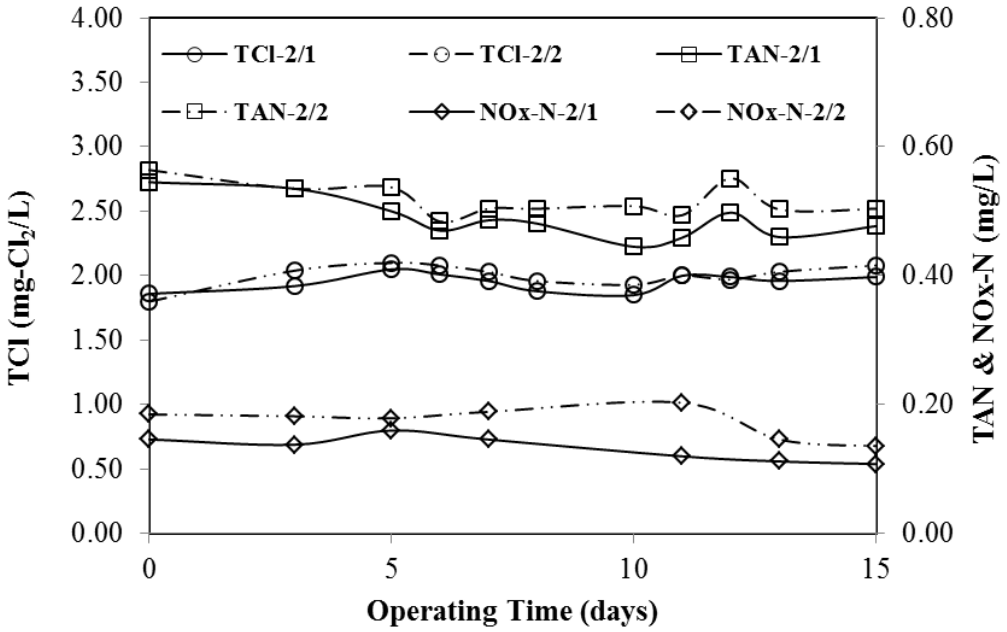

Figure 9.3A: TCl, TAN and NOx-N profiles of Reactor 2 (R-2/1 & R-2/2)

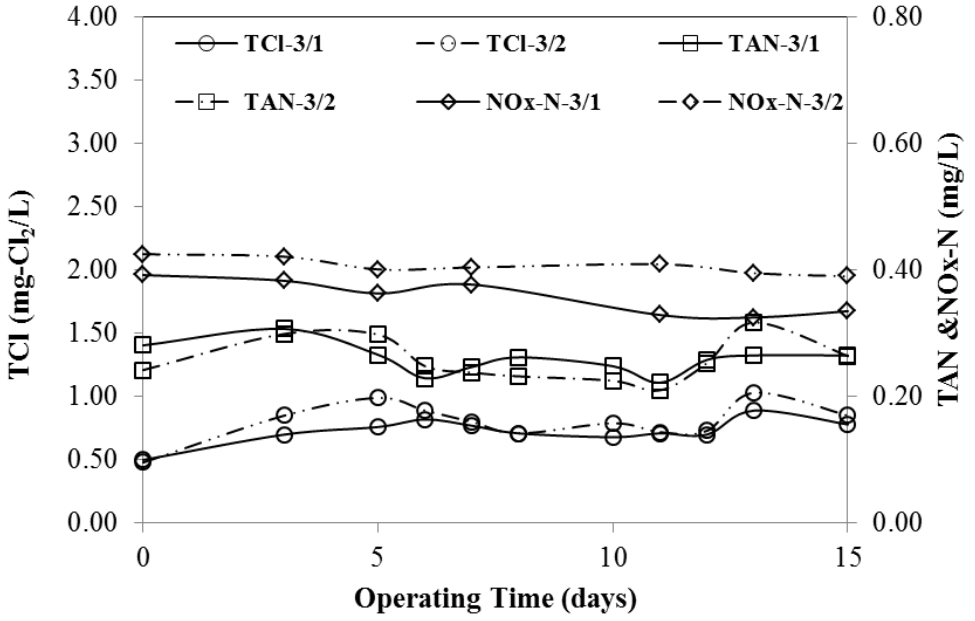

Figure 9.3B: TCl, TAN and NOx-N profiles of Reactor 3 (R-3/1 & R-3/2)

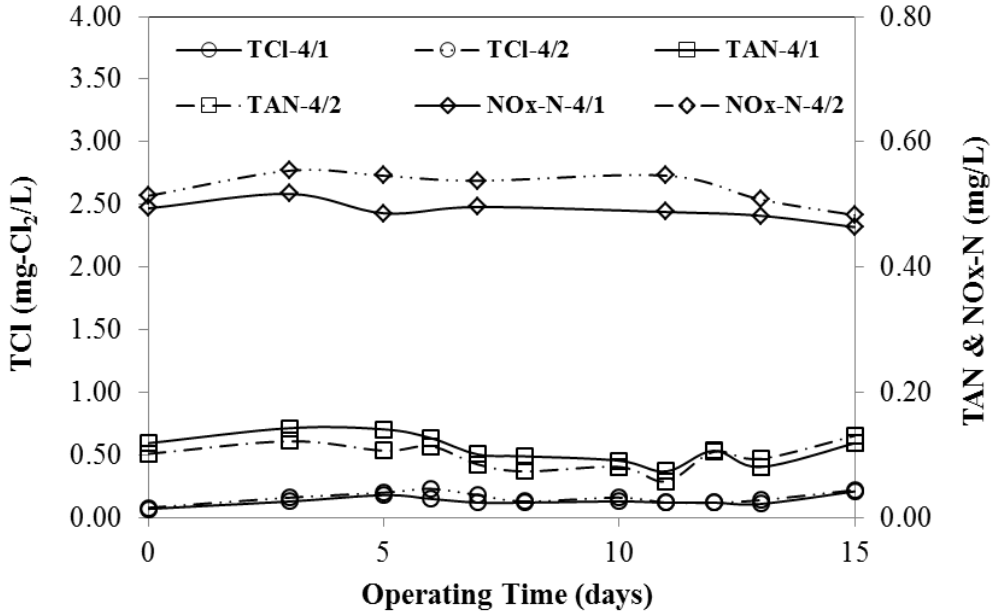

Figure 9.3C: TCl, TAN and NOx-N profiles of Reactor 4 (R-4/1 & R-4/2)

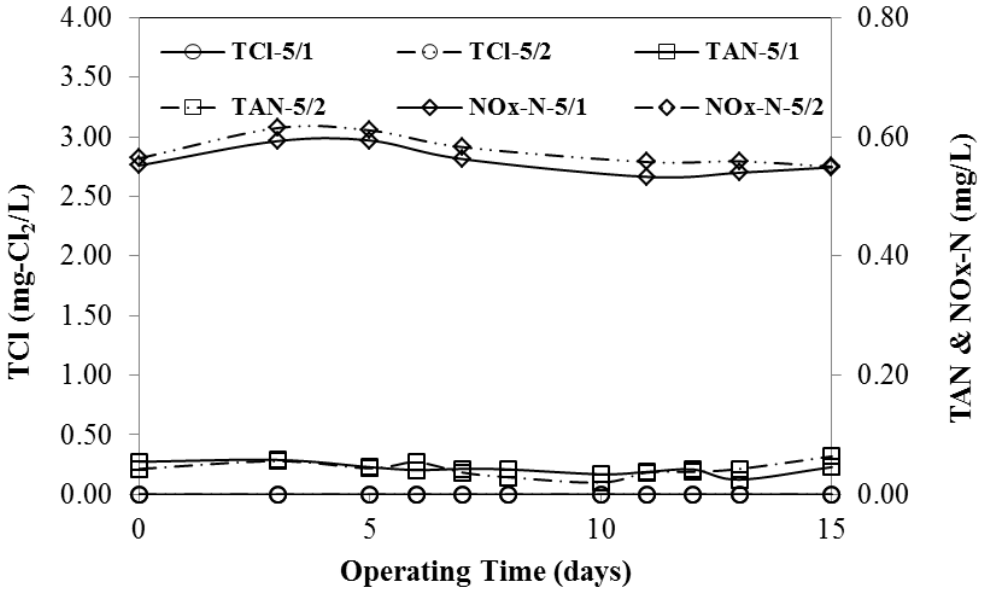

Figure 9.3D: TCl, TAN and NOx-N profiles of Reactor 5 (R-5/1 & R-5/2)

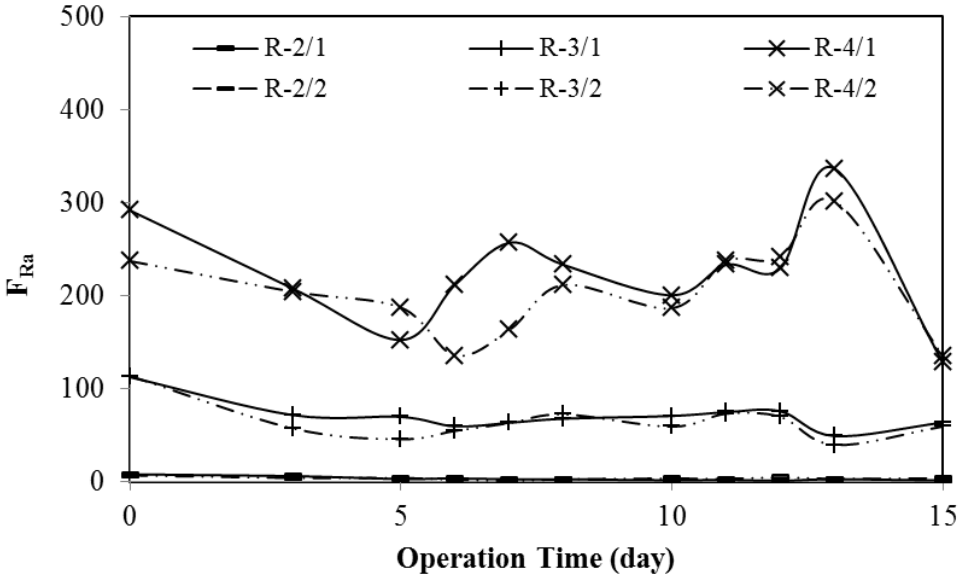

Figure 9.4A: F_{Ra} variation along the Reactors (R-2 to R-3)

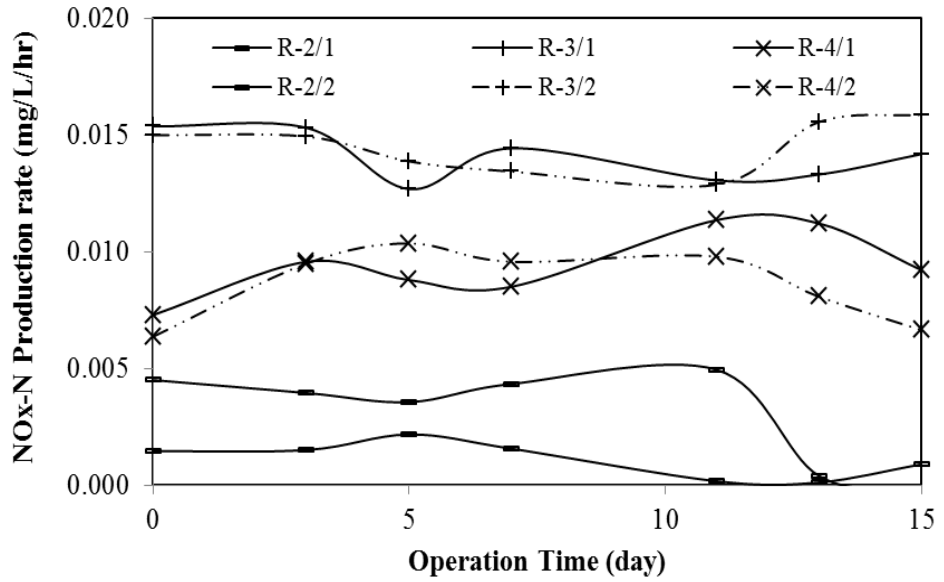

Figure 9.4B: NOx-N production rate along the Reactors (R-2 to R-3)

9.3.2 Behaviour of Severely Nitrified Bulk Waters when Copper Dosed in Severely Nitrified Reactor

Equation 6.7 in Chapter 6 describes the growth and killing rates if copper and chloramine are present. At the start of copper dosing TAN and TCl were 0.115 mg-Cl_2/L and 0.2 mg-Cu/L respectively. Hence, the free ammoniacal nitrogen is 0.115-0.2/5=0.065 mg/L.

Growth rate = $\mu_m.\left(\frac{N}{N+Ks}\right)$

Killing or Inhibition rate = $k_d.TCl + a.TCl.Cu + b.Cu$

If both are divided by k_d, the resulting relationship will be;

Growth rate = $k_d.\frac{\mu_m}{k_d}.\left(\frac{N}{N+Ks}\right)$

Killing or Inhibition rate = $\left(TCl + \alpha.TCl.Cu + \beta.Cu\right).k_d$

The left hand side is therefore, 2*0.065/(0.18+0.065)=(0.53).k_d

Killing or Inhibition rate = (0.05+18.2*0.05*Cu+3.4*Cu).k_d

The copper concentration has to be 0.11 mg-Cu/L to make both of them same or to increase the killing/inhibition rate the copper concentration has to be more than 0.11 mg-Cu/L. However, it could be noted that AOB activity would be suppressed at least to an extent if copper is dosed. This in turn would increase either individual concentrations of the free ammonia or total chlorine. Depending on this situation the balance would change which would lead to a different equilibrium. Hence, as the first dosing, 0.1 mg-Cu/L was chosen then gradually copper dose was increased depending on the outcome.

9.3.2.2 Effect of copper in dosing reactor

The first set of experiment was carried out to observe the behavior of severely nitrified bulk waters when copper was dosed continuously in the same reactor (R-4/2). Initially 0.10 mg-Cu/L copper was dosed for 20 days and then increased to 0.20 mg-Cu/L for 12 days, especially because the copper did not improve total chlorine although it suppressed the AOB activity well. TCl, TAN and NOx-N levels of R-3/1, R-4/1, R-5/1, R-3/2, R-4/2 and R-5/2 were monitored during the copper dosing

period (32 days). As copper was dosed in R-4/2, the reactors R-4/1 and R-4/2 were 'control reactor' and 'copper dosed reactor', respectively; while concentration of the monitoring parameters of R-3/1, R-3/2 and R-4/1, R-4/2 were 'influent' and 'effluent' respectively. The profiles of TCl, TAN and NOx-N of R-4/1 and R-4/2 are shown in Figure 9.5A. It is observed that the trend of TCl, TAN and NOx-N of R-4/1 and R-4/2 followed the same pattern. Though there was no visible improvement in chloramine residual, a marginal improvement was found in TAN level and NOx-N reduction. This is in alignment with what was expected from the experiments. Therefore, an attempt was made to see the impact of copper inhibition on chloramine decay and nitrification by calculating the ratio of effluent/influent of TCl and TAN, F_{Ra} and NOx-N production rate inside the reactor (Figure 9.5B, C & D). From Figure 9.5B, it is noticed that the effluent to influent ratio of TCl is almost same, but a significant improvement of the ratio of TAN (closer to one) is observed in R-4/2 as compared to R-4/1. Furthermore, it is seen that the trend of F_{Ra} (Figure 9.5C) and NOx-N production rate (Figure 9.5D) profiles decreased along with copper dosing time in R-4/2. Therefore, it could be said that copper was effective for controlling nitrification, but marginally effective for improving chloramine residual.

As the reactors were connected in series and there was a reactor downstream of the copper dosing reactor, the copper dosing might influence the downstream reactor due to continuous flow of feed water.

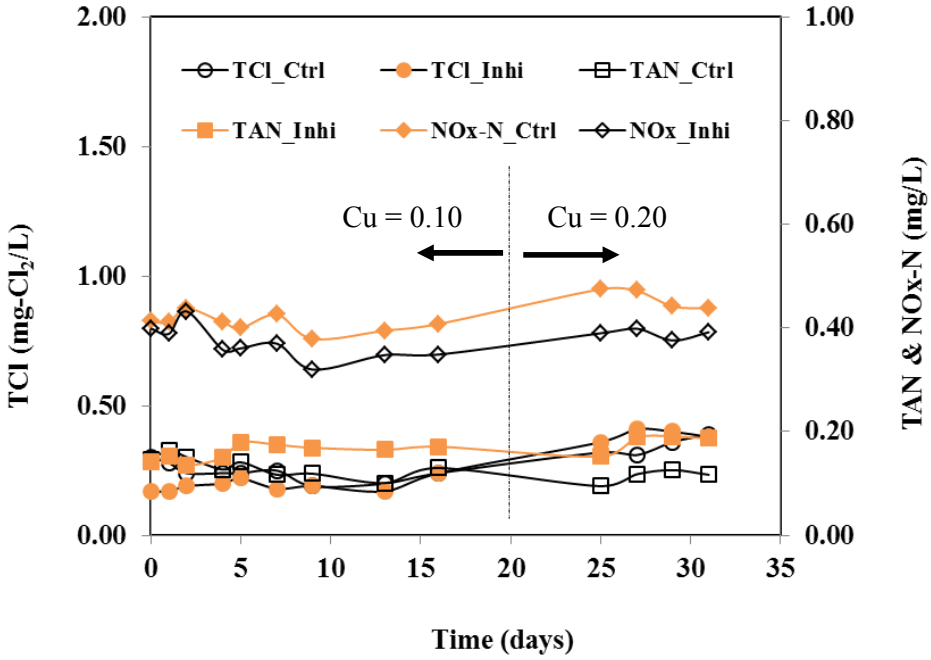

Figure 9.5A: TCl, TAN and NOx-N profiles of R 4/1 and R 4/2 (unit of copper is mg-Cu/L)

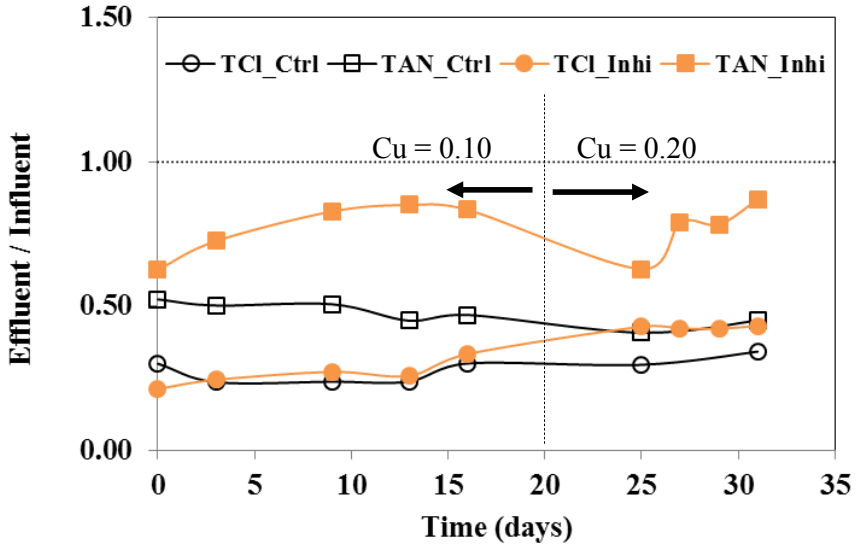

Figure 9.5B: Effluent to influent ratio of TCl and TAN of R 4/1 and R-4/2 (unit of copper is mg-Cu/L)

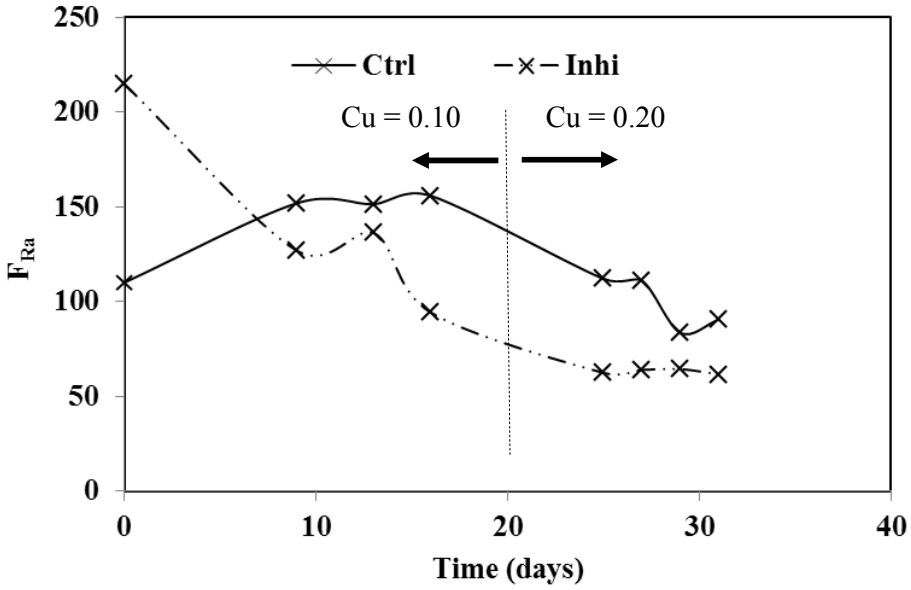

Figure 9.5C: Variation of F_{Ra} of R-4/1 and R-4/2 along the operating time (unit of copper is mg-Cu/L)

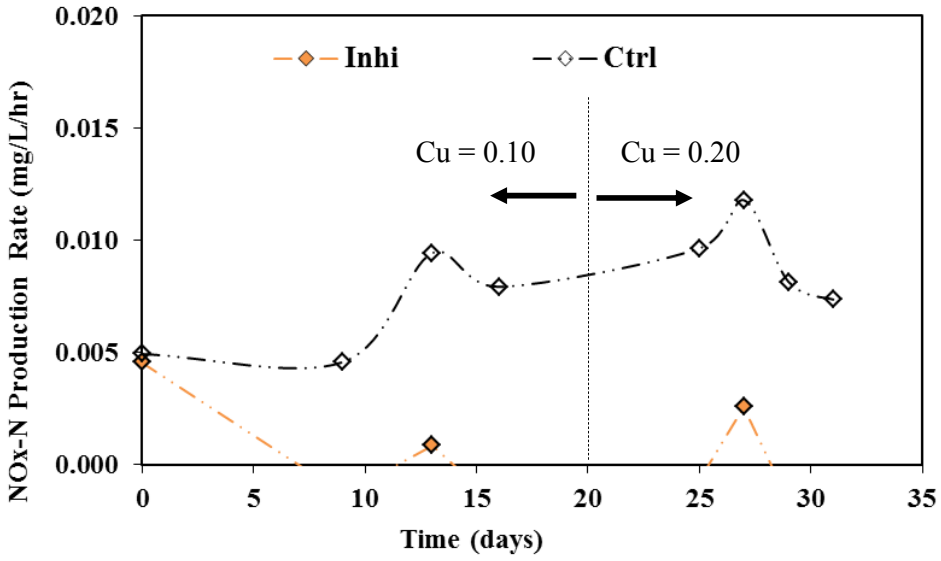

Figure 9.5D: Comparison of NOx-N production rate between R-4/1 and R-4/2 (unit of copper is mg-Cu/L)

9.3.2.3 Effect of Copper Dosing in R-4 on the Succeeding Reactor (R-5)

Due to continuous copper dosing in R-4/2, the reactor R-5/2 was also inhibited. In this case, the reactors R-5/1 and R-5/2 were used as 'control reactor' and 'copper dosed reactor' respectively while concentration of the monitoring parameters of R-4/1, R-4/2 and R-5/1, R-5/2 were used as 'influent' and 'effluent' respectively. Same calculations as defined in Section 9.3.2.2 were made from the experimental value of TCl, TAN and NOx-N of the reactors (R-4/1, R-4/2, R-5/1 and R-5/2) when copper was dosed in R-4/2. Comparing with the control reactor (R-5/1), it is observed that there was an improvement of TAN level (Figure 9.6A) and the effluent/influent ratio is higher (Figure 9.6B) in the inhibited reactor (R-5/2), but the TCl profiles of these two reactors followed closely. In addition, the same F_{Ra} values of R-5/1 and R-5/2 (Figure 9.6C) indicated that copper was not effective for improving chloramine residual. Therefore, it could be said that copper could effectively control nitrification but not chloramine decay in the succeeding reactor, when copper was dosed in the severely nitrified reactor.

Conversely, it was remarked that copper was significantly effective in inhibiting NOx-N production (Figure 9.6D), but not completely as in R-4. The probable reason would be less availability of TCl and TAN concentrations as depicted in the Equation 6.7 could have contributed to less inhibition. However, TCl concentration in R-5 was only 0.05 mg-Cl_2/L which was much lower than that in R-4. This condition would have shifted the equilibrium towards higher growth rates or nitrification activity.

Similar calculation as that for R-4 could be done to understand the killing and growth rates. Initial TCl and TAN levels were 0.05 mg-Cl_2/L and 0.07 mg/L respectively.
Hence, free ammoniacal nitrogen = 0.07-0.05/5= 0.06 mg/L
Growth rate = 2*0.06/(0.18+0.06)*kd=0.5kd
Inhibition/killing rate = (0.05+18.2*0.05*Cu+3.4*Cu)*kd
The required Cu concentration to effect any net inhibition would be more than 0.102 mg-Cu/L. Considering the error in copper measurement of ±0.02 mg-Cu/L, it is necessary to measure more than 0.12 mg-Cu/L. Added copper (0.1 mg-Cu/L) was therefore marginally sufficient to control ammonia oxidizing bacterial activity. When added ammonia concentration was increased in the upstream reactor, the inhibitory

effects of copper in the reactor itself has increased nitrogen concentration, but not the chloramine or copper concentrations, especially within the 20 days of 0.1 mg-Cu/L dosing. Increase in TAN, but not in TCl would increase the growth rate although killing/inhibition rate would remain the same. In turn we should expect a much higher nitrification activity. Probably, switching of this balance could be the reason for fluctuating NOx-N production rate.

The results, therefore, indicated that the proposed Equation 6.7 using severely nitrified samples could be used to understand how copper would inhibit ammonia oxidizing bacterial activity.

Is AOB responsible for accelerating chloramine decay?

Substantial reduction of AOB activity in both R-4/2 and R-5/2 was noted but not the chloramine residual. If AOB activity within these reactors were responsible for degrading chloramine residuals then one would expect an increase in chloramine residual to at least closer to the influent TCl concentration. In these cases, TCl loss of 70% was noted within R-4/2. This was a substantial reduction and hence this again re-confirmed our previous batch experimental results that copper could control AOB activity, but not the chloramine decay. It again throws doubt on traditional belief that AOB activity is mainly responsible for accelerating chloramine decay.

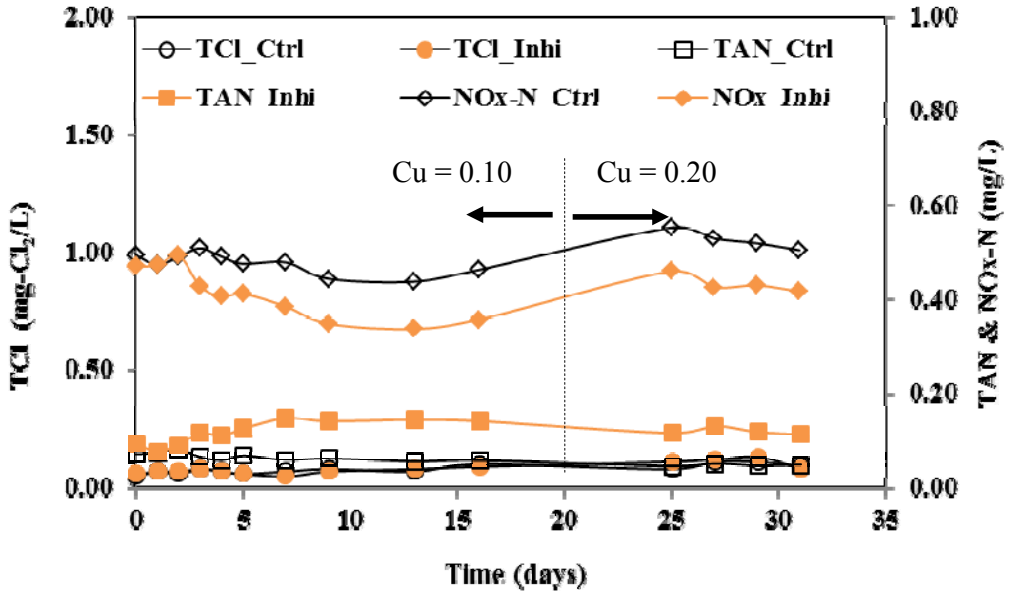

Figure 9.6A: TCl, TAN and NOx-N profiles of R-5/1 and R-5/2 (unit of copper is mg-Cu/L)

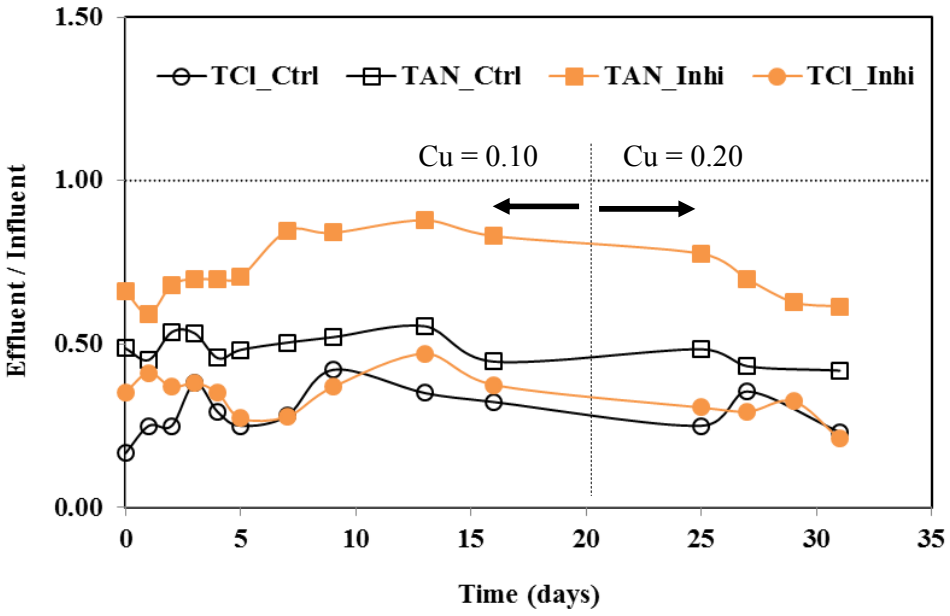

Figure 9.6B: Effluent to influent ratio of TCl and TAN of R 5/1 and R-5/2 (unit of copper is mg-Cu/L)

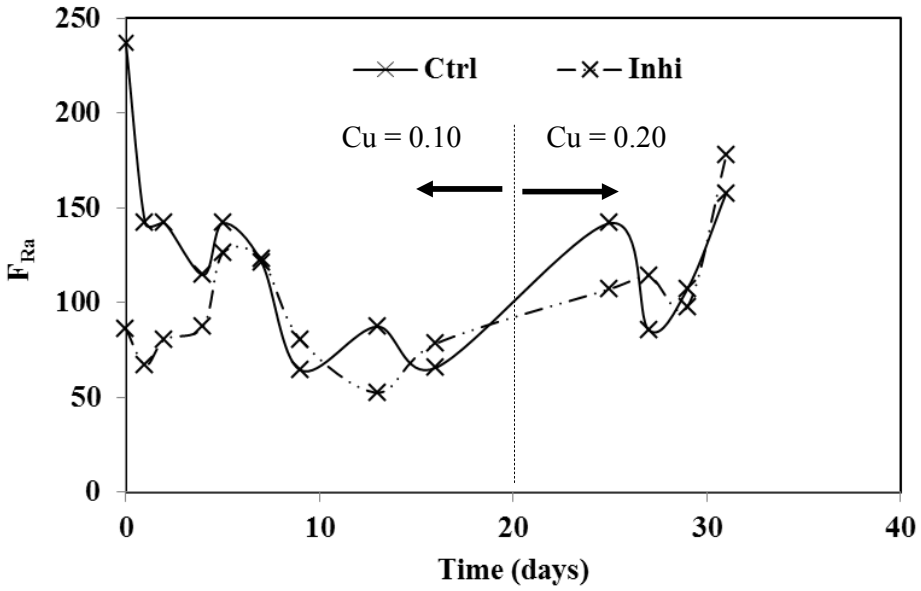

Figure 9.6C: Variation of F_{Ra} of R-5/1 and R-5/2 along the operating time (unit of copper is mg-Cu/L)

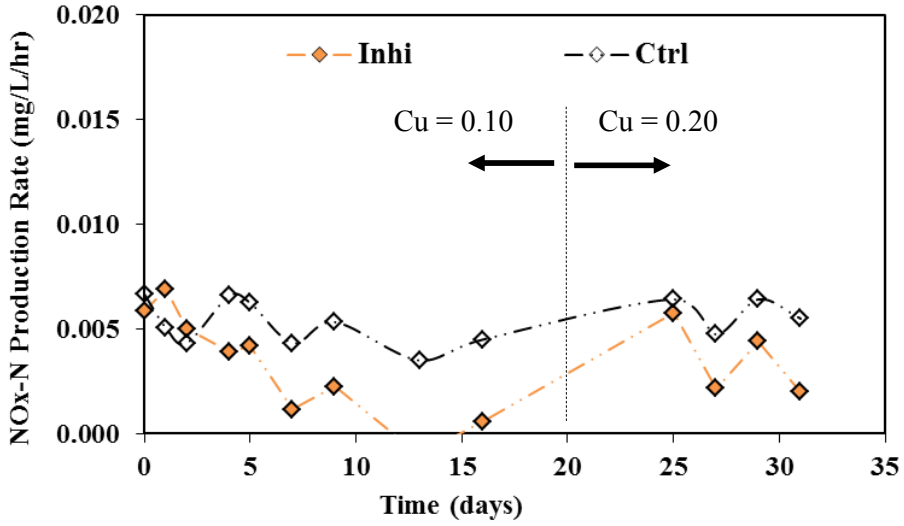

Figure 9.6D: Comparison of NOx-N production rate between R-5/1 and R-5/2 (unit of copper is mg-Cu/L)

9.3.3 Behaviour of Bulk Waters When Copper was Dosed in the Reactor Where Onset of Nitrification has Taken Place (R 3/2)

The results above indicated that dosing of copper under severely nitrified conditions could control AOB activity but couldn't improve the chloramine residual**.** In other words, chloramine loss and nitrification are two separate processes in the severely nitrified conditions whether they are batch tested or in a continuous flow reactors. The mild, onset and severe nitrification could host different microbes and hence it is vital to understand how copper could be effective in each stage. Hence, the reactor R-3, which was located upstream of R-4, was chosen. The experimental results were discussed in the following Sections (9.3.3.1 to 9.3.3.3).

9.3.3.1 Effect of copper inhibition on chloramine decay and nitrogenous compounds in the copper dosed reactor (R-3/2) itself

Figure 9.7A shows the profiles of TCl, TAN and NOx-N of R 3/1(control reactor) and R 3/2 (copper dosed reactor) while 0.10 mg-Cu/L copper was dosed in R 3/2. It is observed that the trend of TCl, TAN and NOx-N of R 3/1 were relatively constant throughout the experimental period. Increased level of NOx-N and reduced TAN than in influent in the R-3 in the control side indicated that there was NOx-N production due to nitrifying bacterial activity. Meanwhile, a significant change is observed in R 3/2. The level of TCl and TAN was upward and the NOx-N was downward in R 3/2 for up to 40 days followed by a stable level during experimental period. More interestingly the chloramine which did not improve when dosed in R-4/2 has substantially increased to cause further killing/inhibition of nitrifying bacteria. It was therefore important to understand why copper did not improve the chloramine residual in the severely nitrified reactor, but did when dosed under onset conditions.

There could be many reasons for the improvement in chloramine residual in R-3/2. First could be, the chloramine residual under this condition could be controlled by the production of NO_2-N and its subsequent impact on chloramine residual. Hence, if NO_2-N production was stopped chloramine residual would increase. Increased chloramine residual would then suppress the AOB subsequently leading to a

complete stopping in AOB activity. The second, there could be some chloramine decaying organisms in the reactor which has become inhibited when a certain chloramine concentration was reached. The third could be different set of microbes performing nitrification in R-3/2 that were inhibited well by copper. The fourth could be that the microorganisms producing SMPs were actually present in R-3/2 which was inhibited that eventually increased the chloramine residual

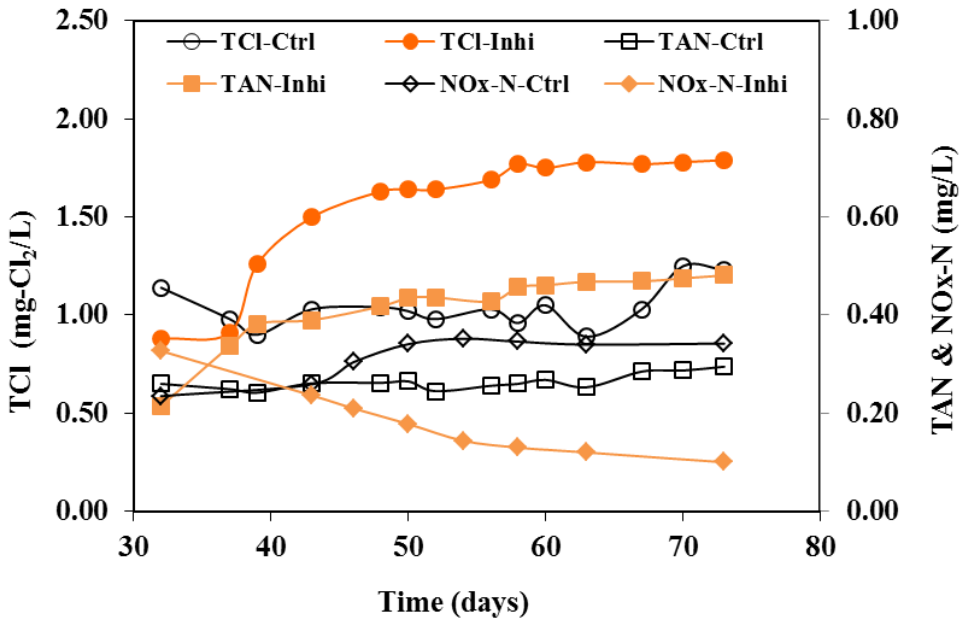

Figure 9.7A: TCl, TAN and NOx-N profiles of R-3/1 and R-3/2.

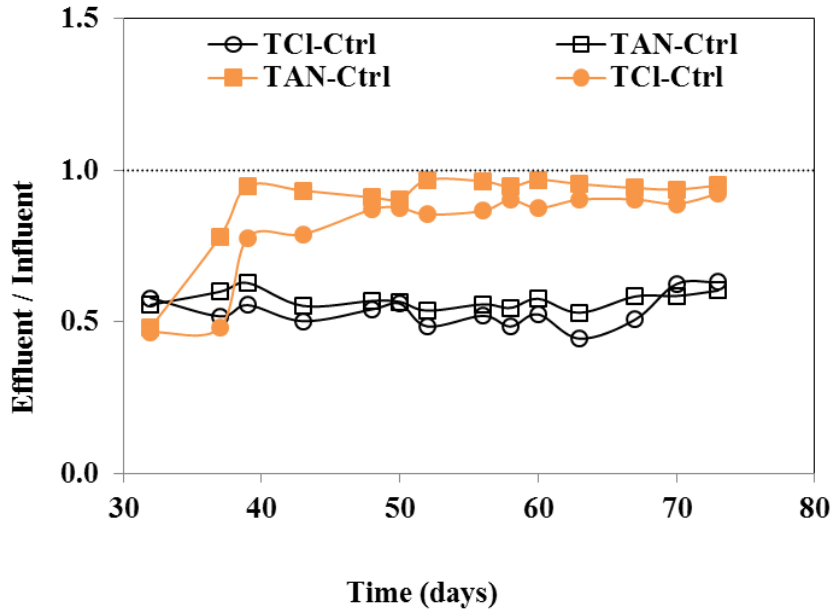

Figure 9.7B: Effluent to influent ratio of TCl and TAN of R-3/2 with R-2/2

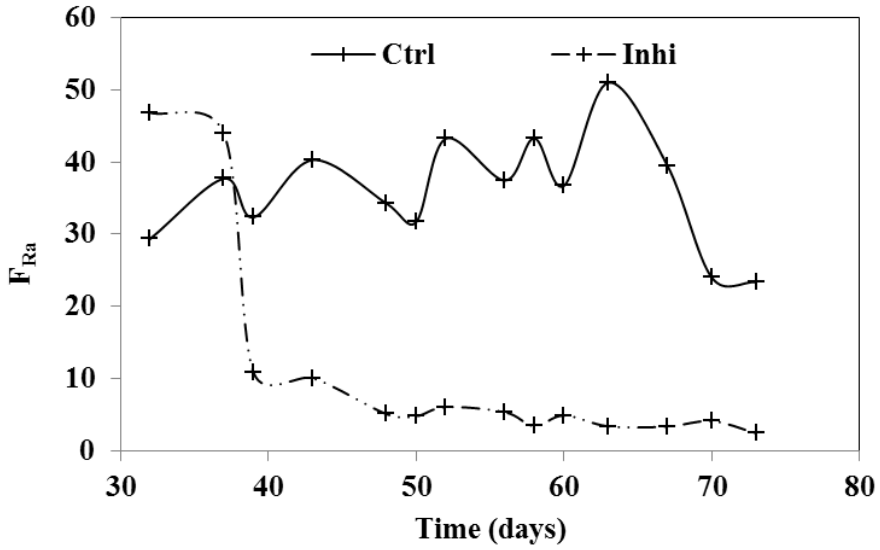

Figure 9.7C: Comparison of F_{Ra} values of R-3/1 and R-3/2

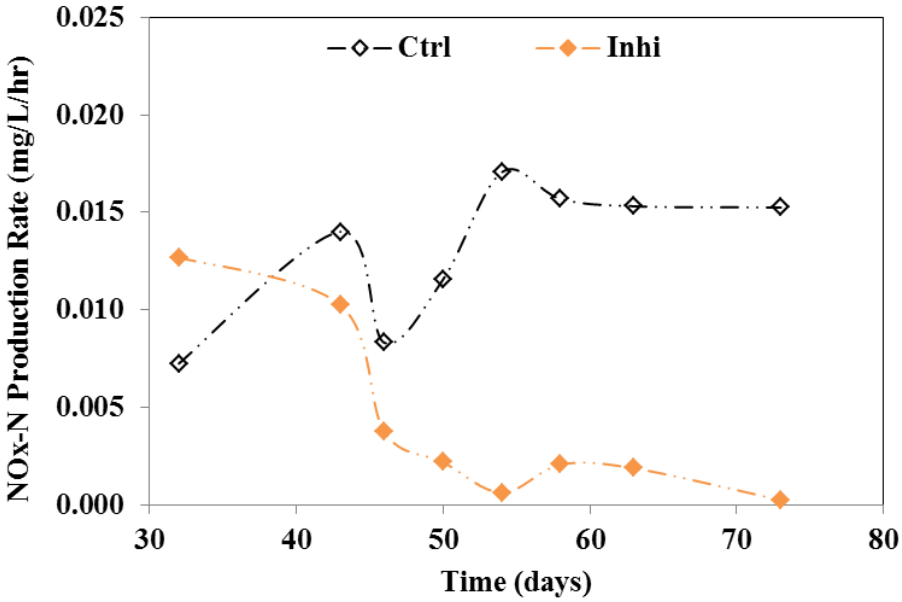

Figure 9.7D: Variation of NOx-N production rate of R-3/1 and R-3/2

A comparative study by calculating effluent to influent ratio of TCl and TAN of R-3/1 and R-3/2 is shown in Figure 9.7B. From Figure 9.7B, it is found that this ratio of TCl and TAN in R-3/1 were approximately 0.6 throughout the experimental period. On the other hand, this ratio increased towards one within 10 days and remained stable in the case of R-3/2. The effluent/influent ratio of one in R-3/2 represented that there was no active nitrifying bacterial activity and the condition of the reactor R-3/2 was tending to the upstream reactor (R-2/2) characteristics. Therefore, copper was effective in controlling nitrification as well as chloramine decay when dosed in the reactor where onset of nitrification was occurred. It was proved by calculating F_{Ra} and NOx-N production rate of R-3/1 and R-3/2 (Figure 9.7C & D). The levels of F_{Ra} and NOx-N production rate of R-3/2 were reduced to zero and lower than that of R-3/1. Lower F_{Ra} value indicates lower chloramine decay and lower NOx-N production rate indicates inhibiting nitrifying bacteria in the reactor. For clear understanding, it would necessary to investigate the impacts of copper inhibition on decay characteristics and F_m values that was described in the following section.

9.3.3.2 Effects of copper inhibition on chloramine decay rates and *Fm*

Figure 9.8 shows the chloramine decay coefficients of the reactor R-3/2 during the copper dose in the same reactor. The k_t, k_c and k_m of R-3/2 before copper doses were 0.0299±0.0030, 0.0155±0.0020 and 0.0144±0.0025 hr^{-1} respectively. Due to maintenance of continuous copper dosing for 38 days in the reactor, these decay rates were reduced to 0.0019±0.0004, 0.0012±0.0004 and 0.0007±0.0004 hr^{-1}, respectively. From Figure 9.8, it is seen that the decay rate gradually reduced significantly due to copper inhibition with copper dosing time. After 32 days of copper dosing, the decay rate coefficients were constant while considering experimental error. Furthermore, F_m value of the reactor gradually reduced from 0.93 (before copper dosing) to 0.58 (after copper dosing).

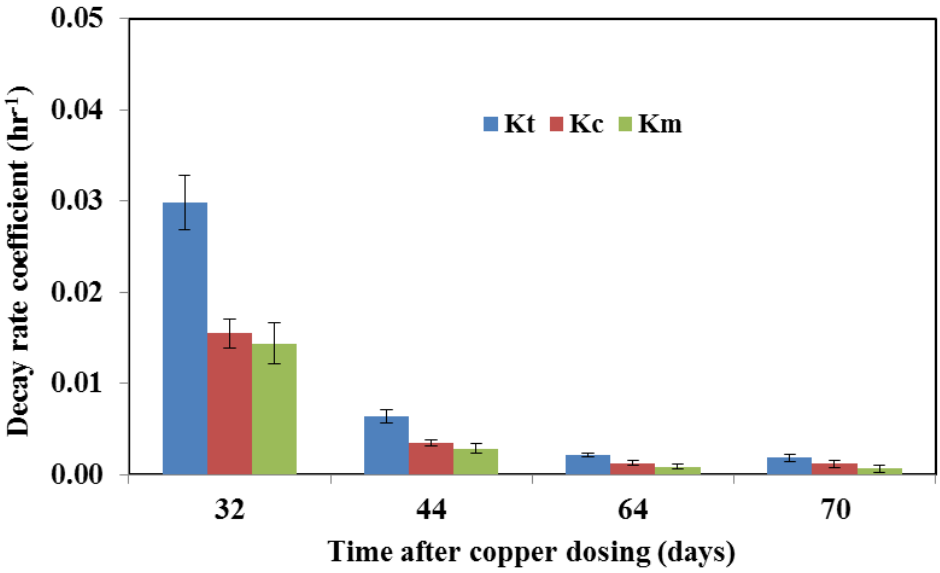

Figure 9.8: Impacts of copper on chloramine decay rates at R-3/2

Therefore, it could be said that dosing of copper continuously at the point of onset of nitrification can significantly help improving the chloramine residual and limit ammonia oxidizing bacterial activity. The reactor in this case represents a service reservoir in the real distribution system, although it should be noted that surface area to volume ratio of the reactor was close to 18 m^{-1}, which represents a pipe line with about 220 mm diameter. To investigate the copper effectiveness in the pipe line downstream of the dosed service reservoir, it was required to study the behaviour of succeeding reactors of the copper dosed reactor.

9.3.3.3 Effects of copper inhibition on immediate succeeding reactor (R-4/2) when dosed in R-3/2

Due to copper dosing in R-3/2, the succeeding reactors R-4/2 and R-5/2 were also inhibited as there was no loss of copper throughout the system. The retention time of R-3/2 was 16 hrs. The impact of copper inhibition after 16 hrs could be examined by monitoring the parameters in the immediate successor reactor (R-4/2) during copper dosing in R-3/2. In this case, R-4/1 and R-4/2 were used as the control and inhibited reactors, respectively. From the experimental results, it was observed that the values of TCl and TAN of copper inhibited reactors increased with the experimental period over control reactors, whereas there was a downward trend of NOx-N levels in the

copper inhibited reactors (Figure 9.9A). Moreover, the ratio between the effluent and influent values of TCl and TAN increased to 0.9 within 15 days that were more than that of R-4/2. On the other hand, from Figure 9.9 C & D, it was observed that the F_{Ra} and NOx-N production rate of R-4/2 started to decrease during copper dosing in R-3/2. Thus, chloramine decay was reduced and nitrifying bacteria was inhibited in the reactor R-4/2.

Similar to copper dosed reactor, the decay rate coefficients of R-4/2 was decreased gradually until a constant value was obtained. The values of k_t, k_c and k_m of R-4/2 at different times after copper dosing in R-3/2 are shown in Figure 9.9E. It was observed that the values of k_t, k_c and k_m of R-4/2 before getting inhibited were 0.0396±0.0160, 0.0142±0.0109 and 0.0254±0.0134 hr^{-1} respectively. Due to copper dosing in R-3/2 for 41 days, these decay coefficients were reduced to 0.0026±0.0007, 0.0012±0.0005 and 0.0014±0.0006 respectively. The F_m value of R-4/2 and R-5/2 was reduced from 1.79 to 1.0 and 0.87 to 0.19 respectively.

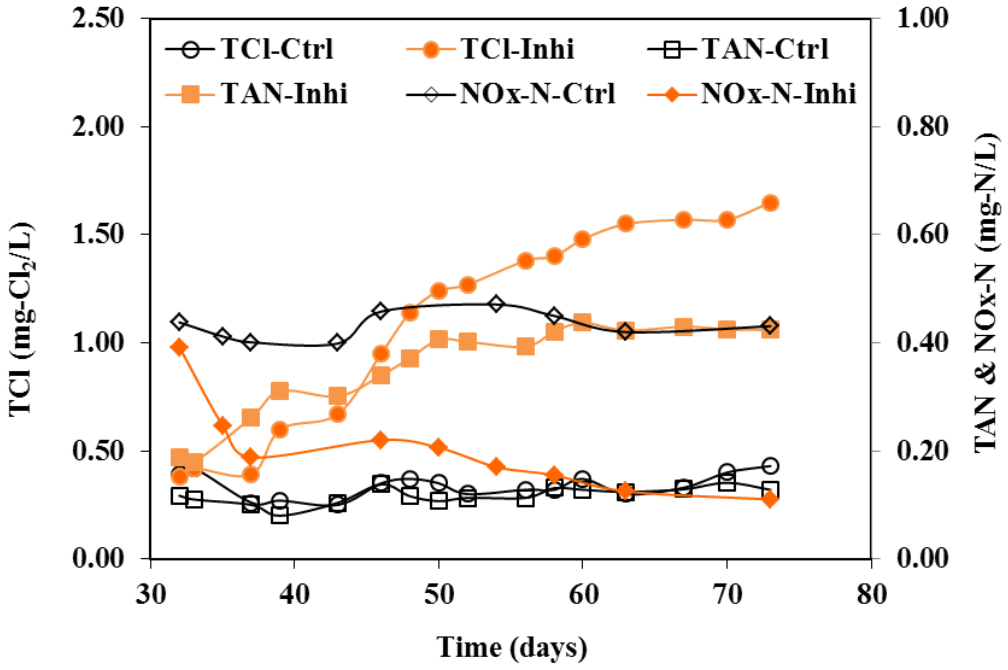

Figure 9.9A: Profiles of TCl, TAN and NOx-N of R-4/1 and R-4/2 when copper dosed in R-3/2

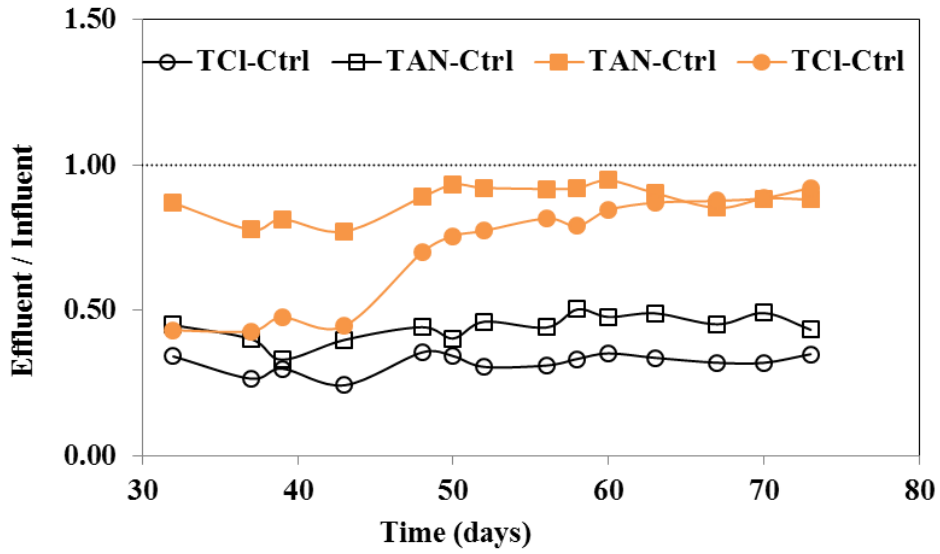

Figure 9.9B: Effluent/Influent ratio of R-4/1 and R-4/2

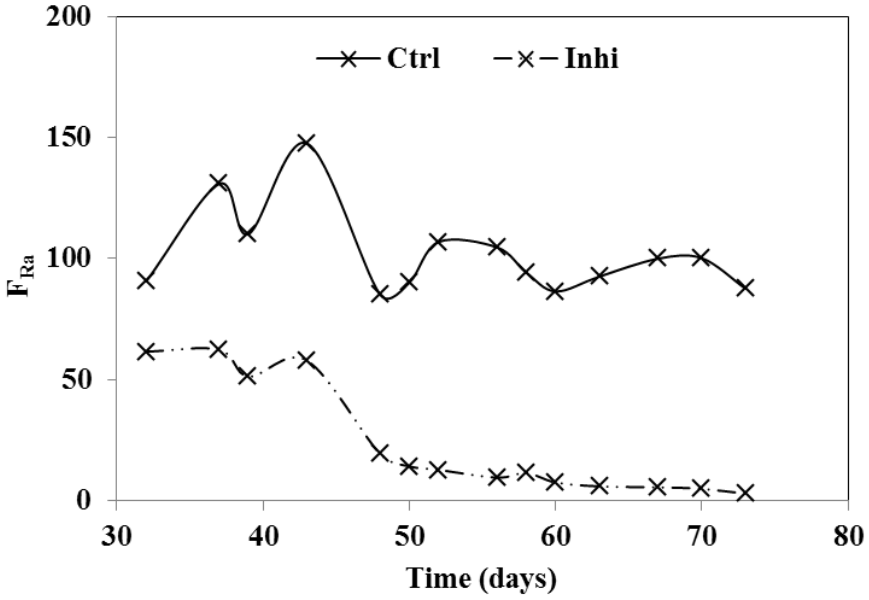

Figure 9.9C: F_{Ra} variation of R-4/1 and R-4/2 along copper dosing period

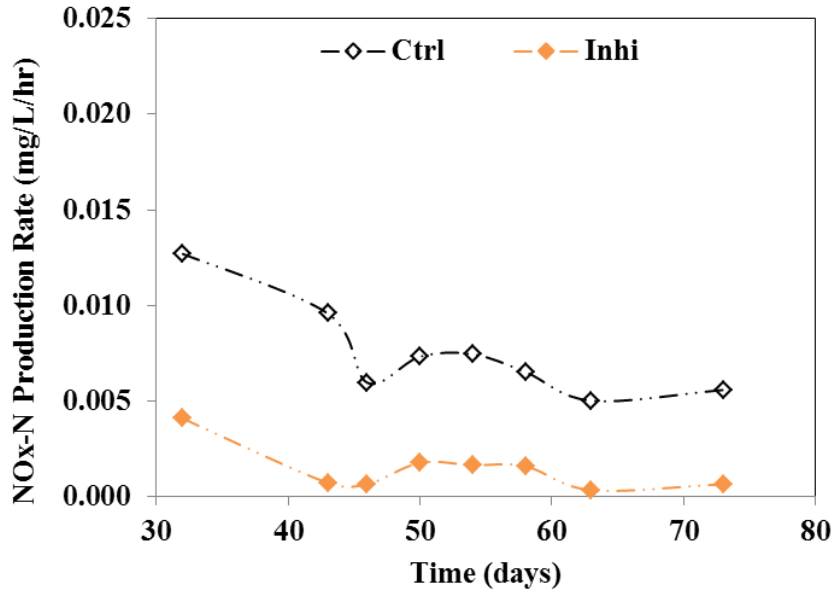

Figure 9.9D: NOx-N production rate of R-4/1 and R-4/2 along the copper dosing time

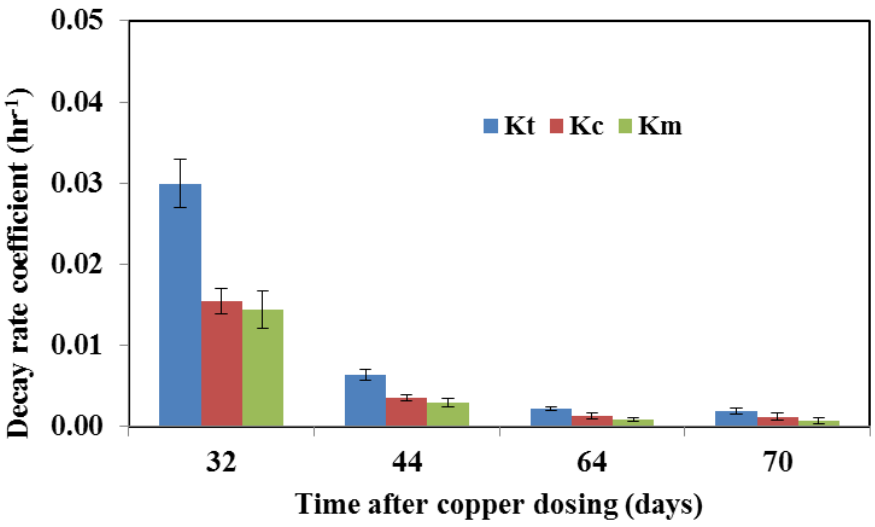

Figure 9.9E: Decay rate coefficients of R-4/2 when copper dosed in R-3/2

Similar to copper dosed reactor, the decay rate coefficients of the succeeding reactors was decreased gradually until a constant value was obtained. The values of k_t, k_c and k_m of R-4/2 at different times after copper dosing in R-3/2 are shown in Figure 9.9E. It was observed that the values of k_t, k_c and k_m of R-4/2 before getting inhibited were 0.0396±0.0160, 0.0142±0.0109 and 0.0254±0.0134 hr^{-1} respectively. Due to copper dosing in R-3/2 for 38 days, these decay coefficients were reduced to 0.0026±0.0007, 0.0012±0.0005 and 0.0014±0.0006 respectively. It should be noted that higher experimental error in decay coefficient estimation was mainly due to difficulty in fitting the first order decay curve rather than the error or scattering in the data points.

9.3.3.4 Effects of copper inhibition on succeeding reactors (R-5/2) when dosed in R-3/2

The retention time of R-3/2 and R-4/2 were 16 and 14 hrs respectively. Therefore, it was possible to check the copper inhibitory effects after 28 hrs by study the behavior of R-5/2 reactor while copper was dosed in R-3/2. In this case, R-5/1 and R-5/2 were used as the control and inhibited reactors respectively. Similar to the previous two Sections (9.3.3.2 and 9.3.3.3), a comparative study was done between the control reactor and the copper inhibited reactor by using the monitoring values of TCl, TAN

and NOx-N, influent/effluent ratio of TCl and TAN, calculated values of F_{Ra} and NOx-N production rate. The graphical representation was shown in Figure 9.10 A, B, C and D. As compared to the control reactor, the inhibited rector had higher levels of TCl and TAN (Figure 9.10A), higher effluent/influent ratio of TCl and TAN (Figure 9.10B), lower levels of F_{Ra} (Figure 9.10C) and NOx-N production rate (Figure 9.10D). Moreover, an increasing trend of TCl and TAN levels, and a downward trend of F_{Ra} and NOx-N production rate were found in the inhibited reactor. Therefore, it could be said that copper was effective for nitrification control and thus improving chloramine residual in the succeeding reactor.

Based on the above discussion, it would be possible to overcome nitrification and chloramine decay in the downstream reactors by complete inhibition at the point when nitrification is first starting to appear (onset of nitrification). Therefore, dosing continuously on nitrification accelerating point, copper was effective in controlling nitrification and chloramine decay in the dosing reactor as well as succeeding reactors.

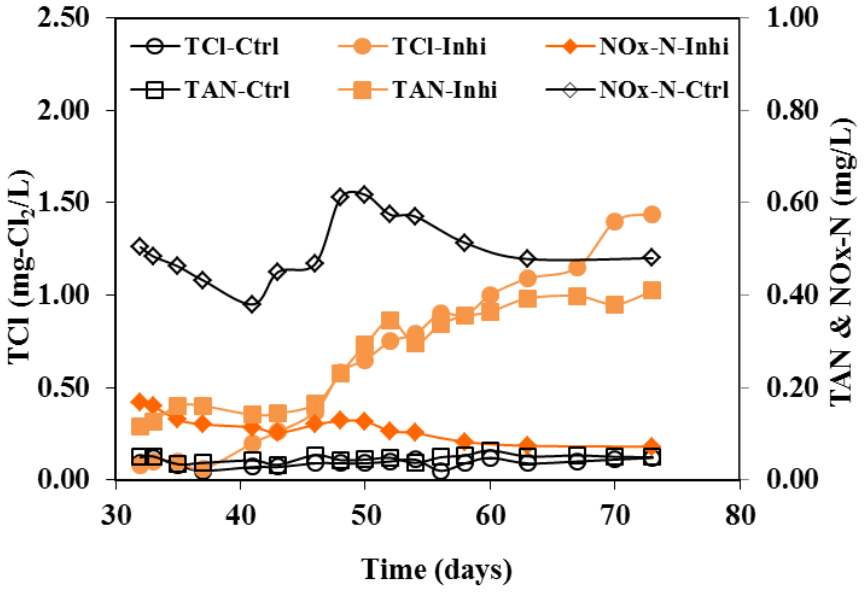

Figure 9.10A: Profiles of TCl, TAN and NOx-N of R-5/1 and R-5/2 when copper dosed in R-3/2

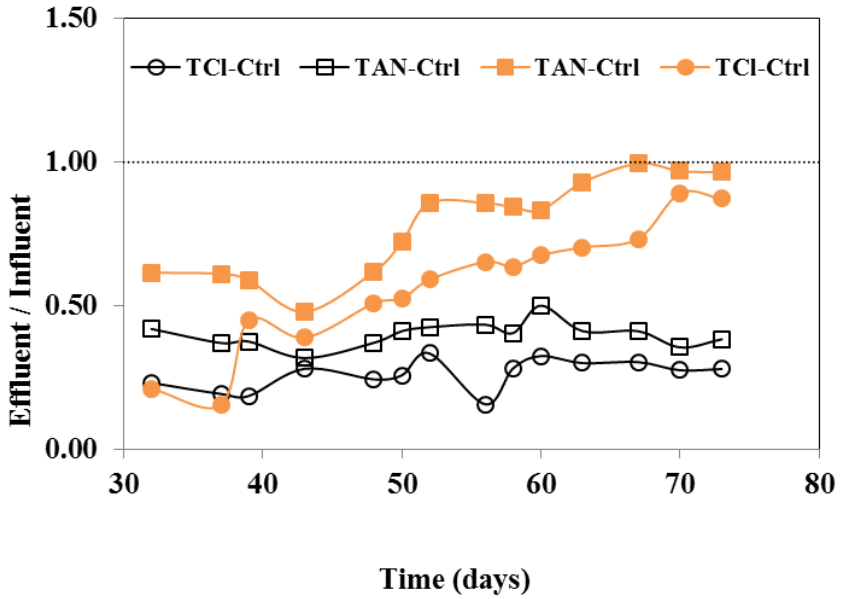

Figure 9.10B: Effluent/Influent ratio of R-5/1 and R-5/2 when copper was dosed in R-3/2

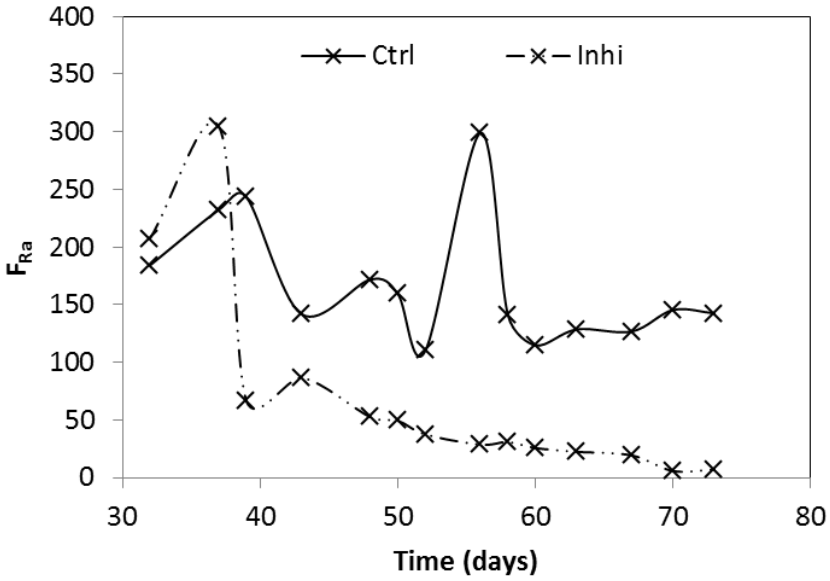

Figure 9.10C: F_{Ra} variation of R-5/1 and R-5/2 when copper was dosed in R-3/2

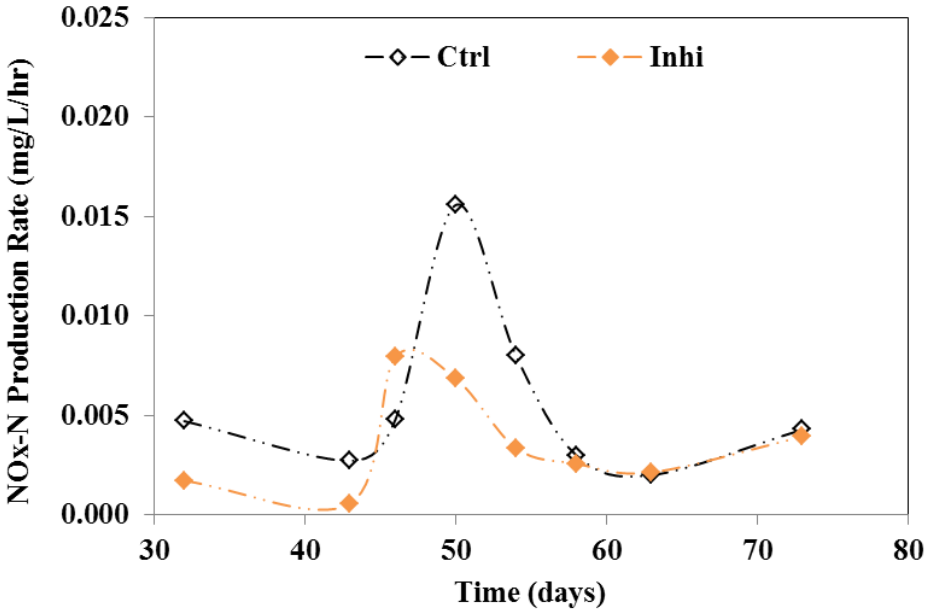

Figure 9.10D: NOx-N production rate of R-5/1 and R-5/2 when copper was dosed in R-3/2

9.3.4 Role of Copper in Controlling Sediment Contribution on Chloramine Decay

The third set of experiment was conducted to investigate the inhibitory ability of copper on chloramine decay due to the presence of sediment. Aryal (2011) reported that sediment besides with bulkwater plays a major role in chloramine loss. The experiment was conducted by collecting samples from reactors R-3/2 and R-4/2, while copper was dosed into reactor R-3/2. The sediment concentration in the reactors R-3/2 and R-4/2 were 3.6 and 2.0 mg-TSS/L respectively. Chloramine decay tests were performed by collecting the samples from R-4/2 in three different operating times during continuous copper dosing in the reactor R-3/2 and the inhibition efficiency was determined by calculating k_t. The variations of k_t of bulkwater and sediment of reactor R-4/2 at different time during copper dosing in R-3/2 are shown in Figure 9.11.

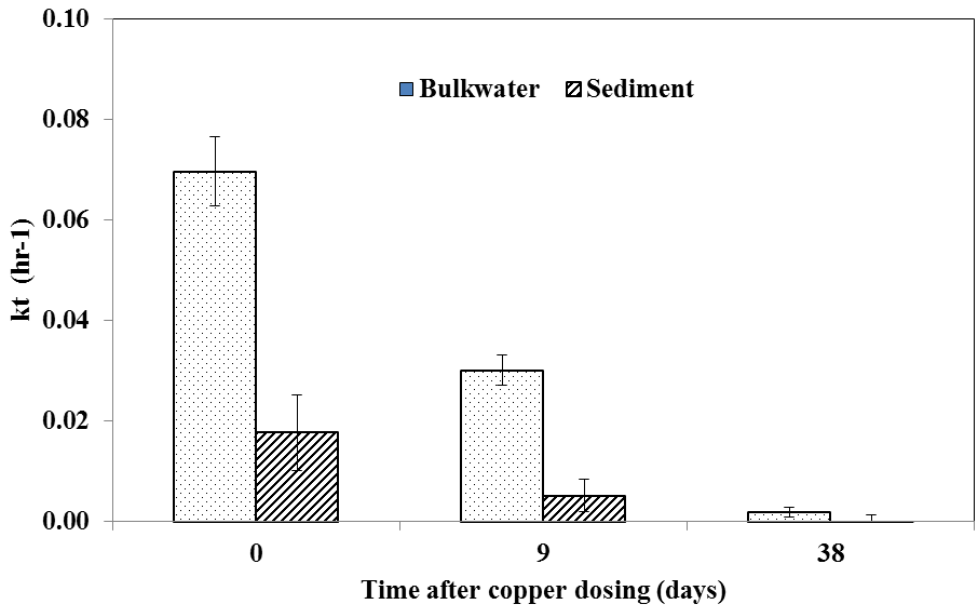

Figure 9.11: Inhibition of bulk water and sediment of R-4/2 at different time interval after copper dosing in R-3/2.

Before starting to dose copper, it was noticed that k_t of bulkwater and sediment were 0.0697±0.020 and 0.0177±0.0041 hr^{-1} respectively, indicating about 25% of chloramine loss was attributed to sediment in R-4/2. From Figure 9.11, it is observed

that k_t values were gradually decreased with increasing copper dosing time for bulk waters and sediments. After 38 days, the k_t values are 0.0018±0.0007 and 0.0004±0.0005 hr^{-1} for bulkwater and sediment respectively. Therefore, it could be said that copper inhbition was effective on reducing chloramine decay rate wherein, about 95% chloramine residual loss due to sediment was minimised by continuous copper dosing.

9.4 Behavior of Inhibited Nitrified Bulk Waters after Stopping Copper Dosing: Investigation of Nitrifying Bacterial Activity Retrieval

The fourth set of experiment was conducted to assess the inhibition efficiency of dosing copper on nitrified samples for a long time. The copper inhibited reactors were monitored for 50 days, after stopping copper dosing. It was noticed that there was no loss of TCl and TAN concentrations; as well there was no increase/decrease in NOx-N concentrations of the reactors (R-3/2 to R-5/2) due to stopping of copper dosing in the reactors. Figure 9.12 shows the profiles of TCl, TAN and NOx-N of copper inhibited reactor R-4/2. From Figure 9.12, it is seen that the trend of TCl, TAN and NOx-N were constant. Stable residual concentration and constant NOx-N profiles proved that nitrification did not return back once it was inhibited with copper at the onset point of nitrification.

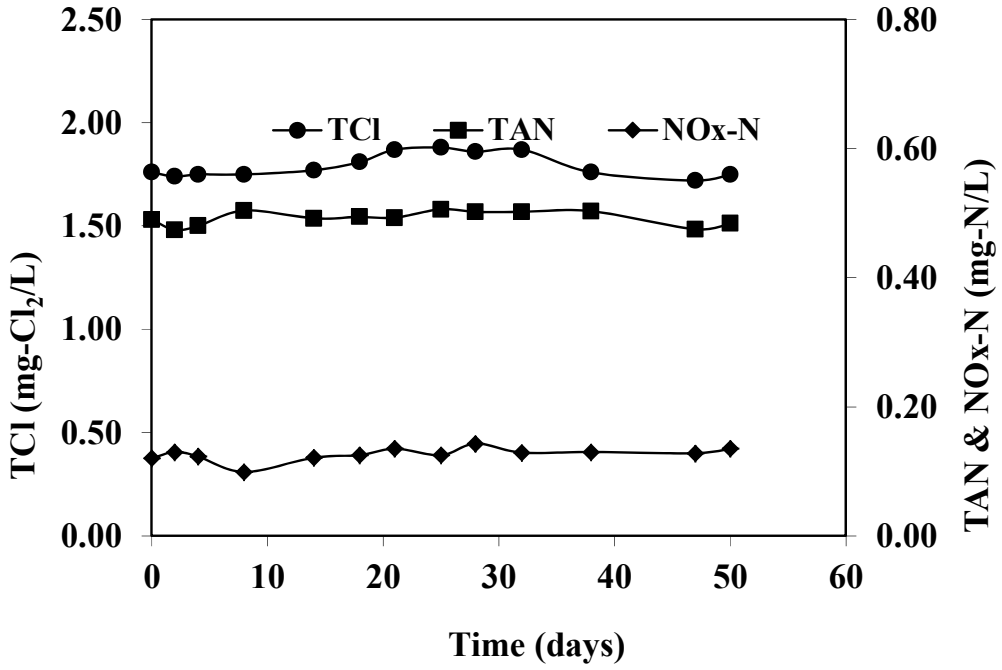

Figure 9.12: TCl and nitrogenous profiles of reactor 4/2 after stopping copper dosing.

The performance in terms of nitrification inhibition and thus chloramine residual improvement inside the reactors by adding copper under different nitrification conditions is presented in Table 9.2

Table 9.2: Effectiveness of copper inhibition when dosed in different nitrification conditions of chloraminated bulk waters

Evaluation Criteria	Sampling conditions at copper dosing points	
	Severely nitrified bulk water	Onset of nitrified bulk water
Monitoring trend of surrogate parameter's profile	No improvement of TCl but a marginal amount of TAN improvement and a slight NOx-N reduction was observed in copper dosed and succeeding reactors.	There was an upward level in TCl and TAN concentration and a downward level of NOx-N concentrations was observed in copper dosed and succeeding reactors.
Comparison of Effluent to influent ratio of surrogate parameters (TCl and TAN) between the control and the copper dosed reactor.	Though this ratio was almost constant for TCl but it was an upward trend for TAN in copper dosed and succeeding reactors.	This ratio increased one within 10 days and remained constant in the copper dosed reactors and succeeding
F_{Ra} and NOx-N production rate	F_{Ra} and NOx-N production rate decreased along with copper dosing time in copper dosed reactors but was not remarkably changed in in succeeding reactors.	The levels of F_{Ra} and NOx-N production rate of copper dosed reactors were reduced to zero and lower than that of control reactor.

9.5 Monitoring of Copper Concentration in the Reactors

Copper concentrations were monitored three times in a week in the dosing reactors and the succeeding reactors to maintain the appropriate concentration. It was noticed that the observed copper concentrations were same as the applied concentrations throughout the experimental periods. The probable reasons might be the continuous copper dosing and the continuously flowing water from the upstream reactors where nitrifying bacteria were not present. The observed copper levels are presented in Figure 9.13.

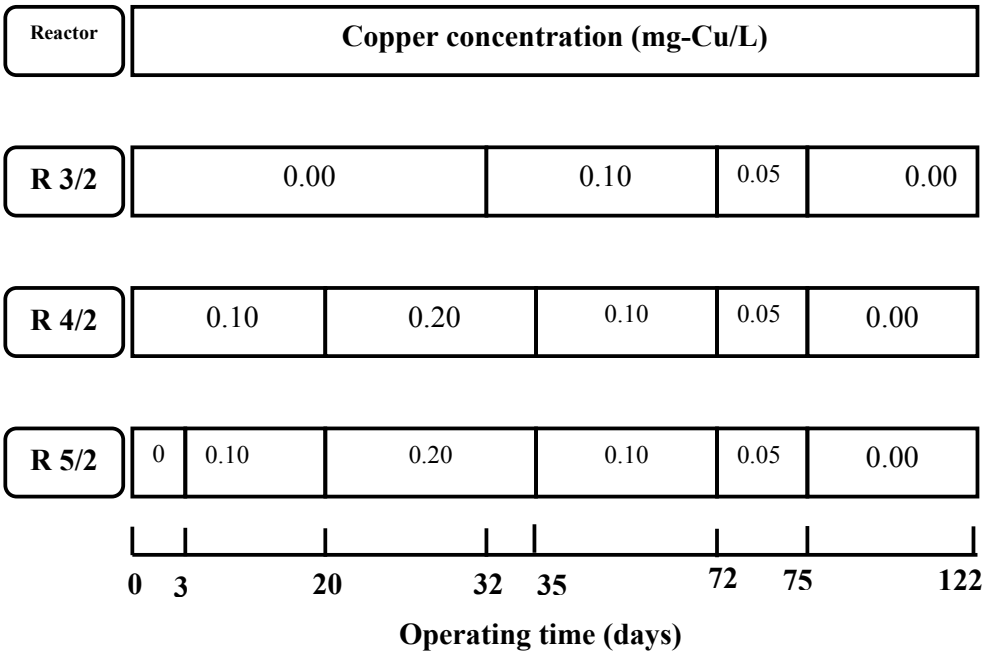

Figure 9.13: Copper concentrations in the reactors along the operating time.

9.6 Conclusions

In order to improve the chloramine residual by inhibiting nitrification, copper was dosed continuously into the reactors at severely nitrifying and onset of severely stages. The experimental results showed the followings:

- In both conditions (onset and severe nitrified), copper inhibited the ammonia oxidising bacterial activities at concentrations less than or equal to 0.20 mg-Cu/L.
- When copper was dosed in severely nitrified samples, there was no noticeable improvement of chloramine residual in the dosing reactor or the succeeding reactors. The probable cause was nitrified water was coming continuously from the upstream reactor.
- When copper was dosed in the reactor where the onset of nitrification had taken place, there was a remarkable improvement of chloramine residual and ammonia in the dosing reactor and even succeeding reactors.
- Copper was found to be effective in controlling chloramine decay due to sediments.
- Copper was effective in nitrification inhibition and not permitting to regain the nitrifying bacterial activity even after stopping copper dosing, especially when the emerging point of nitrification (the reactor with onset of nitrification condition) was targeted.

CHAPTER 10

CONCLUSIONS AND RECOMENDATIONS

The focus of the research was to control nitrification in order to maintain an adequate chloramine residual in the distribution system. Due to limited satisfactory performance of traditional control measures of nitrification, use of copper as a novel nitrification inhibitor was examined in this study. In addition, the effects of pH and temperature that governs chloramine decay were discussed.

A pilot-scale system consisting two set of reactors (five reactors connected in series in each set) were established to obtain various nitrification conditions in the reactors simulating a full-scale distribution system. The reactors were fed continuously with chloraminated water and the nitrification conditions became severe with increasing retention time along the reactors. Based on the measured chloramine concentrations and nitrogenous compounds (TAN, nitrite and NOx-N), it was noticed that higher chloramine decay rate was found in severe nitrification than in mild nitrification. Experiments were conducted for samples collected from the reactors (in batch system) as well as into the reactors (semi- and continuous system). The major conclusions and directions of further research of the study are described below.

Role of pH in Controlling Nitrification in Chloraminated Distribution System

Severely nitrified bulk waters collected from the pilot-scale system were adjusted at different pH (7.5, 8.0 and 8.5) to determine the optimum pH for the better stability of chloramine residual. As an outcome of the study, it can be said that pH adjustment help in preventing nitrification. High amount of free ammonia (energy source for AOBs) and lower chloramine decay was observed in high pH (8.5) when compared to low pH (7.5). As chloramine stability is the prime importance than free-ammonia control, adjusting pH at high level (around 8.5) is better in controlling chloramine decay and suppressing the activity of nitrifiers.

Modelling Temperature Effects to Incorporating into Biostability Concept

Considering temperature effects on maximum growth rate (μ_m) and inactivation rate (k_d) of AOB, an attempt was made to incorporate these effects on μ_m/k_d into biostability concept by developing a model. Batch and continuous flow systems experimental results (obtained from the two full scale distribution systems and the pilot-scale system) were used to validate the model. According to the model, the μ_m/k_d values at 15°C and 27°C temperature are 1.87 and 1.83 mg-Cl_2/L respectively and that are very close to 2 mg-Cl_2/L (traditionally used by other researchers). The maximum value of μ_m/k_d is 2.07 at 23^{o}C which lies within the range. On the contrary, there are remarkable differences of μ_m/k_d values from the proposed model and 2 mg-Cl_2/L at temperatures above 27^{o}C and below 15^{o}C. Therefore, it can be demonstrated that temperature variations will greatly impact on biostable residual concentration (BRC), especially at temperatures above 27^{o}C or below 15^{o}C.

Modelling the Effects of Copper and Chloramine in Chloraminated Bulk Waters

The inhibitory effects of copper were assessed by conducting batch experiments on severely nitrified bulk waters collected from the pilot-scale system. Samples were prepared by 2.0 mg-Cl_2/L chloramine and spiked with different doses (0.10, 0.20, 0.25 and 0.40 mg-Cu/L) of copper. Copper was not effective to control nitrification at very lower concentration (0.10 mg-Cu/L), but was effective at 0.20 mg-Cu/L copper concentration for a short time. Copper concentrations greater than or equal to 0.25 mg-Cu/L could strongly inhibit nitrification. Copper inhibition efficiency also depends on copper concentration. In addition, it was found that copper could delay the onset of nitrification and late onset of nitrification was found in samples inhibited by higher copper concentration. For better understanding of how copper would act as an inhibitor, a model was proposed considering disinfection ability of chloramine and copper individually as well as cumulatively. From the model, it could be said that copper concentration up to 0.25 mg-Cu/L with chloramine or 0.40 mg-Cu/L alone might play an important role in controlling nitrification. In a precise form, it could be said that copper inhibition could act as an early intervention to stop nitrifying bacterial activity and thus to protect chloramine residual.

Assessing Chemical Effects of Copper with Varying pH Conditions and Decay Mechanisms in Severely Nitrified Bulk Waters

The addition of different doses of copper (0.25, 0.50 and 1.00 mg-Cu/L) with 2.0 mg-Cl_2/L chloramine in filtered severely nitrified bulk waters could explain the chemical effects of copper on chloramine decay and nutrients (TAN, NO_2-N and NOx-N) levels. The decay coefficients and nutrients profiles were same for filtered and copper added filtered samples, demonstrated that there was no contribution of copper chemically on chloramine decay and nutrients levels. To see the chemical effects of copper under varying pH, experiments were conducted for filtered severely nitrified bulk waters adjusting at different pH (7.5, 8.0 and 8.5) conditions and 0.25 mg-Cu/L copper was added in each sample. The behavior of the samples under varying pH conditions revealed that residual was improved in higher pH (8.5) samples. Therefore, pH variations affected chloramine decay but copper addition did not affect on chloramine decay and nutrients levels in filtered severely nitrified bulk waters. Nitrogen mass balance was done to explain the decay mechanism in filtered severely nitrified bulk waters. Using stoichiometry, it was found that the major chloramine decay mechanisms in the filtered severely nitrified bulk waters were attributed by auto-decomposition and nitrite oxidation.

Copper Inhibition at Different Nitrification Stages

The inhibitory effects of copper were assessed in different nitrification conditions and different aspects of reactor flow conditions.

Firstly, the reactor was operated in semi-continuous mode feeding with NOM free chloraminated water. The copper concentrations were increased gradually from 0.25 to 1.00 mg-Cu/L in the reactor containing severely nitrified bulk waters. The experimental results showed that copper at low concentration (0.25 mg-Cu/L) was effective for inhibiting nitrification but not effective for controlling chloramine decay even by increasing feed water chloramine concentration from 0.85 to 2.0 mg-Cl_2/L. Desirable nitrification inhibition and residual improvement was observed with higher copper concentration (1.00 mg-Cu/L).

Secondly, copper was dosed in the pilot-scale reactor system, operated in continuous flow condition. Different stages of nitrification were created in the reactors that were similar to full-scale chloraminated distribution system. Copper was dosed at severely nitrified and onset of nitrification stage. Copper was continuously dosed in two phases in severely nitrified bulk waters. First phase - 0.10 mg-Cu/L for 20 days and second phase - 0.20 mg-Cu/L for 12 days. For both phases, reservoir acceleration factor (F_{Ra}) and NOx-N values were reduced and chloramine residual was improved marginally in the copper dosed reactor. Conversely, nitrification was controlled but no residual improvement or F_{Ra} values reduction in the succeeding reactors.

On the other hand, due to continuous dosing of copper (0.10 mg-Cu/L) on the onset of nitrification stage for 41 days, nitrification was fully overwhelmed and noticeable residual improvement was found in the dosing and succeeding reactors. Effluent to influent ratio of TCl, TAN and NOx-N was close to one in the copper dosing reactor and succeeding reactors. The total (k_t), chemical (k_c) and microbial (k_m) decay coefficient of copper dosing reactor were reduced from 0.0299±0.0030, 0.0155±0.0020 and 0.0144±0.0025 hr^{-1} to 0.0019±0.0004, 0.0012±0.0004 and 0.0007±0.0004 hr^{-1} respectively. The F_m value of the copper dosing reactor was reduced from 0.93 (before copper dosing) to 0.58 (after copper dosing) and F_{Ra} was decreased with the increasing time of copper dosing. Copper inhibition efficiency of sediment contribution on chloramine decay was examined by conducting decay test. Results showed that about 95% chloramine residual loss due to sediment was minimised by continuous copper dosing. The identical results were observed in the succeeding reactors.

For checking the effectiveness of copper inhibition for long time whether the nitrification episode returned back or not, copper dosing was stopped and the surrogate parameters (TCl, TAN and NOx-N) in the dosing and succeeding reactors were monitored for 50 days. Constant levels of TCl, TAN and NOx-N concentrations indicated that there was no sign of returning back of nitrification even after stopping copper dosing. It was found that proper selection of copper dosing point in regards of nitrification was crucial for controlling nitrification and residual management. The total outcomes of the study are presented in Table 10.1.

Table 10.1: Overall Summary of Copper Inhibition on Nitrification in chloraminated bulk waters

Objectives	Reactor Condition	Final outcomes
Role of pH effects on nitrification control in severely nitrified bulk waters	Batch Flow	Maintaining high pH (8.5) was found to be effective in controlling chloramine decay and suppressing the activity of nitrifiers.
Incorporating temperature effects on biostability concept for severely nitrified bulk waters	Batch Flow	A model was proposed to show the temperature effects on biostability equation that could be useful in the distribution systems.
Chemical effects on severely nitrified bulk waters	Batch Flow	Copper has no chemical effect on chloramine decay and nutrients concentrations.
Inhibitory effects of copper when dosed in severely nitrified bulk waters	Batch Flow	Minimum 0.25 mg-Cu/L copper was needed to inhibit ammonia oxidising bacterial activities. Onset of nitrification was found late for higher copper dosed samples. The proposed model suggested that copper alone or with chloramine can prevent nitrification thus could help in maintaining residual in the distribution system.
	Semi-continuous flow	0.25 mg-Cu/L copper was effective for inhibiting nitrification but 1.00 mg-Cu/L copper was effective for improving chloramine residual. Soluble microbial products were an important factor causing acceleration of chloramine decay.
	Continuous flow	No noticeable improvement of chloramine residual was found. 0.20 mg-Cu/L was adequate to inhibit the ammonia oxidising bacterial activities.
Inhibitory effects of copper when dosed on onset of nitrification of bulk waters	Continuous flow	0.20 mg-Cu/L was adequate to inhibit the ammonia oxidising bacterial activities. A remarkable improvement of chloramine residual was found in the dosing reactor and succeeding reactors.

Recommendation for Future Studies

- pH adjustment of different nitrification conditions in full scale systems are needed to understand how pH adjustment would help in improving the chloramine residual.
- Change in microbial communities especially nitrifiers due to copper dosing in different nitrification conditions in pilot-scale and full scale distribution systems should be investigated.
- Copper dosing in none/mild nitrification stages of pilot-scale and full scale distribution system should be studied.
- In order to develop an efficient and universal control approach, inhibitory effects of other metals such as silver, zinc for controlling nitrification and chloramine decay should be assessed. The different nitrification conditions should be considered.
- The co-inhibition efficiency of the metals (copper, zinc, silver) within their permissible limits in pilot-scale system and full scale system under different nitrification conditions should be evaluated.
- Copper effectiveness for controlling chloramine decay and nitrification due to biofilm should be investigated.

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APPENDIX-A

MONITORING DATA OF THE PILOT-SCALE SYSTEM

Table A-1: Operational Parameters of the Reactors of Pilot-Scale Reactor System (average values of 60 days)

Operational parameters	Observed Values				
	R-1/1 & R-1/2	R-2/1 & R-2/2	R-3/1 & R-3/2	R-4/1 & R-4/2	R-5/1 & R-5/2
TCl (mg/L)	1.95±0.03	1.89±0.03	0.93±0.03	0.35±0.03	0.11±0.03
TAN (mg/L)	0.460±0.007	0.450±0.007	0.317±0.005	0.159±0.002	0.072±0.002
Temperature (^{o}C)	20±2	20±2	20±2	23±2	23±2
NO_2-N (mg-N/L)	0.003±0.002	0.009±0.002	0.085±0.002	0.218±0.002	0.275±0.002
NOx-N (mg/L)	0.051±0.002	0.070±0.002	0.200±0.002	0.376±0.003	0.470±0.004
TIN (mg/L)	0.511±0.008	0.520±0.009	0.517±0.010	0.535±0.010	0.542±0.010
DOC (mg/L)	2.75±0.20	2.76 ±0.02	2.76± 0.02	2.79±0.02	2.60±0.02
pH	7.9±0.1	7.9±0.1	7.8±0.1	7.6± 0.1	7.6±0.1

APPENDIX-B

EFFECTS OF pH ON CHLORAMINE DECAY AND NITROGENOUS COMPOUNDS FOR SAMPLE B & C

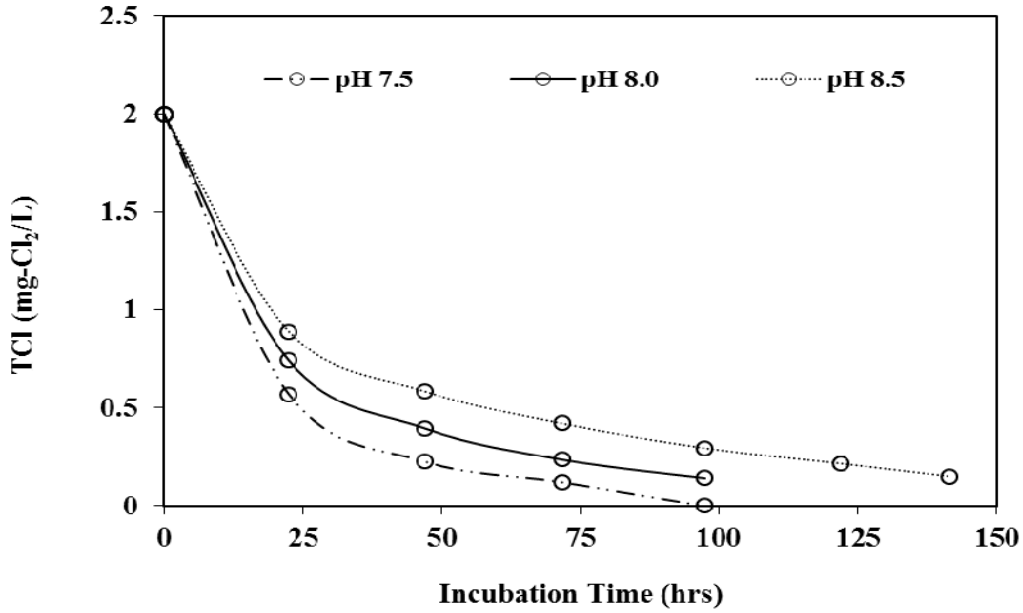

Figure B-1: Effects of pH on TCl profiles at different pH conditions for Sample B

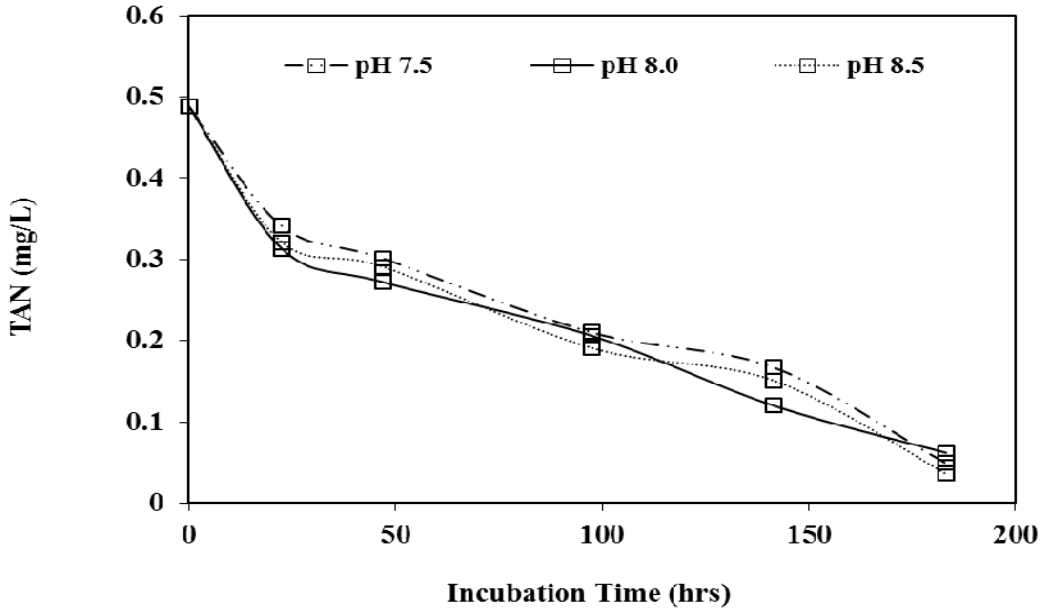

Figure B-2: Effects of pH on TAN profiles at different pH conditions for Sample B

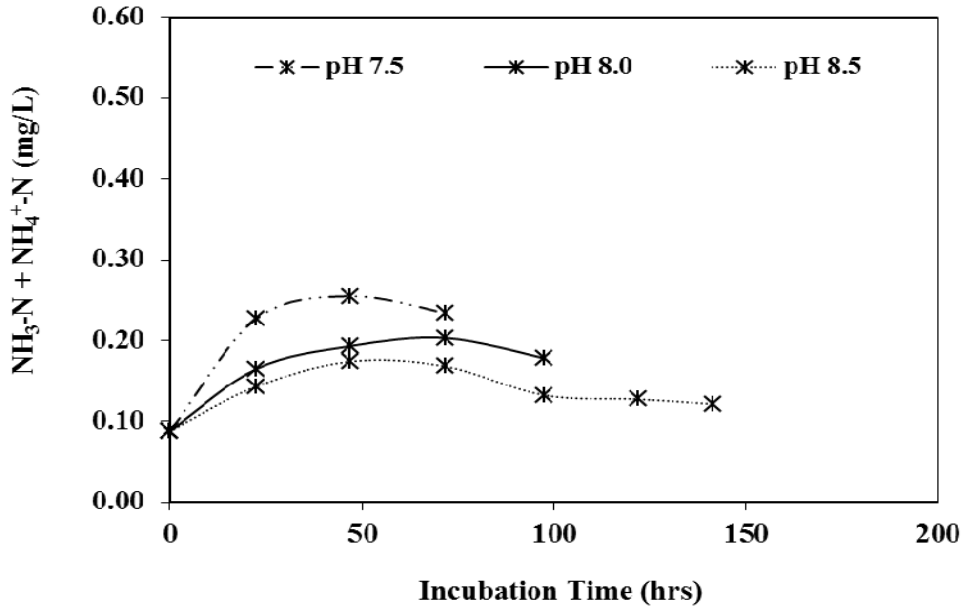

Figure B-3: Effects of pH on NH_3-N+NH_4^+-N profiles at different pH conditions for Sample B

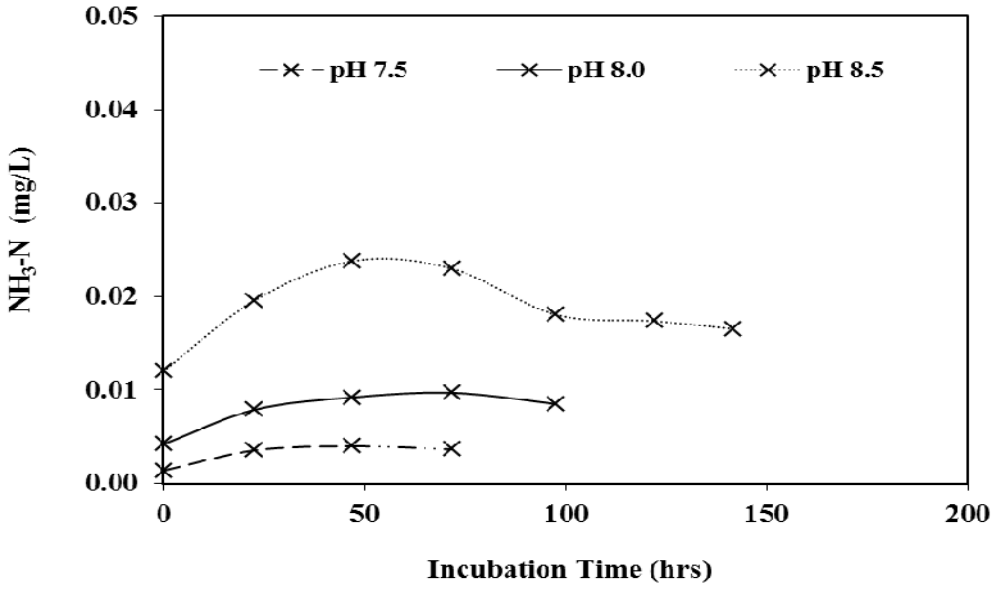

Figure B-4: Effects of pH on NH_3-N profiles at different pH conditions for Sample B

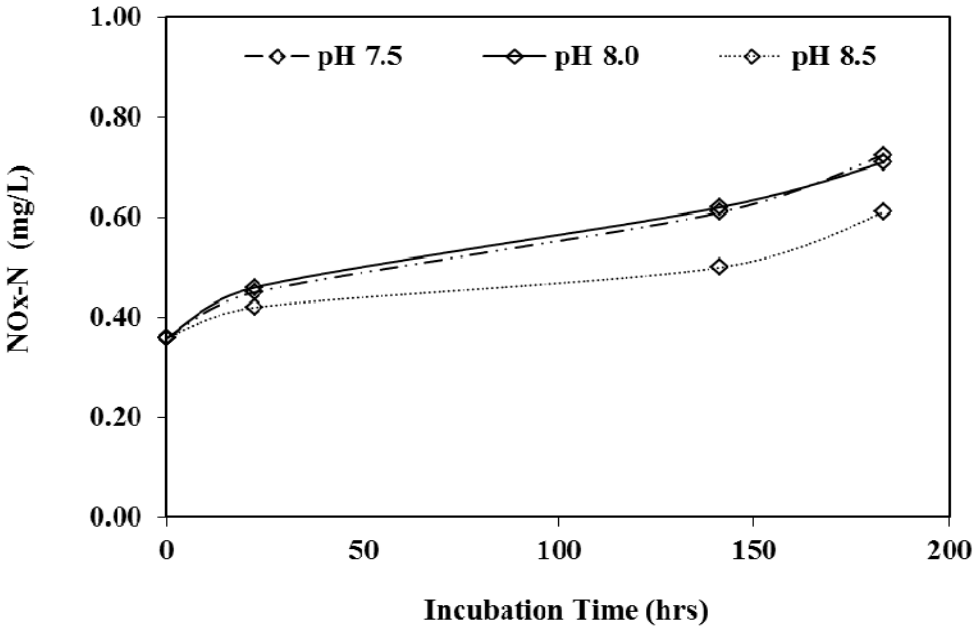

Figure B-5: Effects of pH on NOx-N profiles at different pH conditions for Sample B

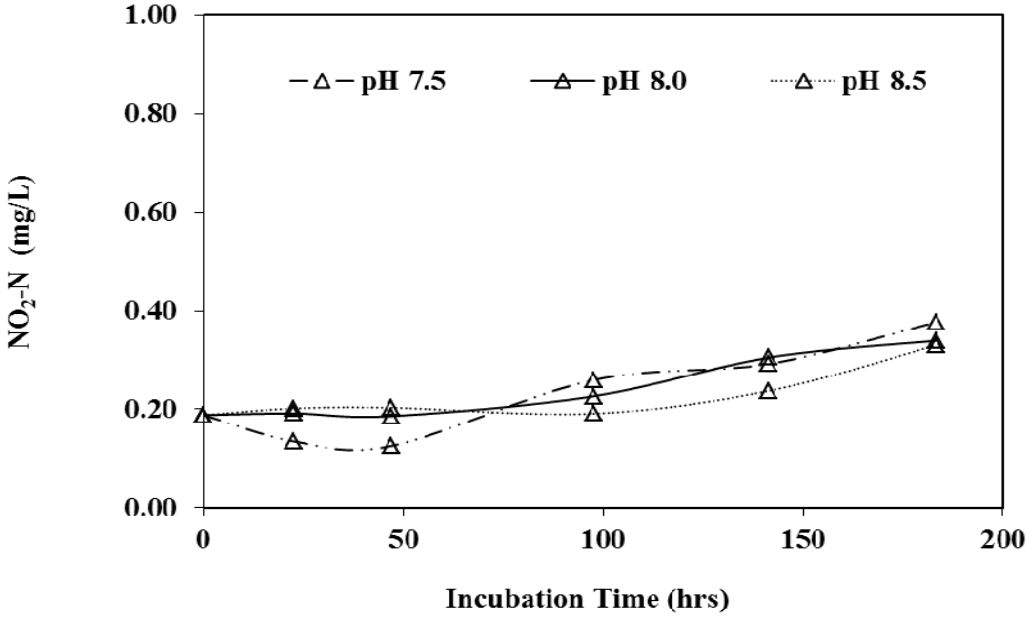

Figure B-6: Effects of pH on NO_2-N profiles at different pH conditions for Sample B

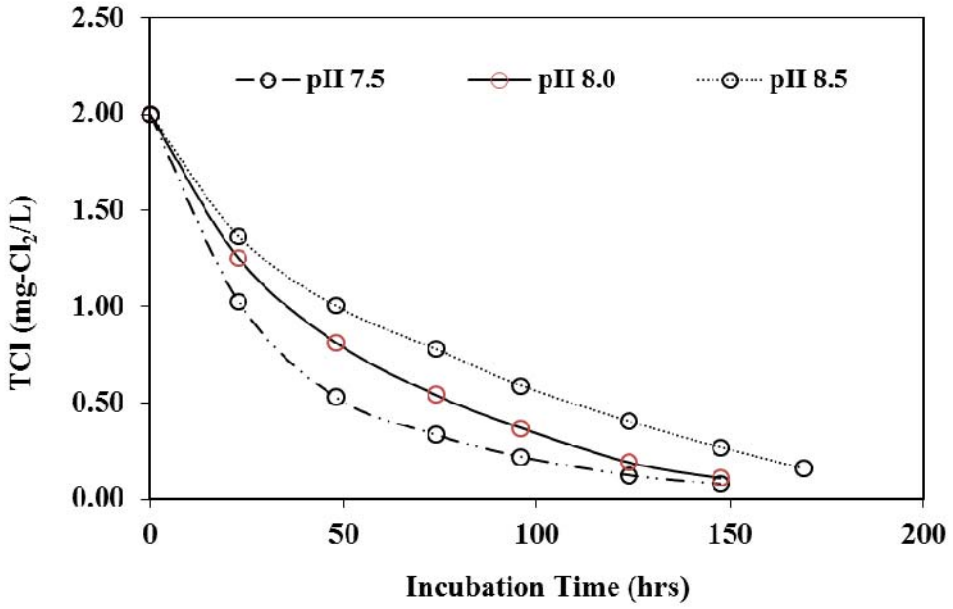

Figure B-7: Effects of pH on TCl profiles at different pH conditions for Sample C

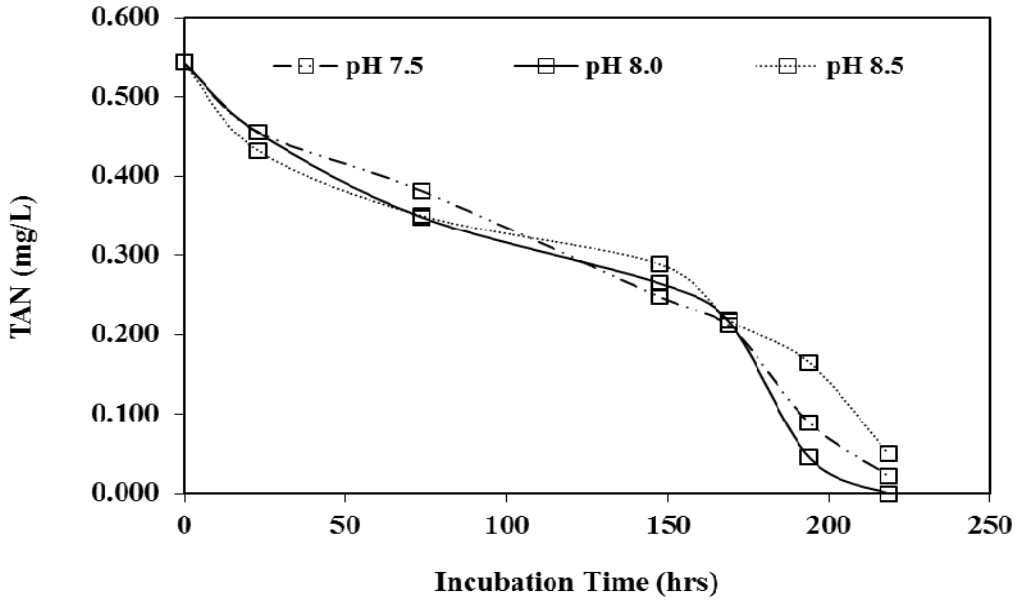

Figure B-8: Effects of pH on TAN profiles at different pH conditions for Sample C

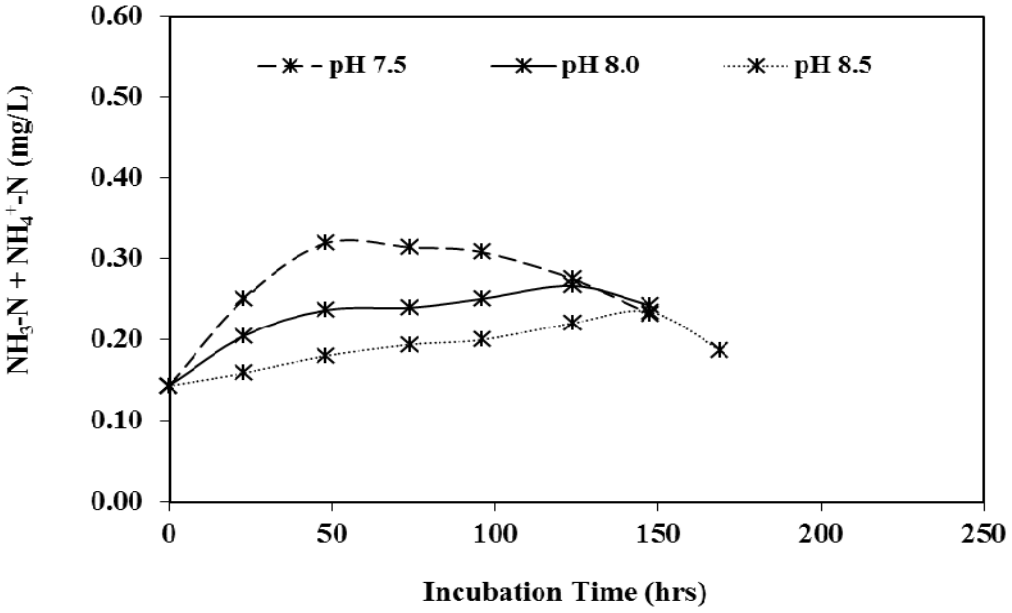

Figure B-9: Effects of pH on NH_3-N+NH_4^+-N profiles at different pH conditions for Sample C

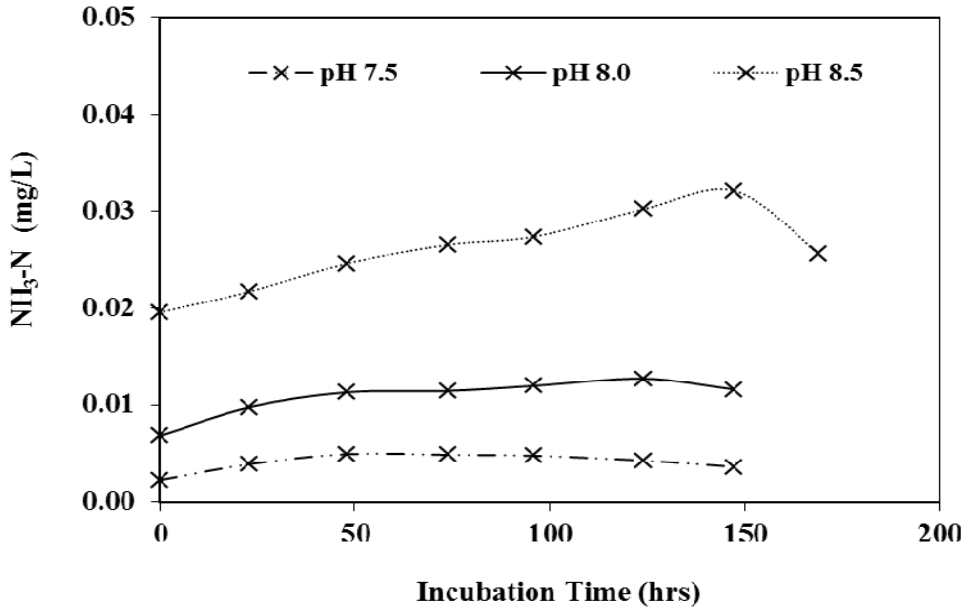

Figure B-10: Effects of pH on NH_3-N profiles at different pH conditions for Sample C

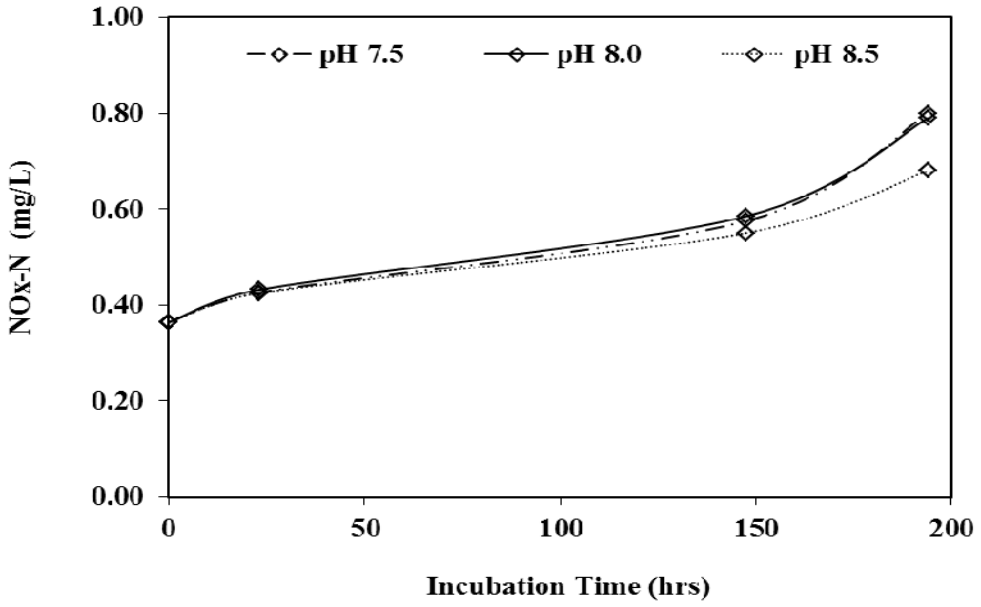

Figure B-11: Effects of pH on NOx-N profiles at different pH conditions for Sample C

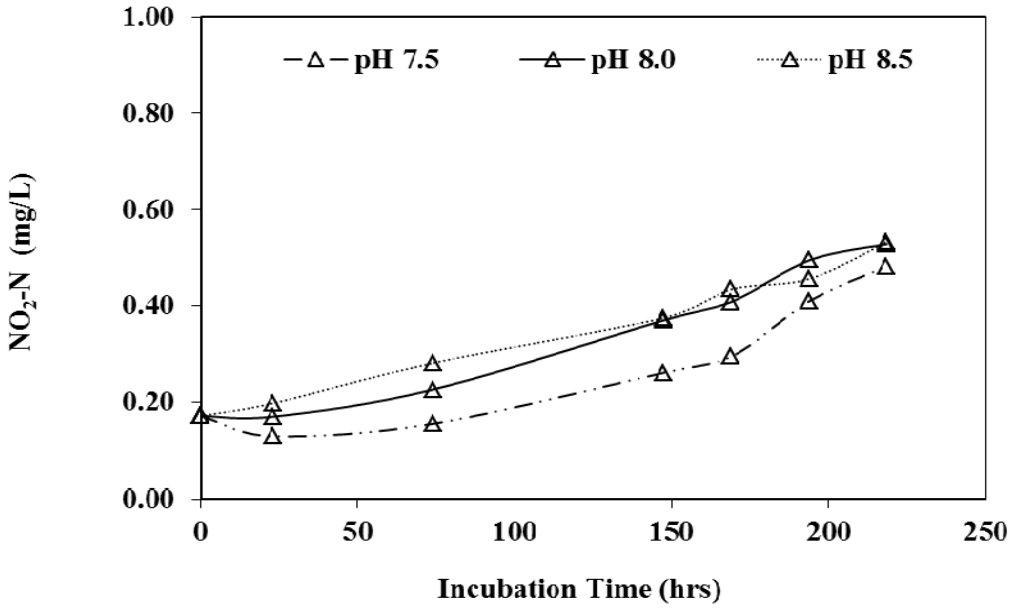

Figure B-12: Effects of pH on NO_2-N profiles at different pH conditions for Sample B

APPENDIX-C

MONITORING DATA OF THE PILOT-SCALE SYSTEM BEFORE COPPER DOSING IN THE REACTOR

Table C-1: Operational parameters of R-1/1 & R-1/2 before copper dosing

Operation period (days)	R-1/1				R-1/2			
	TCl	TAN	NO_2-N	NOx-N	TCl	TAN	NO_2-N	NOx-N
0	2.31	0.595	0.008	0.120	2.18	0.606	0.008	0.104
3	2.28	0.589	0.007	0.111	2.32	0.596	0.008	0.111
5	2.27	0.545	0.009	0.121	2.38	0.534	0.009	0.115
6	2.26	0.512	0.008	…	2.26	0.520	0.008	…
7	2.15	0.518	0.007	0.118	2.13	0.510	0.007	0.111
8	2.06	0.530	0.005	…	2.15	0.518	0.005	…
10	1.98	0.511	0.005	…	2.16	0.523	0.004	…
11	2.16	0.506	0.005	0.117	2.22	0.521	0.005	0.114
12	2.10	0.552	0.006	…	2.27	0.578	0.004	…
13	2.15	0.521	0.008	0.110	2.26	0.524	0.007	0.139
15	2.12	0.516	0.007	0.092	2.35	0.494	0.006	0.139

Table C-2: Operational parameters of R-2/1 & R-2/2 before copper dosing

Operation period(days)	R-2/1				R-2/2			
	TCl	TAN	NO_2-N	NOx-N	TCl	TAN	NO_2-N	NOx-N
0	1.86	0.545	0.015	0.146	1.80	0.564	0.010	0.185
3	1.92	0.535	0.012	0.138	2.04	0.535	0.011	0.182
5	2.05	0.500	0.012	0.160	2.10	0.537	0.012	0.179
6	2.01	0.470	0.010	…	2.08	0.484	0.012	…
7	1.96	0.486	0.009	0.146	2.03	0.504	0.009	0.189
8	1.88	0.481	0.007	…	1.96	0.504	0.008	…
10	1.85	0.445	0.006	…	1.93	0.508	0.007	…
11	2.00	0.459	0.007	0.120	2.00	0.494	0.007	0.203
12	1.99	0.498	0.008	…	1.97	0.551	0.007	…
13	1.96	0.460	0.009	0.112	2.03	0.503	0.009	0.146
15	1.99	0.477	0.010	0.108	2.08	0.504	0.008	0.136

Table C-3: Operational parameters of R-3/1 & R-3/2 before copper dosing

Operation period (days)	R-3/1				R-3/2			
	TCl	TAN	NO_2-N	NOx-N	TCl	TAN	NO_2-N	NOx-N
0	0.50	0.281	0.204	0.392	0.48	0.242	0.235	0.425
3	0.70	0.307	0.198	0.383	0.85	0.299	0.219	0.421
5	0.76	0.266	0.202	0.363	0.99	0.298	0.182	0.401
6	0.82	0.229	0.207	…	0.89	0.248	0.187	…
7	0.77	0.247	0.191	0.377	0.8	0.238	0.190	0.404
8	0.71	0.262	0.178	…	0.71	0.232	0.185	…
10	0.68	0.248	0.166	…	0.79	0.225	0.186	…
11	0.71	0.222	0.192	0.329	0.72	0.210	0.230	0.409
12	0.70	0.258	0.167	…	0.73	0.253	0.186	…
13	0.89	0.265	0.161	0.325	1.03	0.317	0.141	0.395
15	0.78	0.265	0.182	0.335	0.85	0.264	0.177	0.390

Table C-4: Operational parameters of R-4/1 & R-4/2 before copper dosing

Operation period (days)	R-4/1				R-4/2			
	TCl	TAN	NO_2-N	NOx-N	TCl	TAN	NO_2-N	NOx-N
0	0.07	0.119	0.300	0.494	0.08	0.102	0.326	0.514
3	0.13	0.143	0.316	0.517	0.16	0.122	0.346	0.554
5	0.18	0.141	0.289	0.486	0.20	0.107	0.336	0.546
6	0.15	0.127	0.285	…	0.23	0.114	0.308	…
7	0.12	0.101	0.294	0.496	0.18	0.085	0.313	0.538
8	0.12	0.098	0.287	…	0.13	0.074	0.308	…
10	0.13	0.091	0.272	…	0.16	0.081	0.297	…
11	0.12	0.074	0.280	0.488	0.12	0.058	0.327	0.546
12	0.12	0.107	0.261	…	0.12	0.105	0.298	…
13	0.11	0.081	0.288	0.482	0.14	0.094	0.305	0.508
15	0.21	0.119	0.265	0.464	0.22	0.131	0.276	0.483

Table C-5: Operational parameters of R-5/1 & R-5/2 before copper dosing

Operation period (days)	R-5/1				R-5/2			
	TCl	TAN	NO_2-N	NOx-N	TCl	TAN	NO_2-N	NOx-N
0	0	0.055	0.233	0.552	0	0.043	0.184	0.564
3	0	0.058	0.242	0.593	0	0.056	0.201	0.615
5	0	0.046	0.224	0.594	0	0.044	0.205	0.611
6	0	0.041	0.192	…	0	0.054	0.189	…
7	0	0.043	0.222	0.563	0	0.037	0.196	0.583
8	0	0.042	0.222	…	0	0.029	0.164	…
10	0	0.034	0.189	…	0	0.020	0.150	…
11	0	0.038	0.178	0.533	0	0.037	0.135	0.558
12	0	0.042	0.168	…	0	0.038	0.160	…
13	0	0.025	0.190	0.540	0	0.043	0.175	0.559
15	0	0.046	0.223	0.549	0	0.064	0.166	0.550