NITRIFICATION IN PREMISE PLUMBING SYSTEMS

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ABSTRACT

Monochloramine is increasingly used instead of free chlorine as a secondary disinfectant. Ammonia is introduced into water for monochloramine formation or by decay. Nitrification can have deleterious effects on water quality that may lead to regulatory violations. In this project water quality and influence of pipe material on the onset of nitrification and consequences of nitrification in premise plumbing were investigated. Also potential control strategies for nitrification were evaluated.

Initially two types of copper coupons (new and old, i.e., pre-exposed to 0.1N NaOH solution) were used with water of two different carbon (2~4ppm) and ammonia-nitrogen (0.36~0.71ppm) concentrations. In the next experiment, pre-aged copper and PVC coupons were used with high carbon (4 ppm) and two ammonia concentrations (0.36 and 0.71 ppm). When all reactors showed complete signs of nitrification the ammonia concentration in low ammonia (0.36 ppm) feed reactors were raised to the high level (0.71 ppm). The PVC reactors were quicker in adjusting to this change. Next, the effect of copper ion, chlorite and chloramine on nitrifying simulated household plumbing systems was investigated. No significant effect of copper on nitrification was observed. Chlorite was not effective on the PVC system but inhibited the copper system at 20 ppm. Nitrification activity was also impacted significantly at a 5:1 ratio of chlorine to ammonia and ultimately stopped.

To investigate the effect of nutrient conditions on metal release in a nitrifying system and the consequences of change in microbial population, influent humic and ammonia concentrations of two reactors of each set were raised to 8 ppm and 2.13 ppm respectively. Higher ammonia increased only the autotrophs while higher TOC increased only the heterotrophs. For all reactors alkalinity and pH decreased due to nitrification, with lesser effect on copper reactors. Increased TOC or nitrogen increased the copper concentration in the water.

The microbial population was analyzed by PCR and DGGE. The biofilm community composition is influenced by nutrient condition and pipe material and environmental stress (chlorite or monochloramine). The presence of copper in the PVC reactor did not cause any impact on community composition.

CHAPTER 1

INTRODUCTION

Definition of Nitrification

Nitrification is the conversion (oxidation) of reduced forms of nitrogen to nitrite and nitrate. It is a major potential problem in potable water systems that utilize chloramine for secondary disinfection. The problem of nitrification in drinking water supplies in United States was identified as early as 1935 (Faben 1935). Nitrification is believed to be mediated by autotrophic nitrifiers, which obtain energy from ammonia and nitrite. They are usually divided into two groups: ammonia and nitrite oxidizers (i.e AOB and NOB). *Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosolobus,* and *Nitrosovibrio* are common AOB and *Nitrobacter* and *Nitrospina* are common NOB. Heterotrophic bacteria and fungi can also carry out nitrification but at slower rates than autotrophic nitrifying bacteria (Watson et al. 1981).

Regulatory Consequences

Nitrification of chloraminated drinking water can have deleterious effects on water quality, including a decrease in chloramine residual which could lead to an increase in heterotrophic plate count (HPC) bacteria and an increase in nitrite and nitrate, all of which could lead to regulatory violations. Nitrite accelerates the breakdown of the chloramine residual in water (Valentine 1984, Margerum et al. 1994), which could lead to violations of the Surface Water Treatment Rule. An increase in HPC, which might be

indicative of a loss of residual, can lead to violations of the Surface Water Treatment Rule (SWTR). In addition, the maximum contaminant levels (MCL) for nitrite and nitrate are 1 mg NO_2-N/L and 10 mg NO_3-N/L, respectively. The MCL level for these two chemicals may potentially be exceeded because these are released in the nitrification process. Nitrification also reduces pH and alkalinity which can have an effect on Lead and Copper Rule (LCR) compliance.

Many utilities in the US are implementing chloramination instead of chlorination for distribution system disinfection to meet the new regulation for disinfection by products (DBP) (Wolfe et al. 1985, LeChevallier et al. 1990). However, chloramine is not without problems. Chloramination can reduce the biostability of water as ammonia is released from chloramine decomposition or added during chloramine formation. Chloramines degrade in the distribution system as a result of hydrolysis, autodecompositon, and reduction by natural organic matter (NOM) (Harrington et al. 2003, Margerum et al. 1978, Valentine et al. 1988), the presence of nitrite (Vikesland et al. 2001) and reaction with pipe material (Camper et al. 2003, Le Puil et al. 2003, Sung 2003). A 1996 American Water Works Association Research Foundation (AwwaRF) survey indicated that two thirds of drinking water utilities that chloraminate have experienced some degree of nitrification (Wilczak et al. 1996). Despite the understanding of many theoretical aspects of nitrification, practical management/control in distribution systems still poses a challenge to chloraminating utilities.

Conditions Favourable for Nitrification

Distribution systems provide suitable growth conditions for autotrophic nitrifiers. Darkness, long detention time, presence of low or no disinfectant, and continuous supply of even low levels of free ammonia promote growth of nitrifiers. Dead ends and storage reservoir water have long water age due to their long residence time, which is favorable for nitrifiers due to their slow growth rate. Also corrosion and tuberculation of pipes protect the nitrifying biofilm from washout or disinfection.

Potential Mitigation Approaches/Strategies

Possible control methods for nitrification control include reducing detention times, maintaining a chloramine dose throughout the distribution system, increasing the Cl_2: N ratio to minimize excess ammonia, break point chlorination, draining and refilling storage tanks, applying proper corrosion control, and flushing the distribution system routinely (Wolfe et al. 1988, Lieu et al. 1993). Recent studies by McGuire (1999) found that chlorite (ClO_2^-) inhibited nitrification at the Gulf Coast Water Authorities (GCWA). All these control strategies are somewhat site specific; for example nitrification has occurred in distribution systems with chloramine residuals ranging from 0.5 to 5.5 mg/L (Odell et al.1996), and in some systems chlorite did not control nitrification (Karim et al. 2006).

Effect of Nitrification on Premise Plumbing Water Quality

Most research on nitrification has previously been conducted in distribution mains or treatment plants (Wolfe et al. 1990, Cunliffe 1991, Skadsen 1993, Odell et al 1996, Wilczak et al 1996). There is a need to understand if similar processes occur in premise plumbing. Premise plumbing is the part of the distribution system that exist from the property line to the inside of the house, i.e., the part that is associated with schools, hospitals, public and private housing, and other buildings (NRC, 2006). Favorable conditions for nitrification exist in premise plumbing such as low or no disinfectant, long water age and warmer temperature. Premise plumbing not only has a higher surface to volume ratio but also about ten times the length of distribution system mains. The estimated total length of distribution mains in the US is about 1 million miles (Brongers et al. 2002, Grigg 2005), whereas about 5.3 million miles of copper tubing were installed as a part of premise plumbing between 1963 and 1999 (CDA, 2005). According to a study on the distribution system in Columbia, Missouri, premise plumbing had 82 percent of the total pipe length, 24 percent of the total surface area and only 1.6 percent of the total volume of water in the system (Brazos et al. 1985). No particular agency or utility is responsible for premise plumbing water quality other than what is provided for in the Lead and Copper Rule. The only responsible entity for water quality or infrastructure in premise plumbing is the house owner or building supervisor. Therefore problems associated with premise plumbing water quality are a growing concern.

Copper is the most widely used metal for household plumbing systems. According to Oskarsson et al. (1998) in the US more than 90% of domestic plumbing

material is made of copper. PVC pipes are also very common as plumbing material (NSF, 2008). As copper is known to be toxic to bacteria (Kim et al. 2002) there is a general belief that nitrification does not occur in copper plumbing. Conversely PVC is believed to provide a more stress-free environment than copper due to the absence of copper toxicity. Pipe type may also be important because it has been documented that the biological activity in distribution systems is associated with biofilm formation, (Camper et al. 1996, Harrington et al. 2003) and most nitrifying bacteria exist in a biofilm (Lipponen et al. 2002, Stewart et al. 1997, Wolfe et al. 1990) rather than in a planktonic state. Biofilms play an important role in water quality degradation because they can act as a shield for nitrifying bacteria from disinfection present in bulk water (Regan et al 2002). Another important factor may be the relative abundance of organic matter and ammonia. These nutrients play a significant role in controlling microbial populations in distribution systems. Depending on nutrient conditions, autotrophic or heterotrophic populations may dominate the biofilm. Heterotrophic bacteria can influence the corrosion of metal; autotrophic bacteria can also aggravate corrosion by lowering pH and alkalinity through nitrification. The relationship between these two populations can also be competitive or symbiotic. They could compete for space on surfaces and for dissolved oxygen, ammonia and other nutrients. The relationship between these two groups of organisms may also be positive. Heterotrophs can biodegrade organic compounds that are inhibitory to nitrifiers (Richardson 1985). They can also produce organic compounds that stimulate nitifier activity (Steinmüller and Bock 1976, Pan and Umbreit 1972, Hockenbury et al. 1977). Extracellular polymers produced by heterotrophs also improve the aggregation of both

species in a biofilm (Rittmann et al. 1994). Heterotrophs, because of their fast growth form the outer layer of biofilm, and may protect the nitrifiers from detachment (Rittmann et al. 1992, Manem et al. 1992, Furumai et al. 1994, Ohashi et al. 1995) Autotrophs also produce and release soluble microbial products (SMP) that augment heterotrophic substrate supply (Furumai et al. 1992, Rittmann et al. 1994, Kindaichi et al. 2004).

As mentioned earlier, changes in water quality related to nitrification may affect corrosion of metal surfaces in premise plumbing. Corrosion of copper involves both complex chemical and microbiological processes. Natural organic material can also aggravate corrosion by forming soluble complexes or protect the pipe surface through formation of a protective layer. Reduction of pH and alkalinity due to nitrification may influence corrosion. Change in redox potential due to the presence of nitrite produced from incomplete nitrification can influence the corrosion rate (Rozenfeld 1981). Also, microbial corrosion can be enhanced by higher HPC counts resulting from nitrification. According to a study conducted by the city of Willmar, Minnesota, higher copper concentrations in Willmar tap water were due to nitrification (Murphy et al. 1997). Therefore, understanding how water quality parameters interact with microbial processes is beneficial in addressing corrosion concerns in premise plumbing.

Scope and Objectives of This Research

This project is focused on nitrification in premise plumbing, especially on knowing how water quality and pipe material influence the onset of nitrification and the consequence of nitrification on water quality deterioration. Also, potential control

strategies for nitrification were evaluated. Modified CDC reactors were designed to simulate premise plumbing and were operated with 8 hours of stagnation. Preliminary investigations on the effect of different nutrient conditions on biofilm development and metal (copper) release were conducted. In subsequent experiments, four PVC and four copper reactors were used to investigate the influence of pipe material and different nitrogen levels on nitrification. Once nitrification was established in these reactors the efficacy of different control mechanisms was evaluated. These included the influence of copper in a PVC reactor, the inhibitory effect of chlorite, and the efficacy of monochloramine at different chlorine to ammonia ratios. In the final phase of the research, total organic carbon (TOC) and ammonia concentrations were raised and subsequent changes in autotrophic and heterotrophic populations, copper release, pH and alkalinity were evaluated.

Objectives

The focus of this project is to investigate nitrification in a simulated household plumbing system. Specific objectives of the investigation were to:

- Investigate the impact of high and low levels of nutrient (carbon and nitrogen) on the release of copper in simulated home plumbing systems
- Demonstrate the potential for nitrification within a simulated home plumbing system, examine its effect on water quality, and quantify the nitrifying population
- Investigate the impact of metal (copper) ion on nitrification in a premise plumbing system and its effect on microbial populations within the simulated system

- Investigate potential control mechanisms (inhibitor and disinfection) for nitrification and how they affect microbial populations, particularly nitrifying organisms
- Investigate how organic carbon and ammonia levels influence nitrification and corrosion and the subsequent changes in alkalinity and pH

Dissertation Structure

Each chapter of this dissertation represents a separate set of experiments. The dissertation organization is as follows:

- Chapter 2 presents the results of a preliminary investigation on the effect of nutrient conditions on metal release and biofilm development. This chapter also shows the effect of different pipe materials and nitrogen levels on commencement of nitrification. Findings of this chapter were presented at the American Water Works Association 2006 Water Quality Technology Conference.
- Chapter 3 describes the results from the study which evaluated copper toxicity, chlorite inhibition and monochloramine at different chlorine to ammonia ratios on a nitrifying population. This chapter will be submitted as a peer reviewed manuscript.
- Chapter 4 presents the results of an investigation of the effect of nutrient conditions (TOC and ammonia concentrations) on different microbial populations in a nitrifying system and subsequent change in water quality. This chapter will also be submitted as a peer reviewed manuscript.

- Chapter 5 describes the overall findings and conclusions. It also contains suggestions for future work.

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CHAPTER 2

INVESTIGATING THE EFFECT OF DIFFERENT NUTRIENT CONDITIONS AND PIPE MATERIALS ON NITRIFICATION

Abstract

Monochloramine is increasingly used instead of free chlorine as a secondary disinfectant to meet new regulations for disinfection by-products (DBPs). Ammonia is introduced into water by chloramine formation or decay. The ammonia acts as an energy source for ammonia oxidizing bacteria (AOB). Nitrification caused by AOB is a major potential problem in potable water systems that utilize chloramine for secondary disinfection. Nearly two thirds of drinking water utilities that practice chloramination for secondary disinfection have experienced nitrification in their distribution system Nitrification can have deleterious effects on water quality in treatment plants and distribution mains (Valentine 1985, Margerum et al. 1994) that may lead to violations of the Surface Water Treatment Rule. Nitrification also reduces pH and alkalinity which can have an effect on Lead and Copper Rule (LCR) compliance. In this project, the effect of different nitrogen and carbon (TOC) concentrations and premise plumbing materials (copper, PVC) on nitrification were investigated.

A modified version of the commonly used CDC reactor developed at the Center for Biofilm Engineering was used to simulate a household plumbing system in this project. In the preliminary experiment two types of copper coupons (new and old, i.e., pre-exposed to 0.1N NaOH solution) were used. These reactors were fed with water with

different carbon (2~4ppm) and ammonia-nitrogen (0.36~0.71ppm) concentrations and biologically treated tap water to supply the bacterial population. Water in the reactor was stagnant for eight hours and then flowed for five minutes. Copper concentration in reactor water increased with an increase of natural organic matter. Heterotrophic plate counts also showed higher numbers for high carbon reactors. This experiment was conducted for three months but did not show any sign of nitrification. In the next experiment, pre-aged copper and PVC coupons were used with high carbon (4 ppm) and two ammonia concentrations (0.36 and 0.71 ppm) in the feed. After three months of operation, the PVC reactors showed evidence of nitrification, while the copper reactors required five months to exhibit nitrification. This difference in onset may be due to the toxicity of the copper to microbial growth. When all reactors showed complete signs of nitrification the ammonia concentration in low ammonia (0.36 ppm) feed reactors was raised to the high level (0.71ppm). The PVC reactors quickly adjusted to this change by utilizing the excess ammonia, while the copper reactors were slower in utilization. The microbial population in those reactors was analyzed using PCR and DGGE. It was found that biofilm community composition is influenced by nutrient condition and pipe material.

Introduction

Monochloramine is becoming more popular as a secondary disinfectant as facilities attempt to meet the more stringent Disinfection By Products Rule (DBPs) (Seidel et al. 2005). Monochloramine is less reactive than free chlorine and produces less by-products such as trihalomethanes and haloacetic acid (Brodtmannn and Russo

1979,Norman et al. 1980, Mitcham et al. 1983). In spite of the benefits of monochloramine, its use can cause biological instability in distribution systems by promoting the growth of nitrifying bacteria, which oxidize ammonia to nitrite and nitrate. Free ammonia in water can be available to these bacteria through chloramine formation or chloramine decay. Nitrifying bacteria are omnipresent in all surface waters (Faben 1935), so the potential for nitrification in distribution systems using surface water is high if ammonia is present.

Nitrification by autotrophic bacteria consists of two oxidation steps: ammonia is oxidized to nitrite, and nitrite to nitrate. In the first step ammonia oxidizing bacteria (AOB) oxidize ammonia to nitrite according to the following equation. *Nitrosomonas* is the most common AOB, although *Nitrosospira, Nitrosococcus, Nitrosolobus,* and *Nitrosovibrio* are also known AOB. (Kirmeyer et al. 1985).

$$NH_3 + 2H_2O \longrightarrow NO_2^- + 7H^+ + 6e^-$$

In the second step nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate according to the following equation (Doetsch and Cook 1973). *Nitrobacter* is the most common NOB, although *Nitrospina* and *Nitrococcus* species are known NOB (Kirmeyer et al. 1985).

$$NO^-_2 + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^-$$

Most of these nitrifiers are chemoautotrophic, which means that they use energy derived from the oxidation of inorganic compounds.

Certain heterotrophic organisms can also participate in nitrification. For example, heterotrophic bacteria and fungi may also carry out nitrification, but at a lower rate (Watson et al. 1981).

According to the Information Collection Rule (ICR) database, 33% of 353 treatment plants use chloramines (Karim et al. 2006). Chloramine use is expected to be more prevalent in the near future because of its reduced production of disinfectant by products (DBP) (Regan et al. 2002). According to the USEPA, to meet the Stage 2 Disinfection By-Product (DBP) Rule about 65% of the utilities might switch to chloramination (EPA 2000). In a telephone survey (AwwaRF 1995) of 98 utilities which practice chloramination, it was reported that in two thirds of them, nitrification occurs and in one third it causes some type of operational problem.

Nitrification is known to cause changes in chemical and microbiological quality of water. Due to nitrification, chloramine residual, pH, alkalinity and the dissolved oxygen content of water decrease. The nitrifiers produce soluble microbial products (SMP) which may support the growth of heterotrophic bacteria in low nutrient environments (Rittmann et al. 1994). SMP production and depletion of the disinfectant may therefore cause high levels of HPC. The nitrite and nitrate concentration in water also increase. The Safe Drinking Water Act (SDWA) sets primary MCLs for nitrite-N and nitrate-N at the entrance to the distribution system are 1 and 10 mg/L respectively (EPA). Nitrite and nitrate are not regulated by federal rule in the distribution system, but

nitrification can result in elevated levels of these compounds. Nitrite also accelerates the decomposition of chloramines (Margerum et al. 1994, Valentine 1985, Woolschlager et al. 2001). Subsequent depletion of the monochloramine residual may also cause violation of disinfection residual standards, which requires the detection of a disinfection residual in at least 95% of monthly distribution system samples (USEPA 1998).

As stated above, changes in water quality related to nitrification may affect corrosion of metal surfaces in premise plumbing, a primary concern in copper piping. Corrosion of copper involves both complex chemical and microbiological processes. Lowering of pH and destruction of alkalinity due to nitrification may influence corrosion. Nitrite produced from incomplete nitrification can influence the corrosion rate by changing the redox potential (Rozenfeld 1981). Also, higher HPC counts resulting from nitrification can enhance microbial corrosion. According to a study conducted by the city of Willmar, Minnesota, higher copper concentrations in Willmar tap water were due to nitrification (Murphy et al. 1997).

Most nitrifying bacteria exist in a biofilm (Lipponen et al. 2002, Stewart et al. 1997, Wolfe et al. 1990) rather than in a planktonic state. Pipe material can influence biofilm (LeChevallier et al.1990). Studies have showed that surface roughnesses of the pipe material contributes to bacterial attachment and biofilm formation (Pedersen 1990, Percival et al.1998). Schwartz et al. (1998) reported that biofilm grown on copper surfaces were less dense than those grown on plastic. According to Oskarsson et al. (1998) in the US more than 90% of domestic plumbing material is made of copper. PVC pipes are also very common as plumbing material (NSF 2008). As copper is know to be

toxic to bacteria (Thurman and Greba 1989, Straub et al. 1995, Kim et al. 2002, Artz and Killham 2002, Teitzel and Parsek 2003) there is a general belief that nitrification does not occur in copper plumbing. Conversely PVC is believed to provide a more stress-free environment than copper due to the absence of copper toxicity.

In this project, the effect of different nitrogen and carbon (TOC) concentrations and pipe materials (copper, PVC) on nitrification was investigated.

Materials and Methods

Experimental Design

Phase I- the Effect of Water Quality on Copper Release: Eight modified CDC reactors with copper coupons were used to investigate the influence of water quality on copper release. This experiment ran for three months. Two types of copper coupons were used; brand new copper and old (i.e., pre exposed to 0.1N NaOH for eight hours) copper. The experimental matrix is shown in Table 2-1. Two levels (2 and 4 ppm of carbon) of Elliot silt loam (International Humic Substance Society) humics were used in the reactors. Also two levels (0.36 and 0.71 ppm) of NH_3-N in the influent were used. These values represent the concentrations that would be found if approximately 2 and 4mg/L of chloramine respectively decayed to their equivalent amount of ammonia. Alkalinity of the influent was around 35 mg/L as $CaCO_3$ and pH was around 8.15 which represent typical water quality in US distribution systems.

Table 2-1: Experimental matrix for the preliminary experiment

Copper coupon type	TOC (ppm)	NH_3-N (ppm)
New	Low (2ppm)	Low (0.36 ppm)
		High (0.71 ppm)
	High (4ppm)	Low (0.36 ppm)
		High (0.71 ppm)
Old	Low (2ppm)	Low (0.36 ppm)
		High (0.71 ppm)
	High (4ppm)	Low (0.36 ppm
		High (0.71ppm)

PhaseII- the Effect of Nutrient Condition and Pipe Material on Nitrification: At the end of Phase-I, a second experiment was started to investigate the effect of NH3 concentration and pipe material on the onset of nitrification. Two sets of reactors consisting of four in each set were used for this study. In one set copper (old) was used as the coupon material and PVC was used in the other one. Alkalinity of the influent was around 35 mg/L as $CaCO_3$ and pH was maintained around 8.15.

All reactors were fed with four ppm of Elliot humics as the sole carbon source. In the beginning of the experiment two of the reactors were fed with 0.36 ppm of NH_3-N in the influent and the remaining two were fed with 0.71 ppm of NH_3-N. Table 2-2 shows the detailed specifications of all the reactors. The PVC set showed signs of nitrification after three months of operation but in the case of the copper set this took almost five months. When both sets of reactors were nitrifying steadily, influent in the low (0.36 ppm) NH_3 feed reactors was changed to the high (0.71 ppm) level.

Table 2-2: Experimental matrix for the second set of experiments

Type of coupon	TOC(ppm)	NH_3 concentration(ppm)	
		Initial	Final
PVC	4 ppm	0.36	0.71
		0.36	0.71
		0.71	0.71
		0.71	0.71
Copper (Old)	4 ppm	0.36	0.71
		0.36	0.71
		0.71	0.71
		0.71	0.71

Description of Reactor

To simulate a domestic plumbing system the commonly used CDC (Goeres et al. 2005) reactor was modified. These modified reactor's coupons, bottom plate and stirring blades have the same surface to volume ratio as that of a six foot long ¾" diameter domestic copper plumbing pipe. The rotational speed of the blade inside these reactors was 300 rpm. This speed was chosen as it creates 3fps velocity in bulk water which can be found in domestic water lines. Volume of the reactors is 120 ml. Two types of materials were tested, copper and PVC. All coupons were washed with 0.1N NaOH three times to remove any biological materials from their surface prior to use.

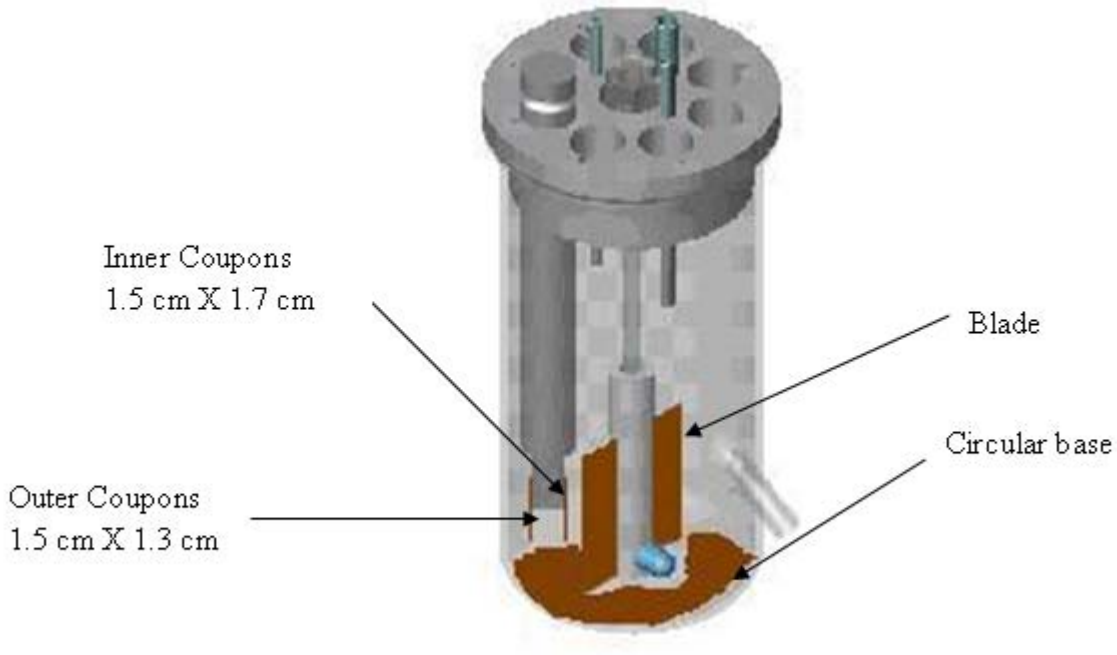


Figure 2-1: Modified CDC reactor

Operation Scheme

To simulate periods of stagnation in home plumbing the reactors were flushed with peristaltic pumps for five minutes and then the water inside the reactors remained stagnant for eight hours. The feed pumps and stirplates were on two different timers, which controlled the power supply. The timers were offset from each other by one minute with the stirplate starting before the pumps. At the end of five minutes the stirplates stopped, followed by the pumps. This cycle was repeated three times a day. As a result, fresh influent mixed with the stagnant bulk water and excess water spilled through the effluent port. Because there is mixing, the effluent water is always diluted with fresh influent feed, which prevents the effluent NH_3 concentration from becoming zero, even

though the bulk NH_3 concentration (the concentration in the reactor) is zero due to nitrification. Because sampling occurred in the both the bulk and the effluent, a mathematical model of the reactor was developed to determine the relation between effluent NH_3 concentration and NH_3 utilization in these reactors. This model was used to calculate the % NH_3-N utilization and the % of ammonia present as NO_2-N, and NO_3-N. As described in Appendix-A, model values were equivalent to those actually measured in the bulk.

Stock/Feed Solution Preparation

Typical setup of these reactors is shown in Figure 2.2. The ratio of flow is RO: humics: BAC= 50:5:1, so these reactors are mostly supplied with RO water. Influent carbon concentration as humics was 4 ppm for all reactors. Lab grade chemicals were added to reverse osmosis (RO) water to give it a target alkalinity of 35 mg/L as $CaCO_3$. The final concentrations of these chemicals are given in

Table 2-3 Chemicals added to influent water.

Chemical	Concentration(mg/L)
$MgSO_4$	39.6
$NaHCO_3$	56.9
$CaSO_4$ $2H_2O$	25
$Al_2(SO_4)$ $18H_20$	0.62
$CaCl_2$ $2H_20$	20.80
Na_2SiO_3 $9H_20$	26

Bozeman tap water was flowed through a biologically activated carbon (BAC) column to remove any residual chlorine. It should be noted that Bozeman water comes

from a surface water source and does not contain ammonia and chlorine is used as a final disinfectant. BAC water was pumped to these reactors to ensure a supply of indigenous bacteria. These organisms were the only inoculum supplied to the reactors. Humics were supplied using a separate pump.

Humics Preparation

50 gm of Elliot silt loam soil (International Humic Substances Society) was added to 500 ml of 0.1 N NaOH and mixed for 48 hours. This solution was then centrifuged at 10,000 X g for 20 minutes. After centrifugation the supernatant was collected in carbon free glassware (prepared by baking at 390^0C for 5 hours) and stored at 4^0 C in the dark. Total organic carbon content of the humics was measured using a Dohrman DC-80® and subsequently diluted to the appropriate concentration using the RO water feeding the reactors.

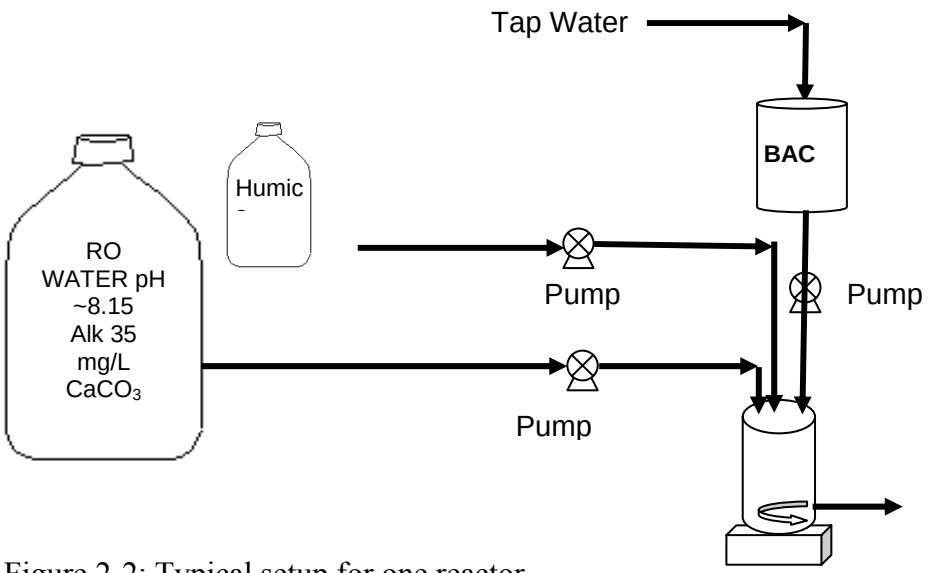


Figure 2-2: Typical setup for one reactor

Analytical Methods (Chemical)

Sampling Technique: Effluent water samples were collected and tested three times (Monday, Wednesday and Friday) every week. Effluent water concentrations were converted to bulk values using the model shown in Appendix-A. Response variables of the water samples shown in Table 2-4 below were measured routinely.

Table 2-4: Response parameters measured in this project

Response parameter	Comment
Copper(Dissolved and total)	Three times a week for effluent sample and once
Ammonia	
Nitrite	
Nitrate	
HPC	Weekly

Copper: Both total and dissolved copper were measured. According to *Standard Methods* (19ed 1995) dissolved copper is operationally defined as the portion of the copper which passes through a 0.45μm pore size syringe filter. It should be noted that in the presence of colloidal species that can pass through the filter, the method represents an upper bound to truly soluble copper. Measurements were done using a portable HACH 2000 spectrophotometer. Copper in the water sample reacts with a salt of bicinchoninic acid contained in the CuVer 1 copper reagent to form a purple colored complex in proportion to the copper concentration. The purple color/copper concentration was measured at 560 nm.

Ammonia Nitrogen: Free NH_3-N was measured using a HACH 2000 spectrophotometer using the salicylate method (HACH method 10023) at 655 nm. Ammonia reacts with salicylate to form 5- aminosalicylate, which is oxidized in the

presence of a sodium nitroprusside catalyst to form a blue colored compound. This blue color is masked by the yellow color from the excess reagent to give a final green-colored solution which is proportional to the amount of ammonia present in the sample. The ammonia was measured immediately when the sample was collected.

Nitrite Nitrogen: After collecting the sample, nitrite nitrogen (NO_2-N) was measured immediately with the HACH 2000 spectrophotometer. The diazotization method was used, where nitrite reacts with sulfanilic acid and forms an intermediate diazonium salt. This intermediate product couples with chromotropic acid to produce a pink colored complex, which is proportional to the amount of nitrite present. This pink color was measured at 507 nm.

Nitrate Nitrogen: NO_3-N measurements were done using a Dionex® ion chromatography system with a CD20 conductivity detector and GP40 gradient pump unit. An AS4A column and DS3 detection stabilizer was also used in this method. Water was filtered through a sterilized 0.2 µm pore size polyethersulfone filter and 5ml BD® syringe to remove any bacteria or suspended particles. The filtered sample was collected in a sterilized 15 ml Falcon® tube and stored in the refrigerator at 4^0C. Stored samples were measured within two weeks of collection. The water sample was loaded using a S40 automated sampler. Before measurement the Dionex® ion chromatography system was first calibrated using five sodium nitrate standards (1, 0.5, 0.2, 0.1, 0 ppm of NO_3-N). To minimize experimental error, after every seven measurements a standard solution of nitrate was measured to check the accuracy of the measurement. If the obtained

measurement of the standard was outside 90 to 110% of the standard value then the calibration was repeated and sample was measured again. This was done according to *Standard Method* (19ed, 1995) 3020.

Total Organic Carbon (TOC): TOC measurements were done using a Dohrman DC-80® carbon analyzer, with potassium hydrogen phthalate as the standard and potassium persulfate as the oxidizing agent.

Analytical Methods (Microbiological Analysis)

Heterotrophic Plate Counts (HPC): Heterotrophic plate counts of the water samples and the biofilms were done according to *Standard Methods* (19th ed, 1995) 9215A using R2A agar plates. Plates were incubated at 20^0C for 7 days, and then the number of colonies in the plates was counted using a Quebec colony counter.

Biofilm Sampling: Biofilm was collected at different time points in each experiment. Autoclaved reverse osmosis (RO) water was filtered through a sterilized 0.2µm polyethersulfone filter to to remove any foreign DNA. Filtered DNA free water was placed in DNA free glass tray (baked at 390^0C for 5 hours). One coupon was removed from the reactor and placed in the glass tray containing the water. The coupon was then scraped using a autoclaved rubber policeman inside a laminar flow hood. After scraping, the biomass with water was poured in a sterilized 50 ml Falcon® tube, which was then homogenized (Biohomogenizer®Model M133/12810, ESGE®) for 30 sec.

From the homogenized biomass, samples were taken to do MPN and HPC analysis. The remaining of the biomass was used for DNA extraction and community analysis.

DNA Extraction: Homogenized biomass was collected on a 0.2µm polycarbonate filter using a three channel manifold (Pall® Life Science) with filter funnels. DNA extraction of the collected biofilm sample was done using a Fast DNA® SPIN Kit for soil (Q-BIOgene catalog #6560-200). Collected DNA was stored in a -30^0 C freezer.

PCR (Polymerase Chain Reaction): GoTag Green® master mix from Promega Inc. was used for amplifying the extracted DNA through PCR using an Eppendorf Mastercycler®. Due to the inhibitory effect of humics present and the low quantity of biomass, a two stage PCR was performed. In the first stage in a 50µl reaction volume was used which contains 25µl master mix, 10 pM of the universal primers 1070F and 1392, 1µl DNA suspension and 20 µl of water. The amplification process involved initial denaturation at 94^0C followed by 15 cycles of 30 second denaturation at 94^0C, 45 second annealing at 52^0 C and 2 minutes of extension at 72^0 C with a final 5 minutes extension at 72^0C. In the second stage, 5 µl of the product from the first sage was used as template. In the second stage similar reaction composition was used except 1392+GC was used instead of the 1392 primer and 20 amplification cycles were used. PCR products were evaluated by agarose gel electrophoresis. Negative controls without template addition were treated identically through the PCR and evaluated by agarose gel to confirm the absence of contaminant DNA.

DGGE: DGGE was performed at 60^0 C with a D-Code Universal Mutation Detection System (Bio-Rad Laboratories). Eight and twelve percent (w/v) acrylamide gels with denaturant gradients of 40 to 70% were used for analyzing fragments amplified using 1070 and 1392+GC. A 25 ml volume denaturing gel was poured and allowed to polymerize prior to pouring of a zero percent denaturant stacking gel for the loading wells. Sixteen hours of electrophoresis was performed for the gels at 60 V. After the electrophoresis the gels were subsequently stained with SYBR Green I (Cambrex Bio Science). Images of the gel were obtained using a FluorChem 8800® Imaging system and AlphaEase FC® software (Alpha Innotech). Composition of all the reagents and conditions used for making DGGE are presented in Appendix-B.

Statistical Analysis: Paired t-test analysis was done using Microsoft Excel on the data to see if there were significant differences between two treatments. The level of significance for all tests was $\alpha=0.05$.

Results

Phase-I-Effect of Water Quality on Copper Release.

For the first set of experiments that ran for three months the effluent NH_3-N concentration did not decrease from the influent over time and no NO_2-N and NO_3-N were detected.

Average effluent total and dissolved copper concentrations are shown in Table 2-5. The ammonia level did not influence the copper release and in general the high carbon reactors had approximately 1/3 more released copper, regardless of their ammonia concentration. Table 2-6 shows the p-values obtained from the paired t-test comparing effluent copper concentrations from the different reactors. Copper concentrations for high carbon dosed reactors are significantly higher than that for low carbon dosed reactors. All copper measurement values are shown in Appendix-C.

Table 2-5: Average effluent copper concentrations

Types of copper used	Carbon nitrogen condition	Average total copper	Average dissolved copper
New	Lo C-Lo N[1]	0.38±0.03	0.31±0.02
	Lo C- Hi N[2]	0.36±0.03	0.29±0.03
	Hi C- Lo N[3]	0.61±0.04	0.51±0.03
	Hi C- Hi N[4]	0.55±0.03	0.45±0.02
Old	Lo C-Lo N[1]	0.32±0.01	0.26±0.01
	Lo C- Hi N[2]	0.31±0.02	0.25±0.01
	Hi C- Lo N[3]	0.56±0.03	0.47±0.02
	Hi C- Hi N[4]	0.65±0.03	0.52±0.02

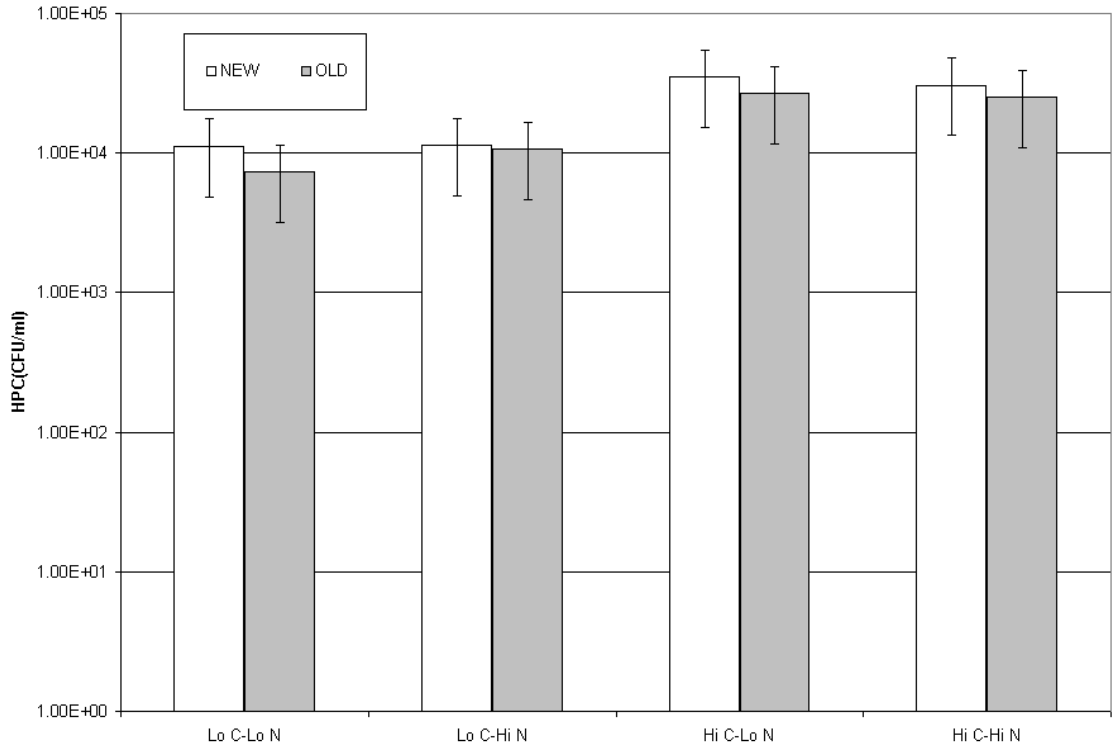
(1: Lo C-Lo N= TOC 2 ppm, influent NH_3-N 0.36 ppm, 2: Lo C-Hi N= TOC 2 ppm, influent NH_3-N 0.71 ppm, 3: Hi C-Lo N= TOC 4 ppm, influent NH_3-N 0.36 ppm, 4: : Hi C-Hi N= TOC 4 ppm, influent NH_3-N 0.71 ppm)

Table 2-6: p-values from paired t-test comparing total and dissolved copper concentration from different reactors

			Lo C-Lo N[1]	Lo C-Hi N[2]	Hi C-Lo N[3]	Hi C-Hi N[4]
Total Copper	New	Lo C Lo N[1]		0.587	0	0
		Lo C-Hi N[2]	0.587		0	0
		Hi C-Lo N[3]	0	0		0
		Hi C-Hi N[4]	0	0	0	
	Comparing Old and New		0	0.005	0.002	0
	Old	Lo C Lo N[1]		0.105	0	0
		Lo C-Hi N[2]	0.105		0	0
		Hi C-Lo N[3]	0	0		0
		Hi C-Hi N[4]	0	0	0	
Dissolved Copper	New	Lo C Lo N[1]		0.47	0	0
		Lo C-Hi N[2]	0.47		0	0
		Hi C-Lo N[3]	0	0		0
		Hi C-Hi N[4]	0	0	0	
	Comparing Old and New		0	0.012	0.007	0
	Old	Lo C Lo N[1]		0.021	0	0
		Lo C-Hi N[2]	0.021		0	0
		Hi C-Lo N[3]	0	0		0
		Hi C-Hi N[4]	0	0	0	

(1: Lo C-Lo N= TOC 2 ppm, influent NH_3-N 0.36 ppm, 2: Lo C-Hi N= TOC 2 ppm, influent NH_3-N 0.71 ppm, 3: Hi C-Lo N= TOC 4 ppm, influent NH_3-N 0.36 ppm, 4: : Hi C-Hi N= TOC 4 ppm, influent NH_3-N 0.71 ppm)

Average HPC populations of the reactors are shown in Figure 2-3. Higher carbon fed reactors had about 1 log higher heterotrophic bacteria in the bulk water. The HPC population was not affected by the type of copper used in the reactors, as there is not much difference in HPC values. In addition, since the high carbon dosed reactors had elevated soluble copper, the released copper did not seem to have a negative effect on HPCs.



(Lo C-Lo N= TOC 2 ppm, influent NH_3-N 0.36 ppm, Lo C-Hi N= TOC 2 ppm, influent NH_3-N 0.71 ppm, Hi C-Lo N= TOC 4 ppm, influent NH_3-N 0.36 ppm, Hi C-Hi N= TOC 4 ppm, influent NH_3-N 0.71 ppm)

Figure 2-3: Average (n=12) bulk water HPC values for the reactors

The CFU/cm^2 values for biofilm grown in different reactors are shown in Figure 2-4 . The CFU/cm^2 values in the new copper reactor biofilm is about 1 log less than the old copper in most cases. The extracted DNA of the biofilm sample from the reactors was amplified by PCR and DGGE was done. The microbial community profile found by DGGE is shown in Figure 2-5. The community composition was affected by the water quality. Some species bands (shown in white circles) were more affected by the conditions tested. Also there are differences between the community compositions in new and old copper reactors.

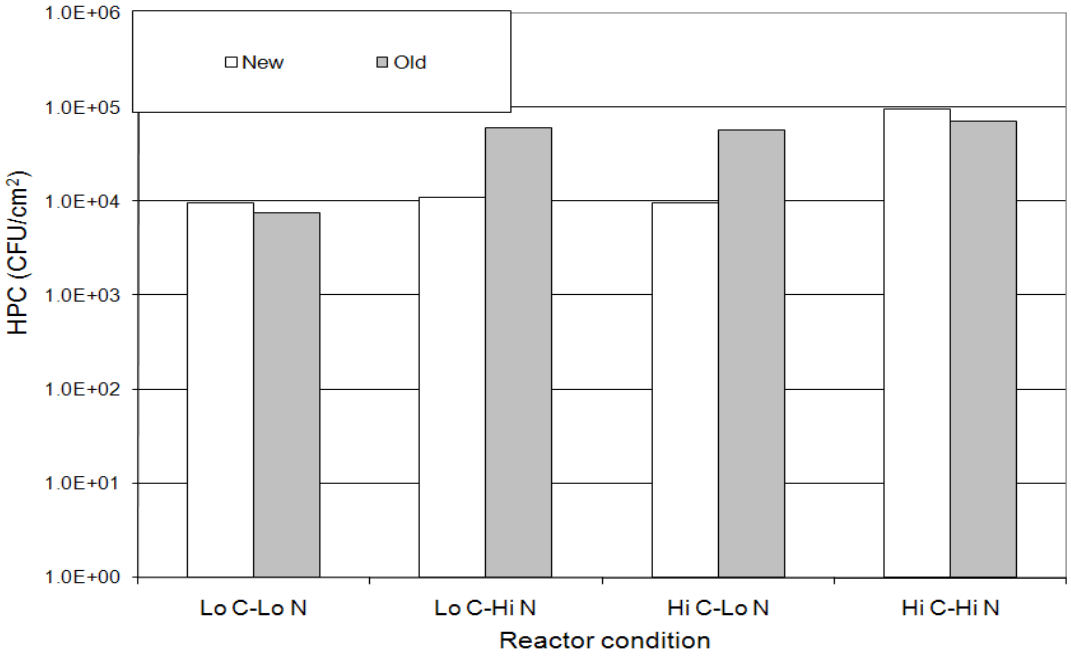


Figure 2-4: CFU/cm^2 values for biofilm from different reactors*

New Copper				Old (pre-exposed) Copper			
Lo-C Lo-N	Lo-C Hi-N	Hi-C Lo-N	Hi-C Hi-N	Lo-C Lo-N	Lo-C Hi-N	Hi-C Lo-N	Hi-C Hi-N
1	2	3	4	5	6	7	8

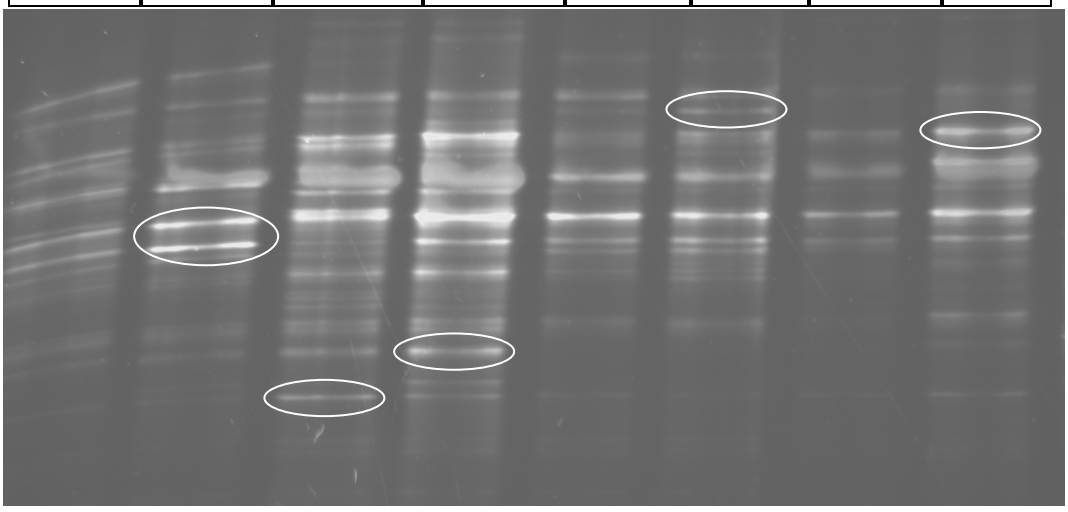


Figure 2-5 DGGE image of the microbial community of the reactors*
(* Lo C-Lo N= TOC 2 ppm, influent NH_3-N 0.36 ppm, Lo C-Hi N= TOC 2 ppm, influent NH_3-N 0.71 ppm, Hi C-Lo N= TOC 4 ppm, influent NH_3-N 0.36 ppm, Hi C-Hi N= TOC 4 ppm, influent NH_3-N 0.71 ppm)
White circles indicate species that are different from other profiles.

Phase-II Effect of Nutrient Condition and Pipe Material on Nitrification

In this study ammonia utilization in the PVC reactors increased after about three months of operation, but in the case of copper reactors a similar trend required almost five months. There was no difference in the onset of nitrification between low and high ammonia feed reactors for each coupon type. These systems were fed with BAC (biologically activated carbon) treated Bozeman tap water, which is dechlorinated and does not contain any ammonia. The organisms in this low nutrient water were able to colonize and begin nitrifying. Percent of ammonia utilization or conversion of ammonia to nitrite and nitrate in the bulk water was calculated from the effluent ammonia, nitrite and nitrate concentrations using the model described in Appendix-A. NH_3 utilization in the PVC and copper reactors are shown in Figure 2-6 and Figure 2-7.

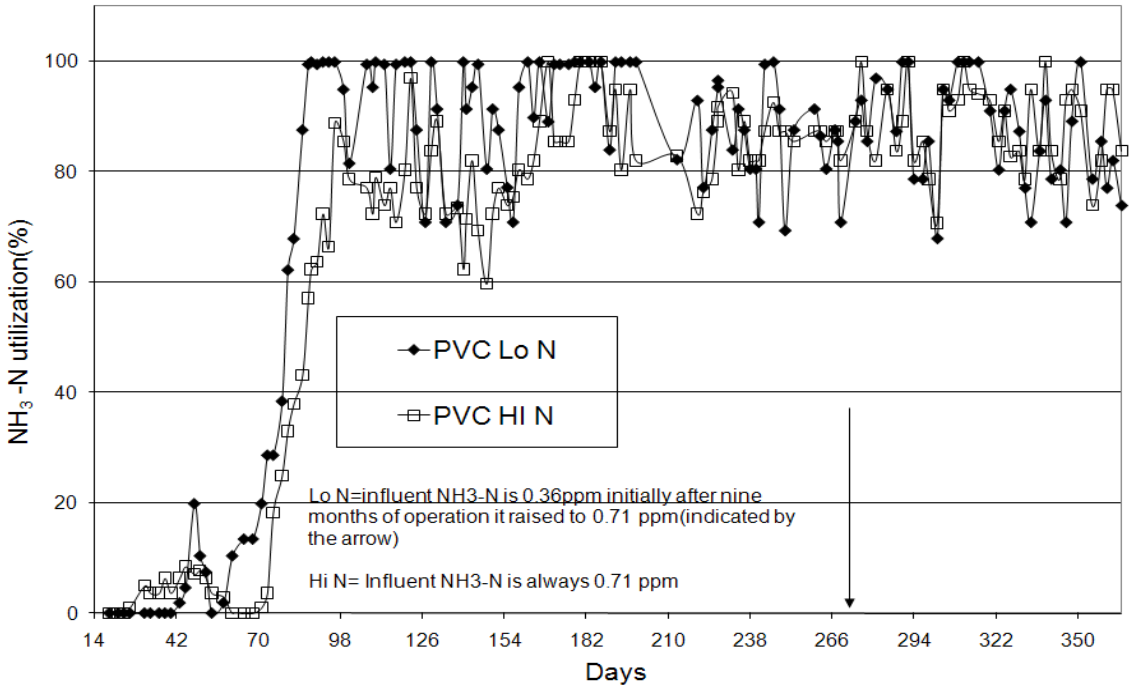


Figure 2-6: NH_3-N utilization in bulk water for PVC reactors

In the case of the copper reactor, the utilization of ammonia was not as stable as in PVC reactors, and sometimes dropped to zero. After nine months of operation, when all the reactors had shown signs of stable nitrification, the influent of the low ammonia feed reactors were changed to the high level. The PVC reactors were found to adjust to the changed condition instantly and the NH_3-N utilization did not change. The copper reactors were slower in adjusting to the high ammonia influent, but gradually the NH_3-N utilization rose to 100%.

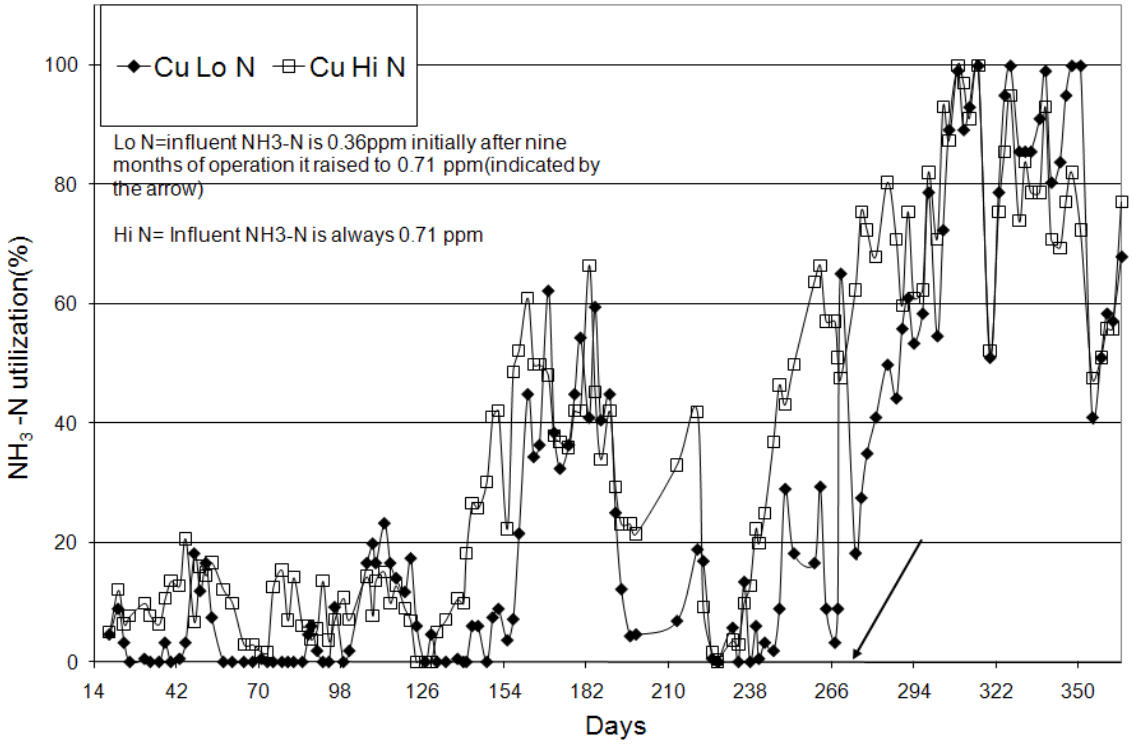


Figure 2-7: NH_3-N utilization in bulk water for copper reactors

Bulk water NO_2-N as percentage of initial NH_3-N concentration in bulk water in PVC and copper reactors are shown in Figure 2-8 and Figure 2-9. One interesting trend was that for low NH_3-N, when the influent NH_3-N was raised to the higher level, the

NO_2-N percentage decreased and became similar to that of the high NH_3-N feed reactors. However, the magnitude of this change is very low; around 1~2% and measured actual concentration of around 7~10 ppb.

Bulk water NO_3-N as percentage of initial NH_3-N in bulk water for PVC and copper reactors are shown in Figure 2-10 and Figure 2-11.Virtually all the NH_3-N was converted to NO_3-N. Considering all these graphs (Figure 2-6 ~ Figure 2-11) another interesting observation is that low ammonia fed copper reactors are slower in adapting to the higher ammonia containing influent than their PVC counterpart.

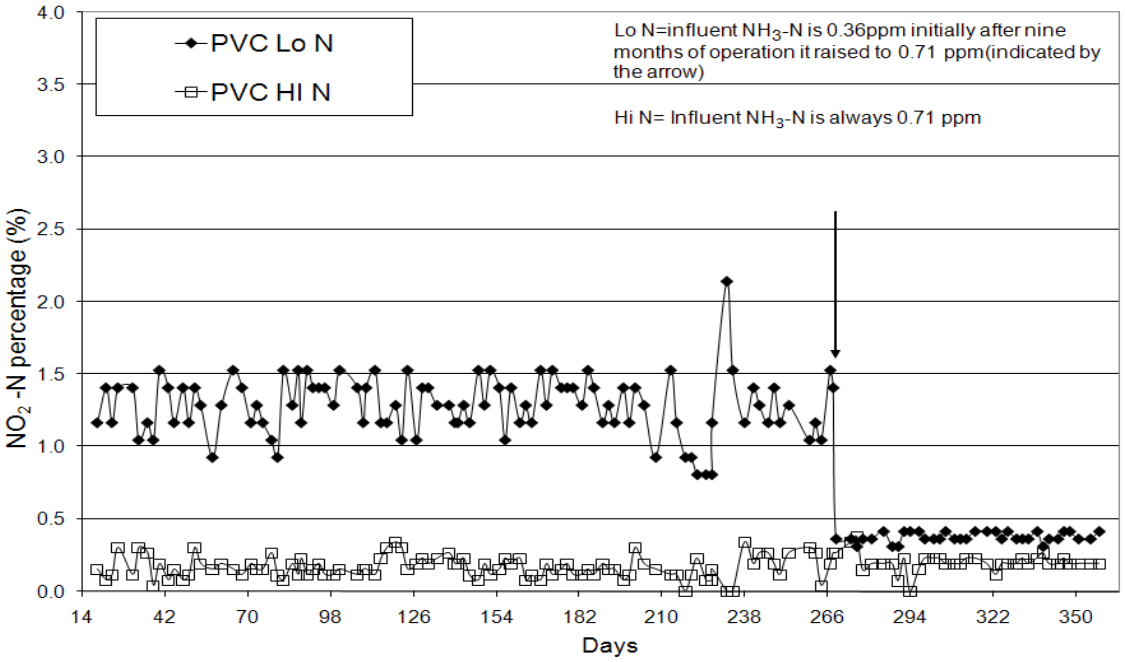


Figure 2-8: Bulk water NO_2-N as percentage (%) of initial NH_3-N for PVC reactors

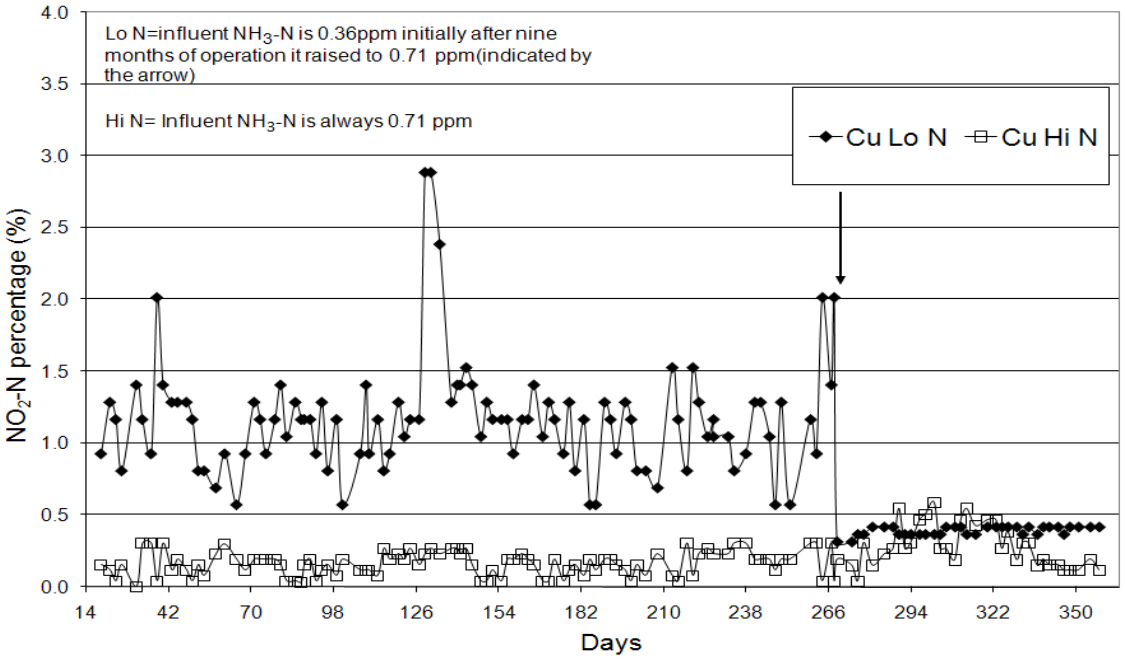


Figure 2-9: Bulk water NO_2-N as percentage (%) of initial NH_3-N for copper reactors

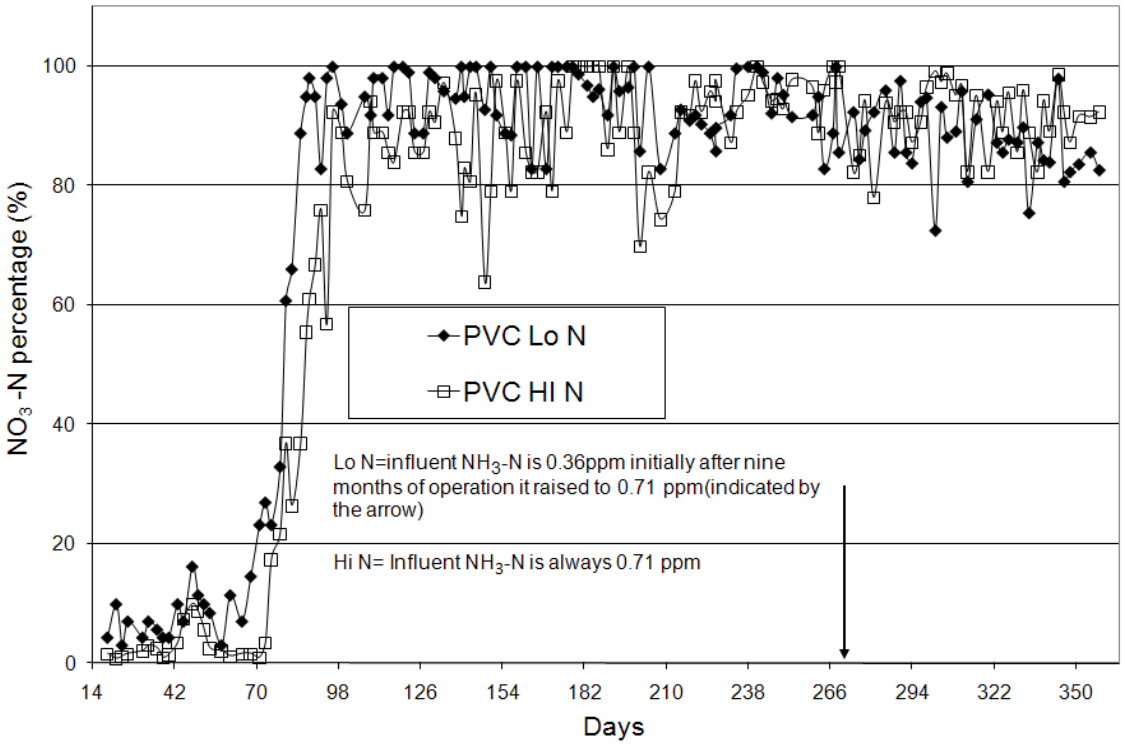


Figure 2-10: Bulk water NO_3-N as percentage (%) of initial NH_3-N for PVC reactors

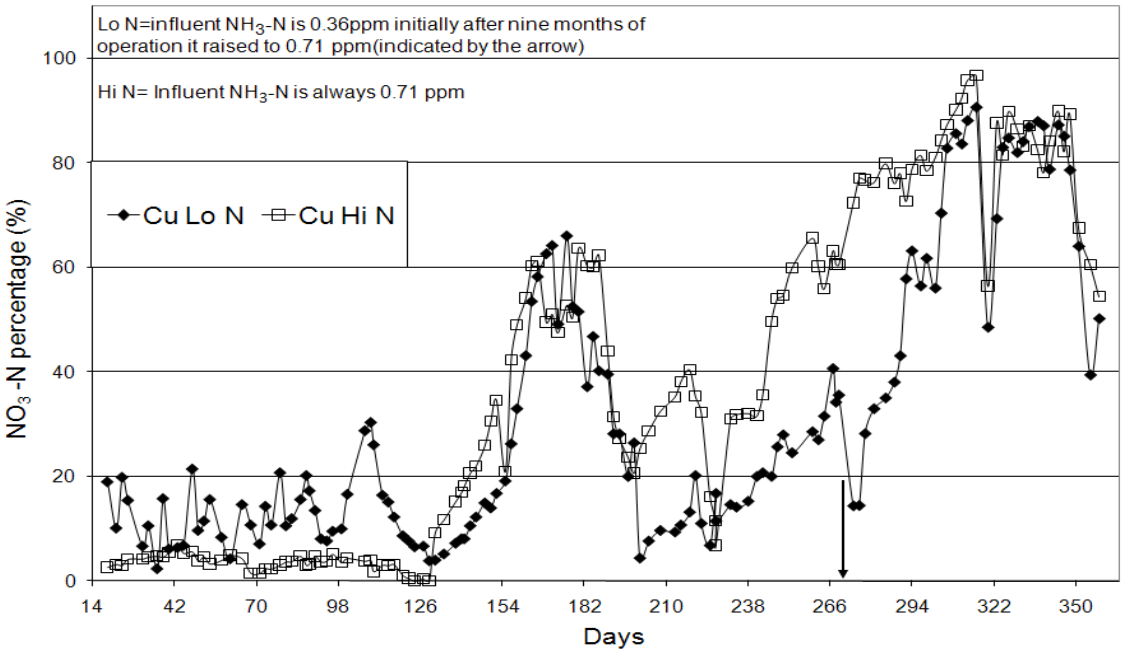


Figure 2-11: Bulk water NO_3-N as percentage (%) of initial NH_3-N for copper reactors

For the first seven months of operation the 95% confidence interval for average total and dissolved copper concentrations of the effluent from the low ammonia fed copper reactors are (0.79±0.05) and (0.55±0.04) respectively. The values for high ammonia feed reactors are (0.83±0.04) and (0.62±0.03). The copper concentration is higher in high ammonia feed reactors as compared to the low ammonia feed reactors. Figure 2-12 and Figure 2-13 shows the NH_3 utilization and total copper concentration for the copper reactors. An interesting trend was that in both cases when the NH_3 utilization increased due to nitrification, the copper concentration increased. This may be due to the lowering of pH through nitrification.

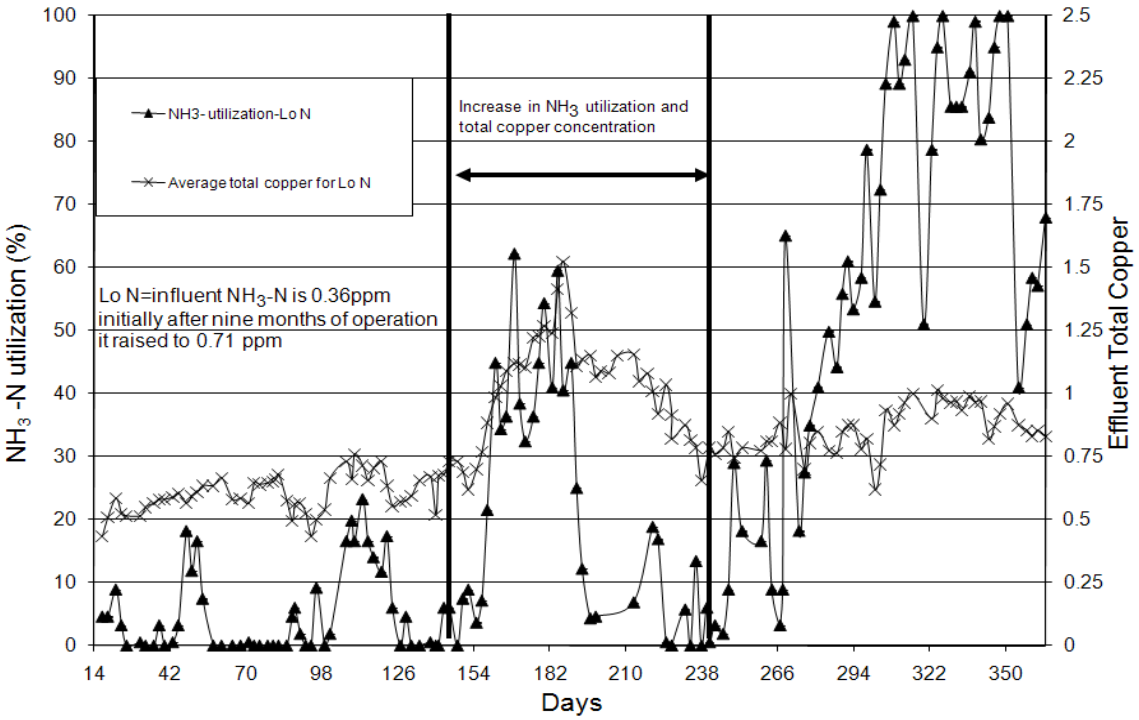


Figure 2-12: NH_3-N utilization (%) in bulk water and effluent total copper concentration for low NH_3 feed copper reactor

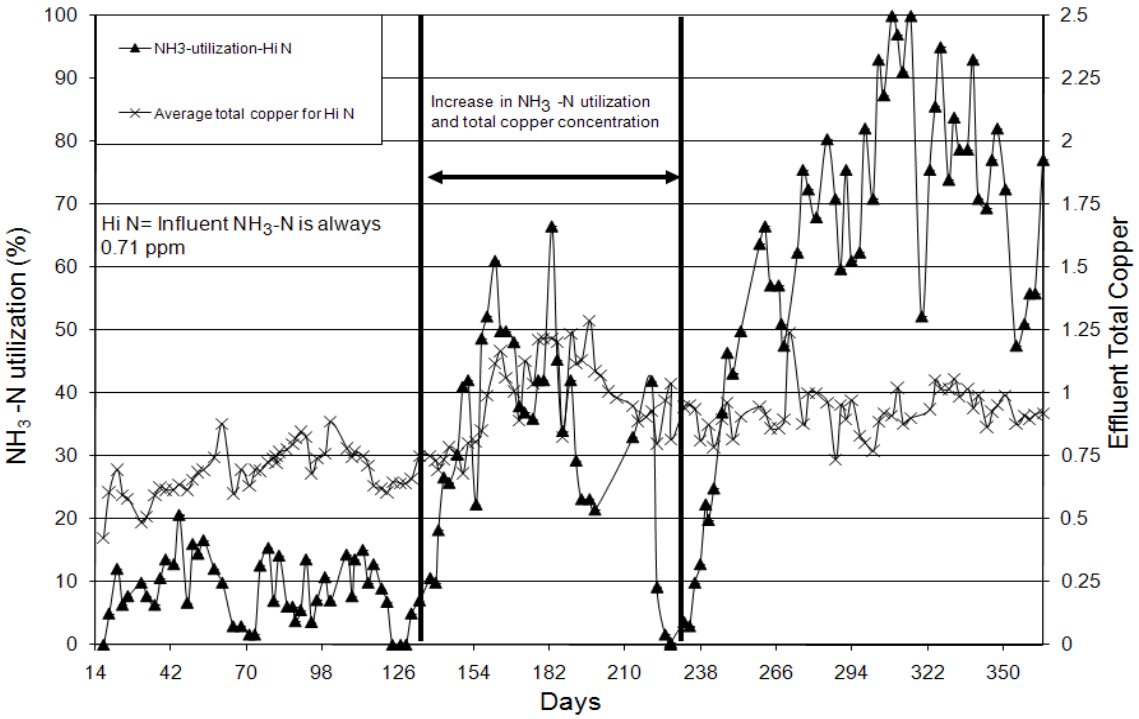


Figure 2-13: NH_3-N utilization (%) in bulk water and effluent total copper concentration for high NH_3 feed copper reactor

The monthly average bulk water HPC of the reactors is shown in Figure 2-14. Also the CFU/cm^2 values for the biofilm in different reactors after nine months of operation (for low ammonia feed reactor before and after raising the ammonia level) are shown in Figure 2-15. The HPC values in PVC and copper reactors did not show any specific trends. There were no apparent effects of soluble copper or increased nitrification on bulk water HPC or biofilm HPC.

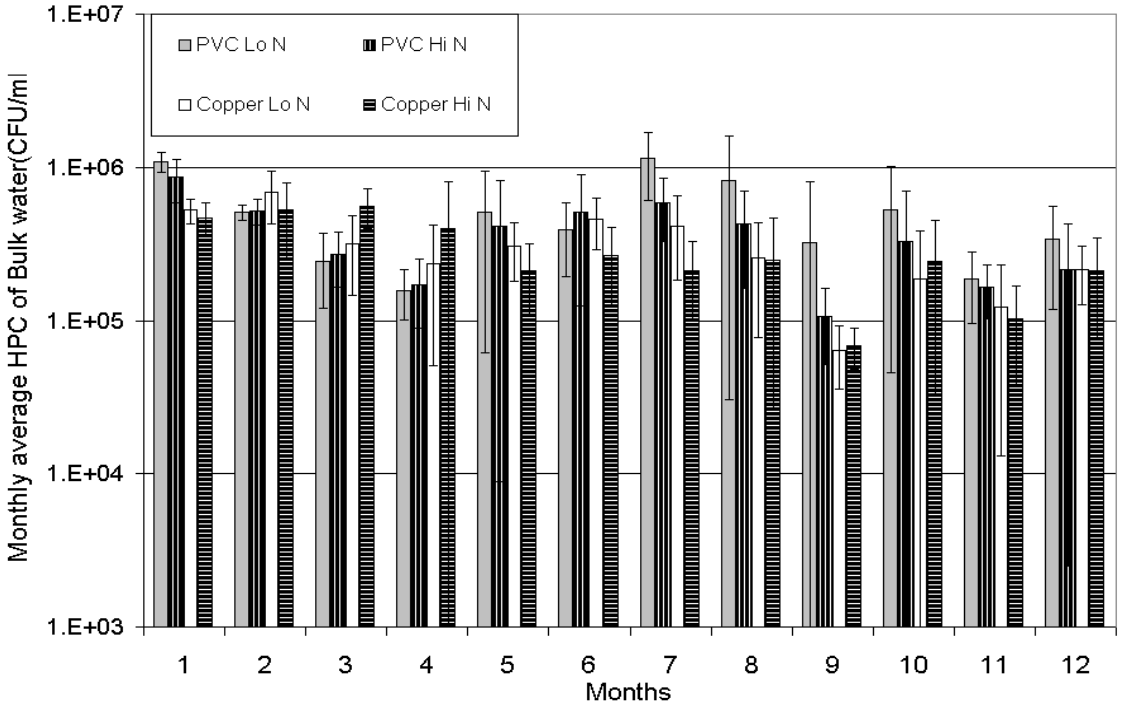


Figure 2-14: Monthly average (n=48) HPC of reactor bulk water.

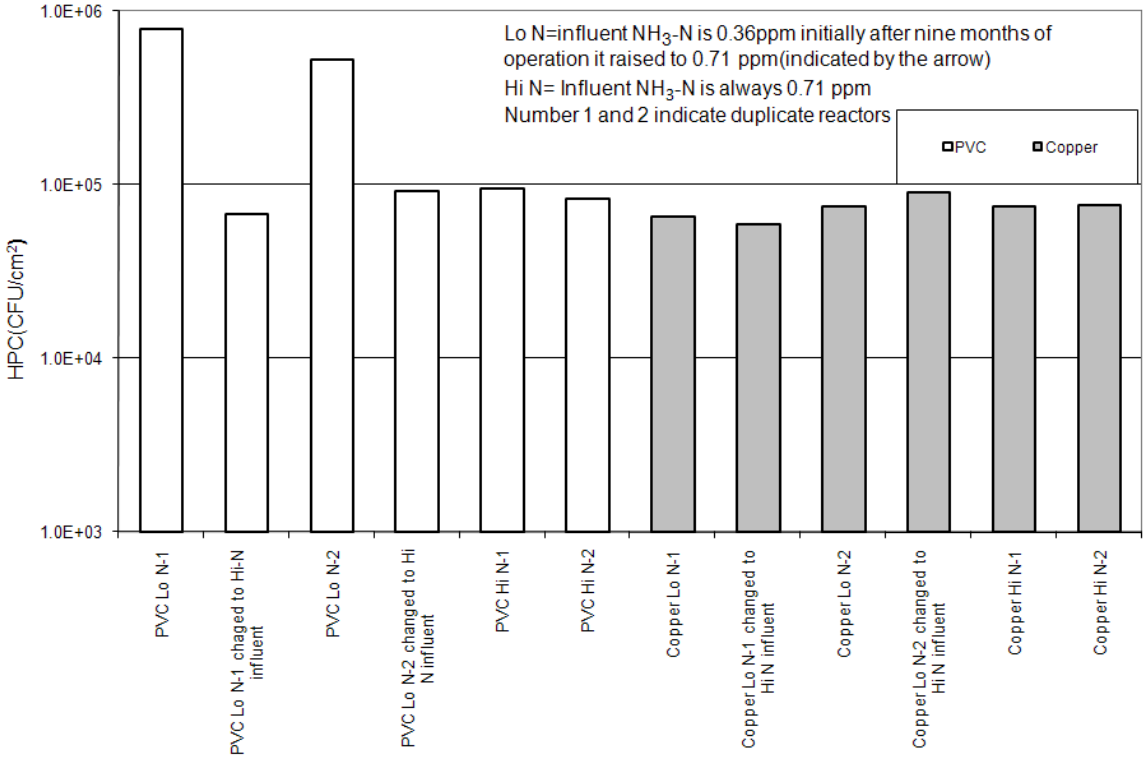
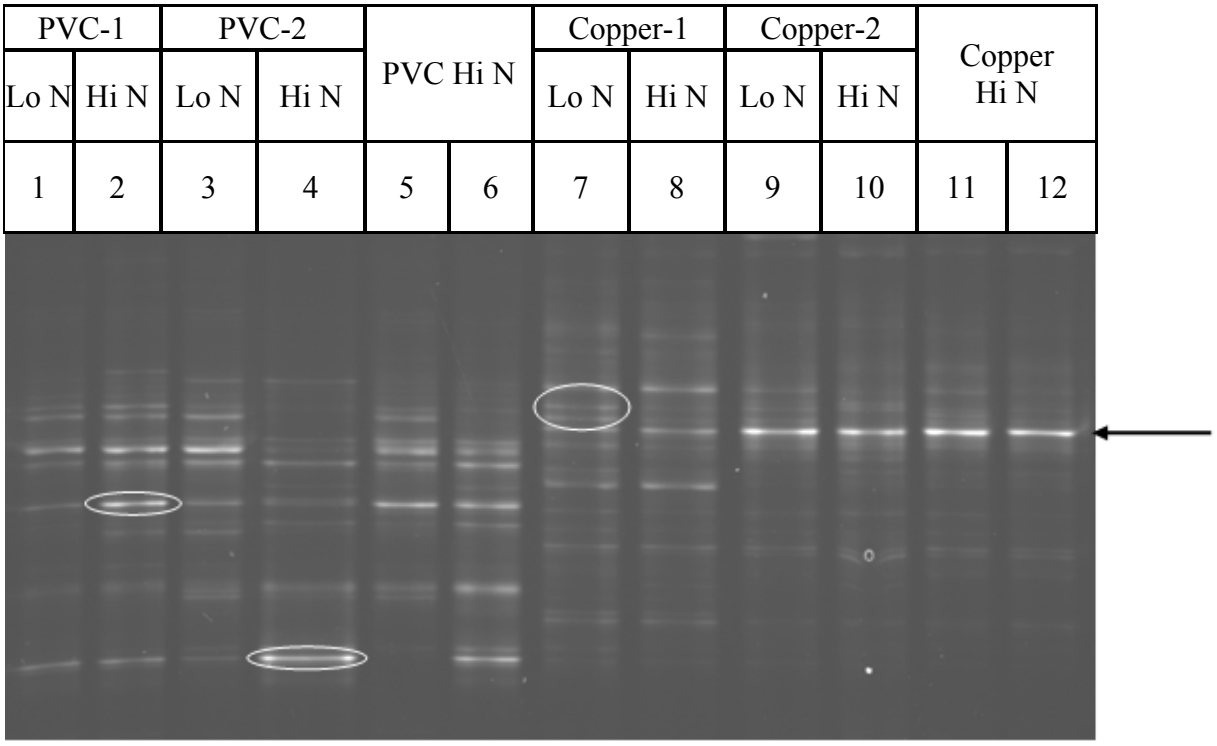


Figure 2-15: CFU/cm^2 for HPC of biofilm in different reactors

A biofilm sample was taken from the reactors just before the low NH_3 feed reactor influent was raised to the high concentration. When the reactors became accustomed to this change, biofilm samples were taken again. The extracted DNA of these biofilm samples was amplified by PCR and DGGE was done. The microbial community profile found by DGGE is shown in Figure 2-16. In this figure, PVC-1 and PVC-2 are community profiles from two duplicate PVC reactors, which are initially low ammonia fed influent. PVC Hi-N indicates the duplicate PVC reactors fed with high ammonia influent throughout the experimental period. Similar notation is applied for the copper reactor's community profile shown on the right side of the figure. Some bands/species are affected by changes in ammonia level as indicated by the circles. Also

another interesting finding is that the pipe material influences the biofilm community profile. For example a bright band shown by the arrow is prominent in all copper reactors, whereas if present in the PVC reactors it is less distinct.



(Lo C-Lo N= TOC 2 ppm, influent NH_3-N 0.36 ppm, Lo C-Hi N= TOC 2 ppm, influent NH_3-N 0.71 ppm, Hi C-Lo N= TOC 4 ppm, influent NH_3-N 0.36 ppm, Hi C-Hi N= TOC 4 ppm, influent NH_3-N 0.71 ppm)

Figure 2-16: DGGE image of the microbial community of the reactors

Comparison between the Two Studies

The first experiment was conducted for only three months and eight different water quality conditions were tested (Table 2-1). In the second set of experiments only two conditions were tested. Three month's average values for total and dissolved copper data from the second set of experiments are plotted against data from first set of

experiments on Figure 2-17. The effluent copper concentration values are comparable. But in comparing average copper concentration for the entire period of phase II the copper concentration increased with increase of added ammonia concentration. This may be due to lower pH by nitrification of the supplied ammonia. Average (n=12) HPC for the first three months of the second set of experiments were also plotted against the average HPC value found at the end of the preliminary experiment in Figure 2-18. Average HPC values obtained in the phase II experiment are about one log higher than the preliminary experiment. Other parameters such as biofilm cell densities or DGGE profiles cannot be compared as they were taken at different time points

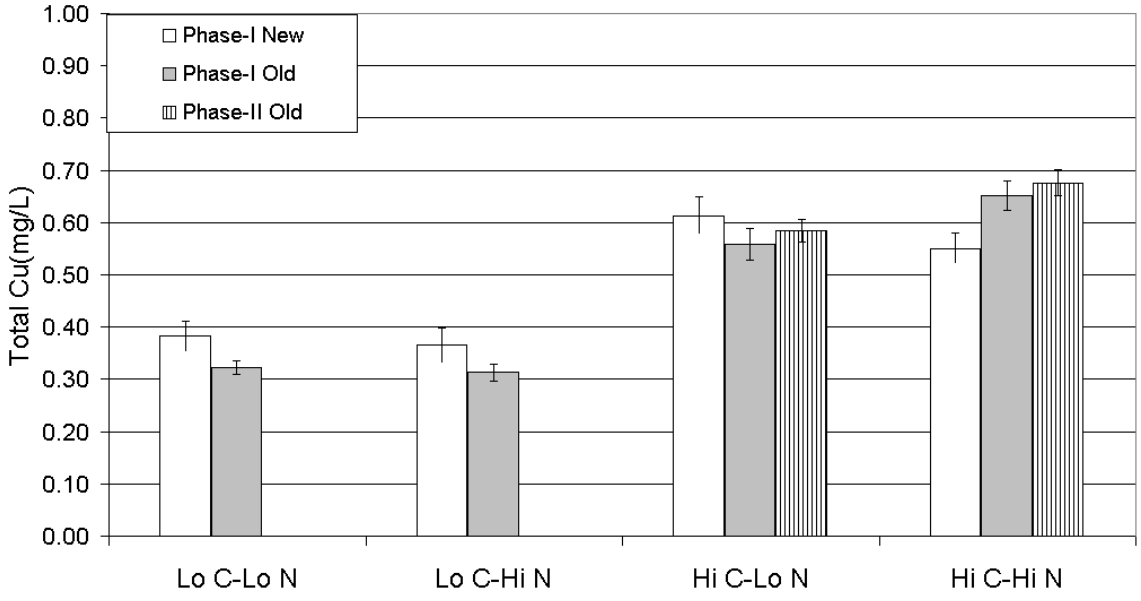


Figure 2-17: Comparison of average total copper values from two sets of experiments

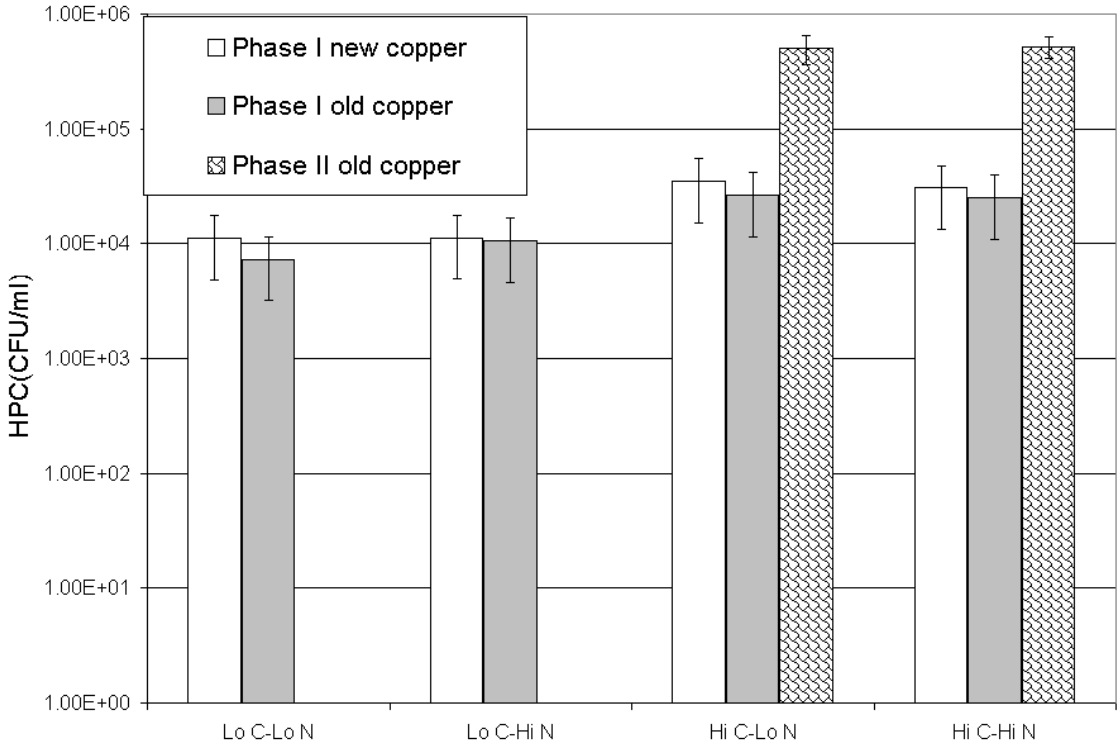


Figure 2-18: Comparison of average HPC values from two sets of experiments

Discussion

In the first experiment with old and new copper (which ran for three months) the effluent NH_3 level did not decrease and NO_2 and NO_3 did not increase, suggesting that there was no nitrification. However copper concentrations in the effluent were affected by the water quality, especially by the carbon concentration. Total carbon concentration (TOC) usually represents the natural organic matter (NOM) present in water. NOM is primarily composed of negatively charged humic and/or fluvic acid (Edwards et al. 1994). These compounds are of relatively high molecular weight, containing oxygenated functional groups such as carboxyls and carbonyls, etc. Copper corrosion can be affected

as these functional groups react with soluble copper ions and oxide solids (corrosion products) (Davis et al. 1984). According to Campbell (1954), NOM can inhibit pitting corrosion of copper. In the presence of NOM he reported that a thick protective cuprous oxide layer formed on the surface. In the absence of NOM, a loose, non protective oxide layer was formed. Edwards et al. (1993) showed that NOM initially increased copper leaching in water at a pH of 9.3, but after aging, copper release eventually decreased. The authors observed that the presence of NOM increases the corrosion rate as measured by a Reiber corrosion cell. Broo et al. (1999) also suggested that NOM increases the corrosion rate and that the corrosion rate is proportional to the logarithm of the free carbon dioxide content and NOM.

The effect of NOM on copper solubility depends on the characteristics of the NOM and the water. According to Davis et al. (1984), NOM forms strong complexes with soluble cupric (Cu^{+2}) and cuprous ion (Cu^{+}). The copper concentration in water is determined by the equilibrium between soluble copper complexes and the meta stable solid cupric hydroxide (Edwards et al. 1996, Mayer and Edwards 1994). Because of the formation of strong complexes between NOM and soluble copper, solubility of the copper corrosion product increases with an increase of NOM content of water. Average copper values in Table 2-5 show that higher NOM content increased the effluent copper concentration.

According to Schock et al. (1995) strong complexations exist between copper ions and NH_3 (up to 8 ppm of NH_3). Therefore the presence of NH_3 should increase copper

leaching. Influence of NH_3 was only observed on higher carbon (TOC= 4 ppm) dosed old copper reactors. In all other cases, copper concentration decreases with increase of NH_3.

The old copper was aged by soaking in 0.1N NaOH solution for eight hours. This process makes it equivalent to copper surface which is used for six months (Edwards 2008). In most cases the new copper reactors have higher copper concentrations than that of old reactors except the Hi C Hi N conditions.

Previous studies (Kim et al. 2002, Lehtola et al. 2004) reported that copper has a toxic effect on heterotrophs in drinking water. In this project no significant effect of copper on the suspended HPC were observed. Again this discrepancy may be due to the difference in experimental conditions and difference in bacterial populations present in the water. The bulk water HPC in high carbon (TOC=4 ppm) feed reactors are about 1 log higher than the low carbon (TOC= 2 ppm) feed reactors (Figure 2-3). The higher count may be attributed to the higher concentration of carbon source in the influent water even though the copper levels were also higher. Microbial community profiles were also impacted by the concentration of NOM. In the DGGE profile shown in Figure 2-5 several bands (in lane 3 and 4) became brighter at higher NOM than those profiles from low NOM (in lanes 1 and 2). No such trend was observed in the case of old copper (in lanes 5 to 8). NH_3 concentration also affected the populations. For example, in higher carbon (TOC=4) new copper reactors (in lanes 3 and 4), two bands became brighter (circled) after the change in NH_3 concentration. Similar changes were observed in the profile of old copper reactors. A similar effect is also found by comparing other lanes (between 1,2

and 5,6 and 7,8) where there were differences in NH_3 concentrations (shown by white circles).

In the second set of experiments where all reactors were operated at 4 ppm TOC, NH_3 utilization in the PVC reactors increased significantly after three months of operation (Figure 2-6). NO_2 in the bulk water was very low but the percentage of NO_3 increased gradually due to complete nitrification. In the case of the copper reactors signs of nitrification were observed after five months of operation. Copper reactors also experienced some type of unknown disturbance/upset as the NH_3 utilization decreased at around 180~240 days of operation (Figure 2-7). During this time period effluent NO_3 concentration also decreased.

When the copper reactors first showed signs of nitrification the effluent total and dissolved copper concentration increased dramatically (up to 1.3~1.7 ppm). With time, the copper concentration decreased to around 1 ppm. The decrease in copper concentration from the maximum concentration occurred mostly when the nitrification in those reactors was hindered to some extent (during days 180~240). The copper concentration may be governed by biofilms. Nitrification occurs predominantly in a biofilm as nitrifiers are mostly found there rather than in the planktonic state (Lipponen et al. 2002, Wolfe et al. 1990). Due to nitrification the pH near the biofilm surface may drop significantly. At a lower pH, the oxide layer on the copper may become soluble and porous (Edwards et al. 1994, Zhang et al. 2002), resulting in higher corrosion/leaching of copper. Excess copper is toxic to both heterotrophic and autotrophic bacteria and the effect of this toxicity on nitrification depends on the amount of free cupric ion (Cu^{+2}),

which is dominant species at low pH (Thurman and Greba 1989, Straub et al. 1995, Kim et al. 2002, Artz and Killham 2002, Teitzel and Parsek 2003). Due to the toxicity of this high copper concentration in water, biological nitrification may be partly hindered, resulting in lower NH_3 utilization. As the nitrification is hindered, the pH change may not be as acute and as a result a thicker protective oxide film may be formed during this time. Also the biofilm might become thicker with time and provide protection to copper toxicity, or it might develop an adaptation to copper. The protective oxide layer might reach some type of equilibrium with the system and exist even after the biofilm recovered from toxicity, as the copper concentration did not show an unusually high value.

When the influent nitrogen concentration of the low NH_3 feed reactors was raised to the high level, the PVC reactors acclimated to this change very quickly. Also the percentage of nitrite in the bulk water dropped after this change, and this may be due to the increased activities of NOB from the increased amount of nitrite formed by the AOB at the higher ammonia concentration. The copper reactors were slower than the PVC in utilizing the excess NH_3. As previously stated most nitrifying bacteria exist in a biofilm and copper is known to be toxic to bacteria. Schwartz et al. (1998) reported that biofilm grown on copper surfaces were less dense than those grown on plastic. Therefore the biofilm in copper reactors may have had weaker nitrification ability because of their thin formation/composition.

Increased HPC values are sometimes reported with nitrification. The bulk water HPC values are shown in Figure 2-14. No increase in the HPC value was found during nitrification. Conversely, no decrease was seen, suggesting that there was minimal impact

of copper ion, even at the highest concentration measured, on suspended HPC. Pipe material can have influence on biofilm (LeChevallier et al.1990). Different researchers (Pedersen 1990, Percival et al.1998) have shown that surface roughness of the pipe material contribute to bacterial attachment and biofilm formation However Paquin et al. (1992) reported no difference in biofilm formation on different plumbing material surfaces. Biofilm cell densities found in different reactors (Figure 2-15) did not show any significant difference, which agrees with these results. This also suggests that if there was an influence of copper on biofilm organisms, it was only towards nitrifiers.

DGGE profiles of different biofilm samples are shown in Figure 2-16. With changing nutrient conditions some species proliferated (shown in the white circles). Pipe materials also were found to influence the biofilm community profile. For example a bright band shown by the arrow is prominent in all copper reactors, whereas if present in the PVC reactors it is less distinct.

In this project, the PVC surface was found to be more susceptible to nitrification than the copper. Nitrification might be responsible for aggravating the corrosion of the copper pipe metals. Nutrient condition and surface properties both play important role in community compositions. In the range of 2~4 ppm TOC, copper leaching increased with increases in TOC.

Conclusion

- Copper leaching increased at higher TOC concentrations
- For conditions tested PVC systems were more prone to nitrification, but nitrification also occurred on copper
- For conditions tested nitrification can increase copper release in water
- Biofilm community composition assessed by DGGE exhibited differences in response to nutrient condition and pipe material
- Leached copper did not influence suspended HPC counts

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CHAPTER 3

CONTROL OF NITRIFICATION IN PREMISE PLUMBING

Abstract

The problem of nitrification in drinking water supplies in United States was identified as early as 1935 (Faben 1935). A 1996 American Water Works Association Research Foundation (AwwaRF) survey indicated that two thirds of drinking water utilities that chloraminate have experienced some degree of nitrification (Wilczak et al. 1996). In this project, the effect of copper ion, chlorite and chloramine on nitrification in a simulated household plumbing system was investigated.

A modified version of the CDC reactors developed at the Center for Biofilm Engineering and the Centers for Disease Control (CDC) was used to simulate a household plumbing system. Two sets of reactors were used; each set consists of four reactors containing either PVC as a coupon material or copper. These reactors were fed water with humics as the organic carbon (4 ppm) source and ammonium sulphate (0.71 ppm) as the nitrogen source and biologically treated tap water to supply the bacterial population. Water in the reactors was stagnant for eight hours and then flowed for five minutes. pH of the influent water was maintained at around 8.15 and alkalinity was around 35 mg/L as $CaCO_3$.

After three months of operation the PVC system began nitrifying and by five months the copper system was also nitrifying. In one PVC reactor, copper was gradually introduced with the influent water as copper sulfate at 15 ppb and gradually it was

increased to 1.3 ppm, allowing a two week exposure time for every concentration. To ensure that most of the copper was in the ionic form, the pH of the influent was then gradually lowered to 6.6. No significant change in nitrification was observed under any of these conditions. Heterotrophic plate count and autotrophic nitrifying bacteria were enumerated using R2A plates and an MPN method. No significant effect of copper on those populations was observed.

In the second stage of the investigation, chlorite, which is known to inhibit autotrophic nitrification in drinking water systems, was introduced in a copper and a PVC reactor. Initially chlorite was added at 0.2 ppm and gradually increased to 2.0 ppm and finally a shock load of 20 ppm was applied. The effect of chlorite on the PVC system was not significant but in the copper system it inhibited nitrification only at 20 ppm. It took about two months for the copper reactor after chlorite was discontinued to restart nitrification. Nitrifiers, especially nitrite oxidizers were more affected by presence of chlorite.

In the final stage of the project, monochloramine was introduced to both PVC and copper reactors. Monochloramine was initially applied at a 0.5:1 chlorine to ammonia ratio. Gradually this ratio was increased to 5:1. Nitrification activity was impacted significantly at a 5: 1 ratio of chlorine to ammonia and ultimately stopped at that ratio. After eight weeks of exposure at the 5:1 ratio, monochloramine was discontinued. The copper reactor regained full nitrification ability within two months but the PVC reactor required three months.

Introduction

Nitrification is the oxidation of ammonia to nitrite and nitrate. Ammonia in water can be present either naturally or from chloramination. Nitrification is believed to be primarily accomplished by a group of autotrophic nitrifying bacteria that obtain energy from ammonia and nitrite. In the first step ammonia oxidizing bacteria (AOB) oxidize ammonia to nitrite according to the following equation.

$$NH_3 + 2H_2O \longrightarrow NO_2^- + 7H^+ + 6e^- \qquad \text{Equation 1}$$

Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosolobus, and *Nitrosovibrio* are common AOB and *Nitrosomonas* is the most common among them (Kirmeyer et al. 1985).

In the second step nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate according to the following equation (Doetsch and Cook 1973).

$$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^- \qquad \text{Equation 2}$$

Nitrobacter is the most common NOB, although *Nitrospina* and *Nitrococcus* species are known nitrite oxidizers (Kirmeyer et al.1985). Heterotrophic bacteria and fungi can also carry out nitrification at a slower rates than autotrophic nitrifying bacteria (Watson et al. 1981)

Chloramine use is expected to be more prevalent in the near future because of its reduced production of DBPs (Regan et al. 2002). According to USEPA, to meet the

Stage 2 Disinfection By-Product (DBP) Rule about 65% of utilities might switch to chloramination (EPA 2000). According to the Information Collection Rule (ICR) database, 33% of 353 treatment plants use chloramines (Karim et al. 2006). Nitrification is one of the most frequent operational problems encountered by drinking water utilities that use chloramine as their distribution systems for secondary disinfection (Feben 1935 Skadesen 1993, Wolf and Lieu 2001). In a telephone survey (AwwaRF 1995) of 98 utilities which practice chloramination, it was reported that in two thirds of them, nitrification occurs and in one third it causes some type of operational problem.

Nitrification causes detrimental changes in water quality. Due to nitrification chloramine residual, pH, alkalinity and dissolved oxygen of water decrease. Reduction in pH and alkalinity can lead to Lead and Copper Rule (LCR) violations. Nitrification can cause biological instability through production of soluble microbial products (SMP) which may support the growth of heterotrophic bacteria in low nutrient environments (Rittmann et al. 1994). SMP production and depletion of disinfectant can cause high levels of HPC. The nitrite and nitrate concentration in water increases because of nitrification. Nitrite accelerates the decomposition of chloramines (Margerum et al. 1994, Valentine 1985, Woolschlager et al. 2001). Theoretically 1 mg/L of NO_2-N could consume 5mg/L of chlorine as monochloramine according to the following reaction.

$$NH_2Cl + NO_2^- + H_2O \longrightarrow NH_3 + NO_3^- + HCl \qquad \text{Equation 3}$$

According to the Safe Drinking Water Act (SDWA), primary MCLs for nitrite-N and nitrate-N are 1 and 10 mg/L respectively (EPA). High levels of nitrite and nitrate have adverse health effects especially on infants. Infants may have symptoms like shortness of breath and blue-baby syndrome (Peavey et al. 1985) or methomoglobinemia (Casarett et al. 1986).

Nitrification, once started in the distribution system, is very hard to control. It can even continue in the presence of higher chloramine residuals (Skaden 1993, Cunlife 1991) The common practices of the utilities to control nitrification include flushing, system cleaning, free chlorination, reducing stagnation and dead ends in the system, ensuring a residual disinfectant etc. If not prevented or controlled nitrification may lead to very expensive and complex operating problems.

Most of the previous studies on nitrification were done in distribution mains or at the treatment plant level. Premise plumbing not only has higher surface to volume ratios but also has about ten times the length of that in mains. Also more favorable conditions for nitrification exist in premise plumbing such as low or no disinfectant, long water age and warmer temperature. Another difference between distribution systems and premise plumbing is its piping material. Copper is the most widely used metal for household plumbing systems and according to Oskarsson et al. (1998) in the US more than 90% of domestic plumbing material is made of copper. PVC pipes are also very common as plumbing material (NSF 2008). Because of the potential of significant nitrification to occur in premise plumbing, and because PVC is used in distribution systems as well, both materials were used in this project. The focus of this research was to evaluate nitrification

control strategies that could be implemented in either the choice of material (copper) or by manipulating the water quality at the treatment plant.

Effect of Copper on Nitrification

Copper is an essential micronutrient in nitrification. AOB bacteria oxidize ammonia to nitrite via hydroxylamine (NH_2OH), nitroxy (NOH), and nitric oxide (N_2O) intermediates (Benmoussa et al. 1986 a and b, Xu et al. 2002). Copper is an essential component for ammonia monooxygenase (AMO) which is the enzyme needed for ammonia oxidation (Anderson 1965, Bedard et al. 1989, Ensign et al. 1993, Richardson and Watmough 1999). A copper protein is also essential for oxidation of hydroxylamine (Nicholas et al. 1962). Shears et al. (1985) proposed a catalytic cycle for AMO, where binding and reduction of oxygen occurs at a binuclear copper site on the enzyme. Oxidation of nitrite to nitrate also involves a copper containing protein (Campell et al. 1965). Conversely copper may have an adverse effect on organisms depending on the concentration. Bernhard (1969) hypothesized noncompetitive enzyme inhibition where copper prevents the enzyme (AMO) from properly catalyzing the chemical reaction of oxidation of ammonia. According to Avery et al. (1996) and Howlett et al. (1997), as a redox-active metal, copper catalyzes the production of hydroxyl radicals and through redox-cycling increases stress, which promotes membrane lipid peroxidation. Other researchers (Avery et al. 1996, Sani et al. 2001, Hu et al. 2003) also described membrane disruption as a possible mode of copper toxicity.

Other studies have investigated the effect of copper in environmental samples. Toxicity towards heterotrophic bacteria in activated sludge was found in a study by Dorussen et al. (1974). An investigation by Braam and Klapwijk (1981) also showed similar levels of toxicity to heterotrophs as that of autotrophic nitrifiers. However, due to low energy availability and low reproduction rates, nitrifying bacteria are generally perceived to be more sensitive to toxicity and environmental conditions (Gerardi 2002). Different researchers (Loveless and Painter 1968, Tomlinson et al. 1966, Skinner and Walker 1961, Tang 1992) reported different ranges of copper concentration for inhibition or stimulation of nitrifier activity. Most of these studies were done in pure cultures or with activated sludge. Loveless and Painter (1968) found that 0.005 to 0.03 ppm of Cu^{+2} stimulated *Nitrosomonas* activity. Another study showed that Cu^{+2} concentrations at higher levels (0.1 to 0.5 ppm) also have a stimulating effect on *Nitrosomonas* (Skinner and Walker 1961). However, Loveless and Painter (1968) reported that 0.05 to 0.56 ppm of Cu^{+2} is inhibitory to *Nitrosomonas* activity. According to Pettet (1965) 1 ppm of copper caused detectable inhibition in a laboratory scale activated sludge process. In a recent study, Zhang et al. (2005) observed slight inhibition of nitrification for pure cultures in presence of 5 ppb copper, 25 ppb copper had a slightly stimulatory effect, and 500 ppb copper significantly inhibited nitrification. They stated that the optimum copper concentration for nitrifying bacterial growth is between 5 ppb and 500 ppb. In general the inhibitory concentration of copper for pure cultures is much lower than for mixed cultures (environmental samples). According to Tomlinson et al. (1966), 4 ppm of copper

could inhibit pure cultures but 150 ppm of copper was needed to achieve the same level of inhibition in activated sludge.

The difference in these results may be due to the organisms, but could also be the result of different system conditions. Copper toxicity depends on exposure time, water hardness, presence of organic matter, alkalinity and pH (Sprague 1985). Schlenk et al. (1994) reported a 30% increase in toxicity from 24 to 96 hours of copper exposure for the ciliate protozoan *Tetrahymena thermophila*. According to Kristen et al. (2004) water hardness and copper toxicity to *Daphnia magna* are inversely related. Hardness cations (e.g. Ca^{+2} and Mg^{+2}) compete with copper ions for active sites, thus reducing copper toxicity (Lauren et al. 1985). However, a study conducted by Karel et al. (2004) did not find any any significant effect of hardness on copper toxicity. Natural organic matter, which includes fluvic and humic acid, can act as a complexion agent for copper in water (Campbel 1995, Sarathy et al. 2005, Kim et al. 2006). Due to this complexation copper may be less bioavailable or less toxic to organisms.

Copper toxicity also depends on the concentration of cupric ion (Cu^{+2}) (Braam and Kapwijk 1981). Cupric ions are very reactive and bind to electron-rich organic molecules (Nriagu 1979), including proteins present in the exoploymeric substance (EPS) of the bacteria and block their function (Geesey and Jang, 1989). According to Alt et al. (1990) cupric ions in the vicinity of the cell membrane may cause damage by depolarization and impairment of receptors or transporter molecules. At lower pH free copper becomes more abundant (Edwards et al. 1996). Free copper is also a direct function of water alkalinity (Edwards et al. 1996). Therefore, water quality can

significantly influence the bacterial nitrification process if copper ion is present by changing the concentration of Cu^{+} or Cu^{+2} ion. Some copper complexes such as copper-ammonia complexes [Cu $(NH_3)_X^{2+}$] are more toxic to nitrifying bacteria (Sato et al. 1988). Lee et al. (1997) suggested that cupric tetraamine [$Cu(NH_3)_4^{+2}$] is mostly responsible for inhibition of *Nitrosomonas* and *Nitrobacter* cultures. They also reported that *Nitrosomonas* species are equally or more sensitive than *Nitrobacter* species and attached nitrifiers are more tolerant than the suspended nitrifiers.

Effect of Chlorite

Chlorite (ClO_2^-) is typically present in a water distribution system as a byproduct of chlorine dioxide (ClO_2) added as a disinfectant (Baribeau et al. 2002, Gordon 2001). ClO_2 degrades by reacting with natural organic matter (NOM) in water forming chlorite. According to Gordon (2001) 30 to 70% of the chlorine dioxide can be degraded to chlorite. Gagnon et al. (2005) reported that unlike chlorine dioxide, chlorite is not an effective disinfectant against heterotrophic bacteria. However chlorite ion has an inhibitory effect on nitrification (O'Conner 2001). Hynes et al. (1983) found that chlorite interfered with the oxidation of ammonia to nitrite by *Nitrosomonas europea* and nitrite to nitrate by *Nitrobacter winogradskyi*.

When AOB oxidize NH_3 to NO_2, hydrogen ions (H^+) are formed (see equation 1). According to Murray et al. (1965), this oxidation process occurs in the cell membrane of AOB. Due to the production of H^+ (equation 1) a temporary acidic environment is

created. In that acidic environment the diffused chlorite reacts with H^+ and forms ClO_2 according to the following reaction (Gates et al. 1989).

$$5NaClO_2 + 5HCl \longleftrightarrow 4ClO_2 + 5NaCl + HCl + 2H_2O \quad \text{Equation 4}$$

Localized ClO_2 can change the permeability of the cell membrane, hinder the enzyme and protein function and destroy nucleic acids (Stewart et al. 1996). Since most of the HPC bacteria do not produce acid (according to equation 1) they remain unaffected by ClO_2^- (McGuire et al. 1999, Gagonon et al. 2005).

McGuire et al. (1999) reported the experience of the Gulf Coast (Texas) Water Authority (GCWA), where chloramine is used as secondary disinfectant with chlorine dioxide as the primary disinfectant. Due to the decay of chlorine dioxide, chlorite was detected in their distribution system in the range of 0.25 to 0.35 ppm. Although the system is in a warm climate and has long detention times, there was no report of nitrification. Robustness of the system against nitrification has been attributed to the inhibitory effect of chlorite present in water. For two utilities in Texas, one using chloramination had high nitrite levels while the second one that uses chlorine dioxide/chloramination had significantly lower nitrite levels. A laboratory test was also conducted by McGuire et al. (1999) to examine the effect of different concentrations of chlorite on AOB populations. Even low levels (0.2 ppm) of chlorite caused a significant reduction in the culturability of AOB. Accoring to another study conducted in plug flow

reactors in Tucson AZ, continuous feed of chlorite at concentrations as low as 0.1 ppm can prevent nitrification (McGuire et al. 2004).

In contrast, a nitrification episode in the Corpus Christi Texas distribution system continued even after dosing chlorite (McGuire et al. 1999). The authors explained this outcome because of the high concentration of ammonia present in water. There are also other reports of chlorite being unable to control nitrification (Karim et al. 2006). According to Karim et al. (2006), 0.5 ppm of chlorite in a pilot system was able to control nitrification for a short period of time and it then reoccurred within two to four weeks. They also reported that the nitrifying population was not affected by the presence of chlorite. According McGuire et al. (2006) the presence of chlorite in water reservoirs prevent the onset of nitrification, but once nitrification started, introducing chlorite was not effective; 0.2 ppm of chlorite dosed in a nitrifying reservoir caused inhibition for only two weeks.

Adding a regulated chemical like chlorite for controlling nitrification in a distribution system is a highly debatable concept. Chlorite is regulated by USEPA Stage 1 D/DBP Rule, with an MCL of 1 ppm with a maximum contaminant level goal of 0.8 ppm. The maximum residual disinfectant level for chlorine dioxide is 0.8 ppm (USEPA 2001). An added problem is that the chlorite concentration could change within the distribution system, so better monitoring practices should be adopted to determine the actual chlorite concentration in water (Baribiau et al. 2002). These issues illustrate that more information is needed before chlorite is acceptable as a nitrification control strategy, particularly in premise plumbing.

Effect of Monochloramine

There is a reasonable amount of information available on the impact of chloramine on nitrifying organisms. The focus of the majority of these studies has been on full scale chloraminated systems, but these lessons can be applied to our understanding of what may happen in premise plumbing. Regan et al. (2001) reported 99% inactivation (CT_{99}) of AOB at 1.5~2 mg/L of chloramine within 30 minutes of exposure. However Wolfe et al. (1990) found CT_{99} for AOB isolated from a treated drinking water reservoir and grown in culture media or dechlorinated tap water to be 3mg.min/L and 33 mg.min/L, respectively. Therefore, AOB grown in dechlorinated tap water are 11 times more resistant than those grown in culture media. Other researchers (Cunlife 1991, Oldenburg et al. 2002) have observed CT_{99} in the range of 760 to 1,9000 mg Cl_2.min/L. This disparity in CT_{99} value may be due to the difference in experimental design, difference in strain, water quality, chlorine to ammonia ratio, enumeration method, temperature and pH. Lieu et al. (1993) conducted a comprehensive investigation of the effect of temperature, chlorine to ammonia ratio and chloramine dose on AOB inactivation. They examined the efficacy of 1.7, 2.0 and 2.5 mg/L of monochloramine at three different chlorine to ammonia nitrogen ratios (3:1, 4:1 and 5:1) at three different temperatures (10^0C, 15^0C and 25^0C). At 10^0C, chlorine to ammonia ratio or incubation time did not affect inactivation, and regrowth of AOB occurred for all cases except at doses of 2.5 mg/L. At 15^0C chloramine dose and incubation time influenced inactivation, but chlorine to ammonia ratio did not play a role. At 25^0C, chloramine dose, chlorine to ammonia ratio and incubation time influenced regrowth of AOB. A dose of 2.5 mg/L of

chloramine was found to be most effective in controlling AOB at this temperature. According to Wolfe et al. (1985) increasing the chloramine residual may be effective in preventing nitrification as higher chloramine concentrations limit AOB regrowth. NOBs, being more sensitive to environmental conditions (Matulewich et al. 1975) are more susceptible than AOB to disinfection by chloramine (Wolfe et al. 2001).

Higher levels of chloramine may not be an effective method to control nitrification episodes that have already started. Skaden (1993) reported that a chloramine dose of 8 mg Cl_2/L was not effective in controlling nitrification in the Ann Arbor, Michigan distribution system. This may be due to the fact that nitrite can degrade chloramine residuals before it can inactivate the nitrifying bacteria (Odell et al. 1996, Wolfe et al. 1988, Kirmeyer et al. 1995). Nitrifying bacteria form protective layers (slime layer or capsules), mainly composed of polysaccharides (Prosser et al. 1986), as a defensive mechanism against unfavorable environmental conditions, such as low pH. These capsules protect organisms so that they are more resistant to disinfectants (Stewart et al. 1996). Cunliffe (1991) detected nitrifiers in 64% of the samples collected in South Australia and of them 20.7% contained more than 5.0 mg Cl_2/L of monochloramine. The author hypothesized that as the nitrifiers grow in aggregates or in biofilm attached to the surface (Watson et al. 1989) or in sediment (Curtis et al. 1975), they remain unaffected by disinfectant and the nitrifiers detected in samples containing high chloramine residual may have been detached shortly before or during the sampling period. Higher AOB have been detected in biofilm than water in the Metropolitan Water District of Southern California distribution system (Stewart et al. 1997). Regan et al. (2001) postulated on the

protective mechanism of biofilms. Monochloramine is mass transport limited in biofilm, so bacteria inside the biofilm are not exposed to the disinfectant. Another protective mechanism may be the relative ratio of growth vs. disinfection. Harrington et al. (2003) stated that if AOB growth rate driven by ammonia concentration exceeds the AOB inactivation rate by monochloramine, then theoretically AOB can grow in the presence of monochloramine. Fleming et al. (2005) proposed nitrification potential curves based on the relative concentration of chlorine (biocide) and free ammonia (food). According to them the threshold chlorine value is 1.6, above which nitrification would be prevented, without any influence from free ammonia concentration. At chlorine concentrations below 1.6, the nitrification potential depends on the ratio of chlorine and free ammonia. In work by Harrington et al. (2002) nitrification did not occur when the total chlorine concentrations were more than 2.2 mg/L and the biocide to food ratio was 1.9 mg Cl_2/mg of N or more.

Other studies (Wolfe et al. 1990, Lieu et al. 1993, Kirmeyer et al. 1995, Odell et al. 1996, Harrington et al. 2002) have recommended maintaining a minimum 2-3 mg Cl_2/L chloramine residual to prevent nitrification. However, once again, if nitrification has started, maintaining a high ratio and high dose may not be sufficient to control it (Skadsen 1993).

The chlorine to ammonia-N weight ratio used to form monochloramine varies from 3:1 to 5:1 (Wilczak et al. 2006). Optimizing the chlorine to ammonia ratio and monochloramine dose is the easiest and most cost-effective way to control/prevent nitrification (Lieu et al. 1993). According to a recent survey by Seidel et al. (2005),

optimizing the chlorine to ammonia ratio is the most common nitrification control technique used. As the AOB use ammonia as their energy source, the main goal of this approach is to minimize the free available ammonia in water.

There are several studies where the chlorine to ammonia ratio has been measured in pilot and full scale systems. There was nitrification in a pilot system where chloramine was applied at 3:1 ratio (McGuire et al. 2004). For two covered finished water reservoirs in southern California which were initially chloraminated at a 3:1 ratio, nitrification was significantly reduced after raising the ratio to 5:1(Wolfe et al.1988). A Florida utility rarely experienced nitrification when the combined chlorine residual was above 1 mg/L at a chlorine to ammonia ratio of 5:1(Liu et al. 2005). A recent study done by Karim et al. (2006) showed that monochloramine applied at a 3:1 ratio was not able to control nitrification, but it was effective at a 5:1 ratio.

There are numerous reports (Kirmeyer et al. 1985, Skadson 1993, Davis 1990) of excess free ammonia causing nitrification in distribution systems. At a 3:1 chlorine to ammonia application ratio, a chloramine dose of 3 mg/L Cl_2/L will theoretically have a free ammonia concentration of 0.41 mg /L as N, whereas at high ratios (such as 5:1), almost no free (0.01 mg/L as N) ammonia exists in water (Kirmeyer et al. 2004). It is recommended that excess ammonia should be less than 0.1 ppm, preferable 0.05 ppm (Skadsen, 2006). Holt et al. (1995) reported that lowering the free ammonia level from 0.11 mg/L to 0.05 mg/L reduced the nitrite level, which is used as an indicator of nitrification. However, studies done by Odel et al. (1996) suggested that nitrification can

take place even when only a small amount of ammonia is available at the entry point to the distribution system.

Maintaining monochloramine at a high ratio close to 5:1 is not always easy, and sometimes associated with dicholoramine formation, and taste/odor problems and higher DBP formation (Skadsen 2006). Finished water pH plays an important role in maintaining monochloramine at the proper chlorine to ammonia ratio. At lower pH, monochloramine is less stable, and chloramine decay doubles for a decrease in pH of 0.70 units (Thomas 1987). At higher pH only a small amount of ammonia is available from monochloramine decay (Valentine et al. 1985). However, the inactivation of *Nitrosomonas europea* by monochloramine decreases as pH increases from 7.0 to 9.0 (Harrington et al. 2003). This may be due to the presence of dichloramine at lower pH, which is a weaker disinfectant than monochloramine. Ward et al. (1984) had similar results, where inactivation of different pure cultures *(Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Klebsiella pneumoniae and Enterobacter cloacae)* increased 1.5~2 times as the chlorine to ammonia ratio increased from 2:1 to 5:1, 5~6 times as pH decreased from 8 to 6 and 5~6 times as chloramine dose increased from 1 to 5 mg/L.

As water travels through the distribution system, chloramine decays, providing more ammonia for nitrification. This excess ammonia can be transformed to monochloramine in the distribution system using booster chlorination to maintain a higher chlorine to ammonia ratio (Wilczak et al. 2006, Wolfe et al. 2001).

Nitrification mitigation at the household system level is difficult at far ends of the distribution system because of monochloramine decay This is even more predominant in

premise plumbing because of the possibility of long period of stagnation. The problem could be even worse because even in houses very close to the treatment plant, monochloramine can rapidly decay through reactions with a copper plumbing system (Edwards et al. 2005). The pipe materials may also have an influence on the biofilm community composition or activities on the surface,as previously mentioned in the section on copper. Therefore pipe material could significantly influence the nitrification process.

Nitrification Control Strategies Investigated In This Project

In this project three different strategies for controlling nitrification in household plumbing were studied on two types of material (i.e. PVC and copper) in the worst case scenario, where all the monochloramine (around 4 ppm) had decayed to ammonia. The inhibitory effect of copper was examined by introducing known amounts of copper into a nitrifying PVC reactor and gradually increasing its concentration. Because Cu^{+2} is more toxic and its concentration depends on pH, at the maximum concentration of copper the pH of the PVC reactor was lowered to 6.6 to ensure that all copper was in this ionic form. In the next step, chlorite ion was introduced to two nitrifying PVC and copper reactors. Initially chlorite was added at a very low dose and gradually it was raised to a very high dose. The effect of monochloramine at various chlorine to ammonia ratios was also studied since it may be possible to either distribute water with a stable residual or re-create chloramine through booster chlorination.

Materials and Methods

Description of Reactor

To simulate a domestic plumbing system the commonly used CDC (Goeres et al. 2005) reactor was modified. These modified reactor's coupons, bottom plate and stirring blades have the same surface to volume ratio as that of a six foot long ¾" diameter domestic copper plumbing pipe. The rotational speed of the blade inside these reactors was 300 rpm. This speed was chosen as it creates 3 fps velocity in bulk water which can be found in domestic water lines. Volume of the reactors is 120 ml. Two types of materials were tested, copper and PVC. All coupons were washed with 0.1N NaOH three times to remove any biological materials from their surface prior to use.

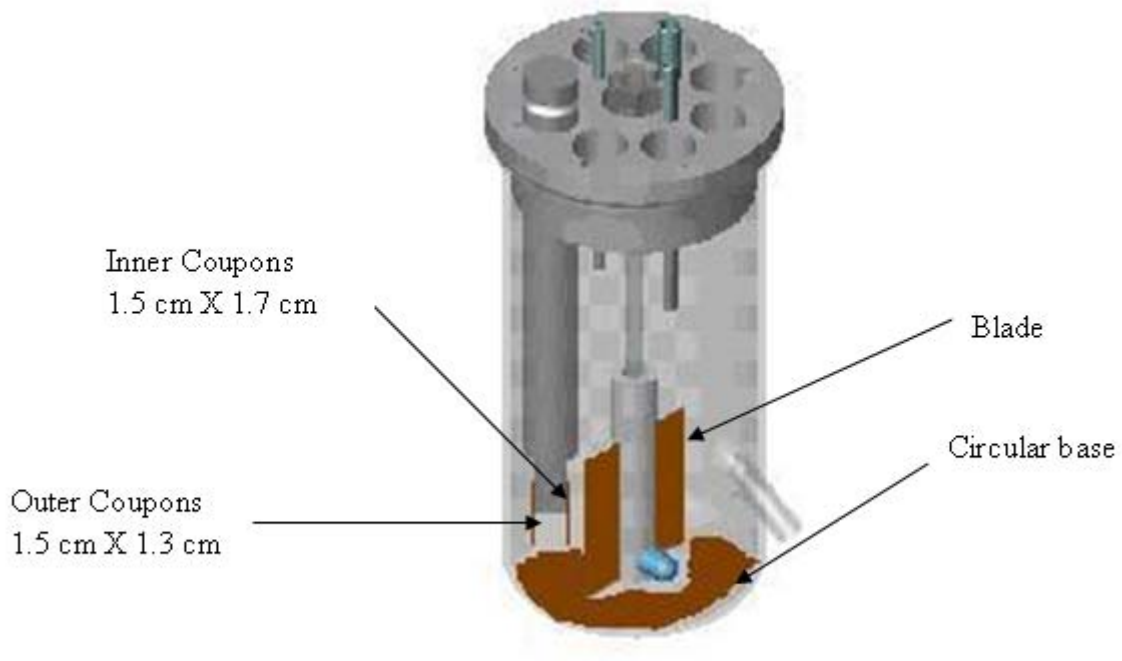

Figure 3-1: Modified CDC reactor

Operation Scheme

To simulate periods of stagnation in home plumbing the reactors were flushed with peristaltic pumps for five minutes and then the water inside the reactors remained stagnant for eight hours. The feed pumps and stirplates were on two different timers, which controlled the power supply. The timers were offset from each other by one minute with the stirplate starting before the pumps. At the end of five minutes the stirplates stopped, followed by the pumps. This cycle was repeated three times a day. As a result, fresh influent mixed with the stagnant bulk water and excess water spilled through the effluent port. Because there is mixing, the effluent water is always diluted with fresh influent feed, which prevents the effluent NH_3 concentration from becoming zero, even though the bulk NH_3 concentration (the concentration in the reactor) is zero due to nitrification. Because sampling occurred in the both the bulk and the effluent, a mathematical model of the reactor was developed to determine the relation between effluent NH_3 concentration and NH_3 utilization in these reactors. This model was used to calculate the % NH_3 utilization and the % of ammonia present as NO_2, and NO_3. As described in the Appendix-A, model values were equivalent to those actually measured in the bulk.

Stock/Feed Solution Preparation

Typical setup of these reactors is shown in Figure 3-2. The ratio of flow is RO: humics: BAC= 50:5:1, so these reactors are mostly supplied with RO water. Influent carbon concentration as humics was 4 ppm for all reactors. Lab grade chemicals were

added to reverse osmosis (RO) water to give it target alkalinity of 35 mg/L as $CaCO_3$. The final concentrations of these chemicals are given in Table 3-1.

Table 3-1: Chemicals added to influent water.

Chemical	Concentration(mg/L)
$MgSO_4$	39.6
$NaHCO_3$	56.9
$CaSO_4$ $2H_2O$	25
$Al_2(SO_4)$ $18H_20$	0.62
$CaCl_2$ $2H_20$	20.80
Na_2SiO_3 $9H_20$	26

Bozeman tap water was flowed through a biologically activated carbon (BAC) column to remove any residual chlorine. It should be noted that Bozeman water comes from a surface water source and does not contain ammonia and chlorine is used as the final disinfectant. BAC water was pumped to these reactors to ensure a supply of indigenous bacteria. These organisms were the only inoculum supplied to the reactors. Humics were supplied using a separate pump.

Humics Preparation

50 gm of Elliot silt loam soil (International Humic Substances Society) was added to 500 ml of 0.1 N NaOH and mixed for 48 hours. This solution was then centrifuged at 10,000 X g for 20 minutes. After centrifugation the supernatant was collected in carbon free glassware (made by baking at 390^0 C for five hours) and stored at 4^0 C in the dark. Total organic carbon content of the humics was measured using a Dohrman DC-80® and subsequently diluted to the appropriate concentration using the RO water feeding the reactors.

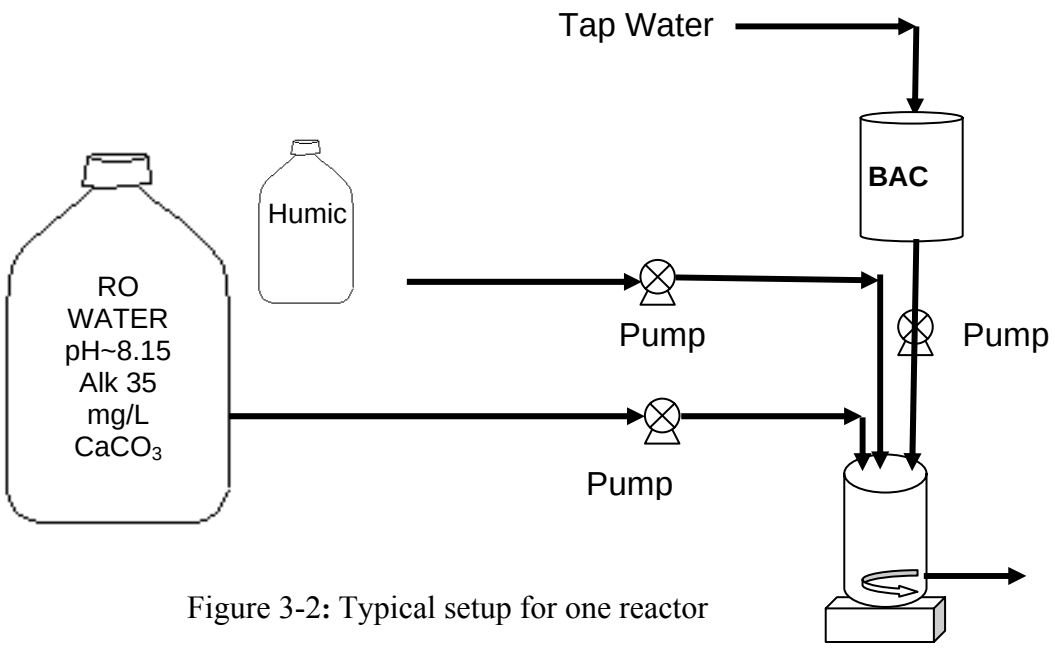

Figure 3-2**:** Typical setup for one reactor

Experimental Approach

Two sets of modified CDC reactors equipped with two different types of coupons (PVC and copper) were used in this investigation. Each set consists of four duplicate reactors. All of these reactors had been running for more than one year and showed stable signs of complete nitrification.

Effect of Copper Ion on Nitrification: To test the effect of copper on nitrification, copper in the form of $CuSO_4$ was added to the influent of one nitrifying PVC reactor. At the beginning 15 ppb copper was dosed and incrementally raised to 1.3 ppm, which is the action limit for copper according to the Lead and Copper Rule. Each concentration of copper was maintained for two weeks. Another PVC reactor was used as the control where no copper was added. Because nitrification is presumed to be more affected by

free copper (Cu^{+2}) (Braam et al. 1981), and because the amount of Cu^{+2} in solution is a function of pH, the pH of the influent with 1.3 ppm of copper was reduced gradually from 8.15 to 6.6 by 0.3 units every two weeks. Another PVC reactor with the influent at the same pH but with no copper was used a control. Heterotrophic bacteria, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in the bulk water were measured weekly. All other response parameters were measured regularly three times (Monday, Wednesday and Friday) every week for the entire period. At the end of the investigation biofilm samples were collected from all reactors for heterotrophic bacteria and nitrifiers. Also DNA was extracted from these biofilm samples for community analysis.

Effect of Chlorite Ion on Nitrification: To investigate its inhibitory effect, laboratory grade sodium chlorite was added to the influent of one PVC and one copper reactor. Initially 0.2 ppm of chlorite was added and gradually the chlorite dose was increased to 2.0 and then a shock load of 20 ppm of chlorite was dosed. Each concentration of chlorite was tested for two weeks. Chlorite was then discontinued and the recovery of both the PVC and copper system from chlorite shock was observed. The response variables listed in Table 3-2 were measured regularly for the entire period of the experiment. Heterotrophic bacterial populations were enumerated every week along with AOB/NOB. Biofilm samples were also taken just before discontinuing chlorite addition.

Effect of Monochloramine on Nitrification: To represent different scenarios that can occur in a distribution system due to chloramine decay, different amounts of chlorine

were added in the ammonia containing influent of two nitrifying copper and PVC reactors. Low chlorine to ammonia ratios represent water at a household where free ammonia is available due to partial decomposition of monochloramine. High chlorine to ammonia ratios represent households that are very close to the treatment plant where the monochloramine has not decomposed. The influent has a fixed amount of ammonia, so different amounts of chlorine added as sodium hypochlorite formed monochloramine at different chlorine to ammonia ratios. Initially a 0.5:1 ratio was applied and gradually it was raised to 5:1. To have a better understanding of how monochloramine is effective against nitrification, bulk water NH_3 measurements were done at regular time intervals over the eight hour stagnation period for each chlorine to ammonia ratio. From these values ammonia utilization rate curves were made. Heterotrophic bacteria, AOB and NOB bacteria were enumerated weekly. The response variables listed in Table 3-2 were measured regularly for the entire period of the experiment. At the end of monochloramine exposure, chlorine was discontinued and recovery of nitrification in PVC and copper reactors was compared. Biofilm samples were also taken for microbial community analysis just before discontinuing chlorine addition.

Analytical Methods (Chemical)

Sampling Technique: Effluent water was collected and tested three times (Monday, Wednesday and Friday) every week. Bulk water samples from the reactors were taken once every week for similar measurements. Effluent water concentrations were converted to bulk values using the model shown in Appendix-A. Response variables of the water samples shown in Table 3-2 were measured routinely.

Table 3-2: Response parameter measured in this project

Response parameter	Comment
Copper(Dissolved and total)	Three times a week for effluent sample and once for bulk sample.
NH_3-N	
NO_2-N	
NO_3-N	
ClO_2^-	
Free and total chlorine	
HPC	Weekly
MPN for AOB and NOB	

Copper: When copper was added to the PVC reactor at lower concentrations (less than 400 ppm) an ICPMS (inductively coupled plasma mass spectrometer) was used to measure the copper. At higher concentrations a HACH 2000 spectrophotometer was used. Both total and dissolved copper in the effluent water were measured. According to *Standard Methods* (19^{th} edition, 1995), dissolved copper is operationally defined as the portion of the copper which passes through a 0.45μm pore size syringe filter. It should be noted that in the presence of colloidal species that can pass through the filter, the method represents an upper bound to truly soluble copper. Measurements were done using a portable HACH 2000 spectrophotometer. Copper in the water sample reacts with a salt of bicinchoninic acid contained in the CuVer 1 copper reagent to form a purple colored complex in proportion to the copper concentration. The purple color/copper concentration was measured at 560 nm.

Cupric Ion (Cu^{+2}): A copper ion selective electrode (Cu-ISE), (Orion Cupric electrode, model, 94-29, Boston, MA) was used to measure the free copper (Cu^{+2}) in the

water. The electrode was calibrated using standard cupric ion solutions according to manufacturer's direction before measuring the sample. Cupric ion at 1.3 ppm total copper and pH 6.6 was measured and was found to be in the 0.90 ± 0.10ppm range.

Ammonia Nitrogen: Free NH_3-N was measured using a HACH 2000 spectrophotometer using the salicylate method (HACH method 10023) at 655 nm. Ammonia reacts with salicylate to form 5- aminosalicylate, which is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. This blue color is masked by the yellow color from the excess reagent to give a final green-colored solution which is proportional to the amount of ammonia present in the sample. The ammonia was measured immediately when the sample was collected.

Nitrite Nitrogen: After collecting the sample, nitrite nitrogen (NO_2-N) was measured immediately with the HACH 2000 spectrophotometer. The diazotization method was used, where nitrite reacts with sulfanilic acid and forms an intermediate diazonium salt. This intermediate product couples with chromotropic acid to produce a pink colored complex, which is proportional to the amount of nitrite present. This pink color was measured at 507 nm.

Nitrate Nitrogen: NO_3-N measurements were done using a Dionex® ion chromatography system with a CD20 conductivity detector and GP40 gradient pump unit. An AS4A column and DS3 detection stabilizer was also used in this method. Water was filtered through a sterilized 0.2 µm pore size polyethersulfone filter and 5ml BD® syringe to remove any bacteria or suspended particles. The filtered sample was collected

in a sterilized 15 ml Falcon® tube and stored in the refrigerator at 4^0C. Stored samples were measured within two weeks of collection. The water sample was loaded using a S40 automated sampler. Before measurement the Dionex® ion chromatography system was first calibrated using five sodium nitrate standards (1, 0.5, 0.2, 0.1, 0 ppm of NO_3-N). To minimize experimental error, after every seven measurements a standard solution of nitrate was measured to check the accuracy of the measurement. If the obtained measurement of the standard was outside 90 to 110% of the standard value then the calibration was repeated and sample was measured again. This was done according to *Standard Method* (19ed, 1995) 3020.

Chlorite: Chlorite was measured by a modification (McGuire el al. 1999) of the US Environmental Protection Agency (USEPA, 1993) method 300 with the Dionex® ion chromatography system described above equipped with an AS9 column and 100µl sampling loop. Samples were filtered through a sterilized 0.2 µm polyethersulfone filter and stored in the refrigerator at 4^0C, and measured within two weeks of sample collection.

Free chlorine and total chlorine: Both free and total chlorine were measured using a Lamotte DC1100® colorimeter with the DPD colorimetric method (*Standard Methods* 4500-Cl). In the absence of iodide ion, *N*,*N*-diethyl-p-phenylenediamine (DPD) reacts with free chlorine to produce a red color. Subsequent addition of a small amount of iodide acts catalytically to cause monochloramine to produce color. For accurate results

careful pH control is essential. Therfore, at the beginning of the measurement phosphate buffer was added to the sample to maintain a pH between 6.2 to 6.5.

Total Organic Carbon (TOC): TOC measurement for the project was done using a Dohrman DC-80® carbon analyzer, with potassium hydrogen phthalate as standard and potassium persulfate as the oxidizing agent.

Analytical Methods (Microbiological Analysis)

Heterotrophic Plate Counts (HPC): Heterotrophic plate counts of the water samples and the biofilms were done according to *Standard Methods* (19th ed., 1995) 9215A using R2A agar plates. Plates were incubated at 20^0 C for 7 days, and then the number of colonies in the plates was counted using a Quebec colony counter.

Biofilm Sampling: Biofilm was collected at different time points in each experiment. Autoclaved reverse osmosis (RO) water was filtered through a sterilized 0.2µm polyethersulfone filter to to remove any foreign DNA. Filtered DNA free water was placed in DNA free glass tray (baked at 390^0C for 5 hours). One coupon was removed from the reactor and placed in the glass tray containing the water. The coupon was then scraped using a autoclaved rubber policeman inside a laminar flow hood. After scraping, the biomass with water was poured in a sterilized 50 ml Falcon® tube, which was then homogenized with a homogenizer (Biohomogenizer® Model M133/12810, ESGE®) for 30 sec. From the homogenized biomass, samples were taken for MPN and

HPC analysis. The remaining biomass was used for DNA extraction and community analysis.

DNA Extraction: Homogenized biomass was collected on a 0.2 µm polycarbonate filter using a three channel manifold (Pall® Life Science) with filter funnels. DNA extraction of the collected biofilm sample was done using a Fast DNA® SPIN Kit for soil (Q-BIOgene catalog #6560-200). Collected DNA was stored in a -30^0 C freezer.

PCR (Polymerase Chain Reaction): GoTag Green® master mix from Promega Inc. was used for amplifying the extracted DNA through PCR using an Eppendorf Mastercycler®. Due to the inhibitory effect of humics present and the low quantity of biomass, a two stage PCR was performed. In the first stage a 50µl reaction volume was used which contains 25µl master mix, 10 pM of the universal primers 1070F and 1392, 1µl DNA suspension and 20 µl of water. The amplification process involved initial denaturation at 94^0C followed by 15 cycles of 30 second denaturation at 94^0C, 45 second annealing at 52^0 C and 2 minutes of extension at 72^0 C with a final 5 minutes extension at 72^0C. In the second stage, 5 µl of the product from the first stage was used as template. In the second stage a similar reaction composition was used except 1392+GC was used instead of the 1392 primer and 20 amplification cycles were used. PCR products were evaluated by agarose gel electrophoresis. Negative controls without template addition were treated identically through the PCR and evaluated by agarose gel to confirm the absence of contaminant DNA.

DGGE: DGGE was performed at 60^0 C with a D-Code Universal Mutation Detection System (Bio-Rad Laboratories). Eight and twelve percent (w/v) acrylamide gels with denaturant gradients of 40 to 70% were used for analyzing amplified fragments using 1070 and 1392+GC. A 25 ml volume denaturing gel was poured and allowed to polymerize prior to pouring of a zero percent denaturant stacking gel for the loading wells. Sixteen hours of electrophoresis were performed for the gels at 60 V. After electrophoresis the gels were subsequently stained with SYBR Green I (Cambrex Bio Science). Images of the gel were obtained using a FluorChem 8800® Imaging system and AlphaEase FC® software (Alpha Innotech). Composition of all the reagents and conditions used for DGGE are presented in Appendix-B.

MPN: AOB and NOB populations were enumerated using the most probable number (MPN) technique (Lipponen et al. 2002) using Costar® Clear-Bottom 96 well microtiter plates. The mineral medium used for AOB contained per liter: $(NH_4)_2SO_4$, 330 mg; KH_2PO_4, 100 mg; $MgSO_4$ $7H_2O$ 40 mg; $CaCl_2$, 15 mg and 1 ml of a trace-element solution. The trace element solution contained per liter: Na_2EDTA, 4292 mg; $FeCl_2$ 4H2O, 1988 mg; $MnCl_2$ H_2O, 99 mg; $NiCl_2$ $6H_2O$,24 mg; $CoCl_2$ $6H_2O$, 24 mg; $CuCl_2$ $2H_2O$, 17 mg; $ZnCl_2$, 68 mg; Na_2MoO_4 $2H_2O$, 24 mg and H_3BO_3, 62 mg. Bromothymol blue (5ml/ l of 0.04% solution in water) was added as a pH indicator. The pH was adjusted to 8 using 1M NaOH before autoclaving at 110^0 C for 15 min. The NOB medium has the same composition except that it did not contain $(NH_4)SO_4$ and bromothymol blue, and was supplemented with 34.5 mg/ l of $NaNO_2$. The pH was adjusted to 6.5 with 1M NaOH before autoclaving at 110^0 C for 15 min. 175µl of media

was poured into each of the 96 wells of the plate. An equal amount of sample was then inoculated into the first column of wells in the plate using a multi channel pipette. The content of the first column of wells was carefully mixed, and 175 µl of inoculated media was transferred to the next column of wells, and this process continued. After inoculation the microtiter plates were sealed with polyester tape to prevent evaporation and incubated for 9 weeks at 20^0C in the dark. After the incubation period AOB presence was determined by detecting the presence of nitrite or nitrate in the medium by adding 40 µl of 0.2% diphenylamine in H_2SO_4 in the well. This reagent reacts with nitrite or nitrate and forms a blue color. The absorbance of the blue color was measured at 630 nm with a microplate reader (EL808 ultra microplate reader BioTek instruments®). The blue color indicated nitrite or nitrate had formed and the well was scored as positive.

Griess Ilosvay reagent was used to detect NOB activity in the samples. This reagent was made by mixing three separate solutions. In the first solution exactly 0.6 gm of sulfanilic acid was dissolved in 70 ml of hot distilled water. After cooling to room temperature, 20 ml of concentrated HCl and 10 ml of distilled water was added. The second solution was made by dissolving 0.6 gm of α-napthylamine in 20 ml of distilled water containing 1 ml of concentrated HCl and then diluting it to 100 ml with H_2O. The third solution is 16.4% (w/V) of $CH_3COONa\ 3H_2O$ in water. All the solutions were made separately in dark bottles and kept in the refrigerator. Equal volumes of these three solutions were mixed and 40 µl of the mixture was added to each well after the incubation period. In the presence of nitrite, the Griess Ilosvay reagent produced a red color within five minutes. The absorbance of the well was measured after five minutes at

540 nm with the microplate reader. If nitrite in the well was detected it was scored as negative. The MPNs were calculated according to Rowe et al. (1977) and finally expressed as cell/ml of sample.

Statistical Analysis: Paired t-test analysis was done using Microsoft Excel on the data to see if there are significant differences between two treatments. The level of significance for all tests was α=0.05.

Results

Effect of Copper on Nitrification

The effect of copper on nitrification was investigated by introducing known concentrations of copper to the nitrifying PVC reactor. After the highest level was reached, to ensure all copper was as Cu^{+2}, the pH of the reactor was gradually lowered to 6.6. Another PVC reactor without copper and low pH was used as a control. The percentage of NH_3 utilization in the copper added PVC, the control and pH adjusted control reactor are shown in Figure 3-3. At the lower copper dose there was little difference in NH_3 utilization, but at higher doses (i.e. around 600 to 1300 ppb) intermittent decreases in NH_3 utilization were observed.

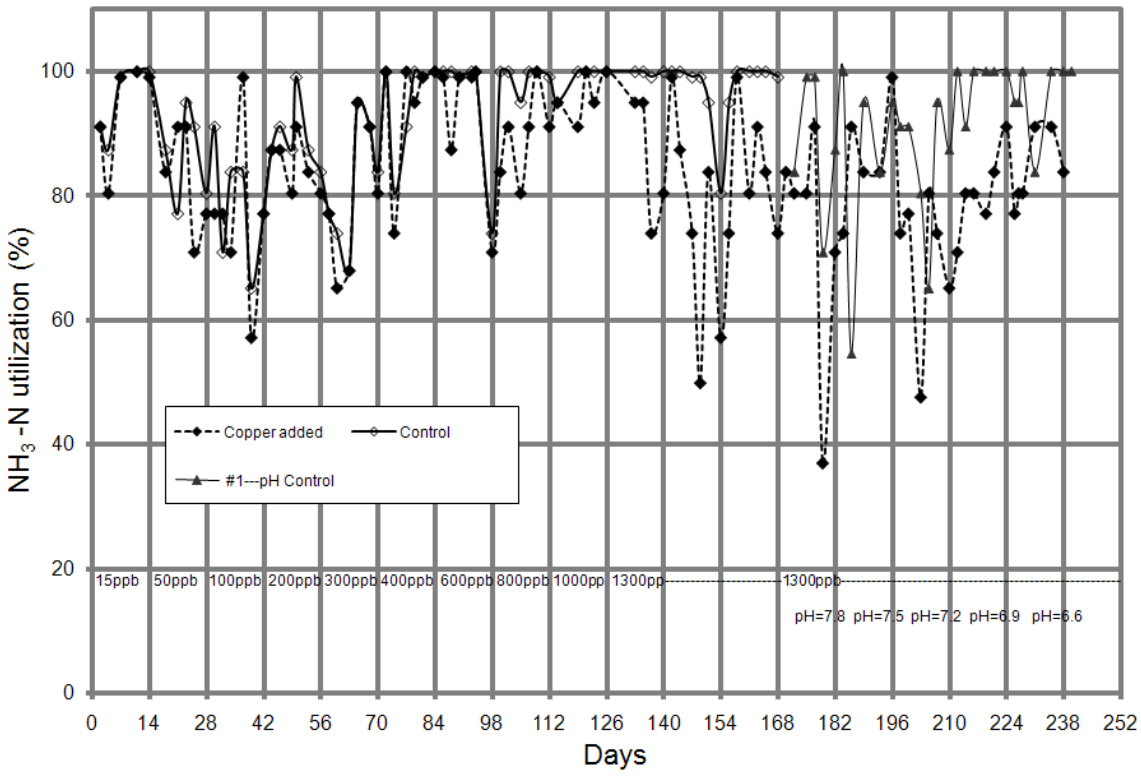

Figure 3-3: NH_3-N utilization in bulk water of copper added PVC and control PVC reactors

The average effluent NH_3-N for different copper doses is shown in Table 3-3 along with the p value found from a paired t-test. These values shows that the effluent NH_3 value in the copper dosed reactors may be statistically significantly different ($p<0.05$) from the control reactor, but the magnitude of the difference is very small, and therefore may not be of any practical importance. A similar trend is also observed between the effluent NH_3-N from the copper added and pH control reactor.

Table 3-3: Average (n=6) effluent NH_3-N at different copper doses and p-value from the paired t-test.

Copper dose	Average effluent NH_3 for copper added PVC reactor	Average effluent NH_3 for Control PVC reactor	Average effluent NH_3 for pH Control PVC reactor	p-value between control and copper dosed PVC reactors	p-value between pH control and copper dosed PVC reactors
15 ppb	0.21	0.20		0.07	
50 ppb	0.25	0.23		0.33	
100 ppb	0.26	0.26		0.90	
200 ppb	0.24	0.23		0.01	
300 ppb	0.25	0.25		0.24	
400 ppb	0.21	0.20		0.89	
600 ppb	0.21	0.17		0.03	
800 ppb	0.22	0.17		0.01	
1000 ppb	0.20	0.18		0.04	
1300 ppb	0.23	0.18		0.00	
pH=7.8		0.20	0.23		0.05
pH=7.5		0.20	0.24		0.87
pH=7.2		0.22	0.23		0.07
pH=6.9		0.16	0.18		0.00
pH=6.6		0.17	0.16		0.01

NH_3-N loss is attributed to bacterial oxidization to NO_2-N and NO_3-N. NO_2-N and NO_3-N in the bulk as a percentage of initial NH_3-N for copper added PVC, pH adjusted control and control reactors are shown in Figure 3-4 and Figure 3-5. Comparing Figure 3-3 through Figure 3-5 it can be concluded that nitrification is complete with all the NH_3-N converted mostly to NO_3-N and that the dosed copper and pH change had little impact on nitrification. The NO_2-N percentage (%) for the copper added PVC shows intermittent increases but the magnitude is very small (ppb range).

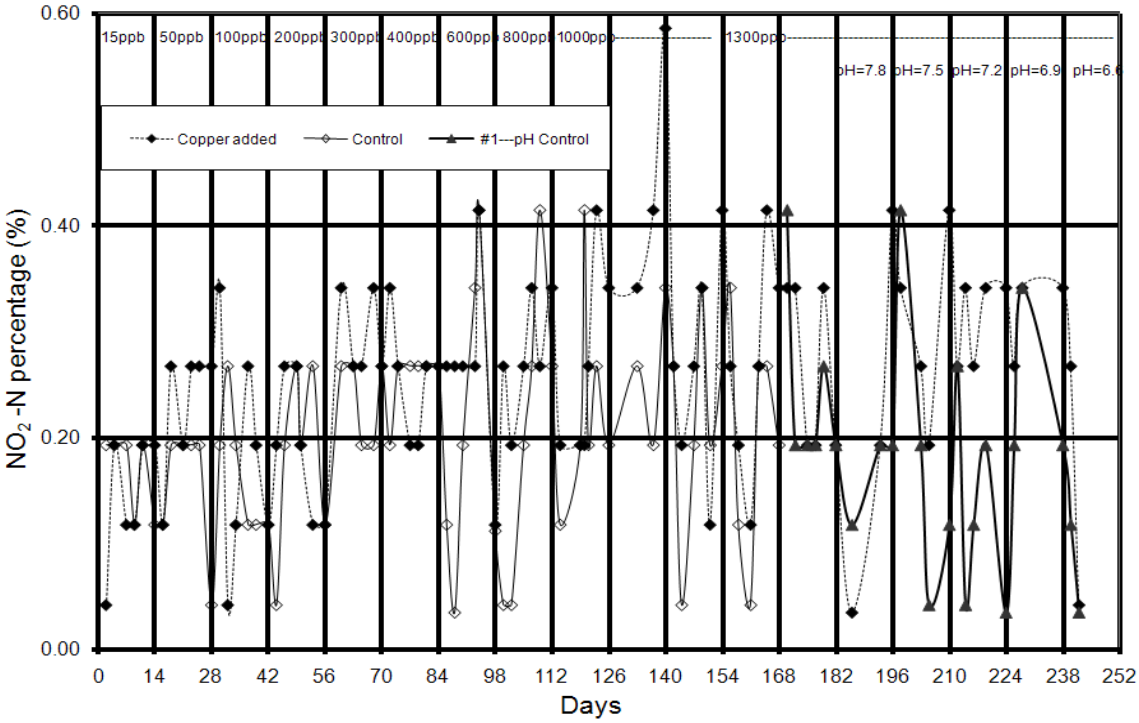

Figure 3-4: Bulk water NO_2-N as percentage (%) of initial NH_3-N for copper added PVC and control PVC reactors

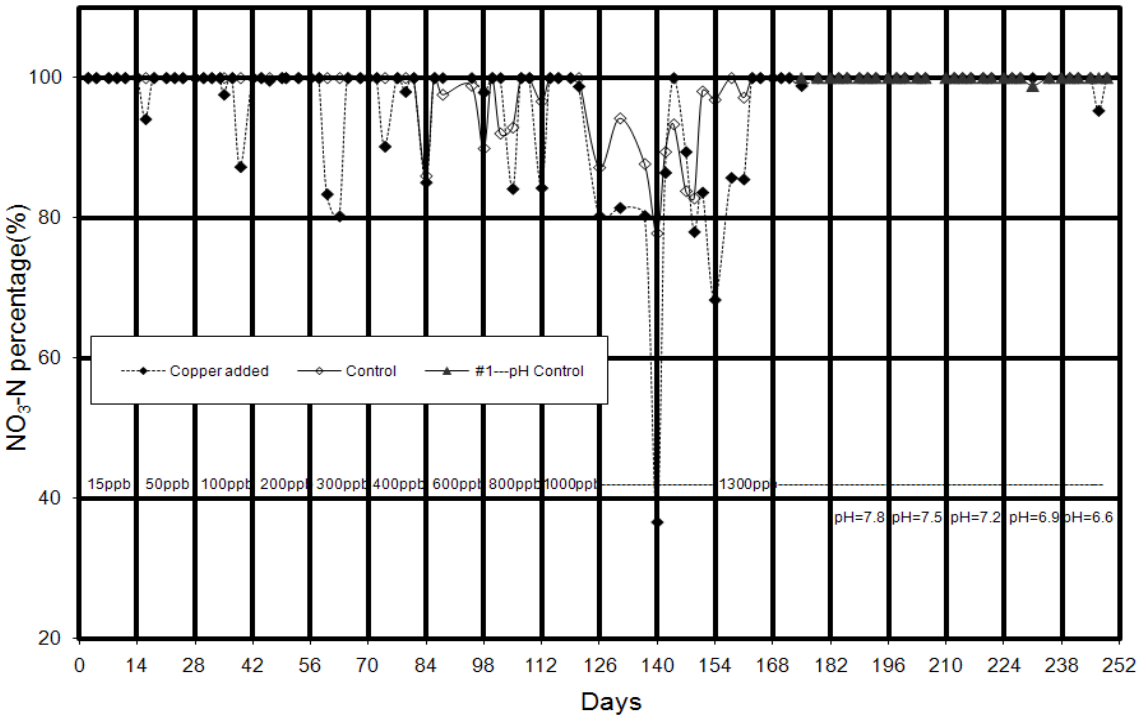

Figure 3-5: Bulk water NO_3-N as percentage (%) of initial NH_3-N for copper added PVC and control PVC reactors

Average effluent NO_3 values for different copper doses and the p-values from paired t-tests are shown in Table 3-4. These values show a similar trend to the NH_3 data.

Table 3-4: Average (n=6) effluent NO_3 at different copper dose and p-value found from the paired t-test.

Copper dose	Average effluent NO_3 for copper added PVC reactor	Average effluent NO_3 for Control PVC reactor	Average effluent NO_3 for pH Control PVC reactor	p-value between control and copper dosed PVC reactors	p-value between pH control and copper dosed PVC reactors
15 ppb	0.63	0.65		0.20	
50 ppb	0.54	0.57		0.19	
100 ppb	0.55	0.57		0.10	
200 ppb	0.49	0.53		0.00	
300 ppb	0.47	0.51		0.00	
400 ppb	0.48	0.53		0.00	
600 ppb	0.48	0.46		0.04	
800 ppb	0.46	0.49		0.00	
1000 ppb	0.44	0.46		0.01	
1300 ppb	0.45	0.43		0.85	
pH=7.8	0.49		0.53		0.00
pH=7.5	0.54		0.53		0.346
pH=7.2	0.56		0.55		0.10
pH=6.9	0.52		0.51		0.21
pH=6.6	0.51		0.55		0.00

The MPN values for AOB, NOB and HPC for heterotrophic populations from the bulk water are shown in Table 3-5. The presence of copper did not appear to influence any of these bacterial populations. Because a previous study (Wolfe et al.1990) reported that HPC counts and AOB are highly correlated, AOB/NOB cell counts are plotted against HPC values in Figure 3-6 and Figure 3-7.The correlation between AOB/NOB and HPC was found to be very weak ($R^2 \approx 0.0002$ to 0.02).

Table 3-5: Average (n=2) MPN value for AOB and NOB and average HPC value for heterotrophic populations

	Average AOB(Cells/ml)			Average NOB(Cells/ml)			Average HPC(CFU/ml)		
Copper dose and pH	pH Control PVC reactor	Cu added PVC reactor	Control PVC reactor	pH Control PVC reactor	Cu added PVC reactor	Control PVC reactor	pH Control PVC reactor	Cu added PVC reactor	Control PVC reactor
15 ppb		22	16.1		39.8	40.9		$6X10^5$	$4\ X10^5$
50 ppb		35.4	30.1		27.3	40.1		$3\ X10^5$	$2\ X10^5$
100 ppb		21.8	26.0		39	38.2		$2\ X10^5$	$2\ X10^5$
200 ppb		18.3	19.2		36.2	32.5		$8\ X10^4$	$5\ X10^4$
300 ppb		16	15.5		19.7	21.8		$6\ X10^5$	$3\ X10^5$
400 ppb		9.1	9.4		19.4	39.2		$3\ X10^5$	$2\ X10^5$
600 ppb		13.6	13.4		23.2	30.6		$8\ X10^4$	$8.\ X10^4$
800 ppb		9.3	7.2		9.7	14.6		$2\ X10^5$	$2\ X10^5$
1000 ppb		10.8	12.8		15.6	14.6		$4\ X10^4$	$5\ X10^4$
1300 ppb		11.1	22		7.7	20.9		$5\ X10^4$	$4\ X10^4$
pH=7.8	10.6	10.5		19.4	21.3		$6X10^4$	$2\ X10^4$	
pH=7.5	46.7	23.7		29	16.6		$2X10^5$	$1\ X10^5$	
pH=7.2	26.9	16.2		31	3.8		$3X10^5$	$2\ X10^5$	
pH=6.9	28.5	13.8		27.8	9.0		$5X10^5$	$3\ X10^5$	
pH=6.6	21.8	25.2		22.2	37.5		$1X10^5$	$3\ X10^5$	

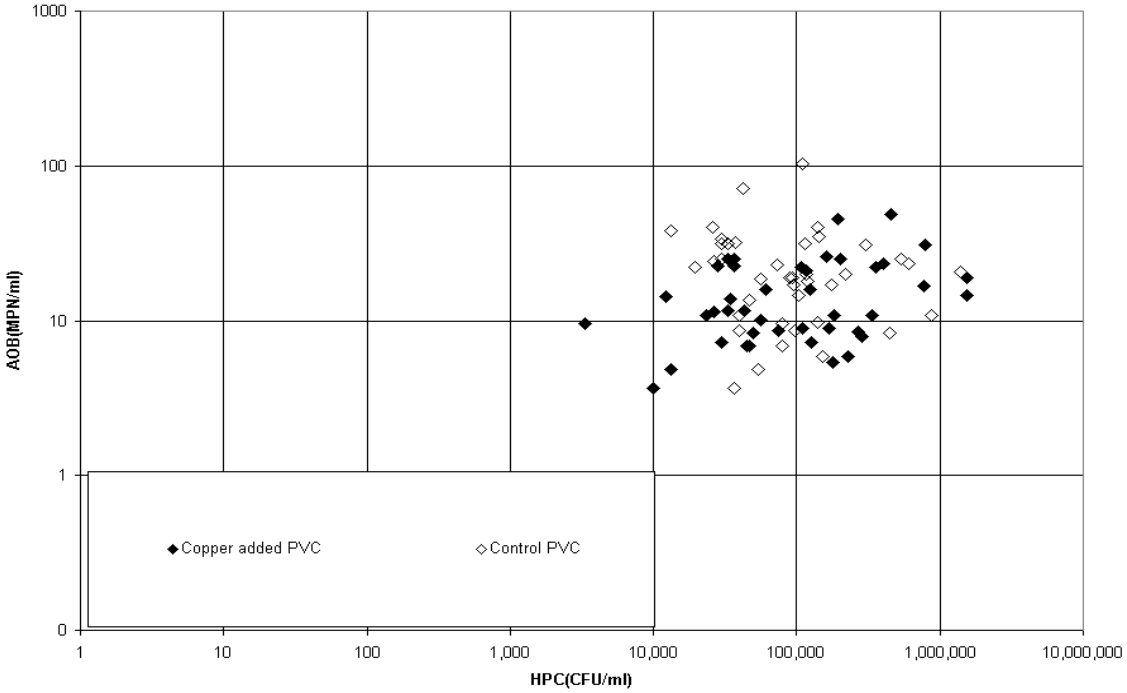

Figure 3-6: Bulk AOB and HPC population in copper added PVC and control reactor.

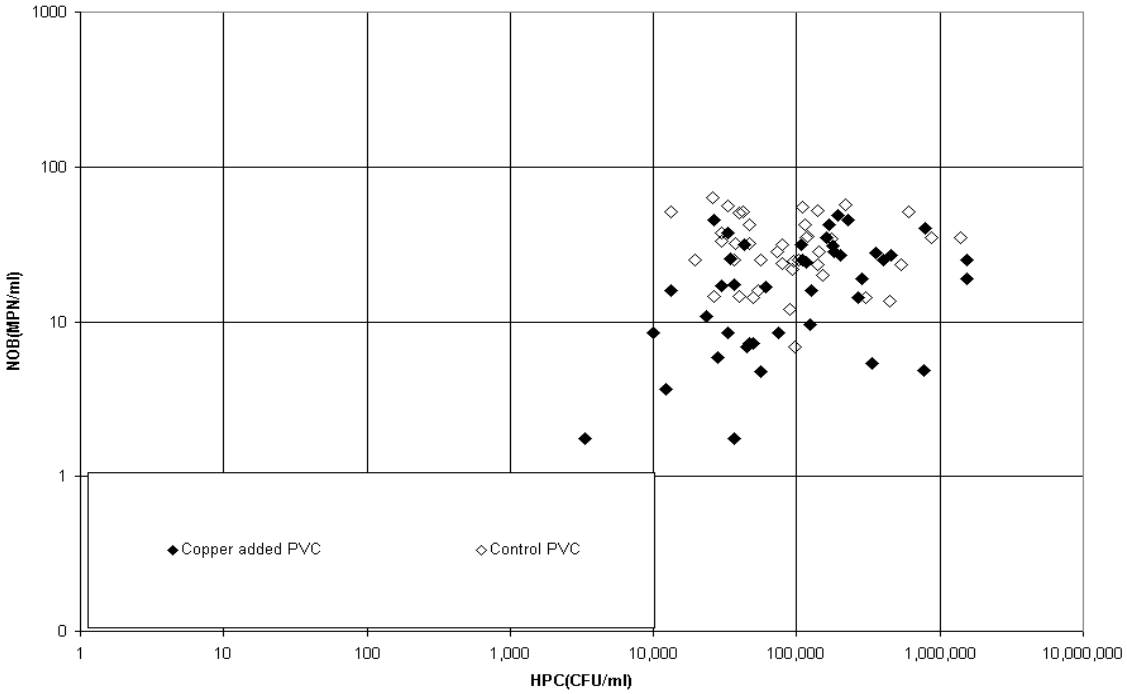

Figure 3-7: Bulk NOB and HPC population in copper added PVC and control reactor.

The biofilm community profile was also analyzed using PCR and DGGE. DGGE gel profiles of these biofilm are shown in Figure 3-8. Each band in the lanes represents one species present in the biofilm. Almost no bands appeared to be significantly brighter or dimmer among these three lanes. Therefore the presence of copper did not appear to significantly affect the community composition.

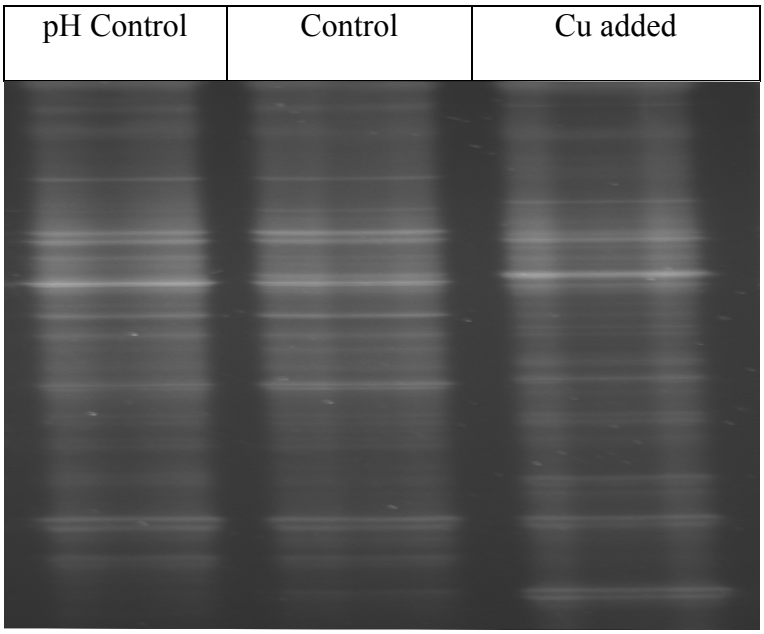

Figure 3-8: DGGE profile of biofilm from copper added PVC and control reactors

Effect of Chlorite on Nitrification

The effect of chlorite on nitrification was investigated by introducing chlorite in nitrifying PVC and copper reactors. Chlorite concentration was gradually increased from 0.2 to 20 ppm. NH_3-N utilization in chlorite added PVC, copper and control reactors are shown in Figure 3-9 and Figure 3-10. Low range chlorite (i.e., 0.2.to 2 ppm) did not

affect nitrification in the PVC reactor. At 20 ppm the PVC reactor was slightly affected as it temporarily had lower NH_3-N utilization but then adapted. In the case of the copper reactors, nitrification was seriously hindered at 20 ppm chlorite. This reactor completely stopped nitrification and the NH_3-N utilization dropped to zero. After chlorite was discontinued it took almost six weeks to re-establish complete nitrification (Figure 3-10).

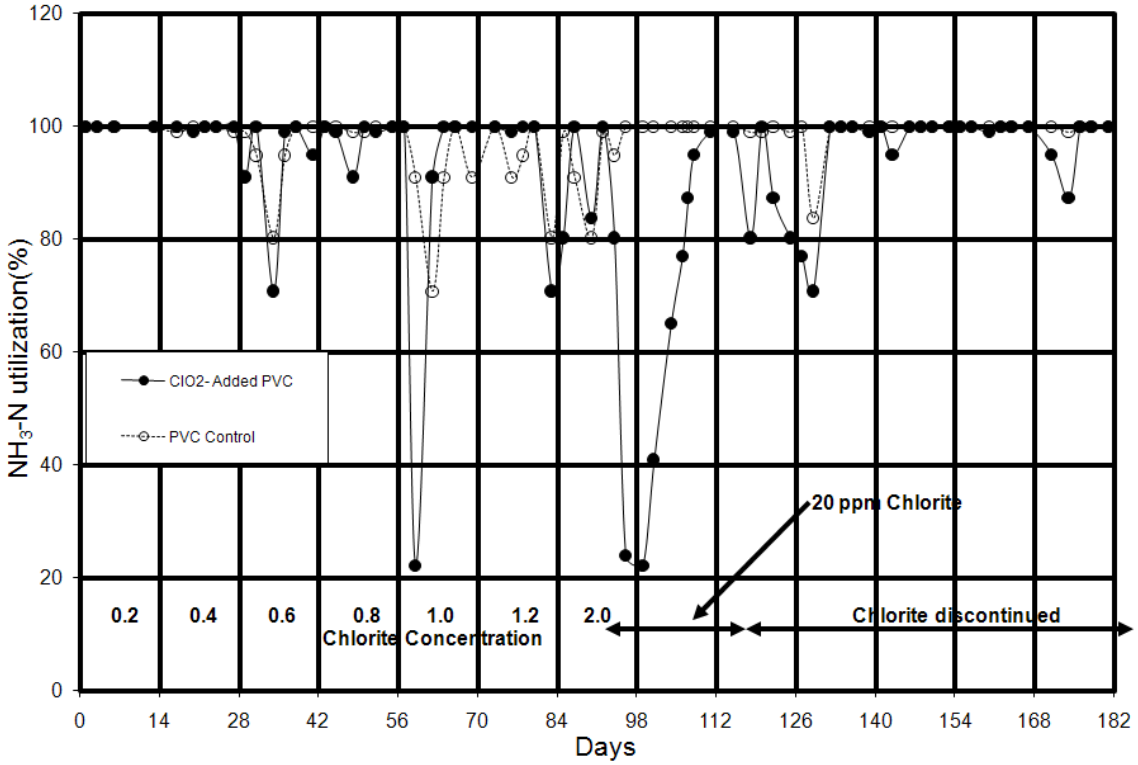

Figure 3-9:NH_3-N utilization (%) in bulk water for chlorite dosed and control PVC reactors

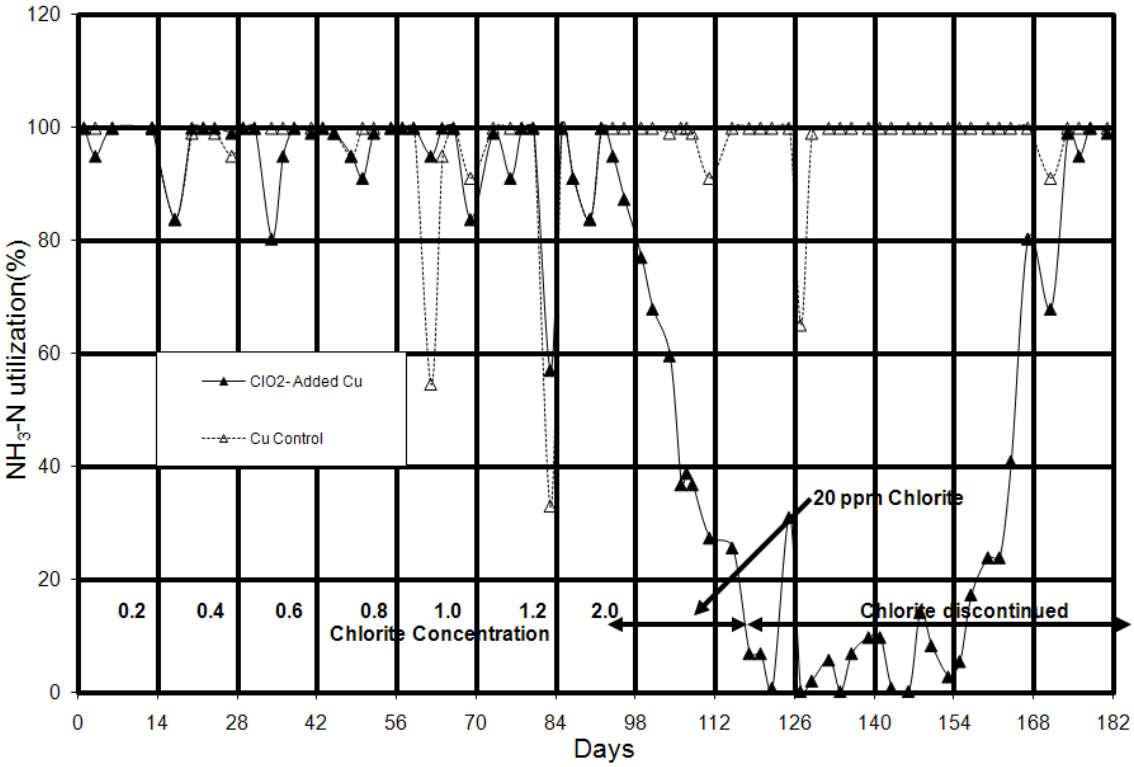

Figure 3-10: NH_3-N utilization (%) in bulk water for chlorite dosed and control copper reactors

NO_2-N and NO_3-N detected in the bulk water as a percentage of initial bulk water NH_3 in chlorite dosed and control reactors are shown in Figure 3-11 through Figure 3-14 . For both PVC and copper reactors chlorite at the low range (0.2 to 2.0 ppm) did not significantly affect the NO_2-N percentage. At 20 ppm chlorite, NO_2-N percentage increased noticeably (about 1~3%), but magnitude of this change is only in the ppb range. NO_3-N percentage in both PVC and copper reactors dropped only at 20 ppm, but in the case of the PVC reactor it quickly returned to the previous level. In the case of copper reactors it took almost two months to detect the same percentage of NO_3-N in the bulk water (Figure 3-14).

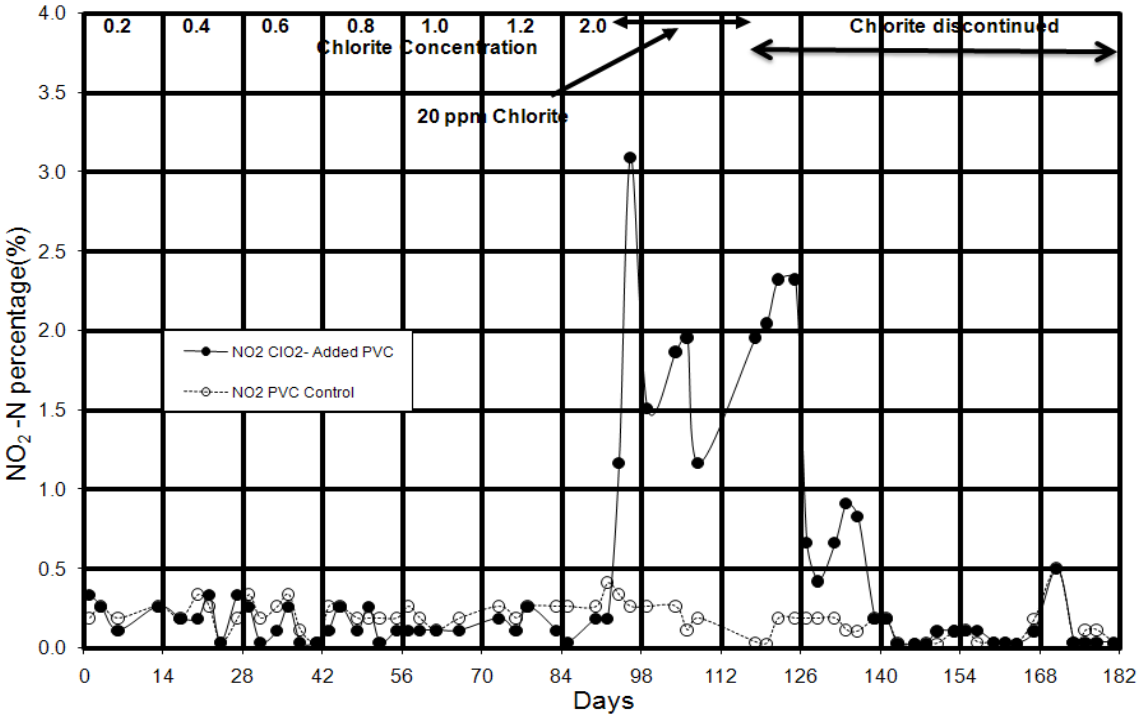

Figure 3-11: Bulk water NO_2-N as percentage (%) of initial NH_3-N for chlorite added PVC and control PVC reactors

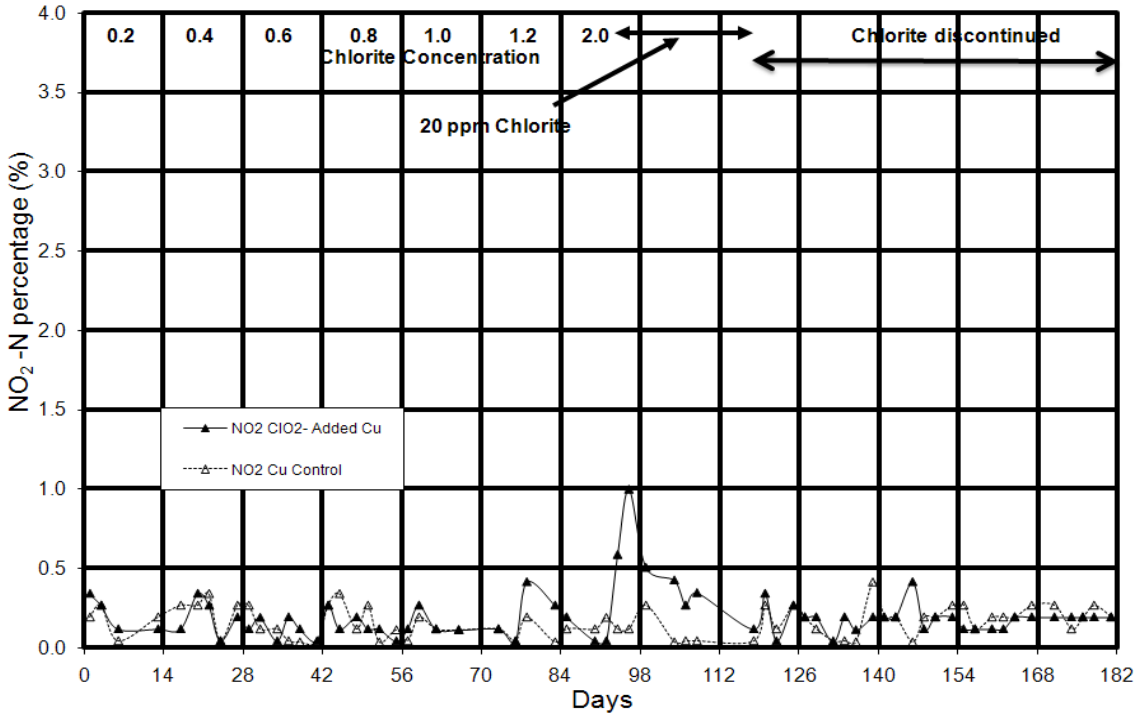

Figure 3-12: Bulk water NO_2-N as percentage (%) of initial NH_3-N for chlorite added copper and control copper reactors

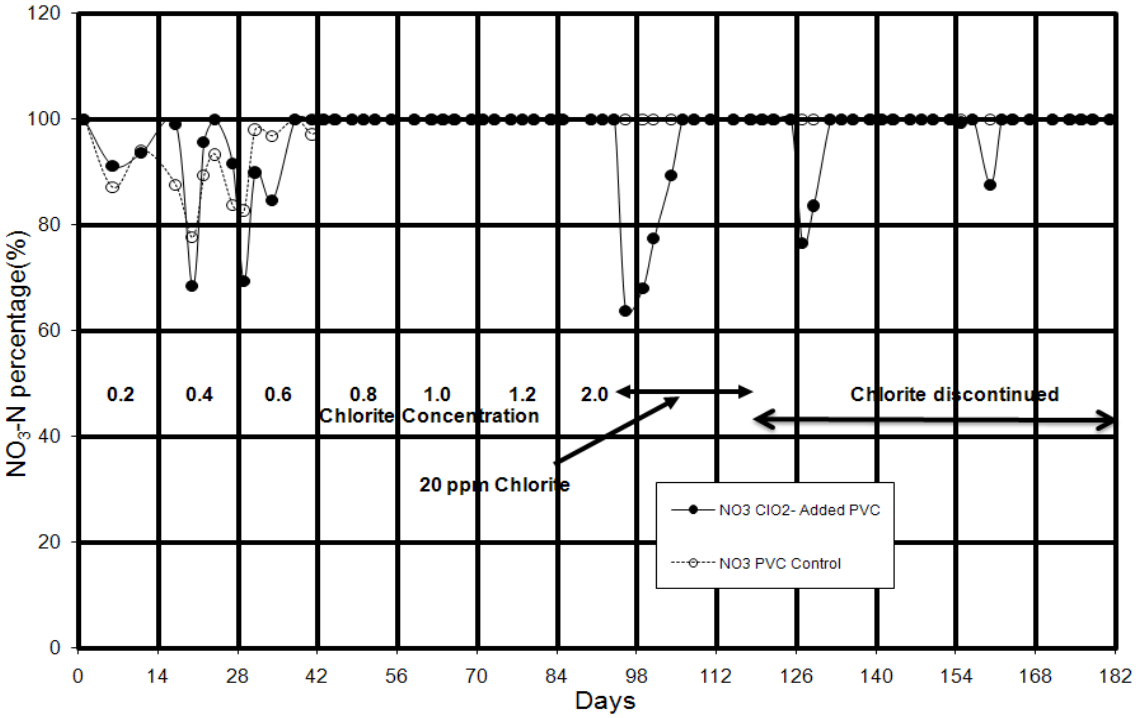

Figure 3-13: Bulk water NO_3-N as percentage (%) of initial NH_3-N for chlorite added PVC and control PVC reactors

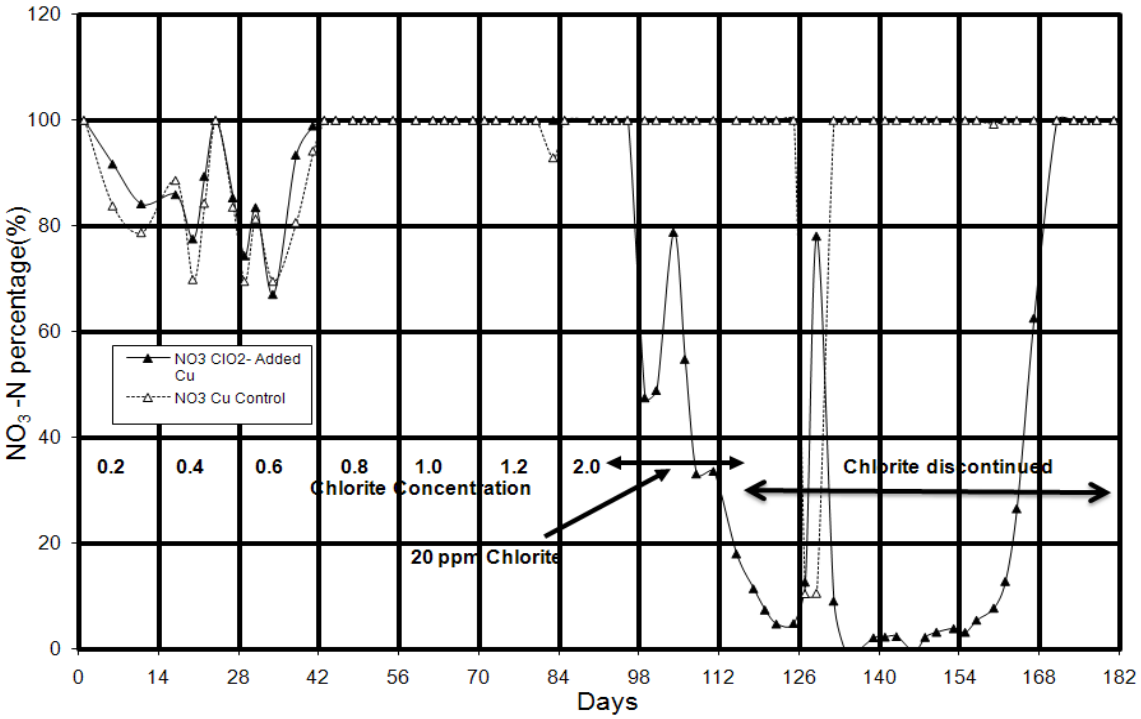

Figure 3-14: Bulk water NO_3-N as percentage (%) of initial NH_3-N for chlorite added copper and control copper reactors

Effluent total and dissolved copper concentrations in chlorite added and control copper reactors are shown in Figure 3-15. The 95% confidence interval for total and dissolved copper for the chlorite dosed reactor (0.8 ±0.04 and 0.69 ±0.03) is higher than that for control reactor (0.71± 0.02 and 0.63±0.02). A paired t-test for both effluent total and dissolved copper was done and the p value are shown in Table 3-6 . It appears that chlorite application resulted in significantly higher effluent copper concentrations.

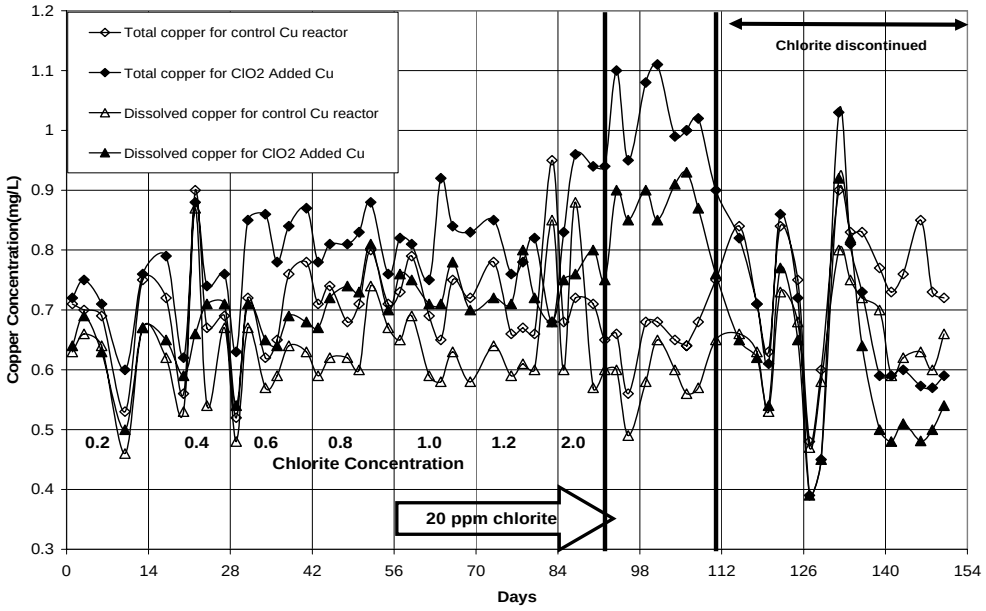

Figure 3-15: Effluent total and dissolved copper concentration for chlorite added and control reactors

Table 3-6: p values from t-test between total and dissolved copper from chlorite added and control reactors

p value comparing effluent total copper concentration	0.2~2.0 ppm chlorite dose	1.39×10^{-6}
	20 ppm chlorite dose	0.002
	Chlorite discontinued	0.011914
p value comparing effluent dissolved copper concentration	0.2~2.0 ppm	3.91×10^{-5}
	20 ppm	0.005
	Chlorite discontinued	0.02

The average MPN values for AOB, NOB and HPC values for the heterotrophic populations for different chlorite doses are shown in Table 3-7. NOB was not detected in the presence of 20 ppm chlorite. Other than that, none these populations were affected.

Table 3-7: Average (n=2) MPN (for AOB/NOB) and HPC (for heterotrophic population) for chlorite dosed and control reactors.

Chlorite Dose(mg/L)		ClO_2^- added PVC reactor	Control PVC reactor	ClO_2^- added copper reactor	Control copper reactor
0.2	MPN(cells/ml) for AOB	11.5	18.0	20.4	31.7
0.4		11.3	18.0	6.3	19.4
0.6		10.2	24.1	2.8	7.4
0.8		17	20.4	8.2	24.6
1		13	32.9	7.3	12
1.2		15.2	48.4	25.9	50.90
2		27.6	40.3	27.6	66.6
20		17.4	33.7	22.3	45.5
0.2	MPN(cells/ml) for NOB	16.2	18	13.3	20.8
0.4		18.2	23.1	6.6	12.2
0.6		11	14.5	4.8	10
0.8		10	25.2	14.7	22.8
1		8.9	32.5	21.6	21.8
1.2		16.6	44.2	8.9	37.2
2		31.5	62.9	35.4	57.8
20		ND	49.8	ND	26.2
0.2	HPC(CFU/ml) for Heterotrophic Population	$5.4X10^4$	$4.1 X10^4$	$2.1 X10^4$	$2.8 X10^4$
0.4		$3.7 X10^4$	$3.3 X10^4$	$1.7 X10^4$	$1.9 X10^4$
0.6		$7.0 X10^4$	$2.3 X10^4$	$4.8 X10^4$	$1.5 X10^4$
0.8		$7.5 X10^4$	$1.1 X10^5$	$1.2 X10^5$	$6.6 X10^4$
1		$1.2 X10^5$	$8.5 X10^4$	$4.1 X10^4$	$9.9 X10^4$
1.2		$6.8 X10^5$	$4.2 X10^5$	$1.0 X10^4$	$1.5 X10^4$
2		$3.7 X10^4$	$4.0 X10^4$	$1.3 X10^4$	$2.3 X10^4$
20		$3.4 X10^5$	$4.7 X10^5$	$6.7 X10^4$	$3.0 X10^5$

No significant correlation was found between the heterotrophic and autotrophic populations (R^2 in the range of 0.007 to 0.30).

A DGGE profile of the biofilm samples collected at the end of the experiment is shown in Figure 3-16. These profiles showed significant differences among them. Chlorite is known to be an autotrophic nitrification inhibitor, though it was not found to be very effective in the case of the PVC reactor. But the presence of chlorite might cause sufficient stress to change the biofilm community. As indicated by the left arrow (Figure 3-16) the species in the control PVC biofilm was not found in chlorite exposed biofilm More bright bands are found in the chlorite exposed copper biofilm (indicated by the right arrow).

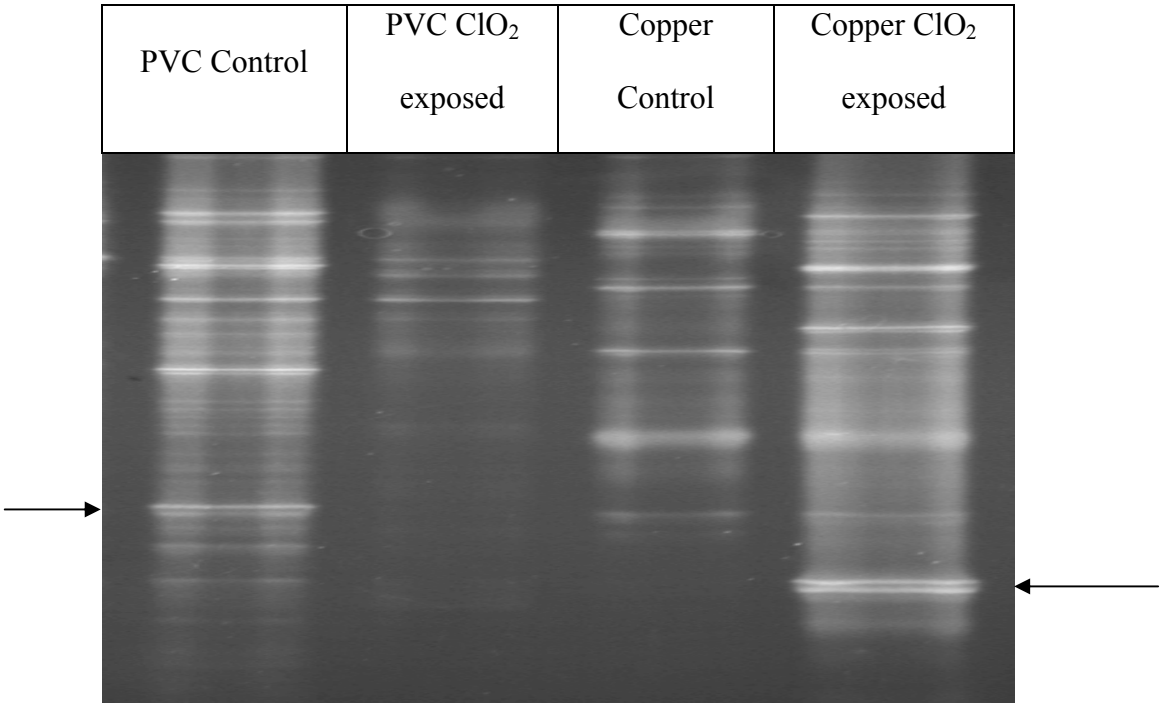

Figure 3-16: DGGE profile of biofilm exposed to chlorite and control reactors..

Effect of Monochloramine on Nitrification

The effect of monochloramine on nitrification was investigated by introducing monochloramine to the nitrifying PVC and copper reactors. Initially monochloramine was applied at a 0.5:1 chlorine to ammonia ratio and gradually raised to a 5:1 ratio, with total/combined chloramine dose of 3.55 mg/L. NH_3 utilization rate in the monochloramine dosed PVC, copper and control reactors are shown in Figure 3-17. Bulk NH_3 utilization decreased significantly at the 5:1 chlorine to ammonia ratio. At lower ratios occasional drops in utilization were observed. When monochloramine was discontinued the copper reactor restarted nitrification after three weeks but the PVC reactor took about six weeks to recover its nitrifying ability.

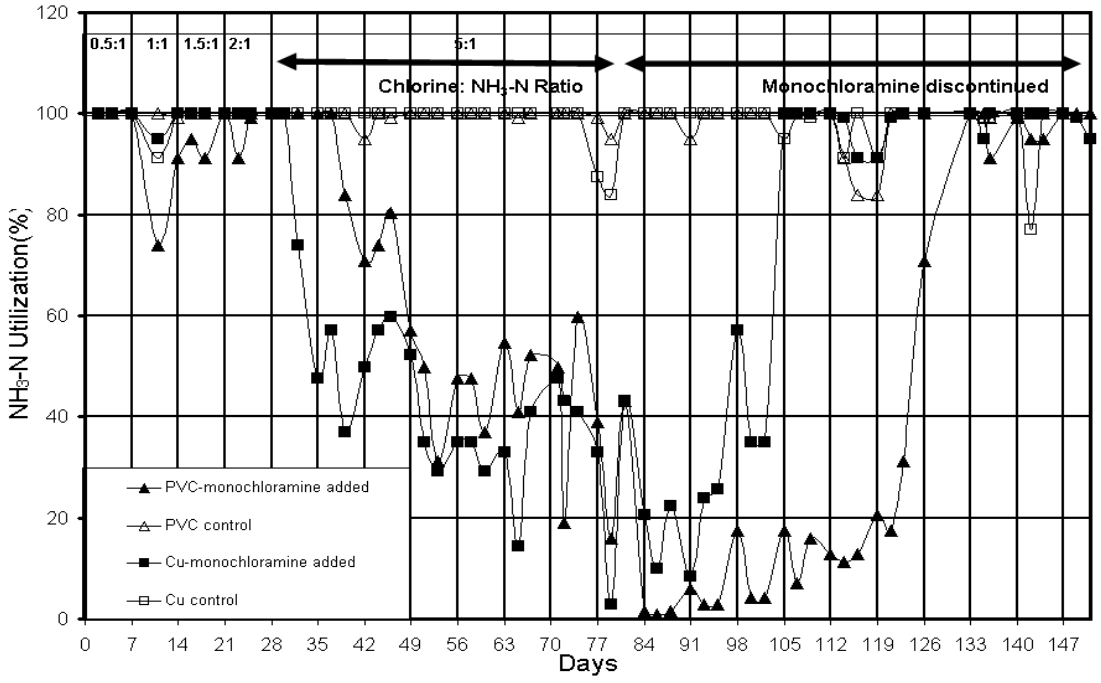

Figure 3-17: NH_3-N utilization (%) in bulk water for monochloramine dosed (PVC, copper) and control (PVC, copper) reactors

NO_2-N and NO_3-N present in the bulk water as a percentage of initial bulk water NH_3 concentration in monochloramine dosed PVC and copper reactors are shown in Figure 3-18 and Figure 3-19. For the PVC reactor the NO_2-N percentage increased at the 5:1 chlorine to ammonia ratio but it then dropped to the usual level. NO_2-N percentage in the copper reactors did not show any notable change.

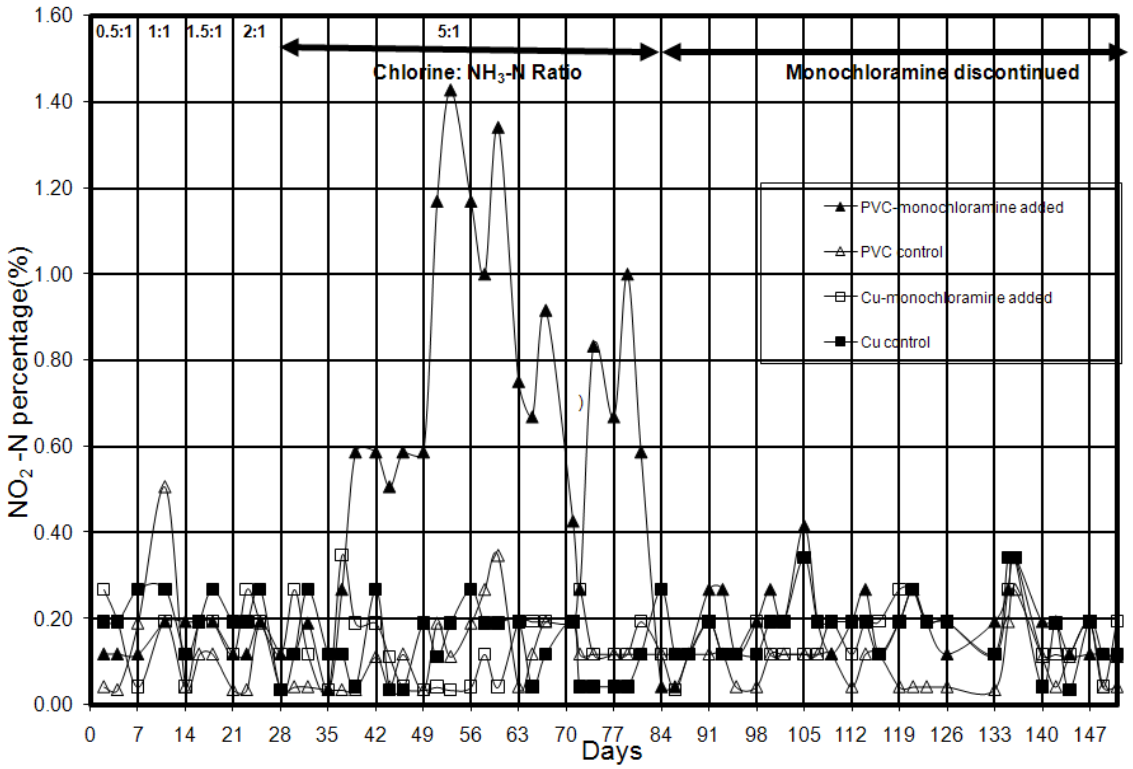

Figure 3-18: Bulk water NO_2-N as percentage (%) of initial NH_3-N for monochloramine dosed (PVC, copper) and control (PVC, copper) reactors

For both reactors the NO_3-N percentage dropped at the 5:1 ratio of chlorine to ammonia. NO_3-N percentage returned to the previous level after monochloramine was

discontinued. Comparing Figure 3-17 and Figure 3-19 it is obvious that most of the NH_3-N was converted to NO_3-N rather than NO_2-N whenever nitrification occurred.

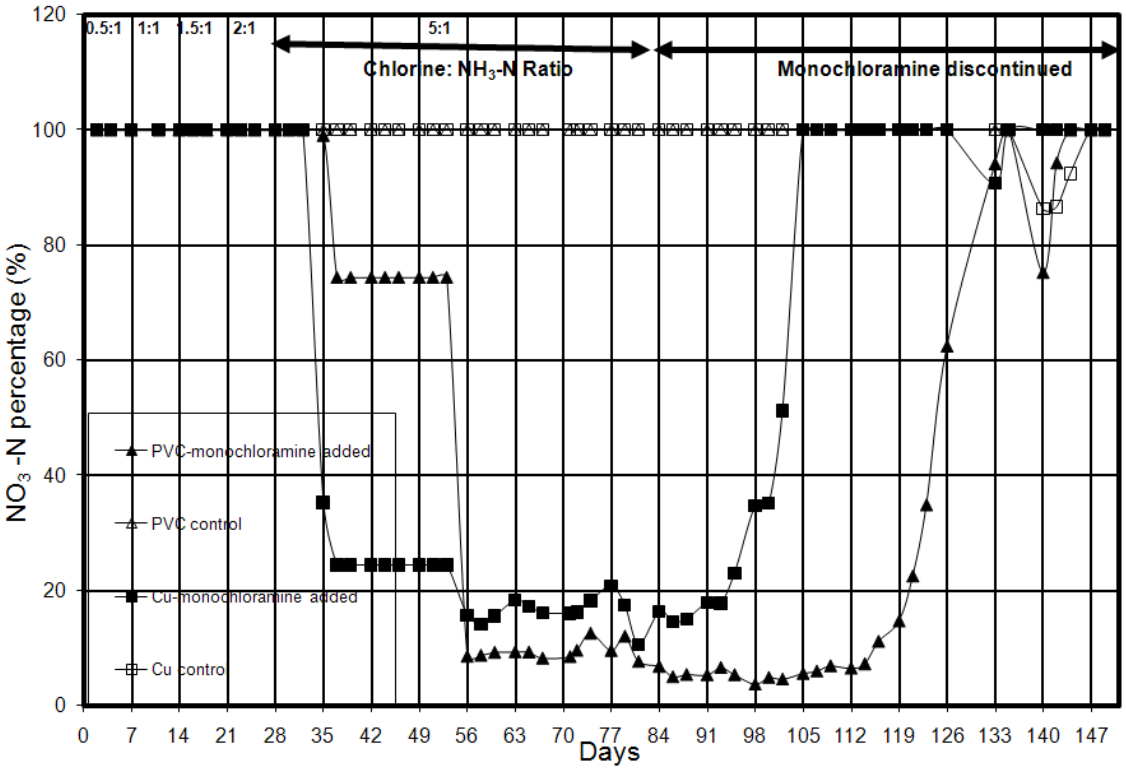

Figure 3-19: Bulk water NO_2-N as percentage (%) of initial NH_3-N for monochloramine dosed (PVC, copper) and control (PVC, copper) reactors

Changes in NH_3-N concentration in the bulk water through the eight hour stagnation period at different chlorine to ammonia ratios are given in Figure 3-20. Compared with the control reactors even at the 0.5:1 ratio NH_3 utilization was noticeably hindered in the presence of monochloramine. The utilization gradually stopped as the ratio increased to 5:1.

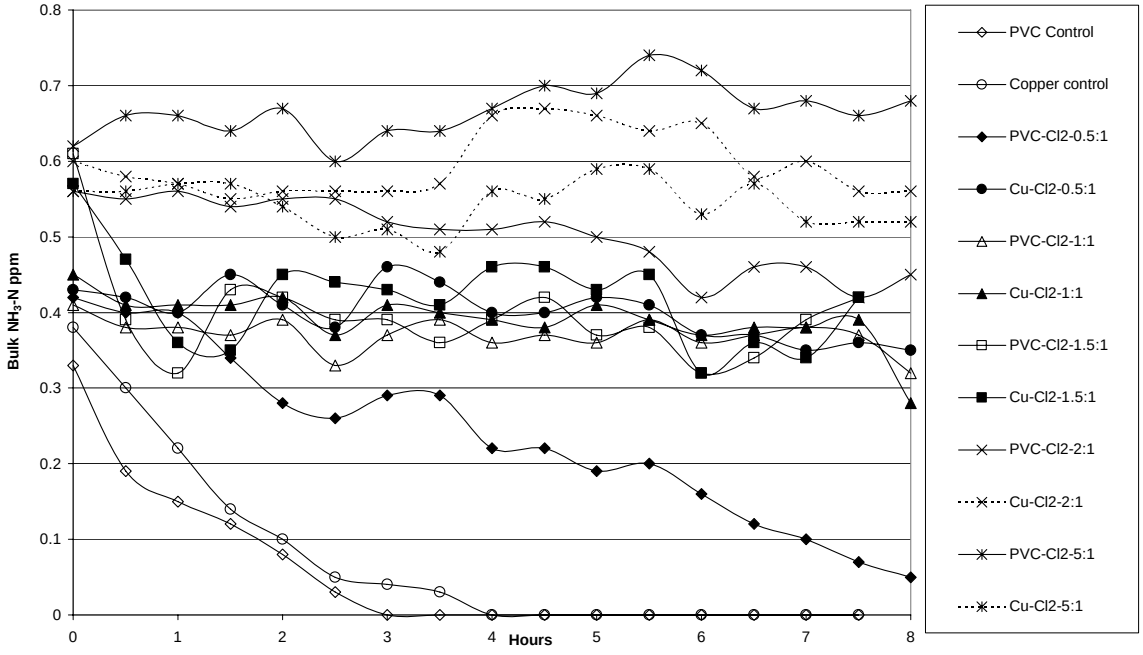

Figure 3-20: Bulk NH_3-N concentrations in the copper and PVC reactors, through the eight hour stagnation period.

Total and dissolved copper concentrations in the effluent of the monochloramine-dosed and control reactors are shown as a time series in Figure 3-21. The 95% confidence interval for total and dissolved copper for the monochloramine dosed reactor (0.83 ±0.02 and 0.73 ±0.02) are higher than that for control reactor (0.75 ± 0.02 and 0.63±0.01). A paired t-test showed that the p values for total and dissolved copper are 0.023 and $1X10^{-4}$ respectively, for control and monochloramine dosed reactors. It appears that the presence of monochloramine significantly increased the copper concentration in the water.

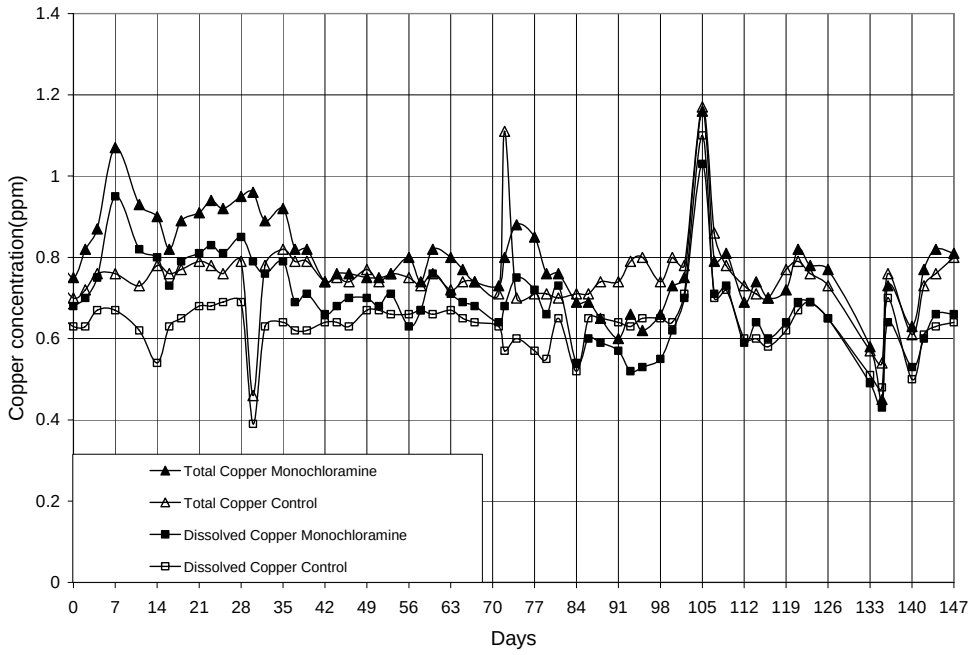

Figure 3-21: Total and dissolved copper concentration in monochloramine dosed and control reactor effluent.

The average MPN value for AOB, NOB and the HPC value for the heterotrophic population at different chlorine to ammonia ratios are shown in Table 3-8. No NOB was detected in the presence of monochloramine at the 5:1 ratio. No significant correlation was found between heterotrophic and autotrophic populations (R^2 in the range of 0.02 to 0.35).

Table 3-8: Average (n=2) MPN for AOB/NOB and HPC for heterotrophic population at different Cl_2 to NH_3-N ratio.

Chlorine to NH_3-N ratio		NH_2Cl added PVC reactor	Control PVC reactor	NH_2Cl added copper reactor	Control copper reactor
0.5:1	AOB	31	37.7	10.7	38.1
1.0:1.0		27.6	31.5	10.4	14.5
1.5:1		9.7	52.2	6.9	27.6
2:01		7.2	9.5	8.7	17
5:01		12.7	31.4	13.2	21.2
0.5:1	NOB	48.8	59.1	106.7	30.3
1.0:1.0		9.7	23.3	23.5	51.5
1.5:1		21	351.9	28.5	170.9
2:01		8.4	793.6	10.2	61.5
5:01		ND	310.1	ND	48.3
0.5:1	HPC	$3.4X10^5$	$4.8\ X10^5$	$1.1\ X10^4$	$4.7\ X10^5$
1.0:1.0		$3.5X10^3$	$9.7\ X10^4$	$1.1\ X10^3$	$2.7\ X10^4$
1.5:1		$3.9X10^4$	$4.7\ X10^5$	$9.3\ X10^3$	$1.0\ X10^4$
2:01		$6.3\ X10^5$	$1.0\ X10^5$	$1.2\ X10^4$	$6.2\ X10^4$
5:01		$3.4\ X10^4$	$1.1\ X10^5$	$2.1\ X10^2$	$8.1\ X10^4$

DGGE gel profiles of the biofilm exposed to NH_2Cl and the control are shown in Figure 3-22. The monochloramine added and control biofilm showed a significant difference in their composition. Bands indicated by white circles show differences in community compositions. Two bands in the control reactors (i.e. both copper and PVC) biofilm community profile shown by circles could not be found in the monochloramine dosed reactor's profile. This species may be very sensitive to monochloramine presence. However two other bands found in the monochloramine dosed reactor's profile were absent or not bright in the control reactors. These bands may represent monochloramine tolerant species. Interestingly, the two bands from the monochloramine dosed copper and PVC profiles were not the same species as they are vertically in different positions.

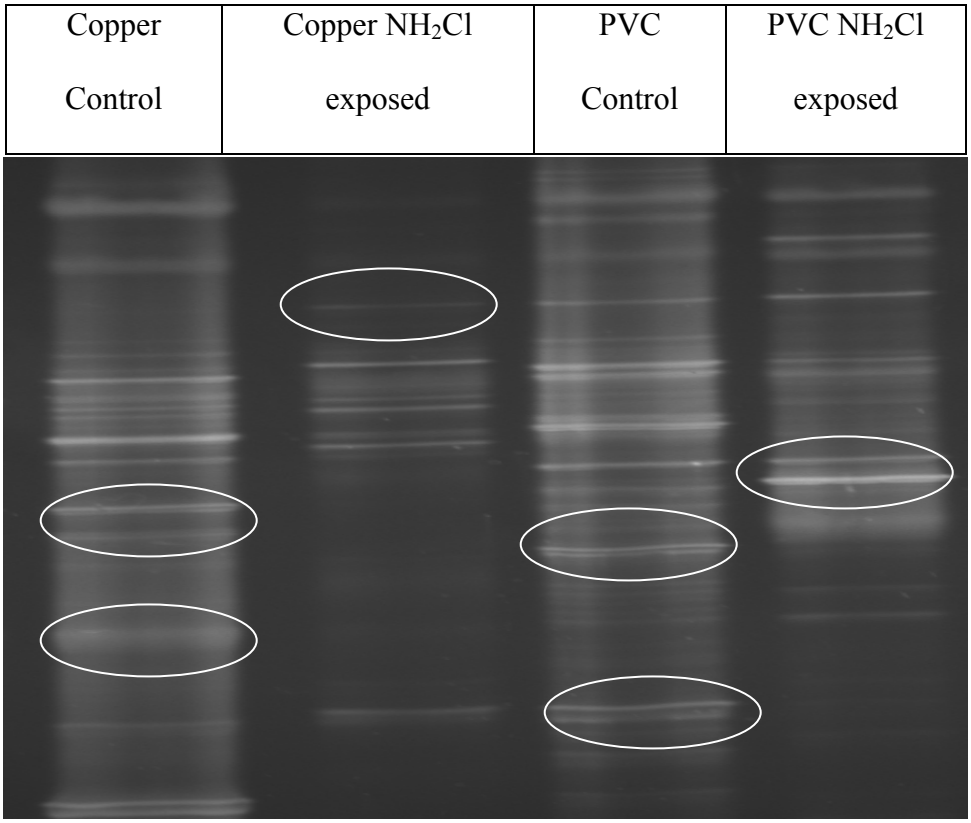

Figure 3-22: DGGE profile of biofilm sampled at the end of the NH_2Cl exposure experiment.

Effect of Copper, Chlorite and Monochloramine on Biofilm Cell Density

AOB/NOB and HPC density in the biofilm were determined at the end of each experiment. These cell densities are given in Table 3-9. AOB/NOB cell density is typically about 3 logs lower than the heterotrophic cell density. In the case of monochloramine in the copper reactor, biofilm AOB cell density is higher than that of the heterotrophs. No NOB was detected in the chlorite and monochloramine dosed copper reactor biofilm.

Table 3-9: Cell densities for autotrophic and heterotrophic population on the biofilm

	AOB (cells/cm^2)	NOB (cells/cm^2)	HPC (CFU/cm^2)
Copper added PVC	$5.7X10^3$	$4.4\ X10^3$	$1.6\ X10^6$
pH Control PVC	$2.8\ X10^3$	$3.2\ X10^3$	$7.7\ X10^5$
Chlorite added PVC	$3.5\ X10^2$	$7.7\ X10^1$	$1.5\ X10^6$
NH_2Cl added PVC	$9.2\ X10^1$	$7.9\ X10^2$	$1.2\ X10^5$
PVC--Control	$2.4\ X10^3$	$2.8\ X10^3$	$1.2\ X10^6$
Chlorite added Copper	$1.3\ X10^1$	0.0	$1.8\ X10^5$
NH_2Cl added Copper	$1.4\ X10^2$	0.0	$9.8\ X10^1$
Copper--Control	$1.1\ X10^3$	$8.0\ X10^2$	$1.8\ X10^5$

Discussion

In this project three different strategies for controlling nitrification in household plumbing were studied. The inhibitory effect of copper and chlorite on nitrification in two types of systems (PVC and copper) was tested. Also the efficacy of monochloramine for controlling nitrification was evaluated.

Copper introduced in the nitrifying PVC reactor did not show any significant effect on the ammonia utilization, nor in nitrate concentration in the reactor. The bacterial population was also not affected by the presence of copper. This observation contradicts the results from other researchers (Pettet 1956, Skinner et al. 1961, Loveless et al. 1968, Martin et al. 1982, Zhang 2005) who reported that lower (5 ppb to 0.56 ppm) copper concentrations inhibited nitrification. However Tomlinson et al. (1966) and Meiklejohn et al. (1950) observed inhibition only at higher concentrations than those tested in our study. This discrepancy may be because the previous work was done with pure cultures or activated sludge, and the copper tolerance for those biological systems may be much different from the biofilm grown in CDC reactors in this project. Another possible

explanation is that organic matter contains many functional groups (Sarathy et al. 2005) that bind to metal to form complexes and make them less bioavailable (Loveless et al. 1968, Ziko et al. 1976, Jenna et al. 1977, Dodge et al. 1979, Crecelius et al. 1982) and therefore less inhibitory (Kim et al. 2006). The humics used in this research may have acted in this capacity although from the free copper measurement most of the added copper was in the ionic form.

Previous studies (Thurman et al. 1989, Artz et al. 2002, Kim et al. 2002, Teitzel et al. 2003, Lehtola et al. 2004) reported that copper has a toxic effect on heterotrophs in drinking water. In this project no significant effect of copper on the HPC was observed. Again this discrepancy may be due to the difference in experimental conditions and difference in bacterial populations present in the water.

Another mechanism for assessing the effect of copper is by using PCR and DGGE. According to Muyzer et al. (1993) bacterial populations that make up a least 1% of the total community can be detected by PCR-DGGE. This suggests that this technique would be reasonably sensitive and should detect any major changes in ecology due to copper. Different researchers (Noda et al. 2002, Yoshie et al. 2002, Kreuzinger et al. 2003, Emtiazi et al. 2004) have successfully used molecular techniques (PCR and DGGE) to show changes in bacterial community diversity due to environmental (i.e. temperature) and operational condition changes and inhibition or toxicity. There are few reports (Emtiazi et al. 2004, Gagnon et al. 2006) available on drinking water biofilm composition. There are a few instances where the presence of copper was reported to influence microbial community composition (Jonas et al. 1989, Webster et al. 2001). In

contrast, in North Sea sediments exposed to copper did not show any immediate effect on the community composition (Gillan et al. 2004). The DGGE profiles of the biofilm from the copper added PVC reactor, control PVC reactor and pH control reactor (Figure 3-8) show that the presence of copper or lower pH did not cause any significant difference in the biofilm community profile.

Previous studies by O'Conner (2001) and McGuire et al. (1999) showed that chlorite ion (0.2 to 1.0 ppm) in distribution systems can inhibit nitrification. Results in this project contradict their reports. The PVC reactor was found to nitrify in the presence of 20 ppm chlorite. McGuire et al. (1999) also mentions that chlorite did not inhibit nitrification in one system, which according to the author may be due to the presence of higher ammonia (1.4 mg/L). Karim et al. (2006) also reported that chlorite was unable to hinder nitrification. All these studies were done with low doses of chlorite (0.2 to 1.0 ppm) and their flow pattern, water quality and bacterial population/biofilm characteristics may be significantly different from this project. Another possible explanation is that chlorite inhibits the activity of *Nitrosomonas europa* and *Nitrobacter winogradski* (Hynes et al. 1983), but may be inactive towards other groups of AOB (*Nitrosospira, Nitrosococcus, Nitrosolobus, Nitrosovibrio*) and NOB (*Nitrospina* and *Nitrococcus)* which may be predominant in the CDC system. These other groups of AOB/NOB might have more tolerance towards chlorite toxicity.

The planktonic and biofilm heterotrophic populations were found to be unaffected by chlorite exposure. The trend in heterotrophic population supports previous work by Gagnon et al. (2005) that states that chlorite at 0.1-0.25 ppm is ineffective in inactivating

heterotrophic bacteria. Similar to HPC, AOB values did not show any effect due to chlorite exposure. The planktonic NOB population remained unchanged for all concentrations of chlorite except at 20 ppm, when they could not be detected. NOB were also not detected in the copper reactor's biofilm. Hynes et al. (1983) reported that pure cultures of AOB (*N.europaea*) and NOB (*N. winogradski*) were inhibited by chlorite, and that the AOBs are 50 times more sensitive to chlorite inhibition than NOB. The opposite effect was seen in this work. Again this may be due to the differences in experimental setup, pure cultures vs environmental biofilm, water quality and other factors.

The biofilm community profile of the chlorite exposed PVC reactor showed fewer bands than that for the control reactor. Some chlorite sensitive species might be outcompeted by other species. In case of the copper reactors the chlorite exposed sample had more bands than the control sample. Due to the presence of chlorite the autotrophic population in the copper reactor might be suppressed allowing the heterotrophic population to thrive without any competition. An interesting observation was that the paired t-test showed that total and dissolved copper concentrations in water from the chlorite dosed reactors were significantly higher than that for control reactors at a 95% confident interval.

As chlorite (ClO_2^{-2}) could be transformed to chlorine dioxide (ClO_2) (Gates et al. 1989) in an acidic environment created by oxidation of ammonia during nitrification by biofilm on the surface, it might increase copper corrosion due to the oxidative nature of chlorine dioxide. There may be an unintended result of elevated copper release when chlorite is applied. For this reason, and because chlorite is a regulated compound, the

utilities should carefully evaluate chlorite before implementing it as a nitrification control strategy.

Halt et al. (1995) showed reduction of nitrification at total chlorine concentrations of more than 0.3 mg/L. At 0.5:1 ratios in these reactors, about 0.35 mg/L of total chlorine was present and nitrification continued. However maintaining a monochloramine residual created at a 5:1 Cl_2 to NH_3-N ratio was found to be effective in decreasing nitrification, which agrees with results from Karim et al. (2006) and Lieu et al. (1993).

The change in NH_3 concentrations in bulk water (Figure 3-20) throughout the stagnation period for different chlorine to ammonia ratiod showed similar trends. AOB cell density was greater than the heterotrophic cell density in biofilm from the monochloramine dosed copper reactor. Higher AOB cell density found in this project supports the result of Stewart et al. (1996) and Cunliffe (1991) who showed that nitifiers are very resistant to disinfection. NO_2-N concentrations were only slightly impacted by monochloramine. No planktonic NOB were detected in monochloramine exposed reactors at the 5:1 ratio. Also NOB were not detected in the biofilm in the presence of monochloramine in the copper reactor. NOB, being more vulnerable to disinfection than AOB (Wolfe et al. 2001), may be the reason for this observation.

In this project copper reactors have lower or equivalent biofilm density than those of PVC reactors. Copper biofilm HPC density in presence of monochloramine was much lower (almost 4 log) than all other biofilms. This may be due to the slower rate of biofilm formation in copper surface (Lehtola et al. 2004). DGGE profiles also showed that monochloramine exposed samples have fewer bands than the control sample. Due to the

presence of monochloramine only the persistant species may be in the biofilm. This is supported by the findings of Redway et al. (1982), who demonstrated the selection or adaptation of more resistant strains to chloramines.

This project also provides some insight on effect of chloramine on copper corrosion. Ammonia, a by-product from chloramines, has strong complexation constant for cupric ion (Schock 1995). Several researchers (Ingleson et al. 1949) found chloramine increased copper corrosion. Enhanced copper solubility during periods of chloramination with excess ammonia present was observed for Champaign IL tap water (AwwaRF 1990). On the other hand, according to MacQuarrie et al. (1997) and Rahman et al. (2007) application of monochloramine would result in a decrease in copper pipe corrosion. In this project the total and dissolved copper concentrations were found to be higher in the monochloramine exposed reactor.

Throughout these experiments there was essentially no correlation between HPC and AOB/NOB MPN values. Wolfe et al. (1990) reported that HPC and AOB population were highly correlated in distribution system water, and that this may be explained due to the dependence of HPC on AOB for carbon fixation. However other researchers (Donnelly et al. 2005) reported that the relationship is very site specific. The experimental results in this project support the concept that there need not be a correlation between HPC and AOB/NOB.

The chlorite exposed copper reactor regained its nitrifying ability after two months from discontinuation of chlorite in the influent. The monochloramine dosed PVC reactor regained nitrification about six weeks after discontinuation of monochloramine.

In contrast the copper reactor took only three weeks from monochloramine discontinuation to gain its nitrification ability.

In this project monochloramine was found to be the most effect solution against nitrification. Copper under the action limit (less than 1.3 ppm) was not found to have any effect on nitrification, which is contrary to the common belief. Addition of chlorite was also not found to be suitable for controlling nitrification.

Conclusions

- Copper did not show any inhibitory action on nitrification
- Autotrophic and heterotrophic populations remained unaffected by the addition of copper up to 1.3 ppm from a range of pH of 8.15 to 6.6
- The biofilm community's DGGE profile did not show any response to copper or pH change
- Chlorite was effective at inhibiting nitrification only at a very high dose (20 ppm)
- Chlorite at 20 ppm exhibited inhibitory effects on the NOB population
- The presence of chlorite may increase copper corrosion
- Monochloramine at a Cl_2 to NH_3-N ratio of 1.5:1 or more is effective in nitrification control
- Both chlorite and monochloramine exposure resulted changes in biofilm community profile as assessed by DGGE
- No strong correlation between HPC and AOB/NOB was found

- Time to recovery after cessation of addition of chlorite and monochloramine was less than it took to initially establish.

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CHAPTER 4

EFFECT OF NUTRIENT CONDITIONS ON NITRIFICATION ALKALINITY, pH AND COPPER RELEASE

Abstract

Nitrification is a major problem for the drinking water industry in the United States. A 1996 American Water Works Association Research Foundation (AwwaRF) survey indicated that two thirds of drinking water utilities that chloraminate have experienced some degree of nitrification (Wilczak et al. 1996). Nitrification causes detrimental changes in water quality, including a decrease in chloramine residual, pH, alkalinity and the dissolved oxygen content of water. Reduction in pH and alkalinity can lead to Lead and Copper Rule (LCR) violations. Nitrification can also cause biological instability through production of soluble microbial products (SMP) which may support the growth of heterotrophic bacteria in low nutrient environments (Rittmann et al. 1994). In this project, the effect of nutrient conditions on the nitrifying population and subsequent changes in pH and alkalinity in a simulated household plumbing system was investigated.

Six nitrifying modified CDC reactors were used to simulate a household plumbing system. Two sets of reactors were used; each set consists of three reactors containing either PVC as a coupon material or copper. Initially these reactors were fed water with humics (4 ppm) as the organic carbon source and ammonium sulphate (0.71 ppm) as nitrogen source and biologically treated tap water to supply the bacterial

population. Water in the reactors was stagnant for eight hours and then flowed for five minutes. pH of the influent water was maintained at around 8.15 and alkalinity was around 35 mg/L as $CaCO_3$. To investigate the effect of nutrient condition on the nitrifying population, influent humics and ammonia of two reactors of each set was raised to 8 ppm of humics and 2.13 ppm as ammonia nitrogen. Alkalinity and pH changes due to nitrification were measured and the heterotrophs and autotrophic nitrifying bacteria were enumerated using R2A plates and an MPN method. Increased ammonia increased the nitrifier population but did not show any effect on the heterotrophic population. Similarly, higher TOC increased only the heterotrophs and did not have much effect on the autotrophic nitrifiers. For all reactors, alkalinity and pH decreased due to nitrification, with lesser effect on copper reactors. Increases in TOC or NH_3 elevated the copper concentration in the water.

Introduction

Nitrification is the oxidation of ammonia to nitrite and nitrate. Ammonia in water can be present either naturally or from chloramination. Nitrification is primarily accomplished by a group of autotrophic nitrifying bacteria that obtain energy from ammonia and nitrite. In the first step ammonia oxidizing bacteria (AOB) oxidize ammonia to nitrite according to equation 1. *Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosolobus*, and *Nitrosovibrio* are common AOB and *Nitrosomonas* is the most common among them (Kirmeyer et al. 1985). In the second step nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate according to equation 2 (Doetsch and Cook 1973).

Nitrobacter is the most common NOB, although *Nitrospina* and *Nitrococcus* species are known nitrite oxidizers (Kirmeyer et al. 1995). Overall the nitrification process can be represented chemically according to equation 3.

$NH_3 + 3/2O_2 \longrightarrow NO_2^- + H^+ + H_2O$ Equation 1

$NO^-_2 + 1/2O_2 \longrightarrow NO_3^-$ Equation 2

$NH_3 + 2\ O_2 \longrightarrow NO_3^- + H^+ + H_2O$ Equation 3

Effect of Nitrification on pH and Alkalinity

Because nitrification produces protons (H^+), the alkalinity of the water is reduced. The destruction of alkalinity will result in a reduction in pH if the water is poorly buffered. Decreases in alkalinity and pH can cause Lead and Copper Rule violations by increasing corrosion and metal release which may exceed the action level of metals in the water (Odell et al. 1996). According to a survey conducted by Kirmeyer et al. (1995), a decrease in pH and alkalinity was not strongly correlated with nitrification in different utilities. However, the the maximum pH change reported in that study was ≤ 1unit and most of the alkalinity change was in the range of 5 ppm as $CaCO_3$. Nitrifying bacteria are very pH sensitive (Srinath et al. 1976) with the optimum pH range for *Nitrosomonas* of 7.0 to 8.0 and *Nitrobacter* being 7.5 to 8.0. Although nitrification is reported to occur over a wide range of pHs outside these optimum (Stankewich, 1972), there are reports of nitrification in the acidic range (Parker et al. 1975, Tarre et al. 2004a and b, Gieseke 2006). The general trend is as the pH drops towards the acidic range nitrification rate decreases (Wilezak et al.1996). Therefore, there may be a larger probability of

nitrification in water with sufficient alkalinity to prevent any significant change in pH over that for weakly buffered water.

It should be noted that most research on changes in alkalinity and pH during nitrification have been done in wastewater. Theoretically 7.14 g/L of alkalinity as $CaCO_3$ is consumed for the oxidation of 1gm/L of NH_3-N to NO_3-N (equation 3). Different experimental and field investigations on fixed film and slurry type reactors reported alkalinity consumption in the range of 6.2 to 7.4 mg/L as $CaCO_3$ (Parker et al. 1975) which is less than theoretical. Benninger et al. (1978) also reported a lower destruction rate than the theoretical value.

pH and alkalinity of water significantly influences pipe corrosion. At higher pHs, copper has less tendency to dissolve and enter drinking water. The formation or solubility of protective films on a metal surface is also pH dependent (Schock 1999). The bicarbonates and carbonate mainly represent the alkalinity of water. These ions affect many important reactions in corrosion chemistry, including the water's ability to form a protective metallic carbonate scale or passivaiting film (Schock 1995). At a constant pH, as the alkalinity increases, copper levels increases. Studies by Edwards et al. (1994) showed that bicarbonate ion has a dual nature that is pH dependent. The researcher found that at $pH \leq 7.0$ it causes activation i.e. increased corrosion and at $pH \geq 8.5$ it causes passivation i.e. reduced copper corrosion.

Effect of Nutrient Conditions on Microbial Populations and the Interaction between Autotroph and Heterotroph Populations

Nutrients, especially carbon and nitrogen, play a significant role in controlling microbial populations. Natural organic matter present in water can act as a carbon source. Heterotrophic bacteria and fungi can carry out nitrification, although these organisms nitrify at a lower rate (Watson et al. 1981). Therefore it is possible that nitrification may be carried out by them in addition to autotrophic organisms. To a limited extent, nitrifiers can also metabolize and incorporate selected organic compounds in the presence of an inorganic energy source (Clark et al. 1967 b). Hommes et al. (2003) reported growth of *N. europea* on fructose or pyruvate as the sole carbon source instead of CO_2. Clark et al. (1966) and Hommes et al. (2003) found that *N. europea* can incorporate pyruvate during growth. Also, some organic compounds are found to increase the growth rate or activity of nitrifiers. Clark et al. (1966) showed that for *N. europea* the presence of pyruvate reduced the lag time for resumption of growth when old cells were used as an inocula. Clark et al. (1967a) reported that the presence of some amino acids increased NO_2^- production by *N. europea*. Abelivich et al. (1992) also observed that the protein content for an *N. europea* culture grown anaerobically with pyruvate as an electron donor and nitrite as an electron acceptor increased by 49% over 12 days. NH_3 was reported to be required for this growth. However, Pan et al. (1972) showed growth of *N. europea* heterotrophically in the absence of NH_3 on media containing 0.2% glucose and 4.7mM $NaHCO_3$.

Conversely, organic carbon may inhibit nitrification. This inhibition could be due to the toxicity of the compound or to competition from heterotrophic populations with higher growth (Hockenbury et al. 1977a). According to Hochster et al. (1963) and Quastel et al. (1951) aliphatic and aromatic amines could cause inhibition by competition with ammonia for the enzyme active site. Hockenbury et al. (1977b) tested fifty two organic compounds for toxicity on nitrifiers. They reported that dodecylamine, aniline and n-methylaniline could cause 50% inhibition at concentrations less than 1 mg/L.

Elevated organic carbon may also stimulate heterotrophs which may adversely affect autotrophs since they compete with heterotrophs for surfaces, dissolved oxygen, ammonia and other nutrients. Autotrophs fix inorganic carbon for cell synthesis (Brock and Madigan 1991, Brock et al. 1991) which is an energy intensive process, causing a low yield and growth rate (Rittmann et al. 1992). Heterotrophs have higher affinity for dissolved oxygen and ammonium (Rosswall 1982). Coexistence and interactions in an environment between autotrophic nitrifiers and heterotrophs should therefore depend on the carbon to nitrogen (C/N) ratio. Below a critical carbon to nitrogen ratio, heterotrophs are carbon limited and two populations can coexist. However at a C/N ratio above the critical value, both populations become nitrogen limited. In that case heterotrophs can out-compete nitrifiers (Jansson 1958). Ohashi et al. (1995) showed decreases in AOB and NOB populations along with dominance of heterotrophs with increasing C/N ratio. They also reported no nitrification at a high C/N ratio in their system during biofilm formation. However nitrification began when the C/N ratio was lowered. Once the nitrification was established their reactors could once again be operated at a higher C/N ratio. The authors

explained that initially at a high C/N ratio, nitrifers faced strong competition from heterotrophs, but once the nitrifying biofilm was formed it was less susceptible to the impact of higher C/N ratio.

Wolfe et al. (1990) reported a strong correlation between heterotrophic and nitrifying populations in water distribution systems. A symbiotic relationship could exist between these two populations. Heterotrophs can biodegrade organic compounds that are inhibitory to nitrifiers (Richardson 1985) but also produce organic compounds that stimulate their activities (Steinmüller and Bock 1976, Pan and Umbreit 1972, Hockenbury et al. 1977a). Heterotrophs also produce extracellular polymers that improve the aggregation of both species in a biofilm (Rittmann et al. 1994). Fast growing heterotrophs form the outer layer of biofilm, which may protect the nitrifiers from detachment (Rittmann et al. 1992, Manem et al. 1992, Furumai et al. 1994, Ohashi et al. 1995). In change, nitrifiers produce and release soluble microbial products (SMP) that augment heterotrophic substrate supply (Furumai et al. 1992, Rittmann et al. 1994, Kindaichi et al. 2004), which may be the reason for strong correlation between these two populations.

Nutrient conditions such as the nitrogen compound (NH_3 or NO_3) or natural organic matter (NOM) in the water can also affect corrosion in several ways. Schock et al. (1995) reported formation of strong complexes between cupric ion and ammonia. Higher copper solubility during chloramination with excess ammonia was observed for Champaign, IL tap water, which decreased when breakpoint chlorination was applied (AwwaRF, 1990). At low oxygen concentrations copper can be oxidized by NO_3

reduction instead of oxygen. However, low concentration of NO_3 can decrease pitting corrosion by modifying the scale characteristics (Edwards et al. 1994), but higher concentrations (>40 mg/L) increase pitting (Lucey 1972, Billiau et al. 1985). Several researchers (Broo et al. 1998) reported that copper release directly increases with NOM concentration. Also Korshin et al. (1996) found that very small amounts (0.1-0.2 mg/L) of NOM produce significant increases (>0.8mg/L) in copper byproduct release, but further increases in NOM concentration did not change the copper release in water. They hypothesized that NOM might cause mobilization of colloidal copper via particle stabilization and detachment. Edwards et al. (2001) conducted a study on copper corrosion byproduct release and organic matter. They concluded that copper corrosion byproduct release increases in the presence of NOM because of complexation and/or colloid mobilization/dispersion. According to the authors the presence of NOM can also reduce the copper corrosion. They explained that NOM can be used as food source for micro-organisms thus leading to DO depletion and subsequent re-deposition of copper onto the pipe wall in the presence of chloride or other ions. Moreover, gradual sorption of soluble NOM on to the scale on copper pipe surfaces decreases soluble copper complexation capacity of water and thus leads to reduced copper concentrations.

In this project the effect of different carbon and nitrogen concentrations on nitrification in simulated household plumbing systems with two types of materials (i.e. copper and PVC) was studied. Soil derived humics substances (Elliot Silt Loam) was used as carbon source and $(NH_4)_2SO_4$ was used as a nitrogen source. Humics were chosen because they occur naturally in water and previous studies showed that biofilm

bacteria can use and grow using humic materials (Volk et al. 1997, Camper 2004). Experiments were conducted to understand the effect of different levels of TOC and NH_3 on autotrophic nitrifiers (AOB/NOB) and heterotrophic populations in simulated household systems which had been nitrifying for almost two years. Also pH and alkalinity changes due to nitrification at different carbon and nitrogen concentrations with different pipe materials was examined.

Materials and Methods

Description of Reactor

To simulate a domestic plumbing system the commonly used CDC (Goeres et al. 2005) reactor was modified. These modified reactor's coupons, bottom plate and stirring blades have the same surface to volume ratio as that of a six foot long ¾" diameter domestic copper plumbing pipe. The rotational speed of the blade inside these reactors was 300 rpm. This speed was chosen as it creates 3fps velocity in bulk water which can be found in domestic water lines. Volume of the reactors is 120 ml. Two types of materials were tested, copper and PVC. All coupons were washed with 0.1N NaOH three times to remove any biological materials from their surface prior to use.

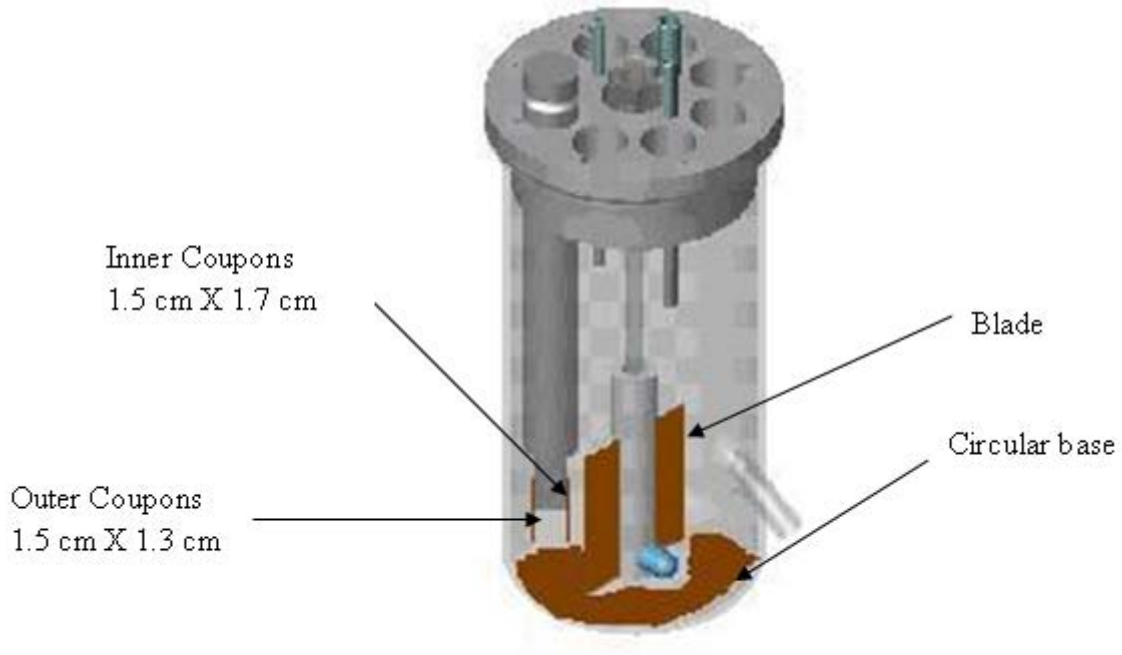


Figure 4-1: Modified CDC reactor

Operation Scheme

To simulate periods of stagnation in home plumbing the reactors were flushed with peristaltic pumps for five minutes and then the water inside the reactors remained stagnant for eight hours. The feed pumps and stirplates were on two different timers, which controlled the power supply. The timers were offset from each other by one minute with the stirplate starting before the pumps. At the end of five minutes the stirplates stopped, followed by pumps. This cycle was repeated three times a day. As a result, fresh influent mixed with the stagnant bulk water and excess water spilled through the effluent port. Because there is mixing, the effluent water is always diluted with fresh influent feed, which prevents the effluent NH_3 concentration from becoming zero, even though the bulk NH_3 concentration (the concentration in the reactor) is zero due to nitrification.

Because sampling occurred in the both the bulk and the effluent, a mathematical model of the reactor was developed to determine the relation between effluent NH_3 concentration and NH_3 utilization in these reactors. This model was used to calculate the % NH_3 utilization and the % of ammonia present as NO_2, and NO_3. As described in Appendix-A, model values were equivalent to those actually measured in the bulk.

Stock/Feed Solution Preparation

Typical setup of these reactors is shown in Figure 4-2. The ratio of flow is RO: humics: BAC= 50:5:1, so these reactors are mostly supplied with RO water. Initially influent carbon concentration as humics was 4 ppm for all reactors. Lab grade chemicals were added to reverse osmosis (RO) water to give it target alkalinity of 35 mg/L as $CaCO_3$. The final concentrations of these chemicals are given in Table 4-1.

Table 4-1: Chemicals added to influent water.

Chemical	Concentration(mg/L)
$MgSO_4$	39.6
$NaHCO_3$	56.9
$CaSO_4\ 2H_2O$	25
$Al_2(SO_4)\ 18H_20$	0.62
$CaCl_2\ 2H_20$	20.80
$Na_2SiO_3\ 9H_20$	26

Bozeman tap water was flowed through a biologically activated carbon (BAC) column to remove any residual chlorine. It should be noted that Bozeman water comes from surface water source, does not contain ammonia, and chlorine is used as the final disinfectant. BAC water was pumped to the reactors to ensure a supply of indigenous

bacteria. These organisms were the only inoculum supplied to the reactors. Humics were supplied using a separate pump.

Humics Preparation

50 gm of Elliot silt loam soil (International Humic Substances Society) was added to 500 ml of 0.1 N NaOH and mixed for 48 hours. This solution was then centrifuged at 10,000 X g for 20 minutes. After centrifugation the supernatant was collected in carbon free glassware (prepared by baking at 390^0C for 5 hours) and stored at 4^0 C in the dark. Total organic carbon content of the humics was measured using a Dohrman DC-80® and subsequently diluted to the appropriate concentration using the RO water feeding the reactors.

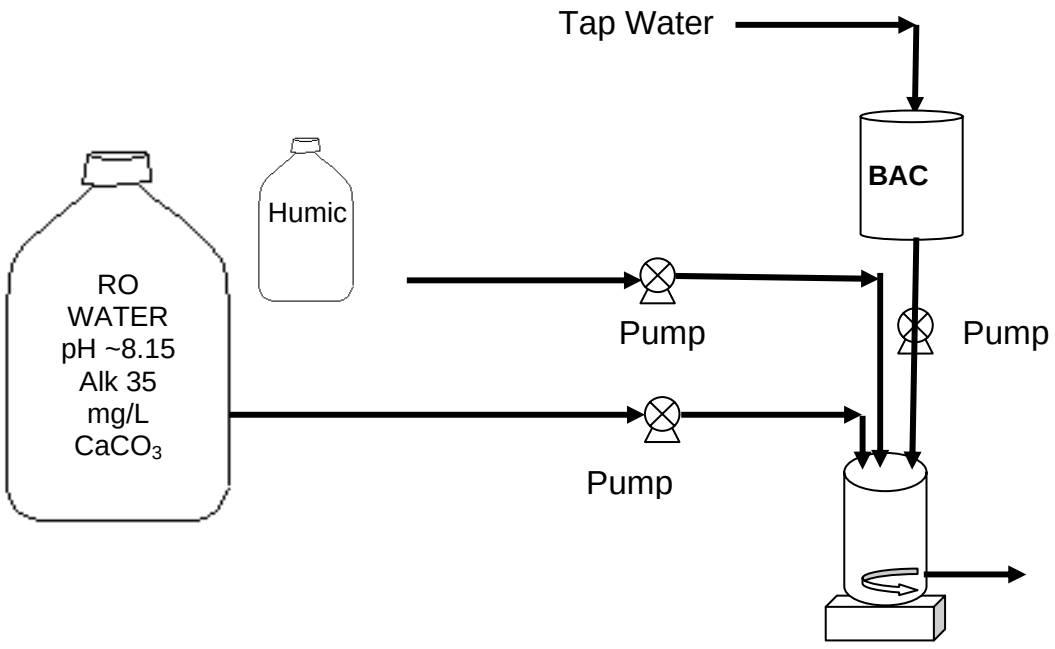


Figure 4-2: Typical setup for one reactor

Experimental Approach

Two sets of modified CDC reactors equipped with two different types of coupons (PVC and copper) were used in this investigation. Each set consists of three duplicate reactors. All of these reactors had been running for all most two years and consistently converted all the added ammonia to nitrite and nitrate. Initially all nitrifying reactors were operated at 0.71 ppm NH_3-N and 4 ppm TOC (humics) in the influent. Regular measurements of all response variables (listed in Table 4-3) were taken throughout the entire time of the project. Influent water pH and alkalinity were always kept at 8.15 and 35 mg/L as $CaCO_3$ respectively. NH_3-N and TOC content of the influent of two reactors from two sets was subsequently raised as shown in the Table 4-2. When the reactors adjusted to the changed conditions as evidenced by stable nitrification, a biofilm sample was collected for analysis. Regular measurement of bulk water alkalinity and pH was done at the end of stagnation period for the entire period. When stable nitrification was observed, regular measurement of bulk water NH_3 concentration was done to estimate the NH_3 utilization pattern in those reactors.

Table 4-2: Influent water quality for different reactors.

Coupon type	Reactor	NH_3-N(ppm)	TOC(ppm)
PVC	1XC-1XN1 Control	0.71	4
	2XC-3XN[2]	2.13	8
	1XC-3XN[3]	2.13	4
Copper	1XC-1XN[1] Control	0.71	4
	2XC-3XN[2]	2.13	8
	1XC-3XN[3]	2.13	4

1: influent NH_3-N 2.13ppm and TOC=8ppm; 2: influent NH_3-N 2.13ppm and TOC=4ppm;1: influent NH_3-N 0.71ppm and TOC=4ppm

Analytical Methods (Chemical)

Sampling Technique: Effluent water was collected and tested three times (Monday, Wednesday and Friday) every week. A bulk water sample from the reactors was taken once every week for similar measurements. Effluent water concentrations were converted to bulk values using the model shown in Appendix-A. Response variables of the water samples shown in Table 4-3 were measured routinely.

Table 4-3 : Response parameter measured in this project

Response parameter	Comment
Copper (Dissolved and total)	Three times a week for effluent sample
Ammonia	
Nitrite	
Nitrate	
HPC	Weekly
MPN for AOB and NOB	

Copper: Both total and dissolved copper were measured. According to Standard Methods (19th ed, 1995) dissolved copper is operationally defined as the portion that passes through a 0.45μm pore size syringe filter. It should be noted that in the presence of colloidal species that can pass through the filter, the Standard Methods approach represents an upper bound to truly soluble copper. Measurements were done using a portable HACH 2000 spectrophotometer. Copper in the water sample reacts with a salt of bicinchoninic acid contained in the CuVer 1 copper reagent to form a purple colored complex in proportion to the copper concentration. The purple color/copper concentration was measured at 560 nm wavelength.

Ammonia Nitrogen: Free NH_3-N was measured using a HACH 2000 spectrophotometer using the salicylate method (HACH method 10023) at 655 nm. Ammonia reacts with salicylate to form 5- aminosalicylate, which is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. This blue color is masked by the yellow color from the excess reagent to give a final green-colored solution which is proportional to the amount of ammonia present in the sample. The ammonia was measured immediately when the sample was collected.

Nitrite Nitrogen: After collecting the sample, nitrite nitrogen (NO_2-N) was measured immediately with the HACH 2000 spectrophotometer. The diazotization method was used, where nitrite reacts with sulfanilic acid and forms an intermediate diazonium salt. This intermediate product couples with chromotropic acid to produce a pink colored complex, which is proportional to the amount of nitrite present. This pink color was measured at 507 nm.

Nitrate Nitrogen: NO_3-N measurements were done using a Dionex® ion chromatography system with a CD20 conductivity detector and GP40 gradient pump unit. An AS4A column and DS3 detection stabilizer was also used in this method. Water was filtered through a sterilized 0.2 µm pore size polyethersulfone filter and 5ml BD® syringe to remove any bacteria or suspended particles. The filtered sample was collected in a sterilized 15 ml Falcon® tube and stored in the refrigerator at 4^0C. Stored samples were measured within two weeks of collection. The water sample was loaded using a S40 automated sampler. Before measurement the Dionex® ion chromatography system was

first calibrated using five sodium nitrate standards (1, 0.5, 0.2, 0.1, 0 ppm of NO_3-N). To minimize experimental error, after every seven measurements a standard solution of nitrate was measured to check the accuracy of the measurement. If the obtained measurement of the standard was outside 90 to 110% of the standard value then the calibration was repeated and sample was measured again. This was done according to *Standard Method* (19ed, 1995) 3020.

pH: An Accumet AP-63P pH probe was used to measure the pH of the bulk samples.

Alkalinity : Alkalinity was measured according to Standard Methods 2320 (19ed, 1995). Collected water samples were titrated with 0.1 N H_2SO_4 to a pH of 4.9 for measuring the total alkalinity. An Accumet AP-63P pH probe was used to measure the end point pH.

Total Organic Carbon (TOC): TOC measurement for the project was done using a Dohrman DC-80® carbon analyzer, with potassium hydrogen phthalate as standard and potassium persulfate as the oxidizing agent.

Weight Loss Measurement for Copper Reactors: Initially the coupons were rinsed three times with 0.1 N NaOH and then rinsed thoroughly five times with RO water. When the coupons were dry, they were weighed. At the end of the experiment coupons were removed from the reactors and the oxide layer over the coupon was carefully removed using a rotary tool (DREMEL® MultiPro™ Model 395, Type 5) and thoroughly

washed with RO water. Then the coupons were weighed for the final weight. From these two weights the weight loss of the coupon was calculated.

Analytical Methods (Microbiological Analysis)

Heterotrophic Plate Counts (HPC): Heterotrophic plate counts of the water samples and the biofilms were done according to Standard Methods (19th ed. 1995) 9215A using R2A agar plates. Plates were incubated at 20^0 C for 7 days, and then the number of colonies in the plates was counted using a Quebec colony counter.

Biofilm Sampling: Biofilm was collected at different time points in each experiment. Autoclaved reverse osmosis (RO) water was filtered through a sterilized 0.2 µm polycarbonate filter to to remove any foreign DNA. Filtered DNA free water was placed in a DNA free glass tray (baked at 390^0C for 5 hours). One coupon was removed from the reactor and placed in the glass tray containing the water. The coupon was then scraped using a autoclaved rubber policeman inside a laminar flow hood. After scraping, the biomass with water was poured in a sterilized 50 ml Falcon® tube, which was then homogenized with a homogenizer (Biohomogenizer® Model M133/12810, ESGE®) for 30 sec. From the homogenized biomass, samples were taken for MPN and HPC analysis. The remaining biomass was used for DNA extraction and community analysis.

DNA Extraction: Homogenized biomass was collected on a 0.2 µm polycarbonate filter using a three channel manifold (Pall® Life Science) with filter funnels. DNA

extraction of the collected biofilm sample was done using a Fast DNA® SPIN Kit for soil (Q-BIOgene catalog #6560-200). Collected DNA was stored in a -30^0 C freezer.

PCR (Polymerase Chain Reaction): GoTag Green® master mix from Promega Inc. was used for amplifying the extracted DNA through PCR using an Eppendorf Mastercycler®. Due to the inhibitory effect of humics present and the low quantity of biomass, a two stage PCR was performed. In the first stage a 50µl reaction volume was used which contains 25µl master mix, 10 pM of the universal primers 1070F and 1392, 1µl DNA suspension and 20 µl of water. The amplification process involved initial denaturation at 94^{0}C followed by 15 cycles of 30 second denaturation at 94^{0}C, 45 second annealing at 52^0 C and 2 minutes of extension at 72^0 C with a final 5 minutes extension at 72^{0}C. In the second stage, 5 µl of the product from the first stage was used as template. In the second stage a similar reaction composition was used except 1392+GC was used instead of the 1392 primer and 20 amplification cycles were used. PCR products were evaluated by an agarose gel electrophoresis. Negative controls without template addition were treated identically through the PCR and evaluated by agarose gels to confirm the absence of contaminant DNA.

DGGE: DGGE was performed at 60^0 C with a D-Code Universal Mutation Detection System (Bio-Rad Laboratories). Eight and twelve percent (w/v) acrylamide gels with denaturant gradients of 40 to 70% were used for analyzing fragments amplified using 1070 and 1392+GC. A 25 ml volume denaturing gel was poured and allowed to polymerize prior to pouring of a zero percent denaturant stacking gel for the loading

wells. Sixteen hours of electrophoresis were performed for the gels at 60 V. After electrophoresis the gels were subsequently stained with SYBR Green I (Cambrex Bio Science). Images of the gel were obtained using a FluorChem 8800® Imaging system and AlphaEase FC® software (Alpha Innotech). Composition of all the reagents and conditions used for DGGE are presented in Appendix-B.

MPN: AOB and NOB populations were enumerated using the most probable number (MPN) technique (Lipponen et al. 2002) using Costar® Clear-Bottom 96 well microtiter plates. The mineral medium used for AOB contained per liter: $(NH_4)_2SO_4$, 330 mg; KH_2PO_4, 100 mg; $MgSO_4$ $7H_2O$ 40 mg; $CaCl_2$, 15 mg and 1 ml of a trace-element solution. The trace element solution contained per liter: Na_2EDTA, 4292 mg; $FeCl_2$ 4H2O, 1988 mg; $MnCl_2$ H_2O, 99 mg; $NiCl_2$ $6H_2O$,24 mg; $CoCl_2$ $6H_2O$, 24 mg; $CuCl_2$ $2H_2O$, 17 mg; $ZnCl_2$, 68 mg; Na_2MoO_4 $2H_2O$, 24 mg and H_3BO_3, 62 mg. Bromothymol blue (5ml/ l of 0.04% solution in water) was added as a pH indicator. The pH was adjusted to 8 using 1M NaOH before autoclaving at 110^0 C for 15 min. The NOB medium has the same composition except that it did not contain $(NH_4)SO_4$ and bromothymol blue, and was supplemented with 34.5 mg/ l of $NaNO_2$. The pH was adjusted to 6.5 with 1M NaOH before autoclaving at 110^0 C for 15 min. 175µl of media was poured into each of the 96 wells of the plate. An equal amount of sample was then inoculated into the first column of wells in the plate using a multi channel pipette. The content of the first column of wells was carefully mixed, and 175 µl of inoculated media was transferred to the next column of wells, and this process continued. After inoculation the microtiter plates were sealed with polyester tape to prevent evaporation and incubated

for 9 weeks at 20^0C in the dark. After the incubation period AOB presence was determined by detecting the presence of nitrite or nitrate in the medium by adding 40 µl of 0.2% diphenylamine in H_2SO_4 in the well. This reagent reacts with nitrite or nitrate and forms a blue color. The absorbance of the blue color was measured at 630 nm with a microplate reader (EL808 ultra microplate reader BioTek instruments®). The blue color indicated nitrite or nitrate had formed and the well was scored as positive.

Griess Ilosvay reagent was used to detect NOB activity in the samples. This reagent was made by mixing three separate solutions. In the first solution exactly 0.6 gm of sulfanilic acid was dissolved in 70 ml of hot distilled water. After cooling to room temperature, 20 ml of concentrated HCl and 10 ml of distilled water was added. The second solution was made by dissolving 0.6 gm of α-napthylamine in 20 ml of distilled water containing 1 ml of concentrated HCl and then diluting it to 100 ml with H_2O. The third solution is 16.4% (w/V) of $CH_3COONa.\ 3H_2O$ in water. All the solutions were made separately in dark bottles and kept in the refrigerator. Equal volumes of these three solutions were mixed and 40 µl of the mixture was added to each well after the incubation period. In the presence of nitrite, the Griess Ilosvay reagent produced a red color within five minutes. The absorbance of the well was measured after five minutes at 540 nm with the microplate reader. If nitrite in the well was detected it was scored as negative. The MPNs were calculated according to Rowe et al. (1977) and finally expressed as cells/ml of sample.

Statistical Analysis: Paired t-test analysis was done with Microsoft Excel on the data to see if there are significant differences between two treatments. The level of significance for all tests was $\alpha=0.05$.

Results

Two sets (each consisting of three reactors) of modified CDC reactors equipped with two different types of coupons (PVC and copper) were used in this investigation. All of these reactors had been running for more than two years and steadily converted 100% of the added ammonia to nitrite and nitrate (See Chapters 2 and 3). Initially all nitrifying reactors were operated at 0.71 ppm NH_3-N and 4 ppm TOC (humics) in the influent. Influent ammonia and carbon content was raised to investigate the effect of nutrient conditions on the nitrifying population and the changes associated in alkalinity and pH. Percent NH_3-N utilization in different PVC reactors is shown in Figure 4-3 and Figure 4-4. Time 0 days on the x-axis indicates the time when influent ammonia and carbon content was increased. Ammonia utilization in both control reactors was almost always near 100%. The PVC reactors adapted to (achieving 95% utilization) the increase in NH_3-N level in influent by two weeks. Copper reactors needed almost two months to attain 95% utilization. During that time NH_3-N utilization (%) in the copper reactors fluctuated and even dropped to around 60%, while utilization in PVC reactors was never below 85%.

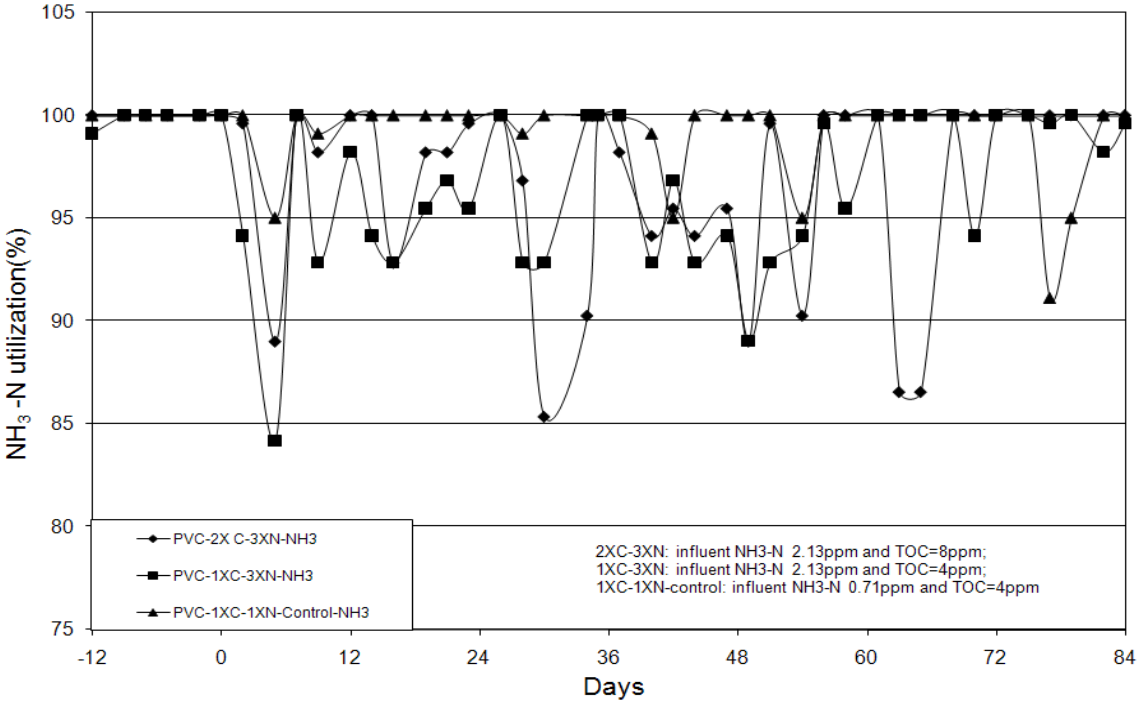


Figure 4-3: NH_3-N utilization in bulk water for PVC reactors

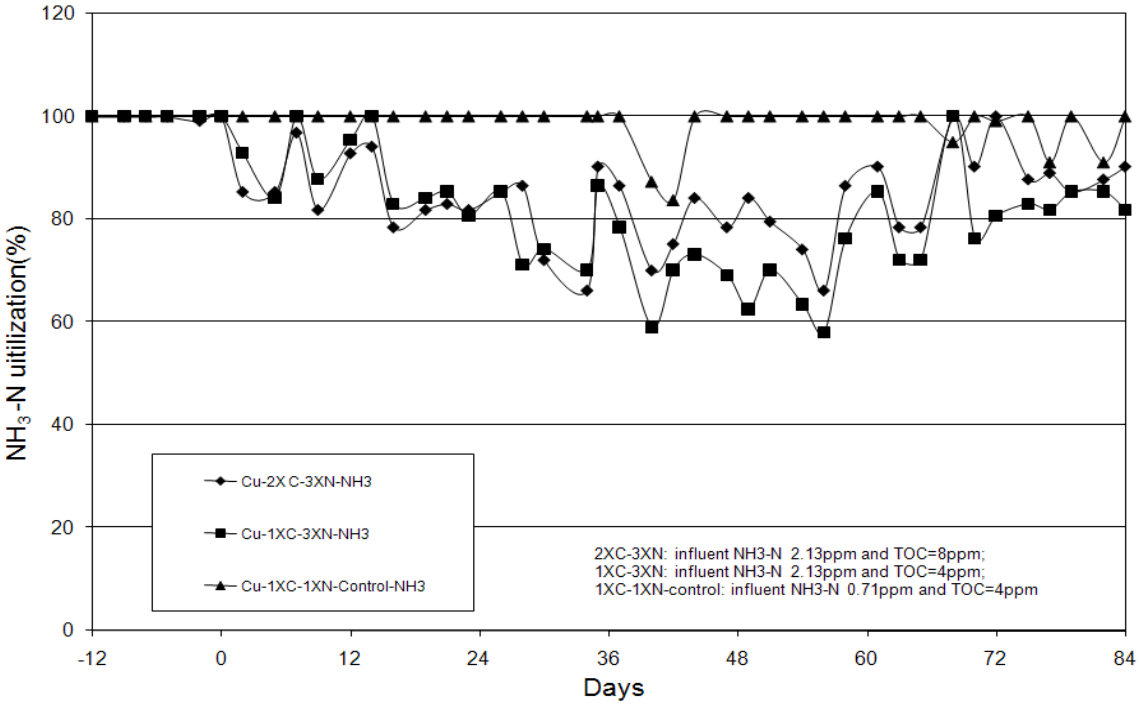


Figure 4-4: NH_3-N utilization (%) in bulk water for copper reactors

Percentage of NO_2-N conversion in the different PVC and copper reactors are shown in Figure 4-5 and Figure 4-6. NO_2-N conversion in all higher (2.13ppm) NH_3-N feed reactors are higher than the control except the copper reactor with 8 ppm TOC fed influent. However the magnitude of this difference (around 1~3%) is very small.

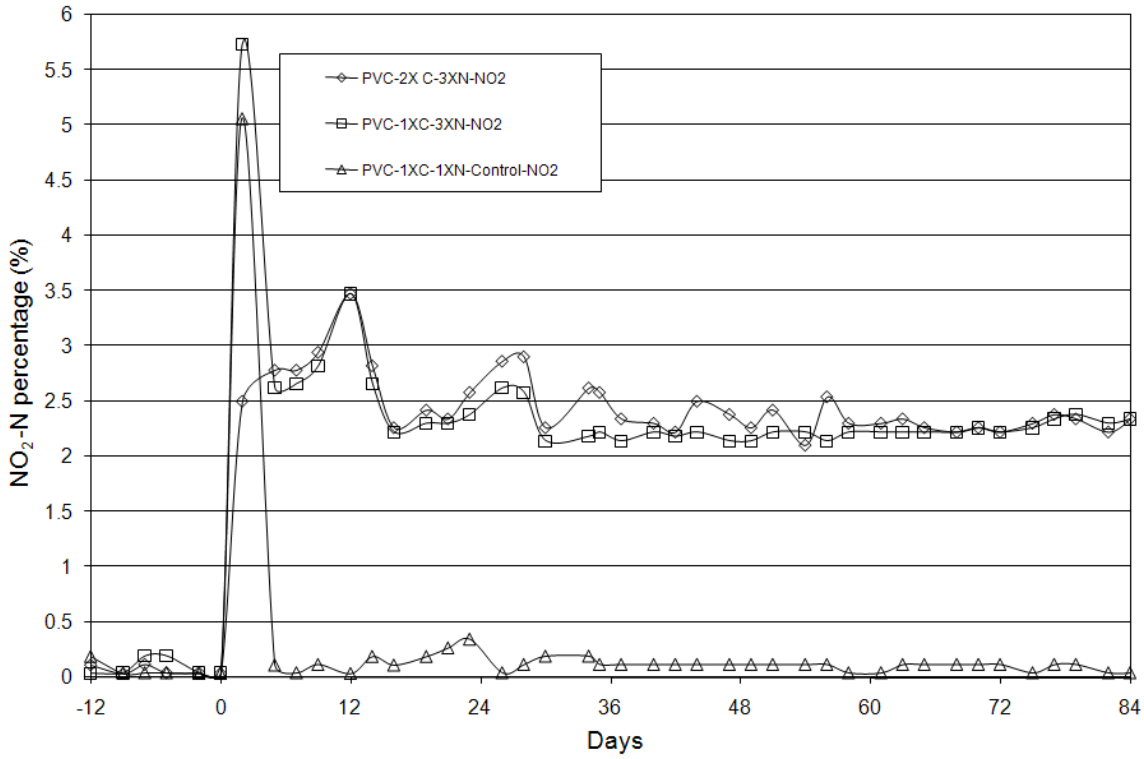


Figure 4-5: Bulk water NO_2-N as percentage (%) of initial NH_3-N in bulk water for PVC reactors

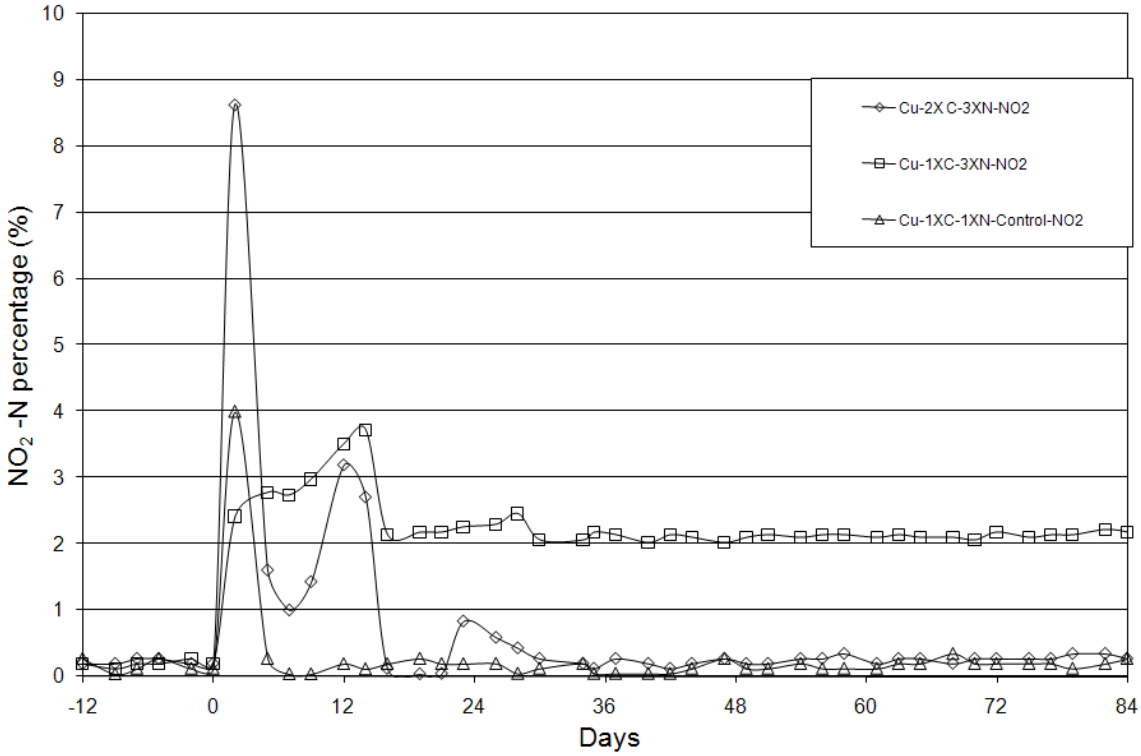


Figure 4-6: Bulk water NO_2-N as percentage (%) of initial NH_3-N for copper reactors

NO_3-N percentages in different reactors are shown in Figure 4-7 and Figure 4-8. NO_3-N in the control reactors (both PVC and copper) are always around 100%. At higher NH_3-N (2.13 ppm), the conversion percentage varied over time. In the case of the PVC reactors the variation in conversion is smaller (around 85~100%) than that for copper reactors (around 60~90%). Comparing all these figures, it becomes obvious that most of the oxidized NH_3 is converted to NO_3-N and only a very small fraction of it remained as NO_2-N. This illustrates that complete nitrification is occurring even with high ammonia concentrations.

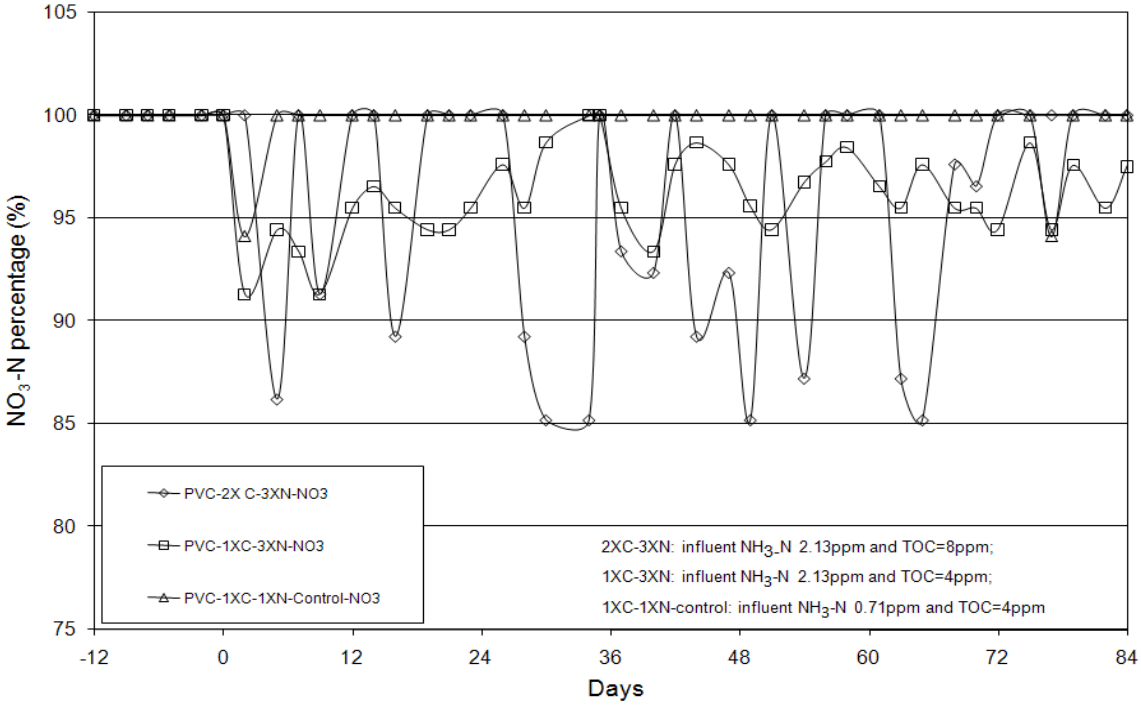


Figure 4-7: Bulk water NO_3-N as percentage (%) of initial NH_3-N for PVC reactors

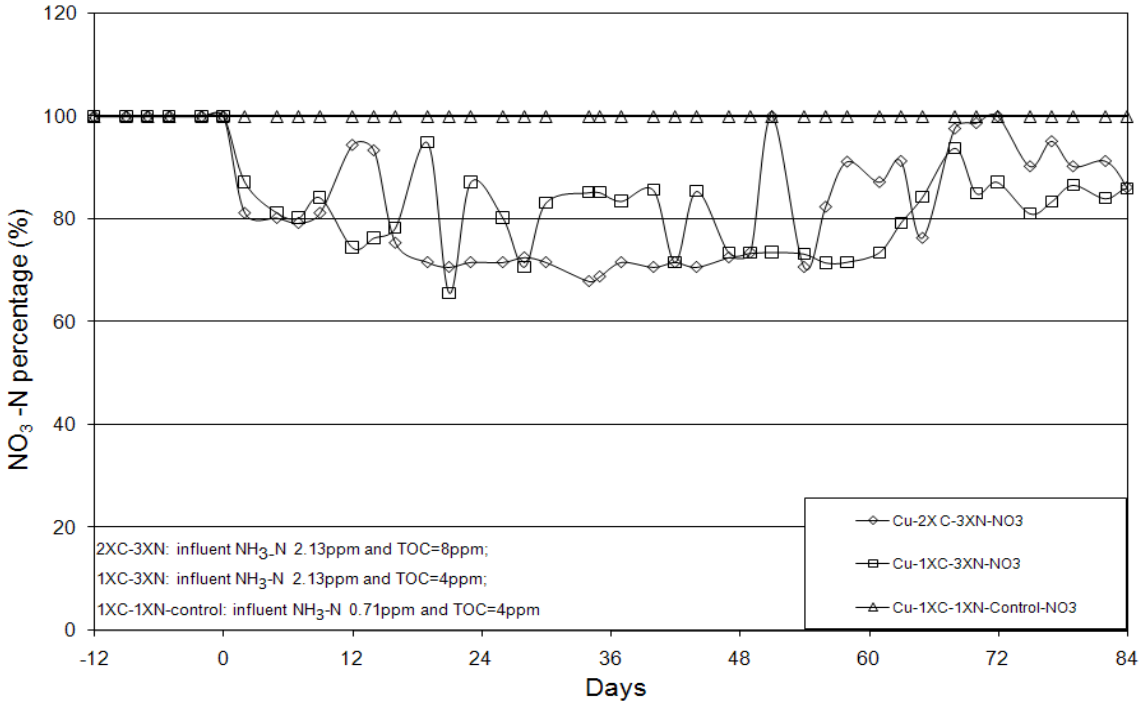


Figure 4-8: Bulk water NO_3-N as percentage (%) of initial NH_3-N for copper reactors

Changes in NH_3-N concentration in the bulk water through the eight hour stagnation period in different reactors is shown in Figure 4-9. Compared with the PVC reactors, copper reactors were slower in using the NH_3-N and some left over ammonia was detected at the end of the stagnation period.

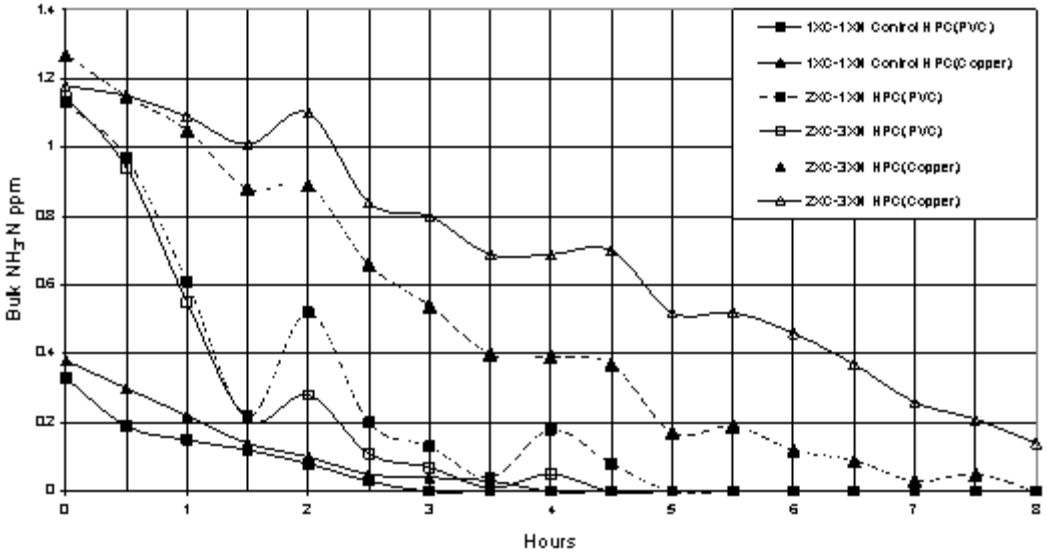


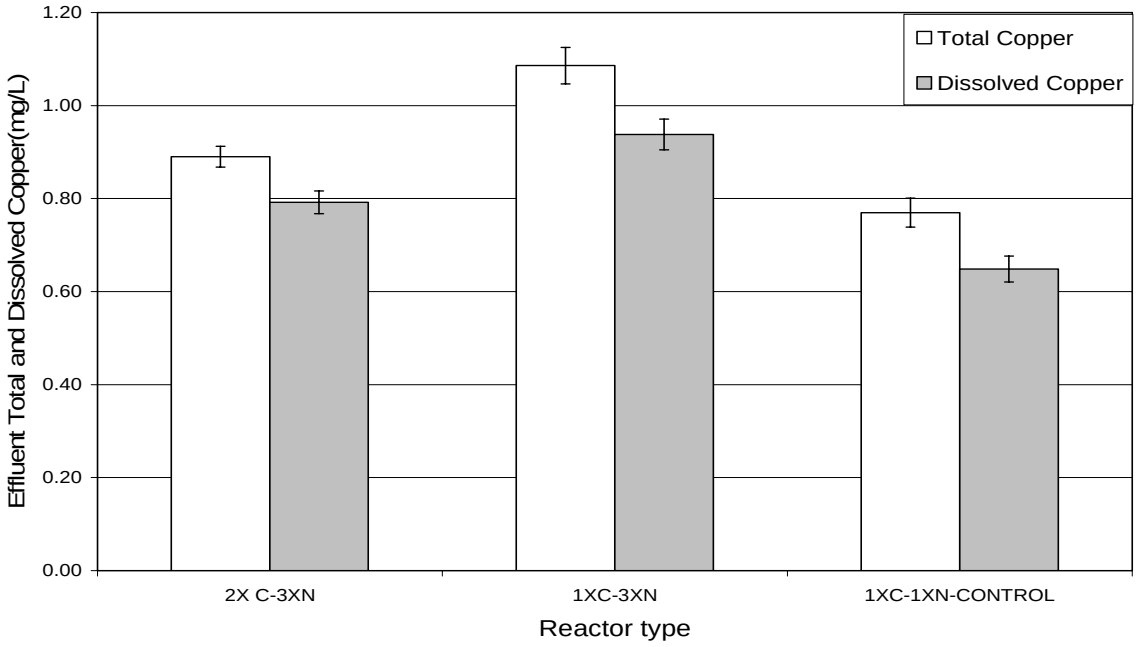
Figure 4-9: Bulk water NH_3-N concentration in different reactors during the eight hours stagnation.

Before the start of this experiment all copper reactors had been exposed to influent containing 4 ppm TOC and 0.71ppm NH_3-N for about two years and had an established nitrifying biofilm. 95% confidence intervals for the effluent average total and dissolved copper concentrations for the copper reactors during this experimental period (days 0 to 84) are shown in Figure 4-10. A paired t-test was done for the total and dissolved copper values and results of these t-tests are shown in Table 4-4. According to the t-test, changes in ammonia and carbon content (i.e. NOM) result in a significant increase in copper concentration. However, the weight loss of the copper coupons after five months of operations was not significant (0.12~0.13%).

Table 4-4 : p-value for the paired t-test on total and dissolved copper values between different reactors.

Total copper	2X C-3XN[1]	1XC-3XN[2]	1XC-1XN[3]-CONTROL
2X C-3XN[1]		$1.94X10^{-11}$	$2.36\ X10^{-5}$
1XC-3XN[2]	$1.94\ X10^{-11}$		$7.41\ X10^{-14}$
1XC-1XN[3]-CONTROL	$2.36\ X10^{-5}$	$7.41\ X10^{-14}$	
Dissolved copper	2X C-3XN[1]	1XC-3XN[2]	1XC-1XN[3]-CONTROL
2X C-3XN[1]		$3.93\ X10^{-11}$	$7.98\ X10^{-8}$
1XC-3XN[2]	$3.93\ X10^{-11}$		$2.70\ X10^{-15}$
1XC-1XN[3]-CONTROL	$7.98\ X10^{-8}$	$2.70\ X10^{-15}$	

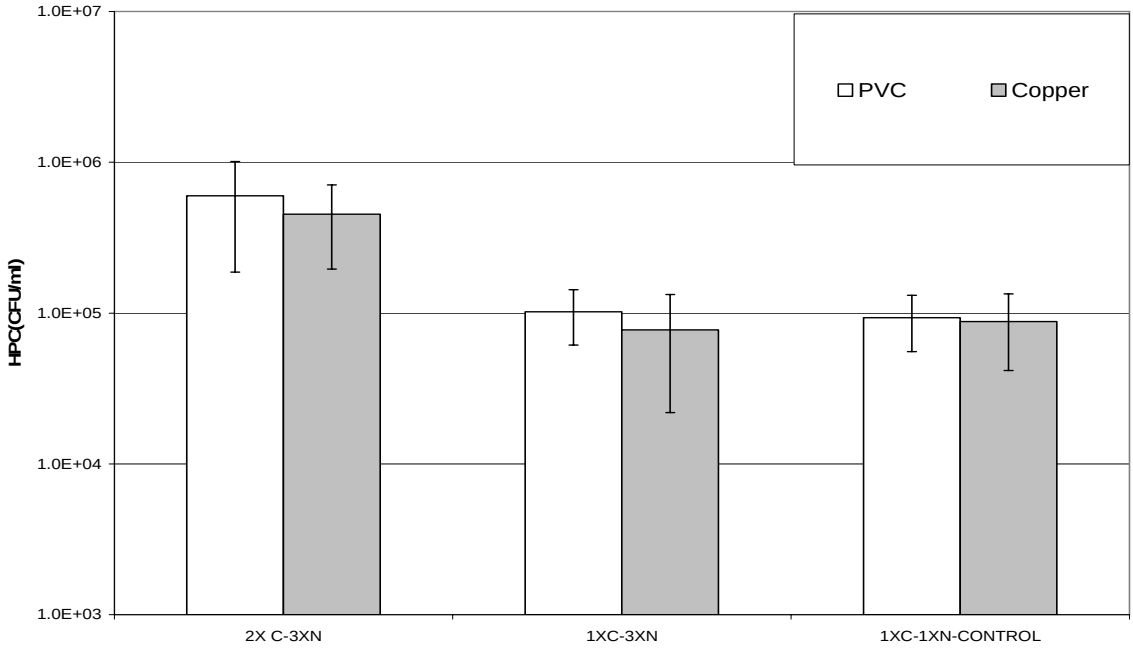
1: influent NH_3-N 2.13ppm and TOC=8ppm; 2: influent NH_3-N 2.13ppm and TOC=4ppm;1: influent NH_3-N 0.71ppm and TOC=4ppm



2XC-3XN: influent NH_3-N 2.13ppm and TOC=8ppm; 1XC-3XN: influent NH_3-N 2.13ppm and TOC=4ppm; 1XC-1XN-control: influent NH_3-N 0.71ppm and TOC=4ppm

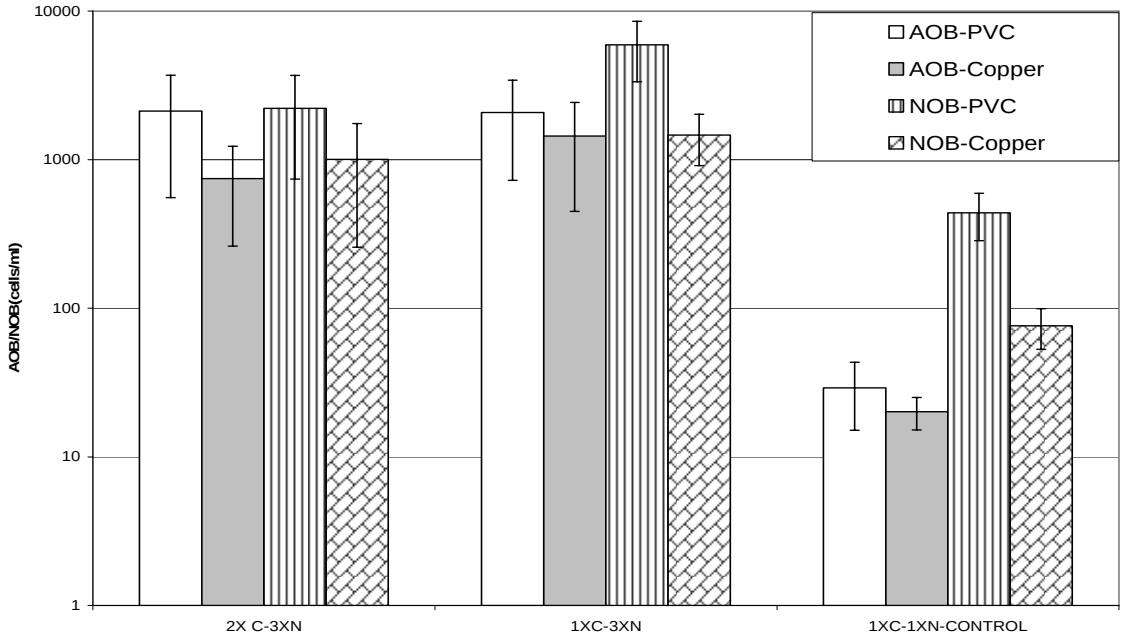
Figure 4-10: Average effluent total and dissolved copper concentrations in different copper reactors (error bar showing 95% confidence interval).

Heterotrophic bacterial populations were counted using R2A and autotrophic nitrifiers (AOB/NOB) were counted using an MPN. Average population counts for these two groups are shown in Figure 4-11 and Figure 4-12. An increase of influent NH_3 had no effect on HPC counts but doubling the TOC to 8 ppm increased the HPC by about 1 log. The increase in NH_3-N level increased the autotrophic population; AOBs are increased by about 2 logs and NOB by 1 log. Change in TOC did not have any apparent effect on autotrophic nitrifiers.



2XC-3XN: influent NH_3-N 2.13ppm and TOC=8ppm; 1XC-3XN: influent NH_3-N 2.13ppm and TOC=4ppm; 1XC-1XN-control: influent NH_3-N 0.71ppm and TOC=4ppm

Figure 4-11: Average (n=12) HPC value for different reactors



2XC-3XN: influent NH_3-N 2.13ppm and TOC=8ppm; 1XC-3XN: influent NH_3-N 2.13ppm and TOC=4ppm; 1XC-1XN-control: influent NH_3-N 0.71ppm and TOC=4ppm

Figure 4-12: Average MPN for AOB and NOB from different reactors

AOB/NOB and HPC densities in the biofilm were determined at the end of each experiment. These cell densities are shown in Figure 4-13. Autotrophic cell density increased (around 1 log) in higher ammonia (2.13 ppm) reactors. HPC cell density in the biofilm was not affected by the change in the TOC level.

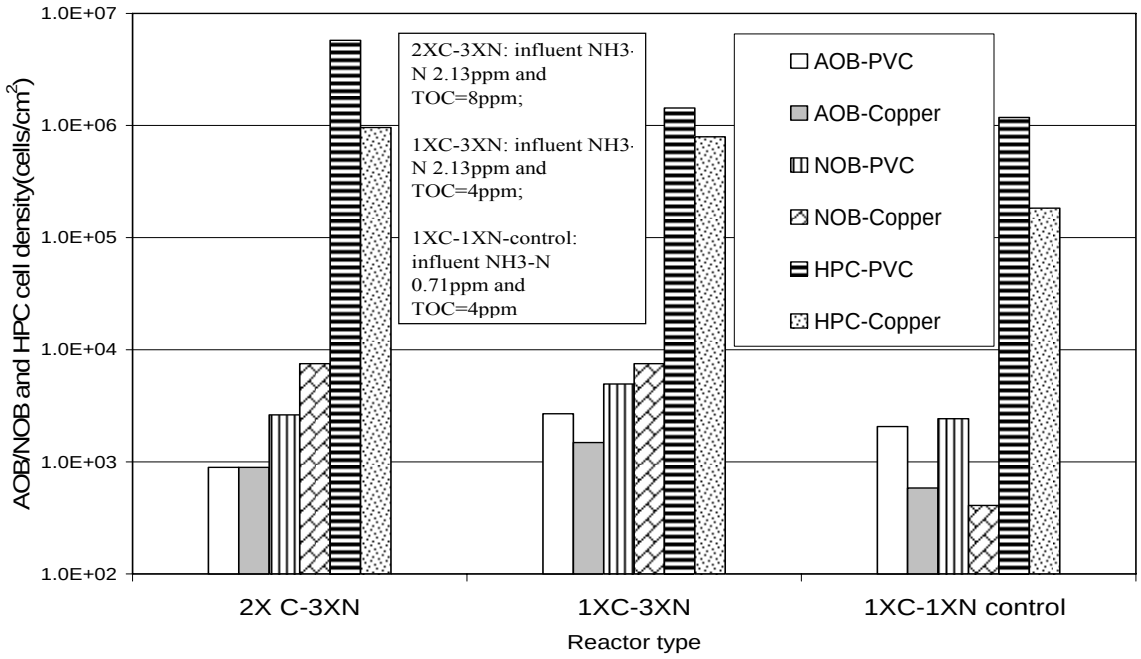
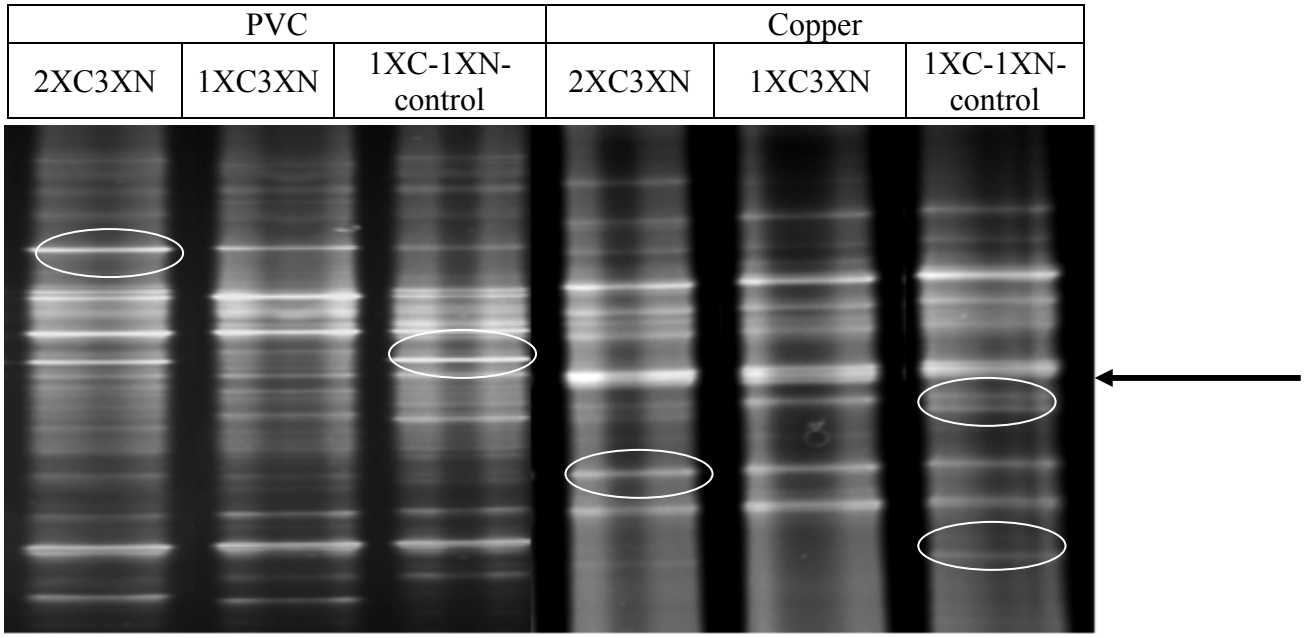


Figure 4-13: Biofilm cell density in different reactors.

No significant correlation was found between heterotrophic and autotrophic populations (R^2 in the range of 0.03 to 0.30). Also the correlation between copper concentration and these populations (AOB/NOB and HPC) was very low (R^2= 0.02 ~ 0.5).

To assess the community composition molecular techniques (PCR and DGGE) were used with the collected DNA from the sampled biofilm. Figure 4-14 shows the DGGE gel for the biofilm from different reactors. By comparing the bands from different nutrient conditions it is obvious that there was overall limited change in the community. Assuming that one band represents one species, a few species (white circles) were significantly influenced by the change in operating conditions. Comparison between the communities from PVC and copper reactors of equivalent operating conditions had

significant differences. In particular the bright band (indicated by arrow) present in all copper reactors is probably absent in the PVC systems.



2XC-3XN: influent NH3-N 2.13ppm and TOC=8ppm; 1XC-3XN: influent NH3-N 2.13ppm and TOC=4ppm; 1XC-1XN-control: influent NH3-N 0.71ppm and TOC=4ppm

Figure 4-14: DGGE profile of biofilm sampled from different reactors

Bulk water average pH after eight hours of stagnation is shown in Figure 4-15. As expected reactors with higher ammonia have lower pH in their bulk water than the control reactors. The amount of TOC did not appear to affect the final pH of the bulk water.

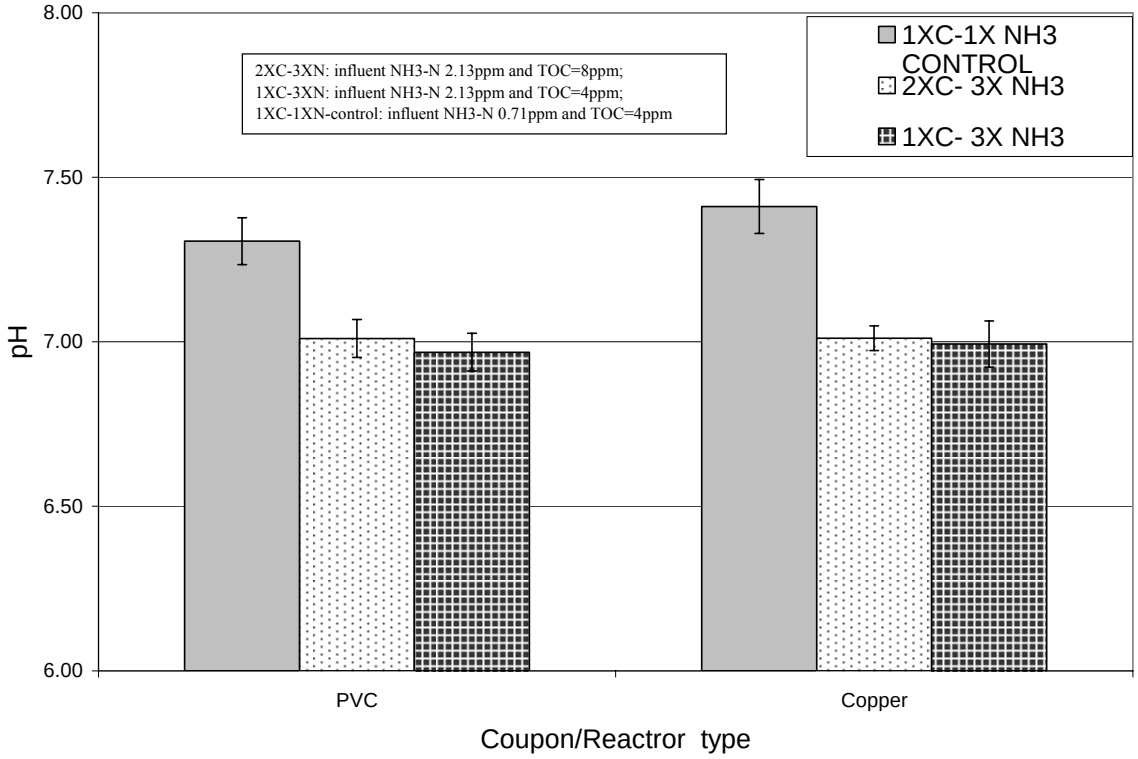


Figure 4-15: Average (n=12) pH of bulk water after eight hours of stagnation

Average alkalinity destroyed per mg/L of NH_3-N oxidized in different reactors is plotted in Figure 4-16. The PVC reactor with 8 ppm TOC and 2.13 ppm NH_3-N has alkalinity destruction rate very similar to the theoretical value. Other reactors have alkalinity destruction rates either higher or lower than the theoretical value. Overall for every condition tested, the copper reactors have a lower destruction rate than the PVC reactors.

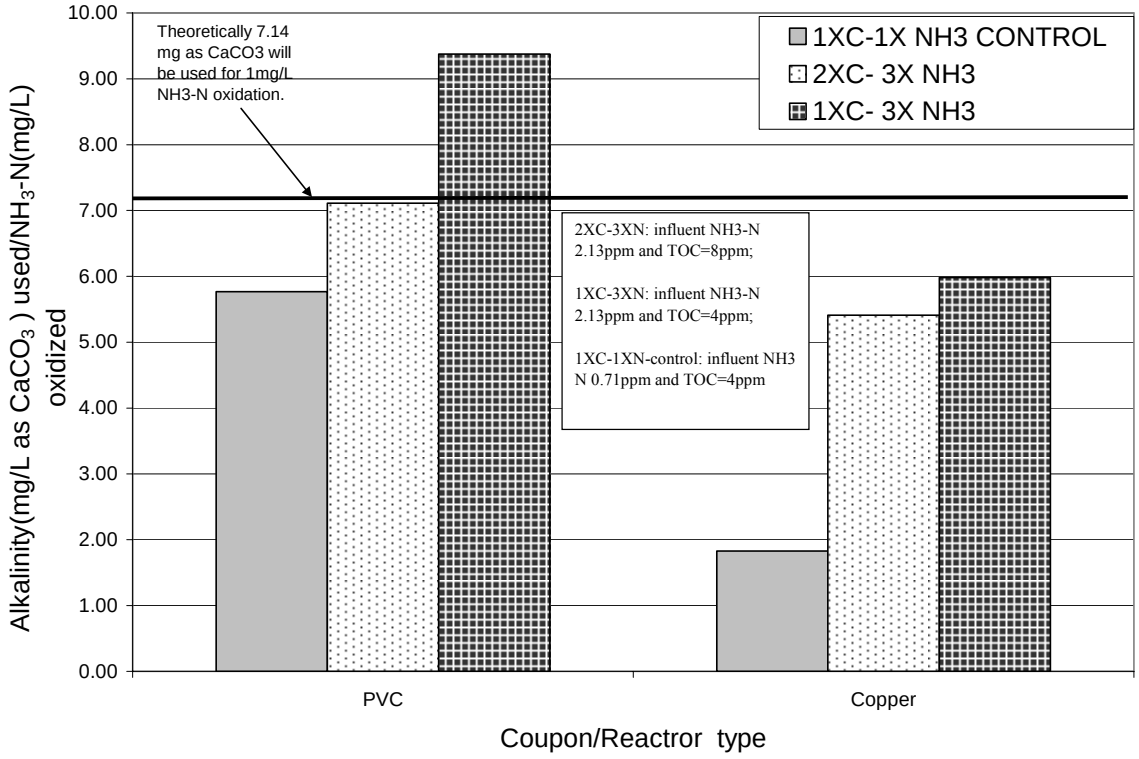


Figure 4-16: Average (n=12) alkalinity used up per mg of ammonia nitrogen oxidized in different reactors.

Discussion

The PVC reactors were more efficient in utilizing excess ammonia nitrogen than the copper reactors. The ammonia nitrogen utilization in PVC reactors was always around 85~100% whereas in case of copper reactors it varied from 60~90%. In both cases nitrite nitrogen (%) in bulk water increased (Figure 4-5and Figure 4-6) as the influent NH_3-N was raised to 2.13 ppm, but the magnitude of this increment is very small (around 1~2%, or around 0.01 ppm), and most of the ammonia nitrogen was converted to nitrate nitrogen with only a very small amount remaining as nitrite nitrogen. The difference between the % conversions may be because of the two types of surfaces Most of the

nitrifiers exist in biofilm rather than the planktonic state (Lipponen et al. 2002, Stewart et al. 1997, Wolfe et al. 1990) and pipe materials are known to influence biofilm formation (LeChevallier et al. 1990). Biofilms formed on copper are reported to be thinner than those on plastic surfaces (Schwartz et al. 1998). Also copper is known to be toxic to bacterial activities. All these may lead to a less competent nitrifying biofilm in copper systems than that in the PVC system. However, the nitrifying biofilm MPN cell density (Figure 4-13) was not significantly different between the copper and PVC reactors. A possible explanation is that the MPN is well known for underestimating the nitrifying community (Both et al. 1990, Rennie et al. 1977) due to their selective nature. According to Belser and Mays (1982) the MPN detects less than 5% of a community, and Feray et al. (1999) reported that it detected 3.1~18%.

Suspended heterotrophic bacterial populations were not influenced by the increase in NH_3 concentration but raising the TOC to 8 ppm increased the HPC by about 1 log in both the copper and PVC system. Higher NH_3 levels increased the autotrophic population in both systems (i.e. copper and PVC). AOBs increased by about 2 logs and NOBs by 1 log. The change in TOC did not appear to have any effect on autotrophic nitrifiers.

The DGGE profile of the biofilm indicates that community composition is influenced by both nutrient condition and pipe surface. This supports the findings from our previous study (Chapter 2). Also similar to the results from the previous study, a brighter band was found in all copper reactor biofilm's DGGE profile. This band may represent some copper tolerant species. Therefore, the pipe surface may have a greater impact on community composition than the nutrient supplied.

For all reactors the final bulk water pH is significantly lower (around 7~7.25) than the influent pH (8.15). However, the higher NH_3-N fed reactors have a pH drop greater than the control reactors, which can be explained because as more NH_3-N is oxidized there is greater the release of H^+. The alkalinity consumption with respect to NH_3-N utilized in the PVC reactor supplied with 8 ppm TOC and 2.13 ppm NH_3-N closely matches the theoretical conversion. The PVC reactor fed with 4 ppm TOC and 2.13 ppm NH_3-N has higher destruction than the theoretical value. The remaining reactors have alkalinity destruction lower than what would be predicted. Parker et al. (1975) found from different experimental and field observations that alkalinity destruction for wastewater is in the range of 6.2 to 7.4 mg/L as $CaCO_3$ for every mg of nitrogen oxidized. Benninger et al. (1978) showed lower destruction than the theoretical value. According to Sherrard et al. (1980) alkalinity destruction is a function of C/N ratio and Benninger et al. (1978) reported that destruction was higher at lower C/N ratio. In this project consistently higher destruction was observed with a lower C/N ratio, in support of previous findings by Benninger et al. (1978). Another interesting observation is that the alkalinity destruction in all copper reactors was lower than that for PVC reactors. One possible explanation is that as copper corroded it reacted with the H^+ produced which leads to less alkalinity destruction (Equation 4).

$$2Cu + 4H^+ + O_2 \longrightarrow 2Cu^{2+} + 2H_2O \qquad \text{Equation 4}$$

Effluent copper concentration for the 4 ppm TOC dosed reactors increased with an increase of influent NH_3-N. This increase may be due to the strong complexes formed between copper and NH_3 (Schock et al. 1995). However effluent copper concentration from reactors with 2.13 ppm influent NH_3-N in the presence of higher TOC (8 ppm) had reduced copper concentrations. Campbell (1950) reported that NOM can inhibit pitting corrosion and the presence of NOM can produce a thick protective cuprous oxide layer. It is possible that the oxide layer formed in the presence of 8 ppm TOC may be thicker than that formed in presence of 4 ppm TOC, which led to lower effluent copper concentrations.

In the range of NH_3-N tested in this project the autotrophic population was found to slightly increase (around 1~2 log) with a tripling of NH_3-N, but the TOC value did not have any effect on autotrophic populations. The suspended heterotrophic population increased slightly (around 1log) with an increase of TOC. The effect of TOC was not observed in biofilm HPC cell density. In all cases pH and alkalinity decreased due to nitrification but alkalinity changes in copper reactors are much lower than that in PVC reactors.

In this project NH_3-N and TOC concentrations of water were found to increase the copper concentration in the water. But in comparing two high ammonia feed reactors, the copper concentration was lower with higher TOC. Therefore at 2.13 ppm of NH_3-N the presence of higher TOC (8 ppm) may produce a protective film/layer which inhibits copper leaching in the water.

Conclusions

- For the condition tested nitrifying biofilm grown on PVC surfaces exhibited higher nitrification ability than biofilm grown on copper surfaces
- An increase in influent NH_3 levels in the reactors slightly increases the AOB/NOB population (1~2 logs), but does not impact the HPC
- Higher concentration of TOC increased the suspended HPC level but it did not affect the autotrophic nitrifiers
- The pipe material (copper/PVC) has a greater influence on biofilm community profiles than the nutrient conditions tested in this project
- Alkalinity and pH decrease in all cases due to nitrification
- Alkalinity consumption due to nitrification was less in copper reactors than in PVC reactors
- Alkalinity consumption was greater for lower C/N value
- In a nitrifying system copper leaching at 2.13 ppm of NH_3-N decreased at higher TOC

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CHAPTER 5

CONCLUSIONS

Summary and Synthesis

The work presented in this dissertation represents a step forward in understanding nitrification in premise plumbing. This is an emerging area of concern as more utilities move towards chloramines as a final disinfectant. Due to the inherent characteristics of premise plumbing that make it's environment conducive to nitrification, greater understanding of how factors interact to induce or control nitrification are needed. There are complex interactions between potential control strategies (chlorite, chloramine residual, choice of plumbing material) and other water quality parameters (organic carbon, alkalinity, pH, free ammonia concentration) that may lead to increased HPC and nitrifiers as well as copper corrosion. These conditions were investigated in the project and are reported herein. This project also addresses the resilience of nitrification by determining how rapidly control options affect the process as well as how rapidly nitrification recovers if an effective mechanism is terminated. Another contribution of this project is to provide a preliminary understanding of the diversity of biofilm organisms on PVC and copper surfaces in these conditions.

One contribution of this work is the development of a reactor system that could simulate premise plumbing without the need to create a pipe rack. The modified CDC reactors had the same surface to volume reactor of a 6 foot long, 1/2" diameter pipe in a

far more compact configuration and with surfaces that are more easily accessed. The use of 8 hr stagnation periods was representative of overnight and day long periods of no flow in households. Overall, the reactors were relatively easy to operate and generated reproducible results without huge consumption of feed.

In the first set of experiments, ammonia was fed to reactors at level that would be the same as that if 2 or 4 mg/L monochloramine had completely decayed to ammonia. Total organic carbon concentrations (2 and 4 mg/L) as humic substances were also used, and these levels are reasonable for surface water sources of drinking water. Alkalinity was set at 35 mg/L as $CaCO_3$ which is typical of some eastern surface waters, and represents a target level if alkalinity is added as a mechanism to control corrosion in distribution systems. A pH of 8.15 was chosen because it is representative of a pH used to stabilize monochloramine in full scale systems. Latter experiments set the ammonia concentration to the equivalent of 4 mg/l decayed monochloramine and 4 mg/L TOC. The final set of experiments doubled the TOC to 8 mg/L and the ammonia to an equivalent of 12 mg/L monochloramine to investigate extreme conditions.

Initial experiments showed that, in the absence of nitrification, higher TOC content increased copper leaching in reactors with copper coupons. As time continued, the copper reactors did eventually begin nitrifying, although it took approximately two months longer than in identical reactors with PVC coupons. The difference in time for nitrification to begin may be due to the surface properties of the copper or toxicity to the attached or suspended organisms. During the time when nitrification began, there was a very close relationship between nitrification and copper leaching/corrosion. When nitrification began, it is probable that the surface pH and alkalinity decreased, thereby

allowing copper release to occur. At this stage, the copper concentration in a nitrifying reactor was positively influenced by increased ammonia concentrations. It is important to note that the source of inoculum for these reactors was Bozeman tap water and no known nitrifiers were deliberately added. Therefore, even systems that do not have high ammonia levels or that are disinfected with chlorine may have the potential to develop nitrification in a relatively short period of time if conditions are conducive to the growth of nitrifiers.

In later experiments when nitrification had fully developed, copper ion added to a PVC reactor did not have any influence on nitrification, even at the maximum level allowed by the Lead and Copper Rule (1.3 mg/L). Lowering the pH to 6.6 to ensure that all of the copper was in the Cu^{+2} form did not change these results. Therefore, once nitrification had stabilized in these reactors, copper no longer had an effect. Based on these experiments, it is not reasonable to believe that copper plumbing is "resistant" to nitrification. When chlorite, a known inhibitor of some autotrophic nitrifiers was added to nitrifying copper and PVC reactors, there was no effect at concentrations below 20 ppm. This unrealistically high level (the regulatory limit is 1 mg/L) had an impact only on the copper reactor. Interestingly, the presence of the high dose of chlorite also increased the soluble copper concentration, suggesting that the inhibitory effect may be due to a combination of both chlorite and copper ion. In a negative light, even though chlorite had an influence on nitrification, it also increased copper corrosion. When chlorite was terminated, the copper reactor regained the ability to nitrify after six weeks, indicating that the loss of nitrification was not permanent.

The only truly effective method for controlling nitrification was the addition of chloramine at a 5:1 chorine to ammonia ratio. Lower ratios were only marginally effective or had no effect. When chloramines were no longer present, the copper reactor began nitrifying sooner than the PVC system. This is the one instance where the copper system was more "robust". For all of these experiments, the ammonia oxidizing nitrifiers as detected using MPN methods were not affected except at very high doses of chlorite or the 5:1 ratio of chlorine to ammonia. Interestingly, the nitrite oxidizing bacteria appeared to be more sensitive to environmental stress.

In the final set of experiments where the influent TOC and ammonia concentrations were raised in some of the reactors, it was found that the PVC systems adapted most quickly and efficiently converted nearly all of the ammonia to nitrate. The copper reactor adapted more slowly. Because nitrification was not as efficient in the copper reactor, there was less loss of alkalinity. The changes in TOC and ammonia resulted in increase in copper concentration in water. Overall, as expected, higher concentrations of ammonia resulted in higher numbers of autotrophic nitrifiers. Also, as expected, elevated levels of TOC increased the heterotrophic populations, but did not influence the autotrophic nitrifiers.

Some additional insights on the impact of the various treatments on the bacterial populations can be made. In contrast to observations made by others, there was no correlation between HPC and autotrophic nitrifier levels. As expected, higher chlorine to ammonia levels decreased suspended bacterial counts. Increased TOC tended to increase HPC in the bulk water and in the biofilms. Because these systems were not nitrogen limited for HPC, elevated ammonia levels did not impact HPC in the bulk or the biofilm.

Neither chlorite nor copper had an effect on the HPC levels. DGGE profiles were obtained from the different experiments and revealed that the community was impacted by both nutrient conditions and pipe material. Chlorite and monochloramine also changed the composition of the communities. Copper added to a nitrifying PVC system did not change the community.

From experiments with low influent NH_3 not much change in pH and alkalinity was detected even though these reactors are nitrifying significantly. From the final set of experiments noticeable alkalinity and pH drop was observed in higher NH_3 feed reactors, but this effect was significantly lower in the copper reactors than in PVC.

Recommendations for Further Work

The work in this dissertation has revealed some new aspects of nitrification, particularly in reference to premise plumbing. As with any research, the results also suggest avenues for further investigations. The toxicity of copper on drinking water nitrifying systems should be investigated further to determine the specific ion responsible for any inactivation. It is possible that Cu^{+} may be more important than Cu^{+2}. It is also important to determine the mode of action of chlorite against nitrifying organisms in more depth. Previous research focused only on pure cultures of autotrophic nitrifiers. In this dissertation, there was limited impact by chlorite, suggesting that other organisms, including heterotrophs or fungi, may be responsible for nitrification. There was also an indication that changes in organic carbon concentrations, ammonia levels, chlorine to ammonia ratios and surfaces (PVC vs. copper) had an impact on the microbial ecology of the reactors. This warrants more investigation, since the changes in ecology give insights

on the performance of the systems in terms of nitrification and potential corrosion mechanisms.

Premise plumbing fixtures consist of lead, brass, and other materials. The effect of these metals on nitrification is therefore of interest. Conversely, the impact of nitrification on the leaching and release of these metals is of critical concern. The influence of corrosion control techniques and changes in disinfection practices may also have an impact on nitrification and its interaction with these metals.

There is a need in the drinking water community for a rapid and easy to use tool for nitrification detection/nitrifier enumeration. Development and application of quantitative PCR directed against a suite of organisms responsible for nitrification could provide the desired solution to this need.

APPENDICES

APPENDIX A

REACTOR MIXING MODEL

Reactor Mixing Model

V_t= Total volume of reactor (120ml)

ΔV= Volume of bulk water spilled in time t=0 to t=1 min when the only the stirplate is ON (12ml)

Q= Influent flow rate (24 ml/min)

C_{in}= Influent water NH_3 concentration (0.71)

C_0= Bulk water NH_3 concentration at the end of eight hour stagnation.

In the beginning at time t=0 both stirplate and pump are OFF.

<u>During time t=0 to time t=1 minutes</u>

Only stirplate is ON. So ΔV ml of water spill out of the reactor. The NH_3 concentration of this water is equal to C_0.

Volume of water inside the reactor after t=1 minutes is $V=V_t- \Delta V=(120-12)=108$ml

<u>During time t=1min to 5min:</u>

Both stirplate and pump are ON. Fresh influent is a mixed with the stagnant bulk water. Excess water also spilled out during this period. Bulk water NH_3 concentration during this time is a function of fresh influent NH_3 concentration and bulk water NH_3 concentration at the beginning of this step (i.e. time t=1min). Applying a mass balance during this time inside the reactor:

$$V\frac{dC}{dt} = QC_i - QC$$

$$\int_{C0}^{C(t)} \frac{dC}{C_i - C} = \int_{1}^{t} \frac{Q}{V} dt$$

$$-\ln\{\frac{C_i - C(t)}{C_i - C_0}\} = (t-1)\frac{Q}{V} = \frac{t-1}{\theta} \qquad \text{where } \theta = \frac{V_t - \nabla V}{Q} = \frac{V}{Q}$$

$$C(t) = C_i - (C_i - C_0)e^{\frac{-(t-1)}{\theta}} \quad \text{----------Equation 1}$$

Now integrating equation 1 for time t= 1 to t=5 min and simplifying we get

$$\int_1^5 C(t)dt = 4C_i + \theta(C_i - C_0)(e^{-4/\theta} - 1)$$ ----------Equation 2

During the time 5~6 minutes the stirplate is OFF and only the pumps are ON. The pump gradually fill up the volume gap created due to the vortex from the stirplate and after that V_x ml of water comes out of the reactors. Let us take C_6 as the average concentration of this volume V_x.

So the effluent NH_3 concentration can be expressed as following equation

$$C_e = \frac{C_0 + \nabla V + q\int_1^5 C(t)dt + C_6 Vx}{4Q + \nabla V + Vx}$$

But $\Delta V+V_x=Q$; so the above equation becomes

$$C_e = \frac{C0 + \nabla V + q\int_1^5 C(t)dt + C6 * Vx}{5Q}$$ ----------Equation 3

The volume of water inside the reactors between time t=5 minutes to t=6 minutes is shown in Figure A-1 below. Now the bulk water NH_3 concentration at the beginning of 5 minutes (5^+) can be approximately expressed by the following equation.

$$C_{5b} = \frac{C(5)(V_t - \nabla V) + C_i \nabla V}{V_t}$$ (Approximately)

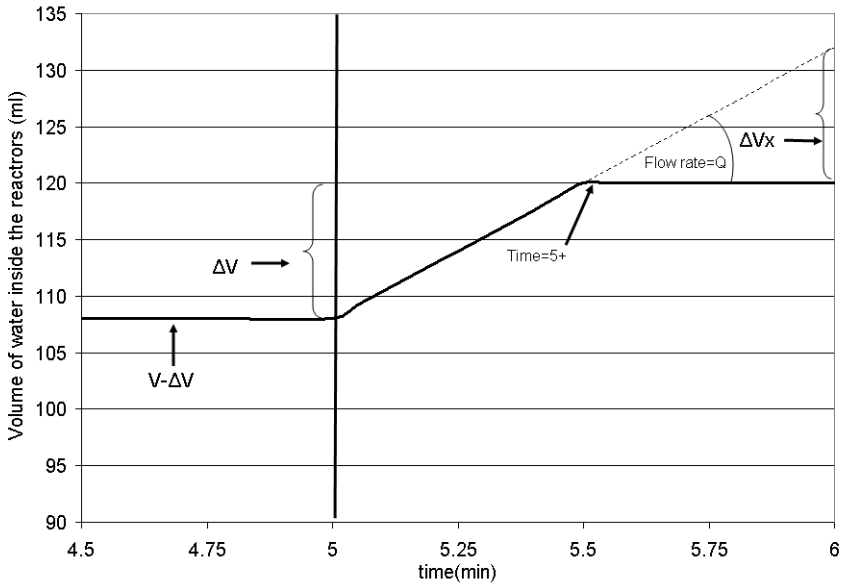


Figure A-1: Volume of water inside the reactors between time 4.5 minute to 6 minutes

Now putting t=5 in equation 1 we get

$$C(5) = C_i - (C_i - C_0)e^{-4/\theta} = C_i(1 - e^{-4/\theta}) + C_0 e^{-4/\theta}$$

Now putting value of C(5) in the above equation we get

$$C_{5b} = \frac{\{C_i(1 - e^{-4/\theta}) + C_0 e^{-4/\theta}\}(V_t - \nabla V) + C_i \nabla V}{V_t}$$

By simplifying the above equation and expressing $A=Q\theta(1-e^{-4/\theta})$ and $B= Q\theta e^{-4/\theta}$ we get

$$C_{5b} = \frac{Ci(\nabla V + A)}{V_t}$$ ----------Equation 4

Now applying mass balance for the time 5^+ to 6 minutes we get

$$V_t \frac{dC}{dt} = QC_i - QC$$

$$\frac{dC}{C_i - C} = \frac{Q}{V_t} dt$$

$$\int_{C_{5b}}^{C(t)} \frac{dC}{C_i - C} = \frac{Q}{V_t}(t - 5^+)$$

$$C(t) = C_i - (C_i - C_{5b})e^{\frac{-Q(t-5^+)}{V_t}}$$

For t=6; $Q(t\text{-}5^+)=V_x$ so the above equation becomes

$$C(6) = C_i - (C_i - C_{5b})e^{\frac{V_x}{V_t}}$$

The average NH_3 concentration during the time t=5~6 minutes

$$C_6 = \frac{1}{2}\{C_{5b} + C(6)\}$$

Putting the value of C_{5b} and C(6) and simplifying with taking $X = 1 + e^{\frac{-V_x}{V_t}}$ and

$Y = 1 - e^{\frac{-V_x}{V_t}}$ we get

$$C_6 = \frac{C_i\{(\nabla V + A)X + 2V_tY\} + C_0BX}{2V_t} \text{---------Equation 5}$$

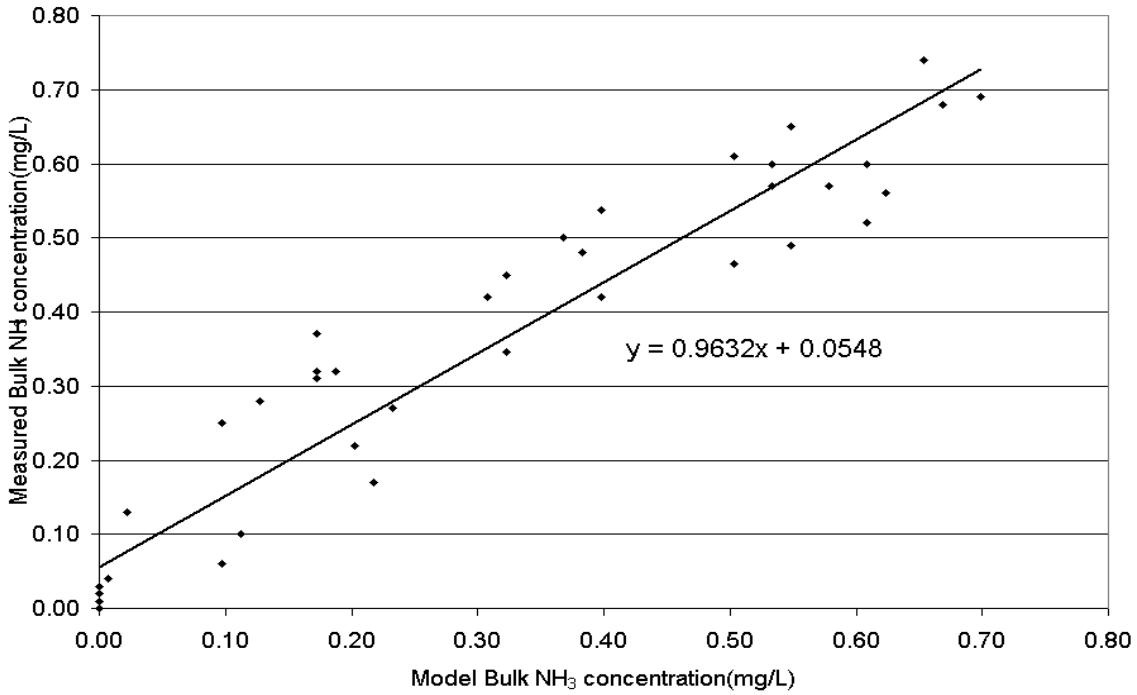
Now putting the value of $\int_{t=1}^{t=5} C(t)dt$ and C_6 from equation 2 and equation 5 in equation 3 and simplifying we get

$$C_0 = \frac{1}{\{(\nabla V + A) + \frac{BXV_x}{2V_t}\}}[5QC_e - C_i\{(4Q - A) + V_xY - \frac{V_x(\nabla V + A)X}{2V_t}] \text{----Equation 6}$$

Now from the measured effluent NH_3 concentration (C_e) bulk NH_3 concentration at the end of stagnation period (C_0) can be calculated using equation 6. From the value of C_0, NH_3 concentration at the beginning of stagnation period (C_6) can be calculated using equation 5.

So the % utilization $= \frac{C_6 - C_0}{C_6} \times 100\%$

The measured bulk NH_3 concentration for different effluent NH_3 concentration is plotted against model bulk NH_3 concentration in FigureA-2.



FigureA-2: Measured and model value for bulk NH_3 concentration.

As shown in Figure A-2 the slope of the trend line between measured and model bulk NH_3 values is almost 1 and the y-axis intercept is almost zero. Therefore the model is valid for the reactors without any further modification.

APPENDIX B

DENATURANT GRADIENT GEL ELECTROPHORESIS (DGGE) REAGENT PROTOCOLS

DGGE REAGENTS

40% ACRYLAMIDE

Reagent	Amount
Acrylamide	38.93 g
Bis-acrylamide	1.07 g
dH2O	to 100.0 ml

Filter through 0.45 um filter and store at 40C

50X TAE BUFFER

Reagent	Amount	Final Concentration
Tris Base	242.0 g 2 M	2M
Acetic acid, glacial	57.1 ml 1 M	1M
0.5 M EDTA, pH 8.0	100.0 ml 50 mM	50mM
dH2O to	1,000.0 ml	

Mix. Autoclave for 20-30 minutes. Stored at room temperature.

40% DENATURING STOCK SOLUTION

Reagent	8%	10%
40 % Acrylamide/Bis	20 ml	25 ml
50x TAE buffer	2 ml	2 ml
Formamide (deionized)	16 ml	16 ml
Urea	16.8 g	16.8 g
dH2O	to 100 ml	to 100 ml

Degas for 10 – 15 minutes. Filter through 0.45 um filter. Stored at 4^0C in a brown bottle for approximately one month.

70% DENATURING STOCK SOLUTION

Reagent	10%	12%
40 % Acrylamide/Bis	25 ml	30 ml
50x TAE buffer	2 ml	2 ml
Formamide (deionized)	28 ml	16 ml
Urea	29.4 g	29.4g
dH2O	to 100 ml	to 100 ml

Degas for 10 – 15 minutes. Filter through 0.45 um filter. Stored at 40C in a brown bottle for approximately one month. Place in warm bath and stir to re-dissolve any crystals that may have formed.

10% AMMONIUM PERSULFATE

Reagent	Amount
Ammonium persulfate	0.1 g
dH2O	1.0 ml

Store at -20^0C for about a week.

2X GEL LOADING DYE

Reagent	Amount	Final Concentration
2% Bromophenol blue	0.25 ml	0.05%
2% Xylene cyanol	0.25 ml	0.05%
100% Glycerol	7.0 ml	70%
dH2O	2.5 ml	
Total Volume	10.0 ml	

Stored at room temperature.

1X TAE RUNNING BUFFER

Reagent	Amount
50x TAE buffer	140 ml
dH2O	6,860 ml
Total volume	7,000 ml

10% ACRYLAMIDE GEL SOLUTION (DGGE)

	Denaturant Concentration	
Reagent	40%	70%
10% Acrylamide stock solution	12.5 ml	12.5 ml
10% Ammonium persulfate	100 ul	100 ul
TEMED	10 ul	10 ul

Add ammonium persulfate and TEMED immediately prior to casting gel.

8 TO 12 % GRADIENT ACRYLAMIDE GEL SOLUTION (DGGE)

	Denaturant Concentration	
Reagent	40%	70%
8% Acrylamide stock solution	12.5 ml	-
12% Acrylamide stock solution	-	12.5 ml
10% Ammonium persulfate	100 ul	100 ul
TEMED	10 ul	10 ul

Add ammonium persulfate and TEMED immediately prior to casting gel.

6% ACRYLAMIDE STACKING GEL

Reagent	Amount
40 % Acrylamide	300 ul
1x TAE buffer	1.7 ml
10% Ammonium persulfate	15 ul
TEMED	2 ul
Total Volume	2 ml

Add ammonium persulfate and TEMED immediately prior to casting gel.

APPENDIX C
RAW DATA FOR THE PROJECT

Table 1: Effluent NH_3-N concentrations (ppm) from different reactors in phase I

Coupon type		New copper				Old copper			
Nutrient conditions		Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N	Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N
Date	Days	NH_3-N concentration (ppm)							
05/09/05	14	0.33	0.74	0.26	0.68	0.35	0.71	0.29	0.45
05/11/05	16	0.3	0.67	0.27	0.69	0.34	0.68	0.28	0.58
05/13/05	18	0.34	0.69	0.28	0.65	0.34	0.71	0.29	0.63
05/16/05	21	0.34	0.66	0.26	0.65	0.35	0.66	0.27	0.56
05/18/05	23	0.28	0.58	0.26	0.67	0.36	0.7	0.24	0.42
05/20/05	25	0.29	0.64	0.27	0.66	0.32	0.62	0.29	0.6
05/23/05	28	0.28	0.67	0.28	0.69	0.33	0.62	0.26	0.58
05/25/05	30	0.36	0.62	0.25	0.67	0.34	0.64	0.29	0.62
05/27/05	32	0.27	0.59	0.26	0.66	0.35	0.68	0.29	0.55
05/30/05	35	0.22	0.45	0.26	0.69	0.37	0.72	0.29	0.63
06/01/05	37	0.29	0.64	0.25	0.67	0.35	0.67	0.29	0.58
06/03/05	39	0.3	0.65	0.26	0.66	0.33	0.63	0.28	0.55
06/06/05	42	0.26	0.64	0.27	0.66	0.31	0.63	0.22	0.38
06/08/05	44	0.26	0.63	0.26	0.65	0.33	0.62	0.28	0.61
06/10/05	46	0.4	0.65	0.23	0.61	0.32	0.63	0.3	0.57
06/13/05	49	0.29	0.59	0.23	0.59	0.32	0.6	0.24	0.56
06/15/05	51	0.31	0.62	0.24	0.66	0.31	0.64	0.25	0.54
06/17/05	53	0.29	0.64	0.25	0.65	0.34	0.62	0.26	0.56
06/20/05	56	0.32	0.66	0.24	0.64	0.33	0.63	0.25	0.55
06/22/05	58	0.28	0.62	0.23	0.65	0.32	0.65	0.26	0.57
06/24/05	60	0.3	0.61	0.24	0.64	0.33	0.61	0.25	0.53
06/27/05	63	0.31	0.59	0.25	0.66	0.34	0.62	0.25	0.55
06/29/05	65	0.3	0.61	0.3	0.62	0.31	0.65	0.32	0.56
07/01/05	67	0.33	0.6	0.24	0.65	0.34	0.64	0.25	0.54
07/04/05	70	0.30	0.57	0.32	0.64	0.30	0.61	0.33	0.57
07/06/05	72	0.29	0.63	0.26	0.65	0.32	0.63	0.27	0.55
07/08/05	74	0.33	0.6	0.24	0.64	0.32	0.62	0.27	0.54
07/11/05	77	0.31	0.61	0.25	0.64	0.33	0.64	0.26	0.53
07/13/05	79	0.29	0.62	0.24	0.64	0.33	0.63	0.25	0.53
07/15/05	81	0.3	0.59	0.25	0.63	0.32	0.63	0.26	0.55
07/18/05	84	0.28	0.62	0.23	0.65	0.33	0.62	0.25	0.54
07/20/05	86	0.29	0.63	0.29	0.63	0.28	0.63	0.33	0.63
07/22/05	88	0.29	0.59	0.25	0.64	0.3	0.64	0.25	0.54
07/25/05	91	0.28	0.59	0.24	0.65	0.33	0.63	0.25	0.55
07/27/05	93	0.31	0.58	0.26	0.62	0.28	0.62	0.26	0.54
07/29/05	95	0.28	0.59	0.25	0.63	0.28	0.64	0.25	0.55

Table 2: Effluent NO_2-N concentrations (ppm) from different reactors in phase I

Coupon type		New copper				Old copper			
Nutrient Conditions		Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N	Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N
Date	Days	NO_2-N concentration(ppm)							
5/9/05	14	0.002	0.003	0.004	0.001	0.002	0.002	0.002	0.005
5/13/05	18	0.001	0.001	0.002	0.003	0.004	0.001	0.001	0.001
5/18/05	23	0.002	0.002	0.002	0.003	0.004	0.001	0.002	0.002
5/20/05	25	0.003	0.003	0.001	0.002	0.003	0.002	0.001	0.002
5/22/05	27	0.003	0.002	0.001	0.002	0.003	0.002	0.001	0.002
5/25/05	30	0.003	0.003	0.002	0.002	0.003	0.002	0.002	0.001
5/30/05	35	0.002	0.002	0.002	0.002	0.003	0.003	0.002	0.001
6/1/05	37	0.003	0.002	0.001	0.001	0.003	0.002	0.001	0.001
6/3/05	39	0.002	0.003	0.003	0.001	0.002	0.004	0.003	0.002
6/6/05	42	0.003	0.003	0.002	0.002	0.003	0.003	0.005	0.003
6/8/05	44	0.003	0.002	0	0.002	0.003	0.003	0.002	0.002
6/10/05	46	0.002	0.002	0.002	0.003	0.003	0.002	0.002	0.002
6/13/05	49	0.004	0.003	0.001	0.001	0.003	0.003	0.001	0.001
06/15/05	51	0.003	0.003	0.002	0.002	0.003	0.003	0.005	0.003
06/17/05	53	0.003	0.002	0	0.002	0.003	0.003	0.002	0.002
06/20/05	56	0.002	0.002	0.002	0.002	0.003	0.003	0.002	0.001
06/22/05	58	0.002	0.002	0.002	0.003	0.003	0.002	0.002	0.002
06/24/05	60	0.003	0.003	0.002	0.002	0.003	0.003	0.005	0.003
06/27/05	63	0.002	0.002	0.002	0.003	0.003	0.002	0.002	0.002
6/30/05	66	0.004	0.003	0.002	0.002	0.003	0.003	0.001	0.002
07/04/05	70	0.002	0.002	0.002	0.003	0.003	0.002	0.002	0.002
07/06/05	72	0.003	0.002	0.001	0.002	0.003	0.002	0.001	0.002
07/08/05	74	0.002	0.002	0.002	0.003	0.003	0.002	0.002	0.002
07/11/05	77	0.003	0.003	0.002	0.002	0.003	0.003	0.003	0.003
07/13/05	79	0.003	0.003	0.002	0.002	0.003	0.003	0.004	0.003
07/15/05	81	0.002	0.003	0.003	0.001	0.002	0.004	0.003	0.002
07/18/05	84	0.005	0.001	0.002	0.002	0.001	0.002	0.002	0.001
07/20/05	86	0.003	0.003	0.002	0.002	0.003	0.003	0.004	0.003
07/22/05	88	0.003	0.003	0.002	0.002	0.003	0.003	0.004	0.003
07/25/05	91	0.002	0.002	0.001	0.001	0.005	0.003	0.002	0.002
07/27/05	93	0.001	0.001	0.002	0.003	0.002	0.004	0.001	0.001
07/29/05	95	0.005	0.001	0.002	0.002	0.001	0.002	0.002	0.001

Table 3: Effluent NO_3-N concentrations (ppm) from different reactors in phase I

Coupon type		New copper				Old copper			
Nutrient Conditions		Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N	Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N
Date	Days	NO_3-N concentration(ppm)							
05/09/05	14	0.1	0	0	0	0	0.1	0.3	0.423
05/11/05	16	0	0.1	0.1	0.1	0	0.1	0.1	0.2
05/13/05	18	0.1	0.1	0.2	0.3	0.1	0	0.3	0.4
05/16/05	21	0	0.1	0.2	0.2	0	0.2	0	0.1
05/18/05	23	0	0	0.1	0.1	0	0	0.3	0.1
05/20/05	25	0.1	0.1	0.3	0.3	0.1	0.1	0.3	0.3
05/23/05	28	0.1	0.1	0.3	0.3	0.1	0	0.2	0.2
05/25/05	30	0	0	0.1	0.2	0	0	0.1	0.1
05/27/05	32	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.2
05/30/05	35	0.2	0.2	0.3	0.3	0	0	0.2	0.2
06/01/05	37	0	0	0.2	0.3	0	0	0.2	0.2
06/03/05	39	0.1	0.2	0.1	0.1	0	0.1	0	0.2
06/06/05	42	0.1	0.1	0.2	0.3	0.1	0	0.4	0.5
06/08/05	44	0.1	0	0.2	0.3	0.1	0	0.2	0.2
06/10/05	46	0.1	0.1	0.3	0.4	0.1	0.1	0.3	0.3
06/13/05	49	0.1	0.1	0.3	0.3	0.1	0.1	0.3	0.3
06/15/05	51	0	0.1	0	0.1	0	0.1	0.1	0.2
06/17/05	53	0.1	0.2	0.1	0.2	0.1	0.2	0	0.3
06/20/05	56	0	0.1	0.2	0.1	0.1	0.3	0	0.1
06/22/05	58	0.1	0.2	0.1	0.2	0	0.2	0.1	0.2
06/24/05	60	0.1	0.1	0.1	0.2	0.1	0.1	0	0.2
06/27/05	63	0	0.3	0.2	0.1	0	0.2	0	0.2
06/29/05	65	0	0.1	0.2	0.2	0	0	0.2	0.4
07/01/05	67	0	0.2	0.2	0.2	0	0.2	0.1	0.3
07/04/05	70	0.2	0.1	0.2	0.1	0.2	0.3	0	0.2
07/06/05	72	0	0.2	0.1	0.2	0	0.2	0.1	0.3
07/08/05	74	0.1	0.2	0.2	0.2	0	0.2	0	0.3
07/11/05	77	0	0.2	0.2	0.2	0	0.2	0.1	0.3
07/13/05	79	0.1	0.2	0.1	0.2	0.1	0.3	0.2	0.3
07/15/05	81	0	0.2	0.1	0.2	0	0.2	0.1	0.4
07/18/05	84	0.1	0.2	0.2	0.3	0	0.2	0.1	0.3
07/20/05	86	0.2	0.1	0.1	0.3	0.3	0.1	0.1	0.3
07/22/05	88	0.1	0.2	0.1	0.2	0	0.2	0.1	0.3
07/25/05	91	0	0.3	0.2	0.1	0	0.2	0.1	0.3
07/27/05	93	0	0.2	0.1	0.2	0.1	0.3	0.2	0.3
07/29/05	95	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.4

Table 4: Effluent total copper concentrations (ppm) from different reactors in phase I

Coupon type		Effluent total Copper concentrations (mg/L)							
Nutrient Conditions		New Copper				Old Copper			
Date	Days	Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N	Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N
05/09/05	14	0.33	0.74	0.26	0.68	0.35	0.71	0.29	0.45
05/11/05	16	0.3	0.67	0.27	0.69	0.34	0.68	0.28	0.58
05/13/05	18	0.34	0.69	0.28	0.65	0.34	0.71	0.29	0.63
05/16/05	21	0.34	0.66	0.26	0.65	0.35	0.66	0.27	0.56
05/18/05	23	0.28	0.58	0.26	0.67	0.36	0.7	0.24	0.42
05/20/05	25	0.29	0.64	0.27	0.66	0.32	0.62	0.29	0.6
05/23/05	28	0.28	0.67	0.28	0.69	0.33	0.62	0.26	0.58
05/25/05	30	0.36	0.62	0.25	0.67	0.34	0.64	0.29	0.62
05/27/05	32	0.27	0.59	0.26	0.66	0.35	0.68	0.29	0.55
05/30/05	35	0.22	0.45	0.26	0.69	0.37	0.72	0.29	0.63
06/01/05	37	0.29	0.64	0.25	0.67	0.35	0.67	0.29	0.58
06/03/05	39	0.3	0.65	0.26	0.66	0.33	0.63	0.28	0.55
06/06/05	42	0.26	0.64	0.27	0.66	0.31	0.63	0.22	0.38
06/08/05	44	0.26	0.63	0.26	0.65	0.33	0.62	0.28	0.61
06/10/05	46	0.4	0.65	0.23	0.61	0.32	0.63	0.3	0.57
06/13/05	49	0.29	0.59	0.23	0.59	0.32	0.6	0.24	0.56
06/15/05	51	0.31	0.62	0.24	0.66	0.31	0.64	0.25	0.54
06/17/05	53	0.29	0.64	0.25	0.65	0.34	0.62	0.26	0.56
06/20/05	56	0.32	0.66	0.24	0.64	0.33	0.63	0.25	0.55
06/22/05	58	0.28	0.62	0.23	0.65	0.32	0.65	0.26	0.57
06/24/05	60	0.3	0.61	0.24	0.64	0.33	0.61	0.25	0.53
06/27/05	63	0.31	0.59	0.25	0.66	0.34	0.62	0.25	0.55
06/29/05	65	0.3	0.61	0.3	0.62	0.31	0.65	0.32	0.56
07/01/05	67	0.33	0.6	0.24	0.65	0.34	0.64	0.25	0.54
07/04/05	70	0.30	0.57	0.32	0.64	0.30	0.61	0.33	0.57
07/06/05	72	0.29	0.63	0.26	0.65	0.32	0.63	0.27	0.55
07/08/05	74	0.33	0.6	0.24	0.64	0.32	0.62	0.27	0.54
07/11/05	77	0.31	0.61	0.25	0.64	0.33	0.64	0.26	0.53
07/13/05	79	0.29	0.62	0.24	0.64	0.33	0.63	0.25	0.53
07/15/05	81	0.3	0.59	0.25	0.63	0.32	0.63	0.26	0.55
07/18/05	84	0.28	0.62	0.23	0.65	0.33	0.62	0.25	0.54
07/20/05	86	0.29	0.63	0.29	0.63	0.28	0.63	0.33	0.63
07/22/05	88	0.29	0.59	0.25	0.64	0.3	0.64	0.25	0.54
07/25/05	91	0.28	0.59	0.24	0.65	0.33	0.63	0.25	0.55
07/27/05	93	0.31	0.58	0.26	0.62	0.28	0.62	0.26	0.54
07/29/05	95	0.28	0.59	0.25	0.63	0.28	0.64	0.25	0.55

Table 5: Effluent dissolved copper concentrations (ppm) in different reactors in phase I

Coupon type		Effluent total copper concentrations (mg/L)							
Nutrient Conditions		New Copper				Old Copper			
Date	Days	Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N	Lo C-Lo N	Lo C-Hi N	Hi C-Lo N	Hi C-Hi N
05/09/05	14	0.26	0.25	0.44	0.44	0.26	0.22	0.33	0.4
05/11/05	16	0.31	0.33	0.47	0.4	0.27	0.21	0.45	0.55
05/13/05	18	0.28	0.22	0.24	0.27	0.19	0.17	0.32	0.4
05/16/05	21	0.25	0.37	0.46	0.42	0.26	0.2	0.44	0.53
05/18/05	23	0.3	0.32	0.42	0.41	0.3	0.27	0.67	0.72
05/20/05	25	0.31	0.34	0.62	0.58	0.33	0.38	0.51	0.56
05/23/05	28	0.35	0.3	0.59	0.53	0.29	0.3	0.47	0.57
05/25/05	30	0.32	0.34	0.64	0.57	0.33	0.31	0.58	0.59
05/27/05	32	0.31	0.35	0.51	0.47	0.25	0.3	0.44	0.54
05/30/05	35	0.345	0.72	0.49	0.46	0.22	0.2	0.47	0.5
06/01/05	37	0.26	0.23	0.31	0.43	0.21	0.23	0.41	0.5
06/03/05	39	0.3056	0.18	0.38	0.33	0.2	0.16	0.35	0.36
06/06/05	42	0.32	0.28	0.56	0.46	0.26	0.26	0.4	0.45
06/08/05	44	0.33	0.29	0.63	0.47	0.31	0.27	0.45	0.49
06/10/05	46	0.28	0.26	0.48	0.42	0.25	0.24	0.43	0.43
06/13/05	49	0.31	0.32	0.59	0.51	0.26	0.23	0.49	0.52
06/15/05	51	0.3	0.25	0.4	0.4	0.25	0.25	0.47	0.51
06/17/05	53	0.29	0.26	0.42	0.42	0.27	0.26	0.48	0.52
06/20/05	56	0.28	0.25	0.52	0.41	0.26	0.25	0.45	0.5
06/22/05	58	0.3	0.24	0.48	0.43	0.26	0.24	0.49	0.49
06/24/05	60	0.28	0.28	0.58	0.44	0.24	0.23	0.47	0.47
06/27/05	63	0.3	0.27	0.55	0.45	0.23	0.23	0.51	0.56
06/29/05	65	0.32	0.35	0.65	0.61	0.31	0.28	0.54	0.65
07/01/05	67	0.28	0.25	0.52	0.51	0.25	0.26	0.49	0.56
07/04/05	70	0.32	0.33	0.62	0.6	0.3	0.32	0.53	0.65
07/06/05	72	0.31	0.26	0.5	0.43	0.26	0.26	0.47	0.59
07/08/05	74	0.32	0.25	0.4	0.4	0.25	0.23	0.46	0.49
07/11/05	77	0.29	0.23	0.51	0.41	0.24	0.22	0.47	0.53
07/13/05	79	0.27	0.26	0.5	0.42	0.28	0.26	0.46	0.56
07/15/05	81	0.3	0.25	0.52	0.41	0.26	0.28	0.43	0.53
07/18/05	84	0.32	0.27	0.58	0.42	0.26	0.26	0.42	0.55
07/20/05	86	0.32	0.35	0.65	0.61	0.31	0.28	0.54	0.65
07/22/05	88	0.31	0.27	0.52	0.41	0.29	0.26	0.49	0.56
07/25/05	91	0.28	0.27	0.5	0.4	0.27	0.25	0.45	0.49
07/27/05	93	0.32	0.28	0.51	0.41	0.25	0.24	0.47	0.41
07/29/05	95	0.33	0.27	0.5	0.4	0.24	0.28	0.52	0.49

Table 6: Effluent NH_3-N concentrations (ppm) from different reactors in phase II

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
09/14/05	17	0.31	0.70	0.31	0.66
09/16/05	19	0.36	0.68	0.31	0.62
09/19/05	22	0.38	0.68	0.29	0.57
09/21/05	24	0.36	0.66	0.31	0.61
09/23/05	26	0.36	0.65	0.34	0.60
09/28/05	31	0.35	0.62	0.32	0.58
09/30/05	33	0.35	0.63	0.36	0.60
10/03/05	36	0.33	0.63	0.35	0.61
10/05/05	38	0.35	0.61	0.31	0.58
10/07/05	40	0.33	0.63	0.33	0.56
10/10/05	43	0.32	0.61	0.32	0.56
10/12/05	45	0.31	0.59	0.31	0.51
10/15/05	48	0.26	0.60	0.26	0.60
10/17/05	50	0.29	0.60	0.28	0.54
10/19/05	52	0.30	0.61	0.27	0.55
10/21/05	54	0.34	0.63	0.30	0.54
10/25/05	58	0.32	0.63	0.33	0.57
10/28/05	61	0.29	0.71	0.35	0.58
11/01/05	65	0.28	0.71	0.34	0.63
11/04/05	68	0.28	0.71	0.34	0.63
11/07/05	71	0.26	0.65	0.32	0.64
11/09/05	73	0.23	0.63	0.33	0.64
11/11/05	75	0.23	0.53	0.34	0.56
11/14/05	78	0.21	0.49	0.35	0.54
11/16/05	80	0.16	0.44	0.35	0.60
11/18/05	82	0.15	0.42	0.35	0.55
11/21/05	85	0.12	0.39	0.36	0.61
11/23/05	87	0.10	0.33	0.31	0.61
11/24/05	88	0.10	0.31	0.30	0.62
11/26/05	90	0.10	0.31	0.32	0.61
11/28/05	92	0.10	0.28	0.33	0.56
11/30/05	94	0.09	0.30	0.36	0.63
12/02/05	96	0.10	0.23	0.29	0.60
12/05/05	99	0.11	0.24	0.33	0.57
12/07/05	101	0.12	0.26	0.32	0.60
12/13/05	107	0.10	0.26	0.27	0.55
12/15/05	109	0.11	0.28	0.26	0.60
12/16/05	110	0.10	0.25	0.27	0.56
12/19/05	113	0.10	0.27	0.25	0.55

Table 6: Effluent NH_3-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
12/21/05	115	0.13	0.26	0.27	0.58
12/23/05	117	0.10	0.28	0.27	0.56
12/26/05	120	0.09	0.25	0.28	0.59
12/28/05	122	0.08	0.21	0.26	0.60
12/30/05	124	0.12	0.26	0.30	0.67
01/02/06	127	0.14	0.28	0.33	0.66
01/04/06	129	0.10	0.24	0.31	0.67
01/06/06	131	0.11	0.23	0.34	0.62
01/09/06	134	0.14	0.28	0.37	0.60
1/13/2006	138	0.14	0.27	0.32	0.58
1/15/2006	140	0.08	0.31	0.34	0.58
1/16/2006	141	0.11	0.28	0.35	0.53
1/18/2006	143	0.11	0.25	0.30	0.48
1/20/2006	145	0.10	0.29	0.30	0.48
1/23/2006	148	0.13	0.32	0.34	0.46
1/25/2006	150	0.11	0.28	0.30	0.40
1/27/2006	152	0.12	0.26	0.29	0.40
1/30/2006	155	0.13	0.27	0.31	0.50
02/01/06	157	0.14	0.27	0.30	0.37
02/03/06	159	0.11	0.25	0.25	0.35
02/06/06	162	0.08	0.26	0.19	0.32
02/08/06	164	0.11	0.25	0.22	0.36
02/10/06	166	0.08	0.23	0.21	0.36
02/13/06	169	0.11	0.19	0.16	0.37
02/15/06	171	0.10	0.24	0.21	0.42
02/17/06	173	0.10	0.24	0.22	0.42
02/20/06	176	0.10	0.24	0.21	0.43
02/22/06	178	0.10	0.22	0.19	0.40
02/24/06	180	0.06	0.19	0.17	0.40
02/27/06	183	0.07	0.16	0.20	0.30
03/01/06	185	0.11	0.18	0.16	0.38
03/03/06	187	0.07	0.20	0.20	0.44
03/06/06	190	0.12	0.23	0.19	0.40
03/08/06	192	0.07	0.21	0.24	0.46
03/10/06	194	0.08	0.25	0.28	0.50
03/13/06	197	0.07	0.21	0.31	0.50
03/15/06	199	0.08	0.25	0.31	0.51
03/29/06	213	0.12	0.24	0.30	0.44

Table 6: Effluent NH_3-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
04/05/06	220	0.11	0.28	0.26	0.40
04/07/06	222	0.13	0.26	0.26	0.59
04/10/06	225	0.12	0.26	0.32	0.64
04/12/06	227	0.11	0.23	0.35	0.67
04/12/06	227	0.10	0.22	0.33	0.65
04/17/06	232	0.12	0.21	0.30	0.63
04/19/06	234	0.11	0.25	0.33	0.63
04/21/06	236	0.12	0.23	0.28	0.58
04/23/06	238	0.13	0.25	0.33	0.56
04/25/06	240	0.13	0.25	0.30	0.50
04/26/06	241	0.14	0.25	0.32	0.52
04/28/06	243	0.10	0.23	0.31	0.49
05/01/06	246	0.10	0.22	0.32	0.42
05/03/06	248	0.11	0.23	0.29	0.38
05/05/06	250	0.14	0.23	0.23	0.39
05/08/06	253	0.12	0.24	0.26	0.36
05/15/06	260	0.11	0.23	0.27	0.31
05/17/06	262	0.12	0.23	0.23	0.30
05/19/06	264	0.13	0.24	0.29	0.33
05/22/06	267	0.12	0.23	0.31	0.33
05/23/06	268	0.24	0.23	0.29	0.36
05/24/06	269	0.28	0.25	0.30	0.37
05/29/06	274	0.23	0.23	0.53	0.31
05/31/06	276	0.22	0.20	0.47	0.27
06/02/06	278	0.24	0.23	0.43	0.28
06/05/06	281	0.21	0.25	0.40	0.29
06/09/06	285	0.21	0.21	0.36	0.25
06/12/06	288	0.23	0.24	0.39	0.28
06/14/06	290	0.17	0.23	0.34	0.32
06/16/06	292	0.18	0.20	0.32	0.27
06/18/06	294	0.26	0.25	0.35	0.32
06/21/06	297	0.26	0.24	0.33	0.31
06/23/06	299	0.24	0.26	0.26	0.25
06/26/06	302	0.29	0.28	0.34	0.28
06/28/06	304	0.21	0.21	0.28	0.22
06/30/06	306	0.22	0.22	0.23	0.23
07/03/06	309	0.20	0.22	0.20	0.17
07/05/06	311	0.20	0.20	0.23	0.21

Table 6: Effluent NH_3-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
07/07/06	313	0.20	0.21	0.22	0.22
07/10/06	316	0.17	0.21	0.19	0.20
07/14/06	320	0.22	0.22	0.36	0.35
07/17/06	323	0.25	0.24	0.26	0.27
07/19/06	325	0.22	0.22	0.21	0.24
07/21/06	327	0.21	0.24	0.20	0.21
07/24/06	330	0.23	0.24	0.24	0.27
07/26/06	332	0.26	0.26	0.24	0.24
07/28/06	334	0.28	0.21	0.24	0.26
07/31/06	337	0.24	0.24	0.22	0.26
08/02/06	339	0.22	0.20	0.20	0.22
08/04/06	341	0.26	0.24	0.25	0.28
08/07/06	344	0.25	0.26	0.24	0.29
08/09/06	346	0.28	0.22	0.21	0.26
08/11/06	348	0.23	0.21	0.19	0.25
08/14/06	351	0.19	0.22	0.19	0.28
08/18/06	355	0.26	0.27	0.40	0.37
08/21/06	358	0.24	0.25	0.36	0.36
08/23/06	360	0.26	0.21	0.33	0.34
08/25/06	362	0.25	0.21	0.33	0.34
08/28/06	365	0.27	0.24	0.29	0.26

Table 7: Effluent NO_2-N concentrations (ppm) from different reactors in phase II

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
09/14/05	17	0.0025	0.03	0.04	0.01
09/16/05	19	0.0025	0.03	0.06	0.04
09/19/05	22	0.0035	0.02	0.04	0.04
09/21/05	24	0.0025	0.02	0.07	0.04
09/23/05	26	0.0035	0.03	0.05	0.05
09/28/05	31	0.0035	0.03	0.02	0.05
09/30/05	33	0.002	0.04	0.04	0.06
10/03/05	36	0.0025	0.04	0.01	0.06
10/05/05	38	0.002	0.02	0.05	0.06
10/07/05	40	0.004	0.02	0.02	0.06
10/10/05	43	0.0035	0.05	0.02	0.08
10/12/05	45	0.0025	0.08	0.02	0.06

Table 7: Effluent NO_2-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
10/15/05	48	0.0035	0.10	0.07	0.06
10/17/05	50	0.0025	0.09	0.03	0.05
10/19/05	52	0.0035	0.07	0.04	0.06
10/21/05	54	0.003	0.04	0.05	0.04
10/25/05	58	0.0015	0.03	0.03	0.05
10/28/05	61	0.003	0.02	0.01	0.06
11/01/05	65	0.004	0.03	0.05	0.05
11/04/05	68	0.0035	0.03	0.04	0.02
11/07/05	71	0.0025	0.02	0.03	0.02
11/09/05	73	0.003	0.05	0.05	0.03
11/11/05	75	0.0025	0.15	0.04	0.03
11/14/05	78	0.002	0.18	0.07	0.04
11/16/05	80	0.0015	0.25	0.04	0.05
11/18/05	82	0.004	0.20	0.04	0.05
11/21/05	85	0.003	0.25	0.05	0.06
11/23/05	87	0.004	0.33	0.07	0.04
11/24/05	88	0.0025	0.35	0.06	0.04
11/26/05	90	0.004	0.37	0.05	0.06
11/28/05	92	0.0035	0.40	0.03	0.05
11/30/05	94	0.0035	0.33	0.03	0.05
12/02/05	96	0.0035	0.45	0.03	0.06
12/05/05	99	0.003	0.44	0.04	0.05
12/07/05	101	0.004	0.41	0.06	0.05
12/13/05	107	0.0035	0.40	0.09	0.05
12/15/05	109	0.0025	0.45	0.09	0.05
12/16/05	110	0.0035	0.44	0.08	0.03
12/19/05	113	0.004	0.44	0.06	0.04
12/21/05	115	0.0025	0.43	0.05	0.04
12/23/05	117	0.0025	0.42	0.04	0.04
12/26/05	120	0.003	0.45	0.03	0.02
12/28/05	122	0.002	0.45	0.03	0.01
12/30/05	124	0.004	0.43	0.02	0.01
01/02/06	127	0.002	0.43	0.02	0.01
01/04/06	129	0.0035	0.45	0.01	0.00
01/06/06	131	0.0035	0.44	0.01	0.09
01/09/06	134	0.003	0.46	0.02	0.11
1/13/2006	138	0.003	0.43	0.03	0.14
1/15/2006	140	0.0025	0.39	0.03	0.15

Table 7: Effluent NO_2-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
1/16/2006	141	0.0025	0.42	0.03	0.15
1/18/2006	143	0.003	0.41	0.04	0.17
1/20/2006	145	0.0025	0.45	0.04	0.18
1/23/2006	148	0.004	0.36	0.05	0.20
1/25/2006	150	0.003	0.41	0.05	0.22
1/27/2006	152	0.004	0.46	0.06	0.24
1/30/2006	155	0.0035	0.44	0.06	0.17
02/01/06	157	0.002	0.41	0.08	0.27
02/03/06	159	0.0035	0.46	0.10	0.30
02/06/06	162	0.0025	0.43	0.12	0.32
02/08/06	164	0.003	0.42	0.15	0.34
02/10/06	166	0.0025	0.42	0.16	0.35
02/13/06	169	0.004	0.45	0.16	0.30
02/15/06	171	0.003	0.41	0.17	0.31
02/17/06	173	0.004	0.46	0.14	0.29
02/20/06	176	0.0035	0.44	0.17	0.32
02/22/06	178	0.0035	0.47	0.14	0.31
02/24/06	180	0.0035	0.47	0.14	0.35
02/27/06	183	0.003	0.47	0.11	0.34
03/01/06	185	0.004	0.48	0.13	0.34
03/03/06	187	0.0035	0.47	0.12	0.35
03/06/06	190	0.0025	0.43	0.12	0.28
03/08/06	192	0.003	0.47	0.09	0.23
03/10/06	194	0.0025	0.44	0.09	0.20
03/13/06	197	0.0035	0.47	0.07	0.19
03/15/06	199	0.0025	0.44	0.08	0.17
03/17/06	201	0.0035	0.38	0.02	0.19
03/20/06	204	0.003	0.42	0.03	0.21
03/24/06	208	0.0015	0.39	0.03	0.23
03/29/06	213	0.004	0.41	0.03	0.24
03/31/06	215	0.0025	0.45	0.04	0.26
04/03/06	218	0.0015	0.44	0.05	0.27
04/05/06	220	0.0015	0.46	0.07	0.24
04/07/06	222	0.001	0.45	0.04	0.23
04/10/06	225	0.001	0.46	0.02	0.14
04/12/06	227	0.001	0.45	0.06	0.11
04/12/06	227	0.0025	0.46	0.04	0.08
04/17/06	232	0.0065	0.43	0.05	0.22

Table 7: Effluent NO_2-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
04/19/06	234	0.004	0.45	0.05	0.23
04/23/06	238	0.0025	0.45	0.05	0.23
04/26/06	241	0.0035	0.47	0.07	0.23
04/28/06	243	0.003	0.46	0.07	0.24
05/01/06	246	0.0025	0.45	0.07	0.30
05/03/06	248	0.0035	0.45	0.08	0.32
05/05/06	250	0.0025	0.45	0.09	0.32
05/08/06	253	0.003	0.46	0.08	0.34
05/15/06	260	0.002	0.46	0.09	0.36
05/17/06	262	0.0025	0.43	0.09	0.34
05/19/06	264	0.002	0.46	0.10	0.33
05/22/06	267	0.004	0.47	0.12	0.35
05/23/06	268	0.0035	0.46	0.10	0.34
05/24/06	269	0.0025	0.47	0.24	0.34
05/29/06	274	0.0025	0.42	0.13	0.38
05/31/06	276	0.003	0.42	0.13	0.40
06/02/06	278	0.0085	0.45	0.21	0.40
06/05/06	281	0.0045	0.40	0.23	0.40
06/09/06	285	0.003	0.45	0.24	0.41
06/12/06	288	0.003	0.44	0.26	0.40
06/14/06	290	0.007	0.45	0.28	0.40
06/16/06	292	0.003	0.45	0.33	0.38
06/18/06	294	0.003	0.43	0.35	0.40
06/21/06	297	0.003	0.44	0.33	0.41
06/23/06	299	0.0035	0.46	0.35	0.40
06/26/06	302	0.0025	0.46	0.33	0.41
06/28/06	304	0.0025	0.46	0.38	0.42
06/30/06	306	0.003	0.46	0.42	0.43
07/03/06	309	0.0035	0.45	0.43	0.44
07/05/06	311	0.0025	0.46	0.42	0.45
07/07/06	313	0.0025	0.42	0.43	0.46
07/10/06	316	0.003	0.45	0.44	0.46
07/14/06	320	0.002	0.42	0.30	0.33
07/17/06	323	0.003	0.45	0.37	0.43
07/19/06	325	0.0025	0.44	0.42	0.41
07/21/06	327	0.003	0.45	0.42	0.44
07/24/06	330	0.0025	0.43	0.41	0.43
07/26/06	332	0.0035	0.46	0.42	0.42

Table 7: Effluent NO_2-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
07/28/06	334	0.0035	0.44	0.43	0.43
07/31/06	337	0.003	0.42	0.43	0.42
08/02/06	339	0.003	0.45	0.43	0.40
08/04/06	341	0.0025	0.44	0.40	0.42
08/07/06	344	0.0025	0.46	0.43	0.44
08/09/06	346	0.003	0.45	0.42	0.41
08/11/06	348	0.003	0.43	0.40	0.44
08/14/06	351	0.0025	0.44	0.36	0.37
08/18/06	355	0.0035	0.44	0.26	0.34
08/21/06	358	0.003	0.45	0.31	0.32
08/23/06	360	0.0035	0.45	0.32	0.29
08/25/06	362	0.003	0.44	0.33	0.31
08/28/06	365	0.003	0.42	0.34	0.37

Table 8: Effluent NO_3-N concentrations (ppm) from different reactors in phase II

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
09/14/05	17	0.02	0.03	0.04	0.01
09/16/05	19	0.02	0.03	0.06	0.04
09/19/05	22	0.04	0.02	0.04	0.04
09/21/05	24	0.01	0.02	0.07	0.04
09/23/05	26	0.03	0.03	0.05	0.05
09/28/05	31	0.02	0.03	0.02	0.05
09/30/05	33	0.03	0.04	0.04	0.06
10/03/05	36	0.02	0.04	0.01	0.06
10/05/05	38	0.02	0.02	0.05	0.06
10/07/05	40	0.02	0.02	0.02	0.06
10/10/05	43	0.04	0.05	0.02	0.08
10/12/05	45	0.03	0.08	0.02	0.06
10/15/05	48	0.06	0.10	0.07	0.06
10/17/05	50	0.04	0.09	0.03	0.05
10/19/05	52	0.04	0.07	0.04	0.06
10/21/05	54	0.03	0.04	0.05	0.04
10/25/05	58	0.01	0.03	0.03	0.05
10/28/05	61	0.04	0.02	0.01	0.06
11/01/05	65	0.03	0.03	0.05	0.05

Table 8: Effluent NO_3-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
11/04/05	68	0.05	0.03	0.04	0.02
11/07/05	71	0.08	0.02	0.03	0.02
11/09/05	73	0.09	0.05	0.05	0.03
11/11/05	75	0.08	0.15	0.04	0.03
11/14/05	78	0.10	0.18	0.07	0.04
11/16/05	80	0.16	0.25	0.04	0.05
11/18/05	82	0.17	0.20	0.04	0.05
11/21/05	85	0.21	0.25	0.05	0.06
11/23/05	87	0.22	0.33	0.07	0.04
11/24/05	88	0.23	0.35	0.06	0.04
11/26/05	90	0.22	0.37	0.05	0.06
11/28/05	92	0.20	0.40	0.03	0.05
11/30/05	94	0.23	0.33	0.03	0.05
12/02/05	96	0.23	0.45	0.03	0.06
12/05/05	99	0.22	0.44	0.04	0.05
12/07/05	101	0.21	0.41	0.06	0.05
12/13/05	107	0.22	0.40	0.09	0.05
12/15/05	109	0.22	0.45	0.09	0.05
12/16/05	110	0.23	0.44	0.08	0.03
12/19/05	113	0.23	0.44	0.06	0.04
12/21/05	115	0.22	0.43	0.05	0.04
12/23/05	117	0.24	0.42	0.04	0.04
12/26/05	120	0.25	0.45	0.03	0.02
12/28/05	122	0.23	0.45	0.03	0.01
12/30/05	124	0.21	0.43	0.02	0.01
01/02/06	127	0.21	0.43	0.02	0.01
01/04/06	129	0.23	0.45	0.01	0.00
01/06/06	131	0.23	0.44	0.01	0.09
01/09/06	134	0.22	0.46	0.02	0.11
1/13/2006	138	0.22	0.43	0.03	0.14
1/15/2006	140	0.24	0.39	0.03	0.15
1/16/2006	141	0.22	0.42	0.03	0.15
1/18/2006	143	0.24	0.41	0.04	0.17
1/20/2006	145	0.25	0.45	0.04	0.18
1/23/2006	148	0.22	0.36	0.05	0.20
1/25/2006	150	0.23	0.41	0.05	0.22
1/27/2006	152	0.22	0.46	0.06	0.24
1/30/2006	155	0.21	0.44	0.06	0.17

Table 8: Effluent NO_3-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
02/01/06	157	0.21	0.41	0.08	0.27
02/03/06	159	0.26	0.46	0.10	0.30
02/06/06	162	0.24	0.43	0.12	0.32
02/08/06	164	0.20	0.42	0.15	0.34
02/10/06	166	0.25	0.42	0.16	0.35
02/13/06	169	0.20	0.45	0.16	0.30
02/15/06	171	0.23	0.41	0.17	0.31
02/17/06	173	0.23	0.46	0.14	0.29
02/20/06	176	0.24	0.44	0.17	0.32
02/22/06	178	0.25	0.47	0.14	0.31
02/24/06	180	0.23	0.47	0.14	0.35
02/27/06	183	0.22	0.47	0.11	0.34
03/01/06	185	0.22	0.48	0.13	0.34
03/03/06	187	0.22	0.47	0.12	0.35
03/06/06	190	0.22	0.43	0.12	0.28
03/08/06	192	0.24	0.47	0.09	0.23
03/10/06	194	0.22	0.44	0.09	0.20
03/13/06	197	0.22	0.47	0.07	0.19
03/15/06	199	0.23	0.44	0.08	0.17
03/17/06	201	0.21	0.38	0.02	0.19
03/20/06	204	0.23	0.42	0.03	0.21
03/24/06	208	0.20	0.39	0.03	0.23
03/29/06	213	0.21	0.41	0.03	0.24
03/31/06	215	0.22	0.45	0.04	0.26
04/03/06	218	0.21	0.44	0.05	0.27
04/05/06	220	0.22	0.46	0.07	0.24
04/07/06	222	0.21	0.45	0.04	0.23
04/10/06	225	0.21	0.46	0.02	0.14
04/12/06	227	0.21	0.45	0.06	0.11
04/12/06	227	0.21	0.46	0.04	0.08
04/17/06	232	0.22	0.43	0.05	0.22
04/19/06	234	0.23	0.45	0.05	0.23
04/23/06	238	0.24	0.45	0.05	0.23
04/26/06	241	0.24	0.47	0.07	0.23
04/28/06	243	0.23	0.46	0.07	0.24
05/01/06	246	0.22	0.45	0.07	0.30
05/03/06	248	0.23	0.45	0.08	0.32
05/05/06	250	0.22	0.45	0.09	0.32

Table 8: Effluent NO_3-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
05/08/06	253	0.21	0.46	0.08	0.34
05/15/06	260	0.22	0.46	0.09	0.36
05/17/06	262	0.22	0.43	0.09	0.34
05/19/06	264	0.20	0.46	0.10	0.33
05/22/06	267	0.21	0.47	0.12	0.35
05/23/06	268	0.45	0.46	0.10	0.34
05/24/06	269	0.43	0.47	0.24	0.34
05/29/06	274	0.45	0.42	0.13	0.38
05/31/06	276	0.42	0.42	0.13	0.40
06/02/06	278	0.44	0.45	0.21	0.40
06/05/06	281	0.45	0.40	0.23	0.40
06/09/06	285	0.46	0.45	0.24	0.41
06/12/06	288	0.43	0.44	0.26	0.40
06/14/06	290	0.46	0.45	0.28	0.40
06/16/06	292	0.43	0.45	0.33	0.38
06/18/06	294	0.42	0.43	0.35	0.40
06/21/06	297	0.45	0.44	0.33	0.41
06/23/06	299	0.45	0.46	0.35	0.40
06/26/06	302	0.38	0.46	0.33	0.41
06/28/06	304	0.45	0.46	0.38	0.42
06/30/06	306	0.43	0.46	0.42	0.43
07/03/06	309	0.44	0.45	0.43	0.44
07/05/06	311	0.46	0.46	0.42	0.45
07/07/06	313	0.41	0.42	0.43	0.46
07/10/06	316	0.44	0.45	0.44	0.46
07/14/06	320	0.45	0.42	0.30	0.33
07/17/06	323	0.43	0.45	0.37	0.43
07/19/06	325	0.43	0.44	0.42	0.41
07/21/06	327	0.43	0.45	0.42	0.44
07/24/06	330	0.43	0.43	0.41	0.43
07/26/06	332	0.44	0.46	0.42	0.42
07/28/06	334	0.39	0.44	0.43	0.43
07/31/06	337	0.43	0.42	0.43	0.42
08/02/06	339	0.42	0.45	0.43	0.40
08/04/06	341	0.42	0.44	0.40	0.42
08/07/06	344	0.46	0.46	0.43	0.44
08/09/06	346	0.41	0.45	0.42	0.41
08/11/06	348	0.42	0.43	0.40	0.44

Table 8: Effluent NO_3-N concentrations (ppm) from different reactors in phase II continued

coupon type		PVC		Copper	
date	Days	Nutrient Conditions			
		Lo N	Hi N	Lo N	Hi N
08/14/06	351	0.42	0.44	0.36	0.37
08/18/06	355	0.43	0.44	0.26	0.34
08/21/06	358	0.42	0.45	0.31	0.32
08/23/06	360	0.41	0.45	0.32	0.29
08/25/06	362	0.42	0.44	0.33	0.31
08/28/06	365	0.43	0.42	0.34	0.37

Table 9: Effluent copper concentrations (ppm) from different reactors in phase II

date	Days	Total copper		Dissolved copper	
		Lo N	Hi N	Lo N	Hi N
09/14/05	17	0.44	0.43	0.41	0.46
09/16/05	19	0.51	0.61	0.40	0.46
09/19/05	22	0.59	0.70	0.42	0.45
09/21/05	24	0.53	0.60	0.40	0.45
09/23/05	26	0.52	0.58	0.38	0.40
09/28/05	31	0.52	0.49	0.37	0.35
09/30/05	33	0.55	0.51	0.39	0.39
10/03/05	36	0.57	0.60	0.39	0.44
10/05/05	38	0.58	0.63	0.39	0.54
10/07/05	40	0.58	0.62	0.39	0.52
10/10/05	43	0.59	0.62	0.38	0.54
10/12/05	45	0.61	0.64	0.40	0.54
10/15/05	48	0.57	0.62	0.43	0.46
10/17/05	50	0.60	0.66	0.47	0.55
10/19/05	52	0.61	0.69	0.47	0.56
10/21/05	54	0.64	0.70	0.44	0.50
10/25/05	58	0.64	0.75	0.39	0.60
10/28/05	61	0.67	0.88	0.45	0.67
11/01/05	65	0.58	0.60	0.41	0.42
11/04/05	68	0.59	0.70	0.35	0.61
11/07/05	71	0.57	0.63	0.37	0.53
11/09/05	73	0.65	0.70	0.34	0.49
11/11/05	75	0.64	0.69	0.37	0.54
11/14/05	78	0.65	0.73	0.44	0.61
11/16/05	80	0.65	0.74	0.44	0.55
11/17/05	81	0.66	0.72	0.45	0.52
11/17/05	81	0.66	0.75	0.43	0.53
11/18/05	82	0.68	0.77	0.41	0.50
11/21/05	85	0.58	0.78	0.29	0.58
11/23/05	87	0.50	0.80	0.28	0.66

Table 9: Effluent copper concentrations (ppm) from different reactors in phase II continued

date	Days	Total copper		Dissolved copper	
		Lo N	Hi N	Lo N	Hi N
11/24/05	88	0.56	0.82	0.32	0.68
11/26/05	90	0.57	0.85	0.39	0.70
11/28/05	92	0.53	0.83	0.33	0.67
11/30/05	94	0.44	0.68	0.29	0.57
12/02/05	96	0.50	0.74	0.35	0.65
12/05/05	99	0.54	0.76	0.33	0.66
12/07/05	101	0.67	0.89	0.44	0.59
12/13/05	107	0.73	0.78	0.41	0.55
12/15/05	109	0.66	0.75	0.38	0.55
12/16/05	110	0.76	0.77	0.40	0.57
12/19/05	113	0.72	0.75	0.54	0.75
12/21/05	115	0.66	0.71	0.33	0.51
12/23/05	117	0.71	0.63	0.31	0.48
12/26/05	120	0.73	0.62	0.21	0.39
12/28/05	122	0.64	0.61	0.34	0.42
12/30/05	124	0.56	0.65	0.37	0.41
01/02/06	127	0.57	0.64	0.41	0.47
01/04/06	129	0.58	0.64	0.43	0.48
01/06/06	131	0.60	0.66	0.45	0.50
01/09/06	134	0.66	0.75	0.48	0.55
1/13/2006	138	0.67	0.75	0.51	0.53
1/15/2006	140	0.52	0.73	0.45	0.37
1/16/2006	141	0.68	0.70	0.50	0.50
1/18/2006	143	0.68	0.74	0.52	0.52
1/20/2006	145	0.73	0.79	0.56	0.57
1/23/2006	148	0.73	0.76	0.55	0.50
1/25/2006	150	0.69	0.68	0.47	0.55
1/27/2006	152	0.62	0.80	0.47	0.55
1/30/2006	155	0.70	0.81	0.40	0.54
02/01/06	157	0.77	0.85	0.46	0.58
02/03/06	159	0.89	0.99	0.52	0.62
02/06/06	162	0.99	1.12	0.76	0.72
02/08/06	164	1.03	1.17	0.63	0.80
02/10/06	166	1.09	1.06	0.63	0.64
02/13/06	169	1.12	1.01	0.63	0.57
02/15/06	171	1.12	0.89	0.64	0.73
02/17/06	173	1.11	1.13	0.66	0.92
02/20/06	176	1.22	1.04	0.93	0.90
02/22/06	178	1.23	1.21	0.90	0.95
02/24/06	180	1.27	1.22	0.90	0.99
02/27/06	183	1.24	1.22	0.91	1.03
03/01/06	185	1.41	1.20	0.96	1.03
03/03/06	187	1.52	0.83	0.76	0.68

Table 9: Effluent copper concentrations (ppm) from different reactors in phase II continued

date	Days	Total copper		Dissolved copper	
		Lo N	Hi N	Lo N	Hi N
03/06/06	190	1.32	1.24	0.91	1.05
03/08/06	192	1.11	1.12	0.89	0.97
03/10/06	194	1.14	1.13	0.90	0.97
03/13/06	197	1.15	1.29	0.93	0.96
03/15/06	199	1.07	1.09	0.91	0.92
03/17/06	201	1.09	1.07	0.93	0.91
03/20/06	204	1.08	1.01	0.77	0.86
03/23/06	207	1.15	0.98	0.97	0.80
03/29/06	213	1.16	0.95	0.90	0.61
03/31/06	215	1.05	0.89	0.80	0.39
04/03/06	218	1.08	0.91	0.85	0.53
04/05/06	220	1.01	0.93	0.81	0.52
04/07/06	222	0.92	0.80	0.70	0.51
04/10/06	225	1.04	0.97	0.86	0.75
04/12/06	227	0.92	1.04	0.68	0.76
04/12/06	227	0.82	0.82	0.63	0.63
04/17/06	232	0.88	0.95	0.69	0.71
04/19/06	234	0.82	0.95	0.65	0.72
04/21/06	236	0.79	0.94	0.66	0.69
04/23/06	238	0.66	0.81	0.60	0.62
04/26/06	241	0.79	0.87	0.61	0.63
04/28/06	243	0.76	0.79	0.59	0.63
05/01/06	246	0.79	0.90	0.62	0.64
05/03/06	248	0.85	0.96	0.68	0.72
05/05/06	250	0.75	0.82	0.60	0.56
05/08/06	253	0.79	0.91	0.65	0.67
05/15/06	260	0.78	0.95	0.61	0.68
05/17/06	262	0.81	0.92	0.62	0.71
05/19/06	264	0.81	0.86	0.58	0.66
05/22/06	267	0.89	0.86	0.59	0.71
05/24/06	269	0.78	0.90	0.55	0.68
05/26/06	271	1.00	1.24	0.80	1.01
05/31/06	276	0.70	0.88	0.49	0.58
06/02/06	278	0.81	1.00	0.55	0.65
06/05/06	281	0.85	1.00	0.61	0.67
06/09/06	285	0.78	0.96	0.58	0.69
06/12/06	288	0.77	0.74	0.62	0.56
06/14/06	290	0.85	0.95	0.61	0.65
06/16/06	292	0.88	0.90	0.64	0.60
06/18/06	294	0.88	0.97	0.55	0.58
06/21/06	297	0.78	0.83	0.55	0.60
06/23/06	299	0.82	0.80	0.53	0.53
06/26/06	302	0.62	0.77	0.42	0.52

Table 9: Effluent copper concentrations (ppm) from different reactors in phase II continued

date	Days	Total copper		Dissolved copper	
		Lo N	Hi N	Lo N	Hi N
06/28/06	304	0.72	0.89	0.52	0.63
06/30/06	306	0.94	0.92	0.61	0.60
07/03/06	309	0.88	0.91	0.61	0.64
07/05/06	311	0.92	1.02	0.64	0.74
07/07/06	313	0.97	0.88	0.66	0.65
07/10/06	316	1.00	0.90	0.69	0.68
07/17/06	323	0.90	0.94	0.57	0.62
07/19/06	325	1.02	1.05	0.65	0.69
07/21/06	327	0.98	1.02	0.64	0.64
07/24/06	330	0.97	1.02	0.65	0.64
07/26/06	332	0.97	1.06	0.62	0.66
07/28/06	334	0.94	0.99	0.63	0.64
07/31/06	337	0.99	1.02	0.77	0.79
08/02/06	339	0.97	0.94	0.74	0.77
08/04/06	341	0.97	0.99	0.74	0.76
08/07/06	344	0.82	0.87	0.64	0.65
08/09/06	346	0.87	0.93	0.67	0.70
08/11/06	348	0.92	0.95	0.76	0.79
08/14/06	351	0.96	0.99	0.84	0.85
08/18/06	355	0.88	0.88	0.74	0.76
08/21/06	358	0.86	0.91	0.64	0.67
08/23/06	360	0.83	0.90	0.59	0.64
08/25/06	362	0.86	0.92	0.62	0.70
08/28/06	365	0.83	0.92	0.60	0.68

Table 10: Effluent NH_3-N concentrations (ppm) from copper added, control and pH control PVC reactors

Copper dose	Date	days	Cupper added PVC	Control PVC	pH Control PVC
15 ppb	09/20/06	2	0.22	0.22	0.27
	09/22/06	4	0.25	0.23	0.38
	09/25/06	7	0.2	0.2	0.23
	09/29/06	11	0.19	0.17	0.28
	10/02/06	14	0.2	0.18	0.23
50ppb	10/06/06	18	0.24	0.23	0.28
	10/09/06	21	0.22	0.26	0.25
	10/11/06	23	0.22	0.21	0.24
	10/13/06	25	0.28	0.22	0.24
	10/16/06	28	0.26	0.25	0.28
	10/18/06	30	0.26	0.22	0.23

Table 10: Effluent NH_3-N concentrations (ppm) from copper added, control and pH control PVC reactors continued

Copper	Date	days	Cupper added	Control	pH Control
100ppb	10/20/06	32	0.26	0.28	0.29
	10/22/06	34	0.28	0.24	0.27
	10/25/06	37	0.2	0.24	0.23
	10/27/06	39	0.33	0.3	0.4
	10/30/06	42	0.26	0.26	0.3
	11/01/06	44	0.23	0.23	0.22
200ppb	11/03/06	46	0.23	0.22	0.24
	11/06/06	49	0.25	0.23	0.26
	11/07/06	50	0.22	0.2	0.24
	11/10/06	53	0.24	0.23	0.24
	11/13/06	56	0.25	0.24	0.25
	11/15/06	58	0.26	0.26	0.26
300ppb	11/17/06	60	0.3	0.27	0.29
	11/20/06	63	0.29	0.29	0.31
	11/22/06	65	0.21	0.21	0.22
	11/25/06	68	0.22	0.22	0.25
	11/27/06	70	0.25	0.24	0.25
	11/29/06	72	0.19	0.18	0.22
400ppb	12/01/06	74	0.27	0.25	0.25
	12/04/06	77	0.17	0.22	0.22
	12/06/06	79	0.21	0.18	0.23
	12/08/06	81	0.2	0.2	0.23
	12/11/06	84	0.19	0.19	0.24
600ppb	12/13/06	86	0.2	0.17	0.22
	12/15/06	88	0.23	0.16	0.2
	12/17/06	90	0.2	0.2	0.264
	12/20/06	93	0.2	0.13	0.15
	12/21/06	94	0.14	0.1	0.1
	12/25/06	98	0.28	0.27	0.2
800 ppb	12/27/06	100	0.24	0.14	0.19
	12/29/06	102	0.22	0.14	0.11
	01/01/07	105	0.25	0.21	0.23
	01/03/07	107	0.22	0.19	0.25
1000ppb	01/10/07	114	0.21	0.21	0.21
	01/15/07	119	0.22	0.17	0.21
	01/17/07	121	0.19	0.15	0.19
	01/19/07	123	0.21	0.17	0.18
	01/22/07	126	0.19	0.18	0.22

Table 10: Effluent NH_3-N concentrations (ppm) from copper added, control and pH control PVC reactors continued

Copper dose or pH	Date	days	Cupper added PVC	Control PVC	pH Control PVC
1300 ppb of copper	01/29/07	133	0.21	0.18	0.22
	01/31/07	135	0.21	0.18	0.22
	02/02/07	137	0.27	0.2	0.21
	02/05/07	140	0.25	0.19	0.19
	02/07/07	142	0.2	0.17	0.17
	02/09/07	144	0.23	0.17	0.19
	02/12/07	147	0.27	0.2	0.2
	02/14/07	149	0.36	0.2	0.21
	02/16/07	151	0.24	0.21	0.25
	02/19/07	154	0.33	0.25	0.27
	02/21/07	156	0.27	0.21	0.22
	02/23/07	158	0.2	0.16	0.17
	02/26/07	161	0.25	0.17	0.21
	02/28/07	163	0.22	0.17	0.29
	03/02/07	165	0.24	0.18	0.22
	03/05/07	168	0.27	0.2	0.23
pH=7.8	03/07/07	170	0.24	0.2	0.22
	03/09/07	172	0.25	0.17	0.24
	03/12/07	175	0.25	0.16	0.2
	03/14/07	177	0.22	0.17	0.2
	03/16/07	179	0.42	0.22	0.28
	03/19/07	182	0.28	0.28	0.23
pH=7.5	03/21/07	184	0.27	0.22	0.19
	03/23/07	186	0.22	0.17	0.34
	03/26/07	189	0.24	0.22	0.21
	03/30/07	193	0.24	0.18	0.24
	04/02/07	196	0.2	0.22	0.21
	04/04/07	198	0.27	0.21	0.22
pH=7.2	04/06/07	200	0.26	0.17	0.22
	04/09/07	203	0.37	0.25	0.25
	04/11/07	205	0.25	0.2	0.3
	04/13/07	207	0.27	0.22	0.21
	04/16/07	210	0.3	0.25	0.23
	04/18/07	212	0.28	0.2	0.19
pH=6.9	04/20/07	214	0.25	0.21	0.22
	04/22/07	216	0.25	0.16	0.19
	04/25/07	219	0.26	0.19	0.16

Table 10: Effluent NH_3-N concentrations (ppm) from copper added, control and pH control PVC reactors continued

Copper dose or pH	Date	days	Cupper added PVC	Control PVC	pH Control PVC
pH=6.9	04/27/07	221	0.24	0.15	0.17
	04/30/07	224	0.22	0.1	0.13
	05/02/07	226	0.26	0.15	0.21
pH=6.6	05/03/07	228	0.25	0.16	0.15
	05/04/07	231	0.22	0.16	0.24
	05/07/07	235	0.22	0.12	0.14
	05/11/07	238	0.24	0.2	0.17
	05/14/07	240	0.18	0.2	0.1
	05/16/07	242	0.23	0.17	0.14

Table 11: Effluent NO_2-N concentrations (ppm) from copper added, control and pH control PVC reactors

Copper dose	Date	days	Cupper added	Control	pH Control
15 ppb	09/20/06	2	0.005	0.003	0.002
	09/22/06	4	0.004	0.003	0.004
	09/25/06	7	0.004	0.004	0.001
	09/29/06	11	0.003	0.004	0.003
	10/02/06	14	0.004	0.004	0.003
50ppb	10/06/06	18	0.003	0.003	0.003
	10/09/06	21	0.002	0.003	0.002
	10/11/06	23	0.002	0.003	0.004
	10/13/06	25	0.002	0.005	0.003
	10/16/06	28	0.001	0.003	0.003
	10/18/06	30	0.005	0.002	0.003
100ppb	10/20/06	32	0.004	0.003	0.003
	10/22/06	34	0.002	0.004	0.002
	10/25/06	37	0.003	0.004	0.003
	10/27/06	39	0.004	0.004	0.004
	10/30/06	42	0.003	0.005	0.002
	11/01/06	44	0.002	0.003	0.005
200ppb	11/03/06	46	0.002	0.002	0.003
	11/06/06	49	0.003	0.003	0.002
	11/07/06	50	0.004	0.002	0.003
	11/10/06	53	0.004	0.004	0.003
	11/13/06	56	0.001	0.002	0.004

Table 11: Effluent NO_2-N concentrations (ppm) from copper added, control and pH control PVC reactors continued

Copper dose	Date	days	Cupper added	Control	pH Control
200ppb	11/15/06	58			
300ppb	11/17/06	60	0.002	0.002	0.002
	11/20/06	63	0.002	0.003	0.001
	11/22/06	65	0.001	0.003	0.002
	11/25/06	68	0.002	0.002	0.004
	11/27/06	70	0.001	0.003	0.006
	11/29/06	72	0.002	0.002	0.004
400ppb	12/01/06	74	0.003	0.009	0.003
	12/04/06	77	0.003	0.002	0.004
	12/06/06	79	0.002	0.002	0.006
	12/08/06	81	0.002	0.002	0.003
	12/11/06	84	0.002	0.004	0.003
600ppb	12/13/06	86	0.002	0.006	0.003
	12/15/06	88	0.002	0.003	0.003
	12/17/06	90	0.002	0.001	0.003
	12/20/06	93	0	0	0
	12/21/06	94	0.004	0.007	0.005
	12/25/06	98	0.002	0.005	0.004
800ppb	12/27/06	100	0.003	0.005	0.004
	12/29/06	102	0.002	0.003	0.003
	01/01/07	105	0.001	0.002	0.003
	01/03/07	107	0.002	0	0.003
	01/05/07	109	0.001	0.002	0.003
	01/08/07	112	0.003	0.004	0.005
1000ppb	01/10/07	114	0.003	0.003	0.002
	01/15/07	119	0.003	0	0.004
	01/17/07	121	0	0.002	0.004
	01/19/07	123	0.001	0.003	0.003
	01/22/07	126	0.001	0.002	0.003
1300ppm	01/29/07	133	0	0.003	0.003
	02/02/07	137	0.013	0.001	0.004
	02/05/07	140	0.002	0.002	0.001
	02/07/07	142	0.003	0.005	0.003
	02/09/07	144	0.002	0.003	0.002
	02/12/07	147	0.001	0.001	0.001

Table 11: Effluent NO_2-N concentrations (ppm) from copper added, control and pH control PVC reactors continued

Copper Dose and pH	Date	days	Cupper added	Control	pH Control
1300 ppb	02/14/07	149	0.004	0.003	0.005
	02/16/07	151	0	0.002	0.004
	02/19/07	154	0.002	0.001	0.003
	02/21/07	156	0.003	0.004	0.003
	02/23/07	158	0.004	0.005	0.004
	02/26/07	161	0.002	0.002	0.003
	02/28/07	163	0	0.002	0.004
	03/02/07	165	0.001	0.003	0.002
	03/05/07	168	0.001	0.003	0
pH=7.8	03/07/07	170	0.001	0.003	0.003
	03/09/07	172	0.003	0.003	0.003
	03/12/07	175	0.003	0.002	0.003
	03/14/07	177	0.001	0.003	0.002
	03/16/07	179	0.003	0.004	0.003
	03/19/07	182	0.006	0.003	0.004
pH=7.5	03/23/07	186	0.003	0.002	0.003
	03/30/07	193	0	0.003	0.003
	04/02/07	196	0.001	0.002	0
	04/04/07	198	0.002	0.002	0.003
pH=7.2	04/09/07	203	0.003	0.002	0.005
	04/11/07	205	0	0.002	0.004
	04/16/07	210	0.002	0	0.002
	04/18/07	212	0.001	0.001	0.005
pH=6.9	04/20/07	214	0.002	0.002	0.004
	04/22/07	216	0.001	0.002	0.003
	04/25/07	219	0.001	0.002	0.006
	04/30/07	224	0.002	0.004	0.003
	05/02/07	226	0.001	0.003	0.001
pH=6.6	05/11/07	238	0.002	0.006	0.004
	05/14/07	240	0.005	0.003	0.006
	05/16/07	242	0.001	0.003	0.002

Table 12: Effluent NO_3-N concentrations (ppm) from copper added, control and pH control PVC reactors

Copper Dose	Date	days	Cupper added	Control	pH Control
15 ppb	09/20/06	2	0.7	0.7	0.6
	09/22/06	4	0.6	0.6	0.5
	09/25/06	7	0.7	0.7	0.6
	09/29/06	11	0.6	0.7	0.6
	10/02/06	14	0.45	0.5	0.6
50ppb	10/06/06	18	0.48	0.47	0.48
	10/09/06	21	0.53	0.52	0.51
	10/11/06	23	0.6	0.6	0.6
	10/13/06	25	0.51	0.55	0.51
	10/16/06	28	0.52	0.56	0.52
	10/18/06	30	0.7	0.7	0.7
100ppb	10/20/06	32	0.6	0.6	0.6
	10/22/06	34	0.46	0.5	0.47
	10/25/06	37	0.43	0.51	0.39
	10/27/06	39	0.56	0.57	0.55
	10/30/06	42	0.48	0.50	0.47
	11/01/06	44	0.47	0.51	0.47
200ppb	11/03/06	46	0.51	0.55	0.50
	11/06/06	49	0.52	0.55	0.50
	11/07/06	50	0.50	0.54	0.50
	11/10/06	53	0.47	0.52	0.46
	11/13/06	56	0.54	0.58	0.61
	11/15/06	58	0.42	0.47	0.46
300ppb	11/17/06	60	0.41	0.47	0.45
	11/20/06	63	0.50	0.52	0.50
	11/22/06	65	0.47	0.51	0.45
	11/25/06	68	0.50	0.56	0.55
	11/27/06	70	0.50	0.55	0.51
	11/29/06	72	0.44	0.50	0.49
400ppb	12/01/06	74	0.49	0.55	0.48
	12/04/06	77	0.46	0.53	0.50
	12/06/06	79	0.47	0.50	0.48
	12/08/06	81	0.42	0.43	0.43
	12/11/06	84	0.48	0.47	0.46

Table 12: Effluent NO_3-N concentrations (ppm) from copper added, control and pH control PVC reactors continued

Copper Dose	Date	days	Cupper added	Control	pH Control
600ppb	12/13/06	86	0.49	0.46	0.42
	12/17/06	90	0.46	0.44	0.40
	12/22/06	95	0.51	0.49	0.45
	12/25/06	98	0.49	0.44	0.45
800ppb	12/27/06	100	0.42	0.45	0.44
	12/29/06	102	0.51	0.56	0.49
	01/01/07	105	0.47	0.48	0.45
	01/03/07	107	0.42	0.46	0.45
	01/05/07	109	0.48	0.51	0.47
	01/08/07	112	0.47	0.50	0.48
1000ppb	01/10/07	114	0.50	0.51	0.48
	01/15/07	119	0.46	0.48	0.46
	01/17/07	121	0.41	0.43	0.43
	01/19/07	123	0.41	0.45	0.44
	01/22/07	126	0.41	0.43	0.43
1300ppb	01/27/07	131	0.25	0.40	0.38
	02/02/07	137	0.43	0.44	0.44
	02/05/07	140	0.83	0.45	0.79
	02/07/07	142	0.44	0.42	0.42
	02/09/07	144	0.40	0.42	0.38
	02/12/07	147	0.42	0.46	0.45
	02/14/07	149	0.37	0.46	0.43
	02/16/07	151	0.43	0.47	0.47
	02/19/07	154	0.42	0.46	0.44
	02/23/07	158	0.49	0.54	0.50
	02/26/07	161	0.49	0.52	0.48
	02/28/07	163	0.48	0.51	0.49
	03/02/07	165	0.48	0.54	0.51
	03/05/07	168	0.47	0.51	0.51

Table 12: Effluent NO_3-N concentrations (ppm) from copper added, control and pH control PVC reactors continued

pH	Date	days	Cupper added	Control	pH Control
7.8	03/07/07	170	0.46	0.49	0.50
	03/09/07	172	0.48	0.53	0.51
	03/12/07	175	0.47	0.53	0.50
	03/16/07	179	0.50	0.53	0.54
	03/19/07	182	0.49	0.55	0.55
7.5	03/21/07	184	0.51	0.59	0.52
	03/23/07	186	0.50	0.57	0.53
	03/26/07	189	0.49	0.57	0.53
	03/28/07	191	0.58	0.54	0.51
	03/30/07	193	0.53	0.57	0.53
	04/02/07	196	0.53	0.58	0.53
7.2	04/06/07	200	0.54	0.57	0.54
	04/09/07	203	0.56	0.58	0.55
	04/11/07	205	0.52	0.58	0.52
	04/16/07	210	0.54	0.57	0.53
	04/18/07	212	0.59	0.63	0.55
6.9	04/20/07	214	0.54	0.61	0.53
	04/22/07	216	0.60	0.73	0.60
	04/25/07	219	0.59	0.70	0.55
	04/27/07	221	0.61	0.75	0.60
	04/30/07	224	0.48	0.62	0.46
	05/02/07	226	0.50	0.69	0.48
6.6	05/04/07	228	0.50	0.75	0.49
	05/07/07	231	0.51	0.54	0.48
	05/11/07	235	0.52	0.56	0.55
	05/14/07	238	0.45	0.49	0.47
	05/16/07	240	0.47	0.52	0.49
	05/18/07	242	0.57	0.61	0.61

Table 13: Effluent NH_3-N concentrations (ppm) from chlorite and control PVC/copper reactors

Chlorite dose	Date	Days	ClO_2^- added PVC reactor	PVC control PVC reactor	ClO_2^- added Cu PVC	Cu control PVC
0.2	01/17/07	1	0.14	0.15	0.17	0.16
	01/19/07	3	0.18	0.17	0.21	0.19
	01/22/07	6	0.17	0.18	0.16	0.19
	01/29/07	13	0.17	0.18	0.18	0.19
	01/29/07	13	0.17	0.18	0.18	0.19
0.4	02/02/07	17	0.18	0.2	0.24	0.24
	02/05/07	20	0.2	0.19	0.19	0.2
	02/07/07	22	0.14	0.17	0.13	0.15
	02/09/07	24	0.18	0.17	0.18	0.2
	02/12/07	27	0.18	0.2	0.2	0.21
0.6	02/14/07	29	0.22	0.2	0.19	0.19
	02/16/07	31	0.18	0.21	0.17	0.18
	02/19/07	34	0.28	0.25	0.25	0.17
	02/21/07	36	0.2	0.21	0.21	0.16
	02/23/07	38	0.16	0.16	0.15	0.12
0.8	02/26/07	41	0.21	0.17	0.2	0.17
	02/28/07	43	0.18	0.17	0.19	0.17
	03/02/07	45	0.2	0.18	0.2	0.2
	03/05/07	48	0.22	0.2	0.21	0.21
	03/07/07	50	0.17	0.2	0.22	0.18
1	03/09/07	52	0.2	0.17	0.2	0.17
	03/12/07	55	0.18	0.16	0.19	0.16
	03/14/07	57	0.17	0.17	0.18	0.15
	03/16/07	59	0.5	0.22	0.15	0.15
	03/19/07	62	0.22	0.28	0.21	0.34
1.2	03/21/07	64	0.13	0.22	0.16	0.21
	03/23/07	66	0.19	0.17	0.19	0.18
	03/26/07	69	0.19	0.22	0.24	0.22
	03/30/07	73	0.16	0.18	0.2	0.15
	04/02/07	76	0.2	0.22	0.22	0.19
2	04/04/07	78	0.18	0.21	0.19	0.15
	04/06/07	80	0.18	0.17	0.19	0.14
	04/09/07	83	0.28	0.25	0.33	0.44
	04/11/07	85	0.25	0.2	0.17	0.17
	04/13/07	87	0.19	0.22	0.22	0.22
	04/16/07	90	0.24	0.25	0.24	0.24
	04/18/07	92	0.19	0.2	0.18	0.18

Table 13: Effluent NH_3-N concentrations (ppm) from chlorite and control PVC/copper reactors continued

Chlorite dose	Date	Days	ClO_2^- added PVC reactor	PVC control PVC	ClO_2^- added Cu PVC reactor	Cu control PVC
2	04/20/07	94	0.25	0.21	0.21	0.18
20	04/22/07	96	0.49	0.16	0.23	0.17
	04/25/07	99	0.5	0.19	0.26	0.15
	04/27/07	101	0.4	0.15	0.29	0.19
	04/30/07	104	0.3	0.1	0.32	0.2
	05/02/07	106	0.26	0.15	0.42	0.17
	05/03/07	107	0.23	0.18	0.41	0.12
	05/04/07	108	0.21	0.16	0.42	0.2
	05/07/07	111	0.2	0.16	0.47	0.22
	05/11/07	115	0.2	0.12	0.48	0.13
	05/14/07	118	0.25	0.2	0.6	0.13
	05/16/07	120	0.18	0.2	0.6	0.14
	05/18/07	122	0.23	0.17	0.66	0.13
	05/21/07	125	0.25	0.2	0.45	0.17
Chlorite discontinued	05/23/07	127	0.26	0.18	0.65	0.3
	05/25/07	129	0.28	0.24	0.67	0.2
	05/28/07	132	0.13	0.1	0.7	0.11
	05/30/07	134	0.1	0.1	0.65	0.1
	06/01/07	136	0.12	0.1	0.6	0.14
	06/04/07	139	0.2	0.18	0.58	0.18
	06/06/07	141	0.18	0.15	0.58	0.1
	06/08/07	143	0.21	0.19	0.66	0.16
	06/11/07	146	0.14	0.13	0.65	0.16
	06/13/07	148	0.09	0.08	0.55	0.11
	06/15/07	150	0.1	0.08	0.59	0.15
	06/18/07	153	0.14	0.12	0.63	0.15
	06/20/07	155	0.19	0.18	0.61	0.15
	06/22/07	157	0.13	0.14	0.53	0.09
	06/25/07	160	0.2	0.11	0.49	0.09
	06/27/07	162	0.1	0.1	0.49	0.1
	06/29/07	164	0.09	0.09	0.4	0.09
	07/02/07	167	0.07	0.05	0.25	0.08
	07/06/07	171	0.21	0.13	0.29	0.22
	07/09/07	174	0.23	0.2	0.2	0.14
	07/11/07	176	0.17	0.19	0.21	0.15
	07/13/07	178	0.18	0.13	0.19	0.13
	07/16/07	181	0.14	0.13	0.2	0.15

Table 14: Effluent NO_2-N concentrations (ppm) from chlorite and control PVC/copper reactors

Chlorite dose	Date	Days	ClO2 Added PVC	PVC Control	ClO2 Added Cu	Cu Control
0.2	01/17/07	1	0.001	0.003	0.001	0.003
	01/19/07	3	0.002	0.002	0.002	0.002
	01/22/07	6	0.004	0.003	0.004	0.005
	01/29/07	13	0.002	0.002	0.004	0.003
0.4	02/02/07	17	0.003	0.003	0.004	0.002
	02/05/07	20	0.003	0.001	0.001	0.002
	02/07/07	22	0.001	0.002	0.002	0.001
	02/09/07	24	0.005	0.005	0.005	0.005
	02/12/07	27	0.001	0.003	0.003	0.002
0.6	02/14/07	29	0.002	0.001	0.004	0.002
	02/16/07	31	0.005	0.003	0.008	0.004
	02/19/07	34	0.004	0.002	0.005	0.004
	02/21/07	36	0.002	0.001	0.003	0.005
	02/23/07	38	0.005	0.004	0.004	0.006
0.8	02/26/07	41	0.005	0.005	0.005	0.006
	02/28/07	43	0.004	0.002	0.002	0.002
	03/02/07	45	0.002	0.002	0.004	0.001
	03/05/07	48	0.004	0.003	0.003	0.004
	03/07/07	50	0.002	0.003	0.004	0.002
1	03/09/07	52	0.005	0.003	0.004	0.006
	03/12/07	55	0.004	0.003	0.005	0.007
	03/14/07	57	0.004	0.002	0.004	0.005
	03/16/07	59	0.004	0.003	0.002	0.003
	03/19/07	62	0.004	0.004	0.004	0.004
1.2	03/23/07	66	0.004	0.003	0.007	0.007
	03/30/07	73	0.003	0.002	0.004	0.004
	04/02/07	76	0.004	0.003	0.005	0.005
	04/04/07	78	0.002	0.002	0	0.003
	04/09/07	83	0.004	0.002	0.002	0.006
2	04/11/07	85	0.005	0.002	0.003	0.004
	04/16/07	90	0.003	0.002	0.005	0.004
	04/18/07	92	0.003	0	0.005	0.008
	04/20/07	94	0.02	0.001	0.013	0.004
	04/22/07	96	0.041	0.002	0.018	0.004
	04/25/07	99	0.024	0.002	0.012	0.009
	04/30/07	104	0.028	0.002	0.011	0.006
	05/02/07	106	0.029	0.004	0.009	0.005

Table 14: Effluent NO_2-N concentrations (ppm) from chlorite and control PVC/copper reactors continued

Chlorite dose	Date	Days	ClO2 Added PVC	PVC Control	ClO2 Added Cu	Cu Control
20 ppm	05/04/07	108	0.02	0.003	0.01	0.005
	05/14/07	118	0.029	0.005	0.004	0.005
	05/16/07	120	0.03	0.006	0.001	0.002
	05/18/07	122	0.033	0.003	0.005	0.004
	05/21/07	125	0.033	0.003	0.002	0.002
	05/23/07	127	0.014	0.003	0.003	0.003
	05/25/07	129	0.011	0.003	0.003	0.004
	05/28/07	132	0.014	0.003	0.006	0.005
Chlorite discontinued	05/30/07	134	0.017	0.004	0.003	0.005
	06/01/07	136	0.016	0.007	0.007	0.006
	06/04/07	139	0.008	0.003	0.003	0
	06/06/07	141	0.008	0.003	0.003	0.003
	06/08/07	143	0.006	0.005	0.003	0.003
	06/11/07	146	0.006	0.006	0	0.006
	06/13/07	148	0.006	0.005	0.004	0.003
	06/15/07	150	0.007	0.006	0.003	0.003
	06/18/07	153	0.007	0.007	0.003	0.002
	06/20/07	155	0.004	0.004	0.004	0.002
	06/22/07	157	0.004	0.005	0.004	0.004
	06/25/07	160	0.005	0.005	0.004	0.003
	06/27/07	162	0.005	0.005	0.004	0.003
	06/29/07	164	0.006	0.006	0.003	0.003
	07/02/07	167	0.007	0.008	0.003	0.002
	07/06/07	171	0.012	0.012	0.003	0.002
	07/09/07	174	0.005	0.005	0.003	0.004
	07/11/07	176	0.005	0.004	0.003	0.003
	07/13/07	178	0.005	0.004	0.003	0.002
	07/16/07	181	0.005	0.006	0.003	0.003

Table 15: Effluent NO_3-N concentrations (ppm) from chlorite and control PVC/copper reactors

Chlorite dose ppm	Date	Days	ClO2 Added PVC	PVC Control	ClO2 Added Cu	Cu Control
0.2	01/17/07	1	0.52	0.48	0.51	0.48
	01/22/07	6	0.44	0.43	0.44	0.42
	01/27/07	11	0.45	0.45	0.42	0.40
0.4	02/02/07	17	0.46	0.43	0.43	0.43
	02/05/07	20	0.37	0.40	0.40	0.38
	02/07/07	22	0.45	0.44	0.44	0.42
	02/09/07	24	0.51	0.45	0.84	0.78
	02/12/07	27	0.44	0.42	0.42	0.42
0.6	02/14/07	29	0.37	0.42	0.39	0.37
	02/16/07	31	0.44	0.46	0.42	0.41
	02/19/07	34	0.42	0.46	0.37	0.37
	02/23/07	38	0.48	0.47	0.45	0.41
0.8	02/26/07	41	0.47	0.46	0.46	0.45
	02/28/07	43	0.54	0.54	0.49	0.49
	03/02/07	45	0.53	0.52	0.51	0.50
	03/05/07	48	0.53	0.51	0.51	0.48
	03/07/07	50	0.58	0.54	0.53	0.52
1	03/09/07	52	0.54	0.51	0.48	0.47
	03/12/07	55	0.55	0.49	0.48	0.48
	03/16/07	59	0.55	0.53	0.52	0.53
	03/19/07	62	0.49	0.53	0.53	0.49
1.2	03/21/07	64	0.55	0.53	0.59	0.53
	03/23/07	66	0.61	0.55	0.56	0.56
	03/26/07	69	0.59	0.59	0.57	0.55
	03/28/07	71	0.60	0.57	0.52	0.54
	03/30/07	73	0.59	0.57	0.55	0.53
	04/02/07	76	0.58	0.54	0.54	0.50
2	04/04/07	78	0.60	0.57	0.56	0.51
	04/06/07	80	0.53	0.58	0.54	0.52
	04/09/07	83	0.55	0.54	0.50	0.45
	04/11/07	85	0.60	0.57	0.55	0.52
	04/16/07	90	0.59	0.58	0.55	0.55
	04/18/07	92	0.56	0.58	0.52	0.54
	04/20/07	94	0.57	0.57	0.54	0.53

Table 15 Effluent NO_3-N concentrations (ppm) from chlorite and control PVC/copper reactors continued

Chlorite dose ppm	Date	Days	ClO2 Added PVC	PVC Control	ClO2 Added Cu	Cu Control
20	04/22/07	96	0.35	0.63	0.53	0.60
	04/25/07	99	0.37	0.61	0.29	0.52
	04/27/07	101	0.40	0.65	0.30	0.55
	04/30/07	104	0.44	0.73	0.40	0.56
	05/02/07	106	0.50	0.70	0.32	0.58
	05/04/07	108	0.54	0.75	0.23	0.59
	05/07/07	111	0.51	0.62	0.24	0.49
	05/11/07	115	0.49	0.69	0.15	0.53
	05/14/07	118	0.49	0.72	0.11	0.54
	05/16/07	120	0.49	0.75	0.08	0.55
	05/18/07	122	0.50	0.54	0.06	0.52
	05/21/07	125	0.47	0.56	0.06	0.56
Chlorite discontinued	05/23/07	127	0.40	0.49	0.12	0.10
	05/25/07	129	0.42	0.52	0.40	0.11
	05/28/07	132	0.56	0.61	0.09	0.55
	05/30/07	134	0.56	0.62	0.00	0.55
	06/01/07	136	0.57	0.61	0.00	0.54
	06/04/07	139	0.47	0.54	0.03	0.50
	06/06/07	141	0.51	0.55	0.03	0.53
	06/08/07	143	0.51	0.56	0.03	0.53
	06/11/07	146	0.52	0.56	0.00	0.53
	06/13/07	148	0.56	0.59	0.03	0.54
	06/15/07	150	0.57	0.64	0.04	0.51
	06/18/07	153	0.63	0.70	0.05	0.52
	06/20/07	155	0.47	0.53	0.04	0.50
	06/22/07	157	0.49	0.53	0.06	0.51
	06/25/07	160	0.43	0.51	0.08	0.46
	06/27/07	162	0.52	0.57	0.12	0.50
	06/29/07	164	0.56	0.60	0.20	0.53
	07/02/07	167	0.58	0.63	0.35	0.51
	07/06/07	171	0.57	0.63	1.00	0.56
	07/09/07	174	0.56	0.55	0.57	0.64
	07/11/07	176	0.53	0.57	0.58	0.64
	07/13/07	178	0.56	0.60	0.60	0.65
	07/16/07	181	0.60	0.63	0.59	0.66

Table 16: Effluent NH_3-N concentrations (ppm) from NH_2Cl added and control PVC/copper reactors

Cl_2 to NH_3-N	Date	days	NH_2Cl added PVC reactor	PVC control reactor	NH_2Cl added copper reactor	Copper control reactor
0.5:1	06/27/07	2	0.16	0.1	0.09	0.1
	06/29/07	4	0.15	0.09	0.1	0.09
0.04	07/02/07	7	0.08	0.05	0.06	0.08
	07/06/07	11	0.27	0.13	0.21	0.22
1.5:	07/09/07	14	0.22	0.2	0.14	0.14
	07/11/07	16	0.21	0.19	0.16	0.15
2:00	07/13/07	18	0.22	0.13	0.13	0.13
	07/16/07	21	0.19	0.13	0.15	0.15
5:01	07/18/07	23	0.22	0.18	0.14	0.13
	07/20/07	25	0.2	0.14	0.19	0.14
	07/23/07	28	0.19	0.15	0.13	0.15
	07/25/07	30	0.12	0.13	0.11	0.12
	07/27/07	32	0.14	0.15	0.27	0.1
	07/30/07	35	0.15	0.15	0.37	0.15
	08/01/07	37	0.09	0.12	0.33	0.11
	08/03/07	39	0.24	0.15	0.42	0.11
	08/06/07	42	0.28	0.21	0.36	0.13
	08/08/07	44	0.27	0.15	0.33	0.13
	08/10/07	46	0.25	0.2	0.32	0.17
	08/13/07	49	0.33	0.18	0.35	0.14
	08/15/07	51	0.36	0.16	0.43	0.17
	08/17/07	53	0.45	0.17	0.46	0.13
	08/20/07	56	0.37	0.13	0.43	0.08
	08/22/07	58	0.37	0.17	0.43	0.12
	08/24/07	60	0.42	0.19	0.46	0.15
	08/27/07	63	0.34	0.13	0.44	0.16
	08/29/07	65	0.4	0.2	0.55	0.17
	08/31/07	67	0.35	0.18	0.4	0.15
	09/04/07	71	0.36	0.12	0.37	0.14
	09/05/07	72	0.52	0.15	0.39	0.13
	09/07/07	74	0.32	0.18	0.4	0.18
	09/10/07	77	0.41	0.2	0.44	0.23
	09/12/07	79	0.54	0.21	0.63	0.24
	09/14/07	81	0.39	0.16	0.39	0.17

Table 16: Effluent NH_3-N concentrations (ppm) from NH_2Cl added and control PVC/copper reactors continued

Cl_2 to NH_3-N	Date	days	NH_2Cl added PVC reactor	PVC control reactor	NH_2Cl added copper reactor	Copper control reactor
NH_2Cl discontinued	09/17/07	84	0.64	0.17	0.51	0.14
	09/19/07	86	0.66	0.17	0.58	0.14
	09/21/07	88	0.64	0.15	0.5	0.16
	09/24/07	91	0.7	0.21	0.59	0.17
	09/26/07	93	0.63	0.13	0.49	0.14
	09/28/07	95	0.63	0.14	0.48	0.14
	10/01/07	98	0.53	0.14	0.33	0.18
	10/03/07	100	0.62	0.19	0.43	0.17
	10/05/07	102	0.62	0.19	0.43	0.17
	10/08/07	105	0.53	0.17	0.18	0.21
	10/10/07	107	0.6	0.17	0.16	0.13
	10/12/07	109	0.54	0.18	0.12	0.2
	10/15/07	112	0.56	0.16	0.12	0.16
	10/17/07	114	0.57	0.22	0.2	0.22
	10/19/07	116	0.56	0.24	0.22	0.18
	10/22/07	119	0.51	0.24	0.22	0.22
	10/24/07	121	0.53	0.16	0.2	0.19
	10/26/07	123	0.45	0.15	0.18	0.16
	10/29/07	126	0.28	0.13	0.1	0.18
	11/05/07	133	0.15	0.07	0.14	0.13
	11/07/07	135	0.18	0.16	0.21	0.17
	11/08/07	136	0.22	0.17	0.19	0.19
	11/12/07	140	0.2	0.13	0.09	0.11
	11/14/07	142	0.21	0.14	0.16	0.26
	11/16/07	144	0.21	0.13	0.11	0.13
	11/19/07	147	0.16	0.14	0.07	0.12

Table 17: Effluent NO_2-N concentrations (ppm) from NH_2Cl added and control PVC/copper reactors

Cl_2 to NH_3-N	Date	days	NH_2Cl added PVC reactor	PVC control reactor	NH_2Cl added copper reactor	Copper control reactor
0.5:1	06/27/07	2	0.004	0.005	0.004	0.003
	06/29/07	4	0.004	0.006	0.003	0.003
1:1	07/02/07	7	0.004	0.008	0.003	0.002
	07/06/07	11	0.003	0.012	0.003	0.002
1.5:1	07/09/07	14	0.003	0.005	0.003	0.004
	07/11/07	16	0.003	0.004	0.003	0.003
2:1	07/13/07	18	0.003	0.004	0.003	0.002
	07/16/07	21	0.004	0.006	0.003	0.003
5:1	07/18/07	23	0.004	0.006	0.002	0.003
	07/20/07	25	0.003	0.008	0.003	0.002
	07/23/07	28	0.004	0.006	0.004	0.006
	07/25/07	30	0.004	0.005	0.003	0.004
	07/27/07	32	0.008	0.005	0.003	0.002
	07/30/07	35	0.006	0.006	0.002	0.004
	08/01/07	37	0.009	0.006	0.003	0.004
	08/03/07	39	0.013	0.006	0.011	0.005
	08/06/07	42	0.013	0.007	0.02	0.009
	08/08/07	44	0.012	0.005	0.019	0.006
	08/10/07	46	0.013	0.004	0.025	0.006
	08/13/07	49	0.013	0.006	0.038	0.008
	08/15/07	51	0.02	0.008	0.043	0.007
	08/17/07	53	0.023	0.007	0.004	0.008
	08/20/07	56	0.02	0.008	0.005	0.009
	08/22/07	58	0.018	0.009	0.005	0.008
	08/24/07	60	0.022	0.01	0.007	0.008
	08/27/07	63	0.015	0.005	0.008	0.003
	08/29/07	65	0.014	0.004	0.012	0.005
	08/31/07	67	0.017	0.003	0.002	0.004
	09/04/07	71	0.011	0.003	0.002	0.003
	09/05/07	72	0.009	0.004	0.005	0.005
	09/07/07	74	0.016	0.004	0.004	0.005
	09/10/07	77	0.014	0.004	0.001	0.005
	09/12/07	79	0.018	0.004	0.004	0.005
	09/14/07	81	0.013	0.004	0.003	0.004

Table 17: Effluent NO_2-N concentrations (ppm) from NH_2Cl added and control PVC/copper reactors continued

Cl_2 to NH_3-N	Date	days	NH_2Cl added PVC reactor	PVC control reactor	NH_2Cl added copper reactor	Copper control reactor
NH_2Cl discontinued	09/17/07	84	0.005	0.004	0.001	0.002
	09/19/07	86	0.005	0.004	0.003	0.004
	09/21/07	88	0.004	0.004	0.004	0.004
	09/24/07	91	0.002	0.004	0.003	0.003
	09/26/07	93	0.002	0.004	0.004	0.004
	09/28/07	95	0.004	0.005	0.004	0.004
	10/01/07	98	0.003	0.005	0.003	0.004
	10/03/07	100	0.002	0.004	0.004	0.003
	10/05/07	102	0.003	0.004	0.003	0.003
	10/08/07	105	0	0.004	0.003	0.001
	10/10/07	107	0.003	0.004	0.002	0.003
	10/12/07	109	0.004	0.004	0.005	0.003
	10/15/07	112	0.003	0.005	0.003	0.003
	10/17/07	114	0.002	0.004	0.004	0.003
	10/19/07	116	0.004	0.004	0.004	0.004
	10/22/07	119	0.003	0.005	0.006	0.003
	10/24/07	121	0.002	0.005	0.005	0.002
	10/26/07	123	0.003	0.005	0.005	0.003
	10/29/07	126	0.004	0.005	0.004	0.003
	11/05/07	133	0.003	0.006	0.004	0.004
	11/07/07	135	0.002	0.003	0	0.001
	11/08/07	136	0.001	0.002	0	0.001
	11/12/07	140	0.003	0.007	0	0.005
	11/14/07	142	0.003	0.005	0	0.008
	11/16/17	144	0.004	0.007	0	0.006
	11/19/07	147	0.004	0.003	0	0.003

Table 18: Effluent NO_3-N concentrations (ppm) from NH_2Cl added and control PVC/copper reactors

Cl_2 to NH_3-N	Date	days	NH_2Cl added PVC reactor	PVC control reactor	NH_2Cl added copper reactor	Copper control reactor
0.5:1	06/27/07	2	0.50	0.57	0.12	0.50
	06/29/07	4	0.51	0.60	0.20	0.53
1:1	07/02/07	7	0.54	0.63	0.35	0.51
	07/06/07	11	0.51	0.71	1.00	0.56
1.5:1	07/09/07	14	0.53	0.72	0.57	0.64
	07/11/07	16	0.50	0.57	0.58	0.64
2:1	07/13/07	18	0.56	0.60	0.60	0.65
	07/16/07	21	0.56	0.63	0.59	0.66
5:1	07/18/07	23	0.61	0.71	0.59	0.64
	07/20/07	25	0.60	0.75	0.59	0.64
	07/23/07	28	0.58	0.71	0.58	0.63
	07/25/07	30	0.57	0.69	0.64	0.69
	07/27/07	32	0.67	0.68	0.60	0.64
	07/30/07	35	0.46	0.65	0.49	0.53
	08/01/07	37	0.39	0.65	1.46	0.51
	08/03/07	39	0.39	0.65	1.48	0.51
	08/06/07	42	0.39	0.65	1.48	0.51
	08/08/07	44	0.39	0.65	1.48	0.51
	08/10/07	46	0.39	0.65	1.48	0.51
	08/13/07	49	0.39	0.65	1.48	0.51
	08/15/07	51	0.39	0.65	1.48	0.51
	08/17/07	53	0.39	0.65	1.48	0.51
	08/20/07	56	0.09	0.63	1.28	0.65
	08/22/07	58	0.09	0.71	0.98	0.73
	08/24/07	60	0.10	0.72	1.30	0.70
	08/27/07	63	0.10	0.54	1.34	0.54
	08/29/07	65	0.10	0.53	1.34	0.54

Table 18: Effluent NO_3-N concentrations (ppm) from NH_2Cl added and control PVC/copper reactors continued

Cl_2 to NH_3-N	Date	days	NH_2Cl added PVC reactor	PVC control reactor	NH_2Cl added copper reactor	Copper control reactor
5:1	08/31/07	67	0.09	0.53	1.21	0.54
	09/04/07	71	0.09	0.53	1.23	0.53
	09/05/07	72	0.10	0.52	1.25	0.53
	09/07/07	74	0.12	0.55	1.17	0.53
	09/10/07	77	0.10	0.54	1.19	0.53
	09/12/07	79	0.12	0.56	1.30	0.57
	09/14/07	81	0.08	0.53	1.19	0.52
NH_2Cl discontinued	09/17/07	84	0.08	0.52	1.07	0.49
	09/19/07	86	0.06	0.50	1.07	0.50
	09/21/07	88	0.06	0.51	1.07	0.48
	09/24/07	91	0.06	0.50	1.07	0.51
	09/26/07	93	0.07	0.50	1.05	0.51
	09/28/07	95	0.06	0.50	1.12	0.51
	10/01/07	98	0.05	0.51	1.17	0.53
	10/03/07	100	0.06	0.51	1.13	0.52
	10/05/07	102	0.06	0.54	1.18	0.51
	10/08/07	105	0.06	0.50	1.27	0.52
	10/10/07	107	0.07	0.54	1.19	0.55
	10/12/07	109	0.08	0.54	1.37	0.54
	10/15/07	112	0.07	0.53	1.15	0.52
	10/17/07	114	0.08	0.56	1.17	0.54
	10/19/07	116	0.11	0.54	1.20	0.55
	10/22/07	119	0.13	0.53	1.18	0.53
	10/24/07	121	0.18	0.53	1.20	0.53
	10/26/07	123	0.24	0.53	1.18	0.53
	10/29/07	126	0.35	0.52	1.20	0.52
	11/05/07	133	0.45	0.54	1.11	0.48

Table 19: Effluent NH_3-N concentrations (ppm) from different PVC/copper reactors operated under different nutrient conditions

Nutrient Conditions		2XC-3XN[1]		1XC-3XN[2]		1XC-1XN[3]	
date	Days	PVC reactor	Copper reactor	PVC reactor	Copper reactor	PVC reactor	Copper reactor
07/20/07	-12	0.17	0.19	0.20	0.18	0.14	0.14
07/23/07	-9	0.17	0.19	0.19	0.18	0.15	0.15
07/25/07	-7	0.14	0.16	0.19	0.14	0.13	0.12
07/27/07	-5	0.16	0.16	0.19	0.14	0.15	0.10
07/30/07	-2	0.17	0.20	0.18	0.18	0.15	0.15
08/01/07	0	0.10	0.17	0.13	0.16	0.12	0.11
08/03/07	2	0.60	0.71	0.64	0.65	0.15	0.11
08/06/07	5	0.68	0.71	0.72	0.72	0.21	0.13
08/08/07	7	0.56	0.62	0.55	0.58	0.15	0.13
08/10/07	9	0.61	0.74	0.65	0.69	0.20	0.17
08/13/07	12	0.59	0.65	0.61	0.63	0.18	0.14
08/15/07	14	0.59	0.64	0.64	0.58	0.16	0.17
08/17/07	16	0.65	0.77	0.65	0.73	0.17	0.13
08/20/07	19	0.61	0.74	0.63	0.72	0.13	0.08
08/22/07	21	0.61	0.73	0.62	0.71	0.17	0.12
08/24/07	23	0.60	0.74	0.63	0.75	0.19	0.15
08/27/07	26	0.58	0.71	0.56	0.71	0.13	0.16
08/29/07	28	0.62	0.70	0.65	0.84	0.20	0.17
08/31/07	30	0.71	0.83	0.65	0.81	0.18	0.15
09/04/07	34	0.67	0.89	0.59	0.85	0.12	0.14
09/05/07	35	0.57	0.67	0.58	0.70	0.15	0.13
09/07/07	37	0.61	0.70	0.58	0.77	0.18	0.18
09/10/07	40	0.64	0.85	0.65	0.97	0.20	0.23
09/12/07	42	0.63	0.80	0.62	0.85	0.21	0.24
09/14/07	44	0.64	0.72	0.65	0.82	0.16	0.17
09/17/07	47	0.63	0.77	0.64	0.86	0.17	0.14
09/19/07	49	0.68	0.72	0.68	0.93	0.17	0.14
09/21/07	51	0.60	0.76	0.65	0.85	0.15	0.16
09/24/07	54	0.67	0.81	0.64	0.92	0.21	0.17
09/26/07	56	0.59	0.89	0.60	0.98	0.13	0.14
09/28/07	58	0.53	0.70	0.63	0.79	0.14	0.14
10/01/07	61	0.51	0.67	0.53	0.71	0.14	0.18
10/03/07	63	0.70	0.77	0.59	0.83	0.19	0.17
10/05/07	65	0.70	0.77	0.59	0.83	0.19	0.17
10/08/07	68	0.39	0.52	0.53	0.56	0.17	0.21

1: 2XC-3XN: influent NH3-N 2.13ppm and TOC=8ppm; 2:1XC-3XN: influent NH3-N 2.13ppm and TOC=4ppm; 3:1XC-1XN-control: influent NH3-N 0.71ppm and TOC=4ppm

Table 19: Effluent NH_3-N concentrations (ppm) from different PVC/copper reactors operated under different nutrient conditions continued

Nutrient Conditions		2XC-3XN[1]		1XC-3XN[2]		1XC-1XN[3]	
date	Days	PVC reactor	Copper reactor	PVC reactor	Copper reactor	PVC reactor	Copper reactor
10/10/07	70	0.58	0.67	0.64	0.79	0.17	0.13
10/12/07	72	0.52	0.59	0.56	0.75	0.18	0.20
10/15/07	75	0.55	0.69	0.56	0.73	0.16	0.16
10/17/07	77	0.57	0.68	0.60	0.74	0.22	0.22
10/19/07	79	0.55	0.71	0.57	0.71	0.21	0.18
10/22/07	82	0.53	0.69	0.61	0.71	0.19	0.22
10/24/07	84	0.56	0.67	0.60	0.74	0.16	0.19

1: 2XC-3XN: influent NH3-N 2.13ppm and TOC=8ppm; 2:1XC-3XN: influent NH3-N 2.13ppm and TOC=4ppm; 3:1XC-1XN-control: influent NH3-N 0.71ppm and TOC=4ppm

Table 20: Effluent NO_2-N concentrations (ppm) from different PVC/copper reactors operated under different nutrient conditions

Nutrient conditions		2XC-3XN[1]		1XC-3XN[2]		1XC-1XN[3]	
Date	Days	PVC reactor	Copper reactor	PVC reactor	Copper reactor	PVC reactor	Copper reactor
07/20/07	-12	0.007	0.003	0.006	0.003	0.008	0.002
07/23/07	-9	0.006	0.003	0.005	0.004	0.006	0.006
07/25/07	-7	0.004	0.002	0.003	0.003	0.005	0.004
07/27/07	-5	0.005	0.002	0.003	0.003	0.005	0.002
07/30/07	-2	0.005	0.003	0.005	0.002	0.006	0.004
08/01/07	0	0.006	0.003	0.005	0.003	0.006	0.004
08/03/07	2	0.013	0.09	0.09	0.011	0.06	0.05
08/06/07	5	0.02	0.025	0.016	0.02	0.007	0.009
08/08/07	7	0.02	0.018	0.017	0.019	0.005	0.006
08/10/07	9	0.024	0.023	0.021	0.025	0.004	0.006
08/13/07	12	0.037	0.042	0.037	0.038	0.006	0.008
08/15/07	14	0.021	0.037	0.017	0.043	0.008	0.007
08/17/07	16	0.007	0.004	0.006	0.004	0.007	0.008
08/20/07	19	0.011	0.006	0.008	0.005	0.008	0.009
08/22/07	21	0.009	0.005	0.008	0.005	0.009	0.008
08/24/07	23	0.015	0.016	0.01	0.007	0.01	0.008
08/27/07	26	0.022	0.013	0.016	0.008	0.005	0.003
08/29/07	28	0.023	0.011	0.015	0.012	0.004	0.005
08/31/07	30	0.007	0.002	0.004	0.002	0.003	0.004

Table 20: Effluent NO_2-N concentrations (ppm) from different PVC/copper reactors operated under different nutrient conditions

Nutrient conditions		2XC-3XN[1]		1XC-3XN[2]		1XC-1XN[3]	
Date	Days	PVC reactor	Copper reactor	PVC reactor	Copper reactor	PVC reactor	Copper reactor
09/04/07	34	0.016	0.003	0.005	0.002	0.003	0.003
09/05/07	35	0.015	0.004	0.006	0.005	0.004	0.005
09/07/07	37	0.009	0.002	0.004	0.004	0.004	0.005
09/10/07	40	0.008	0.003	0.006	0.001	0.004	0.005
09/12/07	42	0.006	0.004	0.005	0.004	0.004	0.005
09/14/07	44	0.013	0.003	0.006	0.003	0.004	0.004
09/17/07	47	0.01	0.002	0.004	0.001	0.004	0.002
09/19/07	49	0.007	0.003	0.004	0.003	0.004	0.004
09/21/07	51	0.011	0.003	0.006	0.004	0.004	0.004
09/24/07	54	0.003	0.002	0.006	0.003	0.004	0.003
09/26/07	56	0.014	0.002	0.004	0.004	0.004	0.004
09/28/07	58	0.008	0.001	0.006	0.004	0.005	0.004
10/01/07	61	0.008	0.003	0.006	0.003	0.005	0.004
10/03/07	63	0.009	0.002	0.006	0.004	0.004	0.003
10/05/07	65	0.007	0.002	0.006	0.003	0.004	0.003
10/08/07	68	0.006	0.003	0.006	0.003	0.004	0.001
10/10/07	70	0.007	0.002	0.007	0.002	0.004	0.003
10/12/07	72	0.006	0.002	0.006	0.005	0.004	0.003
10/15/07	75	0.008	0.002	0.007	0.003	0.005	0.003
10/17/07	77	0.01	0.002	0.009	0.004	0.004	0.003
10/19/07	79	0.009	0.001	0.01	0.004	0.004	0.004
10/22/07	82	0.006	0.001	0.008	0.006	0.005	0.003
10/24/07	84	0.009	0.002	0.009	0.005	0.005	0.002

1: 2XC-3XN: influent NH3-N 2.13ppm and TOC=8ppm; 2:1XC-3XN: influent NH3-N 2.13ppm and TOC=4ppm; 3:1XC-1XN-control: influent NH3-N 0.71ppm and TOC=4ppm

Table 21: Effluent NO_3-N concentrations (ppm) from different PVC/copper reactors operated under different nutrient

Nutrient conditions		2XC-3XN[1]		1XC-3XN[2]		1XC-1XN[3]	
date	Days	PVC reactor	Copper reactor	PVC reactor	Copper reactor	PVC reactor	Copper reactor
07/20/07	-12	0.87	0.60	0.83	0.59	0.90	0.64
07/23/07	-9	0.81	0.60	0.77	0.58	0.83	0.63
07/25/07	-7	0.63	0.61	0.63	0.64	0.69	0.69
07/27/07	-5	0.66	0.58	0.62	0.60	0.68	0.64
07/30/07	-2	0.54	0.50	0.53	0.49	0.56	0.53
08/01/07	0	1.62	1.49	1.60	1.46	0.47	0.51
08/03/07	2	1.57	1.15	1.25	1.21	0.45	0.51
08/06/07	5	1.20	1.14	1.28	1.15	0.47	0.51
08/08/07	7	1.57	1.13	1.27	1.14	0.47	0.51
08/10/07	9	1.25	1.15	1.25	1.18	0.47	0.51
08/13/07	12	1.57	1.28	1.29	1.08	0.47	0.51
8/15/2007	14	1.57	1.27	1.3	1.1	0.47	0.51
8/17/2007	16	1.23	1.09	1.29	1.12	0.47	0.51
08/20/07	19	1.50	1.05	1.28	1.28	0.63	0.65
08/22/07	21	1.51	1.04	1.28	0.98	0.71	0.73
08/24/07	23	1.46	1.05	1.29	1.21	0.72	0.70
08/27/07	26	1.50	1.05	1.31	1.14	0.54	0.54
08/29/07	28	1.23	1.06	1.29	1.04	0.53	0.54
08/31/07	30	1.19	1.05	1.32	1.17	0.53	0.54
09/04/07	34	1.19	1.01	1.35	1.19	0.53	0.53
09/05/07	35	1.38	1.02	1.38	1.19	0.52	0.53
09/07/07	37	1.27	1.05	1.29	1.17	0.55	0.53
09/10/07	40	1.26	1.04	1.27	1.19	0.54	0.53
09/12/07	42	1.62	1.05	1.31	1.05	0.56	0.57
09/14/07	44	1.23	1.04	1.32	1.19	0.53	0.52
09/17/07	47	1.26	1.06	1.31	1.07	0.52	0.49
09/19/07	49	1.19	1.07	1.29	1.07	0.50	0.50
09/21/07	51	1.38	1.51	1.28	1.07	0.51	0.48
09/24/07	54	1.21	1.04	1.30	1.07	0.50	0.51
09/26/07	56	1.38	1.16	1.31	1.05	0.50	0.51
09/28/07	58	1.43	1.25	1.32	1.05	0.50	0.51
10/01/07	61	1.46	1.21	1.30	1.07	0.51	0.53
10/03/07	63	1.210	1.250	1.290	1.130	0.514	0.521

1: 2XC-3XN: influent NH3-N 2.13ppm and TOC=8ppm; 2:1XC-3XN: influent NH3-N 2.13ppm and TOC=4ppm; 3:1XC-1XN-control: influent NH3-N 0.71ppm and TOC=4ppm

Table 21: Effluent NO_3-N concentrations (ppm) from different PVC/copper reactors operated under different nutrient

Nutrient conditions		2XC-3XN[1]		1XC-3XN[2]		1XC-1XN[3]	
date	Days	PVC reactor	Copper reactor	PVC reactor	Copper reactor	PVC reactor	Copper reactor
10/05/07	65	1.190	1.100	1.310	1.181	0.536	0.510
10/08/07	68	1.310	1.310	1.290	1.275	0.503	0.517
10/10/07	70	1.300	1.320	1.290	1.189	0.538	0.546
10/12/07	72	1.360	1.420	1.280	1.210	0.542	0.544
10/15/07	75	1.442	1.240	1.320	1.149	0.533	0.524
10/17/07	77	1.462	1.287	1.280	1.172	0.450	0.543
10/19/07	79	1.446	1.240	1.310	1.204	0.541	0.549
10/22/07	82	1.488	1.250	1.290	1.178	0.525	0.531
10/24/07	84	1.431	1.200	1.309	1.198	0.527	0.531
10/26/07	86	1.464	1.100	1.342	1.184	0.530	0.532

1: 2XC-3XN: influent NH3-N 2.13ppm and TOC=8ppm; 2:1XC-3XN: influent NH3-N 2.13ppm and TOC=4ppm; 3:1XC-1XN-control: influent NH3-N 0.71ppm and TOC=4ppm

Table 22: Effluent total copper and dissolved copper concentrations (ppm) from different copper reactors operated under different nutrient conditions

		Total copper			Dissolved copper		
date	Days	2XC-3XN[1]	1XC-3XN[2]	1XC-1XN[3]	2XC-3XN[1]	1XC-3XN[2]	1XC-1XN[3]
07/20/07	-12	0.65	0.82	0.76	0.57	0.72	0.69
07/23/07	-9	0.66	0.82	0.79	0.58	0.73	0.69
07/25/07	-7	0.68	0.48	0.46	0.56	0.41	0.39
07/27/07	-5	0.68	0.83	0.78	0.56	0.67	0.63
07/30/07	-2	0.68	0.88	0.82	0.56	0.7	0.64
08/01/07	0	0.65	0.85	0.79	0.53	0.7	0.62
08/03/07	2	0.9	0.94	0.79	0.8	0.76	0.62
08/06/07	5	0.93	0.94	0.74	0.81	0.85	0.64
08/08/07	7	0.92	1.04	0.75	0.91	0.94	0.64
08/10/07	9	0.95	1.06	0.74	0.82	0.96	0.63
08/13/07	12	0.82	1.08	0.77	0.71	0.98	0.67
08/15/07	14	0.94	1.06	0.74	0.85	0.94	0.67
08/17/07	16	0.93	1.08	0.76	0.85	0.99	0.66
08/20/07	19	0.94	1.12	0.75	0.85	1.03	0.66
08/22/07	21	0.93	1.15	0.73	0.88	1.05	0.67
08/24/07	23	0.98	1.14	0.76	0.89	1.03	0.66
08/27/07	26	0.96	1.11	0.72	0.89	1.05	0.67
08/29/07	28	0.92	1.11	0.74	0.84	1	0.65
08/31/07	30	0.97	1.12	0.74	0.86	0.97	0.64

Table 22: Effluent total copper and dissolved copper concentrations (ppm) from different copper reactors operated under different nutrient conditions continued

		Total copper			Dissolved copper		
date	Days	2XC-3XN[1]	1XC-3XN[2]	1XC-1XN[3]	2XC-3XN[1]	1XC-3XN[2]	1XC-1XN[3]
09/04/07	34	0.99	1.11	0.71	0.87	0.93	0.63
09/05/07	35	0.96	1.16	1.11	0.81	0.92	0.57
09/07/07	37	0.96	1.12	0.7	0.82	0.92	0.6
09/10/07	40	0.89	1.15	0.71	0.79	0.9	0.57
09/12/07	42	0.78	0.99	0.71	0.66	0.81	0.55
09/14/07	44	0.92	1.19	0.7	0.82	1.05	0.65
09/17/07	47	0.87	1.11	0.71	0.7	0.83	0.52
09/19/07	49	0.9	1.11	0.71	0.82	0.99	0.65
09/21/07	51	0.85	1.13	0.74	0.78	1.02	0.65
09/24/07	54	0.88	1.1	0.74	0.79	1	0.64
09/26/07	56	0.87	1.48	0.79	0.81	0.96	0.63
09/28/07	58	0.88	1.11	0.8	0.81	0.98	0.65
10/01/07	61	0.95	1.14	0.74	0.78	0.93	0.65
10/03/07	63	0.9	1.12	0.8	0.82	0.99	0.64
10/05/07	65	0.81	1.08	0.78	0.71	0.97	0.71
10/08/07	68	0.85	1.12	1.17	0.8	1.04	1.1
10/10/07	70	0.85	1.15	0.86	0.78	1	0.7
10/12/07	72	0.89	0.62	0.78	0.81	0.55	0.72
10/15/07	75	0.79	1.08	0.73	0.67	0.91	0.6
10/17/07	77	0.85	1.07	0.71	0.74	0.94	0.6
10/19/07	79	0.8	1.06	0.7	0.7	0.89	0.58
10/22/07	82	0.82	1.05	0.77	0.72	0.92	0.62
10/24/07	84	0.93	1.13	0.79	0.8	1	0.67

1: 2XC-3XN: influent NH3-N 2.13ppm and TOC=8ppm; 2:1XC-3XN: influent NH3-N 2.13ppm and TOC=4ppm; 3:1XC-1XN-control: influent NH3-N 0.71ppm and TOC=4ppm