NITRIFICATION IN PREMISE PLUMBING AND ITS EFFECT ON CORROSION AND WATER QUALITY DEGRADATION

Yan Zhang

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Marc Edwards, Chair
Andrea Dietrich
Nancy Love
Ann Stevens
Peter Vikesland

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ABSTRACT

Nitrification is increasingly of concern in US potable water systems, due to changes from chlorine to chloramine as a secondary disinfectant in order to comply with new regulations for disinfectant by-products. The ammonia that is released from the chloramine decay supports nitrification.

A comprehensive literature review systematically examined the complex inter-relationships between nitrification, materials corrosion and metals release. That analysis suggested that nitrification could accelerate decay of chloramine, enhance corrosion of water distribution system materials, and increase leaching of lead and copper to potable water under at least some circumstances. Moreover, that certain plumbing materials would inhibit nitrification, but that in other situations the plumbing materials would enhance nitrification.

Experiments verified that nitrification could affect the relative efficacy of chlorine versus chloramine in controlling heterotrophic bacteria in premise plumbing. Without nitrification, chloramine was always more persistent and effective than chlorine in controlling biofilms. But with nitrification and in pipe materials that are relatively non-reactive with chlorine, chloramine was much less persistent and less effective than chlorine. In materials that are reactive with chlorine such as iron pipes, the relative efficacy of chloramine versus chlorine depends on the relative rate of corrosion and rate of nitrification. High rates of corrosion and low rates of nitrification favor the use of chloramine versus free chlorine in controlling bacteria.

Plumbing materials had profound impacts on the incidence of nitrification in homes. Effects were due to toxicity (i.e., release of Cu^{2+}), recycling of nitrate back to ammonia substrate by reaction (zero-valent iron, lead or zinc materials), or release of nutrients that are essential to nitrification by leaching from concrete or other materials. As a general rule it was determined that concrete and iron materials encouraged growth of nitrifiers in certain oligotrophic waters, materials such as lead, PVC/plastic pipe, glass and surfaces of other materials were readily colonized by nitrifiers, and materials such as copper and brass were very toxic and relatively resistant to nitrifier colonization.
Dependent on circumstance, nitrification had no effect, increased or decreased aspects of materials corrosion. Nitrification markedly increased lead contamination of low alkalinity potable water by reducing the pH. In some cases nitrification dramatically decreased leaching of zinc to potable water from galvanized iron, because of lowered dissolved oxygen and reduced pH. Nitrification did not affect copper solubility in low alkalinity water, but is expected to increase copper solubility in higher alkalinity waters. Finally, nitrification in homes plumbed with PVC or plastics can drop the pH and increase leaching of lead from downstream brass materials in faucets. This can explain why some modern homes plumbed with PVC can have more lead in water when compared to homes plumbed with copper pipe.

Phosphate had profound impacts on the incidence of nitrification and resulting effects on water quality. While phosphate levels below about 5 ppb could strongly inhibit nitrification due to a nutrient limitation, nitrifiers can obtain sufficient phosphate from plastic, concrete, copper and iron pipe materials to meet nutritional needs. High levels of phosphate inhibitor can reduce the concentration of Cu$^{+2}$ ions and make nitrification more likely, but phosphate can also sometimes lower the corrosion rate and increase the stability of disinfectant and its efficacy in controlling nitrifiers. Phosphate plays a key role in determining where, when and if problems with nitrification will occur in a given water distribution system.

This work provides some new fundamental and practical insights to nitrification issues through a comprehensive literature review, lab experiments, solubility modeling and field studies. The results and practical tools developed can be used by utilities and consumers to predict nitrification events and resulting water quality problems, and to make rational decisions about practices such as inhibitor dosing, plumbing material selection and use of whole house filters.
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AUTHOR’S PREFERENCE

Each chapter of this dissertation is a separate manuscript that is formatted according to the specifications of the journal to which it was submitted or published. Chapter 1, “Nitrification in Drinking Water Systems”, has been published in Critical Reviews in Environmental Science and Technology, 39(3), 153-208. Chapter 2, “Effect of Ambient Nutrients and Metals on Nitrification Occurrence in Premise Plumbing”, will be submitted to Water Research. Chapter 3, Nitrification in Premise Plumbing: Role of Phosphate, pH and Pipe Corrosion” has been published in Environmental Science & Technology 42(12), 4280-4284. Chapter 4, “Lead Contamination of Potable Water Due to Nitrification”, has been published in Environmental Science & Technology, 43(6), 1890-1895. Chapter 5, “Accelerated Chloramine Decay and Microbial Growth Resulting from Nitrification in Premise Plumbing” will be submitted to Journal of American Water Works Association. Chapter 6, “Role of Nitrification in Corrosion of Galvanized iron, Copper and Concrete” will be submitted to Journal of American Water Works Association. Chapter 7, “Effect of Nitrification and GAC Filtration on Copper and Lead Leaching in Home Plumbing Systems” has been published in ASCE Journal of Environmental Engineering, 134(7), 521-530. Chapter 8, “Overview, Practical Synthesis and Key Conclusions” summarized the key findings of the dissertation and provided practical guide for water utilities to use this dissertation for nitrification problem diagnose and mitigation.

Although each chapter is a separate manuscript, this dissertation is a comprehensive study of nitrification in drinking water systems, particularly under premise plumbing conditions. Chapter 1 is a thorough review of nitrification in drinking water systems: factors affecting nitrification occurrence, nitrification impacts on water quality and corrosion, nitrification monitoring and control methods are emphasized. Chapter 2 investigated effects of significant trace micro and macro nutrients, and emphasizes the role of different pipe materials in modifying nutrient levels in nitrifier biofilms. Chapter 3 focused on the interaction of phosphorus, pH, and pipe corrosion and their impacts on nitrification. Chapter 4 investigated the effect of nitrification on lead release. Chapter 5 detailed the impact of nitrification on the relative efficacy of chlorine versus chloramine in controlling HPC in premise plumbing. Chapter 6 further addressed the impact of nitrification on the corrosion of other pipe materials.
including galvanized iron, copper and concrete. Chapter 7 is the investigation of nitrification and metal release under a simulated complex home plumbing system. Chapter 8 summarized the key findings of the dissertation and provided practical guide for water utilities to use this dissertation for nitrification problem diagnose and mitigation.
CHAPTER I: NITRIFICATION IN DRINKING WATER SYSTEMS
Yan Zhang, Nancy Love, and Marc Edwards

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ABSTRACT
Nitrification is increasingly a concern in United States potable water distribution systems. This paper reviews research on nitrification as it relates to the ammonia levels and unique environments present in potable water distribution systems. Factors affecting nitrification occurrence, nitrification impacts on water quality and corrosion, nitrification monitoring and control methods are emphasized. The potential role of nitrogen cycling via coupled microbial and electrochemical reactions is also described.
INTRODUCTION
The United States Environmental Protection Agency (USEPA) Stage 1 and Stage 2 Disinfectants and Disinfection By-Products Rule (D/DBPR) has been actively negotiated since 1992. The use of combined chlorine (e.g., chlorine with ammonia or chloramine) rather than free chlorine at water utilities is often the lowest cost means of complying with D/DBPR. About 30\% of surface water treatment plants currently use chloramines versus 20\% in 1990, and as many as 40-65\% of surface water treatment plants might ultimately use chloramines after implementation of Stage 2 (Seidel et al. 2005; USEPA 2005).

In addition to reduced formation of Disinfectant By-Products (DBP), use of chloramines produces less chlorinous and chloro-phenolic tastes and odors relative to chlorine (Feben 1935). Chloramines are also generally considered to be much more persistent in water than free chlorine (Neden et al. 1992; Norton and LeChevallier 1997). However, recent laboratory studies and practical utility experience have revealed that chloramines can sometimes decay as fast as chlorine (Powell 2004; Zhang 2005; Zhang and Edwards 2007). Chloramines are also a much less effective disinfectant when compared to the same concentration of free chlorine, but the D/DBPR was crafted to ensure "that the reduction of potential health hazards of DBPs does not compromise microbial protection" (Federal-Register 2006). The lower disinfectant efficacy of chloramines is partly offset by use of higher doses (compared to chlorine) and its greater stability (Neden et al. 1992; Norton and LeChevallier 1997).

The ammonia released during chloramine decay can trigger nitrification incidence and reduced pH or higher nitrite. Nitrification problems in drinking water systems and their link to chloramination were identified as early as the 1930’s (Feben 1935; Hulbert 1933). A recent telephone survey indicated that two thirds of medium and large US systems that chloraminate experience nitrification to some degree (Wilczak et al. 1996). In Australia, using most-probable-number (MPN) procedure, nitrifying bacteria were detected in 64\% of samples from five chloraminated water supplies and in 21\% of samples containing more than 5 mg/L of monochloramine (Cunliffe 1991).

Nitrification and nitrifying bacteria are also widespread in drinking water systems in Finland (boreal region). Fifteen drinking water distribution systems were tested for ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) using MPN and Nitrification Potential methods, both AOB and
NOB were detected at the end of the distribution systems in at least eleven systems including non-
disinfection and chlorinated systems (Lipponen et al. 2002).

**Nitrification and Physiology of Nitrifying Bacteria**

Nitrification is usually accomplished through a two-step microbiological process. The first step is the
oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB), also known as ammonia
oxidizers. The stoichiometry of this reaction, assuming a biomass yield of 0.2 mg biomass formed per
mg ammonia oxidized on a theoretical oxygen demand basis (Grady et al. 1999), is:

\[
\text{NH}_4^+ + 1.38 \text{O}_2 + 0.069 \text{CO}_2 + 0.0172 \text{HCO}_3^- \rightarrow 0.0172 \text{C}_5\text{H}_7\text{O}_2\text{N} + 0.983 \text{NO}_2^- + 0.966 \text{H}_2\text{O} + 1.97 \text{H}^+ (1)
\]

The second step in nitrification is the oxidation of nitrite to nitrate carried out by nitrite-oxidizing
bacteria (NOB), also known as nitrite oxidizers. The stoichiometry of this reaction, assuming a biomass
yield of 0.1 mg biomass formed per mg nitrite oxidized on a theoretical oxygen demand basis (Grady et
al. 1999), is:

\[
\text{NO}_2^- + 0.00875 \text{NH}_4^+ + 0.035\text{CO}_2 + 0.00875 \text{HCO}_3^- + 0.456 \text{O}_2 + 0.00875 \text{H}_2\text{O} \rightarrow 0.00875 \text{C}_5\text{H}_7\text{O}_2\text{N} + 1.0 \text{NO}_3^- (3)
\]

Ammonia oxidizers have been reclassified based on phylogenetic methods. AOB relevant to freshwater
or low salinity systems fall into the *Nitrosomonas* (including ‘*Nitrosococcus mobilis*’) and *Nitrosospira*
(including *Nitrosolobus* and *Nitrosovibrio*) lineages and are members of the β subclass of the
proteobacteria (Purkhold et al. 2003). The most thoroughly studied ammonia oxidizer is *Nitrosomonas
europaea*, because it has been available as a pure culture for more than 50 years (Engel and Alexander
1958; Lewis and Pramer 1958; Meiklejohn 1950). The rapid development of more reliable, culture-
independent methods has allowed researchers to identify which bacterial species are present in natural
and engineered systems, although generalizations as to which strains predominate in which systems have
not been successfully established (Geets et al. 2006). For example, while *Nitrosomonas europaea* has
been found to be the predominant AOB in some wastewater treatment biofilm systems (Gieske et al.
2003), *Nitrosomonas oligotropha* (Lydmark et al. 2006) and bacteria of the genus *Nitrosospira*
(Coskuner and Curtis 2002) have also been found to predominate in biofilm and activated sludge
bioreactors, respectively. Using culture independent analysis, Regan (Regan et al. 2003) found
Nitrosomonas oligotropha to predominate in chloraminated systems whereas Eichler (Eichler et al. 2006) found Nitrosospira briensis to predominate in chlorinated distribution system water. Although these cultures are of different genera, both are known to be among the AOB that have high affinity for ammonia, thereby enhancing their ability to survive under low ammonia environments (Bollmann et al. 2002; Bollmann et al. 2005).

Like all microbes, the growth of nitrifiers can be limited by nutrients, and the extent of this limitation is often described by the Monod equation:

\[ \mu = \frac{\mu_{\text{max}} S}{K_s + S}, \]

where \( \mu \) is the specific growth rate at substrate concentration \( S \), \( \mu_{\text{max}} \) is the maximum specific growth rate, and \( K_s \) is the half-saturation coefficient which is defined as the substrate concentration at which \( \mu \) is equal to half of \( \mu_{\text{max}} \). Lower \( K_s \) values reflect cultures that have a higher affinity for a given substrate. The half-saturation coefficient for AOB found in drinking water distribution systems, Nitrosomonas oligotropha and Nitrosospira briensis, are below 5 \( \mu\text{M} \) (<0.07 mg/L as N) (Bollmann et al. 2005; Koops and Pommerening-Roser 2001). In contrast, the \( K_S \) value for pure culture strains that are typically found in ammonium rich environments (such as wastewater treatment plants) are an order of magnitude higher (Koops and Pommerening-Roser 2001). Accordingly, the lower half-saturation constant of Nitrosomonas oligotropha and Nitrosospira briensis versus Nitrosomonas europaea might explain the relative dominance of these strains at the relatively low ammonium concentrations (between 0 to 1 mg/L as N) (Odell et al. 1996) often present in drinking water. Furthermore, one study by Gieseke (Gieseke et al. 2001) suggested that Nitrosomonas oligotropha might also have a higher affinity for oxygen than other Nitrosomonas species, which would enable it to better compete with heterotrophs for oxygen in multispecies biofilms such as those found in potable water systems. Nitrosomonas oligotropha was speculated to retain more ribosomes than Nitrosomonas europaea during inactivation (Gieseke et al. 2001), which might partly explain in situ detection of Nitrosomonas oligotropha rather than Nitrosomonas europaea by fluorescent in situ hybridization (FISH), though there is no evidence for this hypothesis yet (Gieseke et al. 2001).

The NOB are found in four phylogenetic groups, two of which are associated with marine environments (Koops and Pommerening-Roser 2001). Two non-marine genera of NOB have been widely reported.
The first genus of NOB is *Nitrobacter*, which belongs to the α-proteobacteria and was historically identified as the predominant NOB in many natural and engineered environments (Painter 1977). More recently, the other genus was identified through culture-independent methods, which showed that the phylogenetically distinct *Nitrospira* are more common in nitrifying wastewater bioreactors (Burrell et al. 1998; Juretschko et al. 2002), freshwater aquaria (Hovanec et al. 1998) and in both pilot (Regan et al. 2002) and full-scale (Regan et al. 2003) drinking water distribution systems than previously thought. In the drinking water distribution systems studied by Regan and colleagues, *Nitrospira moscoviensis* was most prominent with few *Nitrobacter* detected. This is not surprising given that this strain was first isolated from a corroded iron pipe in a heating system (Ehrich et al. 1995). Recently, researchers (Daims et al. 2006; Maixner et al. 2006) showed that *Nitrospira* can be segregated into sublineages, and that sublineage II grows more effectively than sublineage I in environments with a low concentration of nitrite, further supporting the notion that subgroups of *Nitrospira* can tolerate and may actually prefer low nitrite growth environments, such as those found in drinking water distribution systems.

**FACTORS AFFECTING NITRIFICATION IN DRINKING WATER SYSTEMS**

In order for nitrifying bacteria to grow, certain physical factors and chemical substrates are required. These factors are likely to affect growth of nitrifying bacteria in drinking water distribution systems (Wolfe and Lieu 2001).

**Presence of Ammonia**

Nitrifying bacteria are chemolithotrophic bacteria with limited ability to utilize organic compounds. Ammonia, nitrite and (in some species) urea are the only exogenous energy source for nitrifying bacteria, although the presence of fructose can provide an energy benefit to *N. europaea* (Hommes et al. 2003).

In drinking water systems, ammonia can be present from the untreated drinking water, released by chloramines decay, or even formed in the distribution system from reactions between nitrate and metals (Table 1.1). The initial chlorine to ammonia ratio used to form chloramines affects the free ammonia levels at the start of the distribution system (Fleming et al. 2005). Excess free ammonia present at lower
ratios of chlorine to ammonia (< 4:1 mass ratio) tend to encourage nitrification (Karim and LeChevallier 2006; Skadsen 1993).

We note that abiotic corrosion reactions with distribution system pipe materials could serve to regenerate ammonium from nitrate at metal pipe surfaces. Complete conversion of nitrate to ammonium has been detected in a new galvanized steel pipe during stagnation (McIntyre and Mercer 1993). These reactions could result in coupled nitrogen cycling between abiotic (Reaction 10 and 11 in Table 1.1) and biotic reactions (equation 1 and 3).

\[
4 \text{Fe}^0 + \text{NO}_3^- + 10 \text{H}^+ = 4 \text{Fe}^{2+} + \text{NH}_4^+ + 3\text{H}_2\text{O} \tag{4}
\]

Consequently, even a small amount of ammonia or nitrate has the potential to support a relatively high level of nitrifying bacteria growth on iron or lead pipe. That is, reaction of nitrate with metallic pipes can create a continuous supply of ammonium for nitrifiers.

**Dissolved Oxygen (DO)**

Nitrifying bacteria are obligate aerobes. During nitrification, 4.33 mg O₂ is consumed for every mg-N of ammonia oxidized to nitrate according to stoichiometry (Grady et al. 1999).

The oxygen half saturation constant of *Nitrosomonas* and *Nitrobacter* is reported to lie between 0.3 and 1.3 mg/L including values established under both pure cultures and activated sludge conditions (Sharma and Ahlert 1977). Considering diffusion resistance, values of 0.5 and 0.68 mg/L have been adopted as typical for *Nitrosomonas* and *Nitrobacter*, respectively (Grady et al. 1999). The limiting dissolved oxygen concentration in activated sludge reactors for nitrifying bacteria ranges from 0.5 to 4 mg/L (Stenstrom and Song 1991) and 2 mg/L has been established as the limiting concentration in other studies (Painter 1977). These values can vary greatly depending on the culture purity, cell residence time and mass transfer resistance conditions (Stenstrom and Song 1991).

Drinking water distribution systems are generally well oxygenated and have sufficient oxygen for nitrifier growth in the bulk water. However, dead ends, stagnation and biofilms, and corrosion may create micro-anaerobic environments where oxygen could be limiting. Ammonia oxidizers may still survive at low DO as they are tolerant of low oxygen or anoxic environments (Kowalchuk and Stephen
They can also use nitrate or nitrite as an alternative electron acceptor when oxygen is low (Alleman and Preston 2005). This process is not thought to support cell growth, but it may provide enough energy to allow survival under anaerobic conditions (Kowalchuk and Stephen 2001).
Table 1.1: Important reactions influencing nitrogen in the distribution system

<table>
<thead>
<tr>
<th>Reaction Description</th>
<th>Overall Reaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Formation of chloramines</td>
<td>( \text{NH}_3 + \text{HOCl} \rightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O} )</td>
<td>Snoeyink and Jenkins 1980</td>
</tr>
<tr>
<td>2. Ammonium and nitrite utilization by nitrifiers</td>
<td>( \begin{align*} \text{NH}_4^+ + 1.9 \text{O}_2 + 0.069 \text{CO}_2 + 0.0172 \text{HCO}_3^- &amp; \rightarrow \ 0.0172 \text{C}_3\text{H}_7\text{O}_2\text{N} + 0.983 \text{NO}_2^- + 0.966 \text{H}_2\text{O} + \ 1.97 \text{H}^+ \end{align*} )</td>
<td>Grady et al. 1999</td>
</tr>
<tr>
<td></td>
<td>( \text{NO}_2^- + 0.00875 \text{NH}_4^+ + 0.035\text{CO}_2 + 0.00875 ) ( \text{HCO}_3^- + 0.456 \text{O}_2 + 0.00875 \text{H}_2\text{O} \rightarrow 0.00875 ) ( \text{C}_3\text{H}_7\text{O}_2\text{N} + 1.0 \text{NO}_3^- )</td>
<td>Grady et al. 1999</td>
</tr>
<tr>
<td>3. Formation of ammonia via chloramines decay</td>
<td>( 3\text{NH}_2\text{Cl} \rightarrow \text{N}_2 + \text{NH}_3 + 3\text{Cl}^- + 3\text{H}^+ )</td>
<td>Vikesland et al. 1998</td>
</tr>
<tr>
<td>4. Release of ammonia through oxidation of organic matter by chloramines</td>
<td>( \begin{align*} 1/10\text{C}_3\text{H}_7\text{O}_2\text{N} + \text{NH}_2\text{Cl} + 9/10 \text{H}_2\text{O} &amp; \rightarrow 4/10\text{CO}_2 + \ 1/10\text{HCO}_3^- + 11/10\text{NH}_4^+ + \text{Cl}^- \end{align*} )</td>
<td>Woolschlager et al. 2001</td>
</tr>
<tr>
<td>5. Release of ammonium through reaction of chloramines with corrosion products at pipe surfaces</td>
<td>( 1/2\text{NH}_2\text{Cl} + \text{H}^+ + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + 1/2\text{NH}_4^+ + 1/2 \text{Cl}^- )</td>
<td>Vikesland and Valentine 2000</td>
</tr>
<tr>
<td>6. Release of ammonia through oxidation of nitrite by chloramines</td>
<td>( \text{NH}_2\text{Cl} + \text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{NO}_3^- + \text{HCl} )</td>
<td>Valentine 1984</td>
</tr>
<tr>
<td>7. Breakpoint reactions with free chlorine</td>
<td>( 2\text{NH}_2\text{Cl} + \text{HOCl} \rightarrow \text{N}_2 + \text{H}_2\text{O} + 3\text{HCl} )</td>
<td>Snoeyink and Jenkins 1980</td>
</tr>
<tr>
<td>8. Direct metabolism by ammonia-oxidizing bacteria</td>
<td>( \text{NH}_2\text{Cl} + \text{O}_2 \rightarrow \text{NO}_2 + 2\text{H}^+ + \text{Cl}^- )</td>
<td>Woolschlager et al. 2001</td>
</tr>
<tr>
<td>9. Ammonia oxidation by anaerobic bacteria</td>
<td>( \text{NH}_3 + \text{NO}_2^- \rightarrow \text{N}_2 + \text{H}_2\text{O} )</td>
<td>Jetten et al. 1997</td>
</tr>
<tr>
<td>10. Cycling of ammonia through corrosion with metallic lead</td>
<td>( \text{Pb} + \text{NO}_3^- \rightarrow \text{NO}_2^- + \text{PbO} )</td>
<td>Uchida and Okuwaki 1998</td>
</tr>
<tr>
<td></td>
<td>( 3\text{Pb} + 2\text{H}_2\text{O} + \text{NO}_2^- \rightarrow \text{NH}_3 + 3\text{PbO} + \text{OH}^- )</td>
<td>Uchida and Okuwaki 1998</td>
</tr>
<tr>
<td></td>
<td>( 3\text{Pb} + \text{H}_2\text{O} + 2\text{NO}_2^- \rightarrow \text{N}_2 + 3\text{PbO} + 2\text{OH}^- )</td>
<td>Uchida and Okuwaki 1998</td>
</tr>
<tr>
<td>11. Cycling of ammonia through corrosion with metallic iron</td>
<td>( 4\text{Fe}^{2+} + \text{NO}_3^- + 10 \text{H}^+ = 4\text{Fe}^{2+} + \text{NH}_4^+ + 3\text{H}_2\text{O} )</td>
<td>Huang and Zhang 2005</td>
</tr>
</tbody>
</table>

**Cell Attachment**

In drinking water systems, most microbes are attached to pipe surfaces in film/slime layers on the pipe. This layer is often referred to as a biofilm, but this living layer is also associated with and integrated into inorganic layers present on the pipe termed “scale.” The inorganic materials can include characteristic
rust layers from the underlying material as well as deposits/precipitates from the water. Nitrifiers can exist as free living cells or attach to a surface as a biofilm and they have been repeatedly shown to prefer surfaces under many circumstances. Based on modeling results, Furumai and Rittman suggested that growth in the biofilm is advantageous because it prevents detachment loss (Furumai and Rittmann 1994); it is also possible that biofilm growth makes the cells less susceptible to inactivation by disinfectants. Attached nitrifiers in biofilms are more active than free living cells and more resistant to substrate limitation (Keen and Prosser 1987; Stein and Arp 1998). Biofilms and aggregates were also reported to facilitate nitrification outside the optimal pH ranges for growth (Tarre and Green 2004).

The presence of nitrifiers in biofilms helps explain the persistence of nitrifiers in drinking water systems (Wilczak et al. 1996). Cunliffe (Cunliffe 1991) and Skadsen (Skadsen 1993) speculated that greater detention times favor the formation of biofilms observed in dead-ends of distribution systems and water storage reservoirs. These are often sites of persistent and highly active nitrification (Cunliffe 1991; Skadsen 1993). Also, sediment areas (Ike et al. 1988), and plumbing systems in premises (Edwards et al. 2005) can support more nitrifier growth due to low disinfectant residuals and more surface area for attachment. Sediment and tubercles in distribution pipes may also exert a chlorine demand and further facilitate nitrifier growth (Odell et al. 1996). Attachment to solid surfaces can also enhance bacteria including nitrifiers’ resistance to potential toxins like disinfectants (Wolfe et al. 1988).

**Temperature**

Temperature can exert an effect on biological reactions in two ways: by influencing the rates of enzymatically catalyzed reactions and by affecting the rate of substrate diffusion to the cells (Grady et al. 1999). Kinetic parameters of nitrification are affected by temperature following the Arrhenius equation in a certain range, the commonly used equation in environmental engineering field is:

$$k_1 = k_2 \theta^{(T_1-T_2)}$$

(5)

where $k_1$ and $k_2$ are the temperature dependent kinetic parameters at temperature $T_1$ and $T_2$, $\theta$ is the temperature coefficient (Grady et al. 1999). For the temperature range in which this equation is valid, the maximum specific growth rate for nitrification doubles for every 6°C increase in temperature if an average reported $\theta$ value ($\theta =1.12$) was used (Characklis and Gujer 1979). However, higher temperature increases ammonia half-saturation coefficients for nitrifiers (Knowles et al. 1965), which would
decrease the growth rate according to the Monod equation. Nevertheless, nitrifier growth and ammonia utilization at 20 °C is significantly lower than at 30 °C, and experiments at these two temperatures noted that this temperature increase doubled substrate consumption rates and biomass yield (Groeneweg et al. 1994). Protein denaturation becomes important at temperature ranges above the range over which equation 5 applies (Neufeld et al. 1986; Neufeld et al. 1980). When the inhibiting effect of denaturation exceeds the higher biological growth rate associated with increasing temperature, nitrifier growth slows or stops. Early work by Gibbs (Gibbs 1920) and Sawyer and Bradney (Sawyer and Bradney 1946) reported 55 °C as very effective for inactivating nitrifiers. Other researchers have found that 45 °C inactivated nitrifiers (Neufeld et al. 1980).

Nitrifiers are known to grow in a wide temperature range of 4-45 °C although the upper limit is unclear (Painter 1977; Wolfe and Lieu 2001). However, the optimal growth for nitrifiers occurs in a very narrow temperature range. In a pure culture study, the optimum temperature for *Nitrosomonas* was reported to be 35 °C (Grunditz and Dalhammar 2001; Painter 1977). For *Nitrobacter*, the optimum lies between 35 - 42 °C (Grunditz and Dalhammar 2001; Laudelot and Tichelen 1960; Painter 1977). In a mixed culture of AOB and NOB found in activated sludge, the optimum temperature for nitrification occurrence was 30 °C (Neufeld et al. 1986; Neufeld et al. 1980). Different optimum temperatures for nitrification have been found by other researchers, including 15 °C (Charley et al. 1980), 21-28 °C (Wong-Chong and Loehr 1975), and no optimum in a range of 15 to 35 °C (Shammas 1971).

Not surprisingly, in drinking water systems nitrification is highly influenced by water temperature. In their survey of nitrification occurrence in drinking water distribution systems, (Wilczak et al. 1996) concluded that nitrification incidences were higher during summer or when temperatures were greater than 15 °C. Wolfe (Wolfe et al. 1990; Wolfe et al. 1988) and Ike (Ike et al. 1988) also found the number of AOB was approximately 100 to 1000 times higher in the summer than in the winter. No AOB were detected in water storage tank in the study of Wolfe (Wolfe et al. 1990) when the water temperature was less than 18 °C; however, above this value, the number of AOB generally increased in relation to temperature. Elevated temperature also increases the rate of chloramine decay and therefore provides more free ammonia for nitrifier growth (Nowlin et al. 2001). But on the other hand, elevated temperature increased the disinfectant efficiency of chloramine (Lieu et al. 1993), which could help control nitrifiers in at least some cases.
In drinking water systems, the optimal temperature for nitrification and nitrifier growth has been cited as 25 to 30 °C (Odell et al. 1996; Skadsen 1993; Wolfe and Lieu 2001). Analysis from a South Australia study indicated that nitrifying bacteria grew in distribution systems with temperatures ranging from 10 to 34 °C (Cunliffe 1991). But even some distribution system sites sampled under cold water conditions (below 10 °C) also showed evidence of nitrification such as increase in nitrite concentrations (Wilczak et al. 1996).

**Light**

Nitrifiers are very sensitive to visible and ultraviolet irradiation and even fluorescent lighting (Wolfe and Lieu 2001). Ammonia oxidation by *Nitrosomonas europaea* is inhibited by sunlight (Alleman et al. 1987) and UV light (Hooper and Terry 1974). Alleman and Preston (Alleman and Preston 2005) also pointed out that when nitrifiers grow as attached biofilm, bacterial layering will provide considerable shading and shelter the organisms deeper in the biofilm from stressful light. Nitrifying bacteria have the capability to recover from light inhibition. Recovery after exposure to light occurs in four to six hours (Alleman et al. 1987; Hooper and Terry 1974). Light is almost completely absent in drinking water distribution system pipes, although plastics including PVC allow some light transmittance to the pipe interior and sunlight falls on open reservoirs. Nonetheless, nitrification in open reservoirs has been noted (Odell et al. 1996).

**pH**

Many studies have reported the effect of pH on nitrification (Table 1.2). Nitrification occurs over a fairly wide pH range, as low as 4.6 (Wolfe and Lieu 2001) and as high as 11.2 (Prakasam and Loehr 1972). Nitrite oxidizers exist in a narrower range of pH values than ammonia oxidizers. The optimum pH for nitrification is between 7 and 8. Accordingly, the neutral to slightly alkaline pHs often found in the bulk water of drinking water systems accommodate nitrification (Odell et al. 1996; Wilczak et al. 1996).
<table>
<thead>
<tr>
<th>pH Range</th>
<th>Optimal pH</th>
<th>Nitrifiers strain or Nitrification Occurrence Environment</th>
<th>Nitrification Indication Parameters</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-10</td>
<td>7-8</td>
<td>Not specific strain, but in pure culture</td>
<td>N/A, generalized from Previous literature</td>
<td>Painter 1977</td>
</tr>
<tr>
<td>4.6-9</td>
<td>7.5-8.1</td>
<td>Not specified</td>
<td>N/A, generalized from Previous literature</td>
<td>Wolfe and Lieu 2001</td>
</tr>
<tr>
<td>&lt;11</td>
<td>6.7-7.0</td>
<td>Pure <em>Nitrosomonas europaea</em> cultured in suspension, fermenter sparged with CO₂ enriched air</td>
<td>Ammonia oxidation rate</td>
<td>Groeneweg et al. 1994</td>
</tr>
<tr>
<td>7.2-9.8</td>
<td>7.2-8.5</td>
<td>Nitrification in Drinking water system</td>
<td>Utility Survey</td>
<td>Odell et al. 1996</td>
</tr>
<tr>
<td>6.5-9.5</td>
<td>n.d.</td>
<td>Nitrification in Drinking water system</td>
<td>Utility Survey</td>
<td>Wilczak et al. 1996</td>
</tr>
<tr>
<td>n.d.</td>
<td>7-8</td>
<td><em>Nitrosomonas</em></td>
<td>N/A, generalized from Previous literature</td>
<td>USEPA 2005</td>
</tr>
<tr>
<td>n.d.</td>
<td>7.5-8</td>
<td><em>Nitrobacter</em></td>
<td>N/A, generalized from Previous literature</td>
<td>USEPA 2005</td>
</tr>
<tr>
<td>n.d.</td>
<td>8.1</td>
<td>Pure <em>Nitrosomonas</em> suspension culture, grown in fermenter with aeration</td>
<td>Ammonia oxidation rate</td>
<td>Grunditz and Dalhammar 2001</td>
</tr>
<tr>
<td>n.d.</td>
<td>7.9</td>
<td>Pure <em>Nitrobacter</em> suspension culture, grown in fermenter with aeration</td>
<td>Nitrite oxidation rate</td>
<td>Grunditz and Dalhammar 2001</td>
</tr>
<tr>
<td>n.d.</td>
<td>7.82</td>
<td><em>Nitrobacter winogradskyi</em> suspension culture, grown in continuous dialysis</td>
<td>Oxygen Uptake Rate</td>
<td>Boon and Laudelout 1962</td>
</tr>
<tr>
<td>high nitrification rate at pH below 4</td>
<td>Mixed culture in both suspended growth and biofilm growth with oxygen</td>
<td>Ammonia oxidation rate</td>
<td>Tarre and Green 2004</td>
<td></td>
</tr>
</tbody>
</table>

It is commonly accepted that pH affects nitrification by changing the concentration of free ammonia, because free ammonia rather than ammonium ions have been identified as the substrate for ammonia oxidation (Suzuki et al. 1974). Consideration of equilibrium ($\text{NH}_3 + \text{H}^+ \rightarrow \text{NH}_4^+$ with a pKa of 9.25) illustrates that very little free ammonia exists at a pH below 6, at which point nitrification has been noted to cease (Painter 1977; Stein and Arp 1998). However, Groeneweg et al. (Groeneweg et al. 1994) observed that ammonia oxidation rate of *Nitrosomonas europaea* was not constant in a pH range of 5-11 when applying a constant free ammonia concentration, indicating that other mechanisms besides free ammonia concentrations are accountable for the pH effect on nitrification. Groeneweg and colleagues preferred the theory of Bock et al. (Bock et al. 1991) as an explanatory hypothesis, which states that at low pH, most energy is consumed to maintain a favorable pH environment inside the cell, thus there is no energy left for growth.

Tarre and Green (Tarre and Green 2004) recently suggested that the effect of pH has little to do with free ammonia concentration; rather, the reduced growth that had been observed at lower pH arose from
an inorganic carbon limitation due to CO₂ stripping occurring in aerated batch culture. Using pure oxygen aeration in a continuous flow reactor, Tarre and Green (Tarre and Green 2004) demonstrated a very high nitrification rate of 5.6 g-N/L-day by *Nitrosomonas* at low pH in both a biofilm reactor (4.3 ± 0.1 pH) and a suspended biomass reactor (3.2-3.8 pH). This rate is similar to that reported for nitrifying reactors at optimum pH. Somewhat consistent with the Tarre and Green hypothesis, Groeneweeg et al. (1994) observed a lower optimal pH for nitrification than others (Table 1.2) when using CO₂-enriched air for aeration at a constant free ammonia concentration. Resolving this debate is important relative to better understanding mitigation of nitrification in potable water systems, since altering bulk water pH is an economically attractive strategy for reducing nitrification occurrence (Harrington et al. 2002; Skadsen 2002). However, adjusting pH might not be effective since the pH near the biofilm on iron pipe walls can be much lower (> 3 pH units) than in the bulk water (McNeill and Edwards 2001).

In drinking water systems, pH can affect nitrification not only by affecting the growth of nitrifiers, but also by affecting ammonia release from chloramine decay and chloramine inactivation rate on nitrifiers (Harrington 2002; Oldenburg et al. 2002). The effect of pH on ammonia release, disinfection decay and nitrifying bacterial growth exhibit contrary trends, so the optimal pH for nitrification occurrence is not always the same as the optimal pH for nitrifier growth (Harrington et al. 2002).

**Alkalinity and Inorganic Carbon**

A large amount of alkalinity is consumed during the oxidation of ammonia to nitrate: 8.62 mg HCO₃⁻ (7 mg/L as CaCO₃) is consumed for every mg NH₄⁺-N oxidized. Most alkalinity that is consumed results from neutralization of hydrogen ions during the oxidation of ammonia, and only a small amount of alkalinity is lost by incorporation of inorganic carbon into cell biomass (Grady et al. 1999). Alkalinity levels which are adequate to stop pH from dropping below a preferred range favor nitrification (Alleman and Preston 2005). Potable water typically has 1 mg/L NH₃-N or less, and alkalinity is usually maintained well above 7 mg/L as CaCO₃ for corrosion control. Therefore, minimum required levels of alkalinity are sufficient to support complete conversion of ammonia to nitrate. Though Wilczak et al. (1996) reported that nitrification did not significantly impact alkalinity; this conclusion might have been different if the waters studied had less than 30 mg/L as CaCO₃.
Nitrifying bacteria are autotrophic bacteria, and they use carbon dioxide as a carbon source which is fixed via the Calvin cycle. Inorganic carbon is not limiting to ammonia oxidizers even in wastewaters (Kowalchuk and Stephen 2001) unless very high levels of nitrogenous substances are present (Jun et al. 2000). When the ratio of inorganic carbon to ammonia, expressed as mg as CaCO₃/ mg ammonia-N ratio is above 8.25, the nitrite oxidizing process is the rate limiting process; while below this level, the ammonia oxidizing process is often the limiting step (Sakairi et al. 1996). This effect of inorganic carbon on the growth of AOB and NOB is more significant in biofilm systems (Tokutomi et al. 2006). In drinking water systems, a ratio of at least 15 (minimum 15 mg/L as CaCO₃ alkalinity and maximum 1 mg/L-N ammonia) is maintained, hence, nitrite oxidation is suspected to be the rate limiting step. This explains the accumulation of nitrite in distribution systems when nitrification occurs.

**Organic Carbon and Competition between Heterotrophic Bacteria and Nitrifying Bacteria**

Organic substrates are ubiquitous in the aquatic environment (Rittmann and Manem 1992) and even predominantly autotrophic nitrifying bacteria can incorporate exogenous organic compounds like acetate, 2-oxalolutarate, succinate, amino acids and sugars into biomass (Clark and Schmidt 1966; 1967; Martiny and Koops 1982; Wallace et al. 1970). In fact, all AOB can metabolize selected organic compounds to a limited extent in the presence of an inorganic energy source (Wolfe and Lieu 2001). *Nitrosomonas europaea* has been reported to grow on fructose or pyruvate instead of CO₂ as the sole carbon source (Hommes et al. 2003).

Beneficial effects of organic compounds on the growth of nitrifying bacteria have been reported. Pyruvate and peptone increased cell yield of a *Nitrosomonas* strain, while formate and acetate shortened the lag phase and enhanced cell yield of a *Nitrospira* strain (Krummel and Harms 1982). The time for a culture of *Nitrospira marina* to double in cell number decreased from 90 hour when grown autotrophically to 23 hours when grown in medium supplemented with pyruvate, yeast extract and peptone (Watson et al. 1986). All the above studies with AOB were conducted with ammonia present as an energy source, although Pan and Umbreit (Pan and Umbreit 1972b) grew *Nitrosomonas europaea* in the presence of glucose and in the absence of ammonia using a dialysis system to remove toxic metabolic products. The cell yield using glucose as an energy source was less than with ammonia;
moreover, the results have been difficult to reproduce and have been criticized by others (Hommes et al. 2003; Krummel and Harms 1982).

Overall organic carbon is generally considered to have a negative effect on nitrification. This effect can be due to the toxic nature of the organic material itself or due to heterotrophic bacteria out-competing nitrifiers for essential co-nutrients (Hockenbury et al. 1977; Sharma and Ahlert 1977).

Nitrifiers and heterotrophs compete for surfaces, dissolved oxygen, ammonium and other nutrients. Nitrifiers must reduce inorganic carbon to the oxidation state of cellular carbon, a reaction that is energy intensive and results in lower yield and growth rates compared to heterotrophs (Rittmann and Manem 1992). Nitrifiers are therefore at a competitive disadvantage versus heterotrophs at high levels of organic carbon. Based on their lower half saturation constants ($K_m$), heterotrophs were generally considered to have a greater affinity for dissolved oxygen (Grady et al. 1999) and ammonium (Rosswall 1982) than nitrifiers, although studies have indicated that specific affinity ($V_{max}/K_m$) rather than $K_m$ should be considered when determining the competition by heterotrophs and nitrifiers for oxygen (Bodelier and Laanbroek 1997). When coexisting in a biofilm, faster growing heterotrophs are believed to dominate at the biofilm’s surface while the slower growing nitrifiers exist deep inside the film because bacteria with higher growth rate can survive the higher detachment rate at the biofilm surface (Furumai and Rittmann 1994); therefore mass transport of key constituents such as oxygen through the biofilm to nitrifiers can be an issue since it must first pass through a layer of heterotrophs (Furumai and Rittmann 1994; Rittmann and Manem 1992). Heterotrophs can also out-compete nitrifiers for ammonia when organic carbon is high (Jansson 1958).

If disinfectant were not considered, the net result is that below a critical organic carbon-to-nitrogen ratio, nitrifiers and heterotrophs coexist. Furthermore, under extreme conditions when no dissolved organic carbon is present in the water, heterotrophs are completely dependent on the lysis and extracellular products of nitrifiers as a source of organic carbon. At higher organic carbon to nitrogen ratios or if ammonia is limited, nitrifiers’ numbers are strongly reduced by heterotrophs (Ohashi et al. 1995; Verhagen and Laanbroek 1991).
Conceptually, it is therefore expected that regimes of organic carbon and ammonia can be demarcated in which nitrifiers dominate in the system, co-exist with heterotrophs, or are negligible based on the levels of organic carbon relative to ammonia. A rough approximation can be made based on the relative growth rate of nitrifiers or by using the data of Verhagen and Laanbroek (Verhagen and Laanbroek 1991) (Figure 1.1 and Appendix 1). If C and N concentrations are below the line deducted by the Monod Model, nitrifiers would be dominant in the system. If C and N concentrations are above this line but below the line deduced from Verhagen and Laanbroek’s study, both nitrifying and heterotrophic bacteria grow in the system, but heterotrophic bacteria dominate. If C and N concentrations are above the line of Verhagen and Laanbroek, nitrifying bacteria would be negligible in the system. However, the work of Verhagen and Laanbroek was performed at very high ammonia concentration (> 28 mg/L-N) and organic carbon (72- 360 mg/L-C) relative to the conditions in drinking water system. The predictions would be more accurate in predicting the occurrence of nitrification in drinking water systems if a critical carbon/nitrogen ratio can be established under conditions more relevant to typical assimilable organic carbon concentrations (between 0.01 – 1 mg/L) and NH3-N levels (between 0 to 1 mg/L as N) in drinking water systems.

![Figure 1.1: Conceptual model illustrating coexistence of nitrifiers and heterotrophs.](image)

The top line (triangle) is plotted by using C/N = 10 of Verhagen and Laanbroek, 1991 directly, and the bottom line (circle) is plotted by equation $C = \frac{0.17N}{0.806 + 0.586N}$, which is deducted by using Monod equation and letting nitrifer growth rate equal to heterotroph growth rate (Appendix 1).
Beneficial Interactions between Heterotrophic Bacteria and Nitrifying Bacteria

Heterotrophic bacteria are always found in association with nitrifying bacteria when nitrification occurs in water distribution systems. This is probably because the two groups can have synergistic effects under some circumstances.

Heterotrophs can produce organic compounds that stimulate nitrifiers and degrade organic compounds that are inhibitory to the nitrifiers. Hockenbury et al. (Hockenbury and Grady 1977) reported filtrate from heterotrophic bacteria had a slightly (e.g., 17%) beneficial effect on nitrifying bacteria growth, and they proposed that heterotrophs might excrete metabolic products useful to nitrifiers. Pan and Umbreit (Pan and Umbreit 1972a) also reported *Nitrosomonas* and *Nitrobacter* growth could be stimulated by a particular heterotrophic bacteria group, as illustrated by shortening of the lag phase and extension of log phase. It was suggested that this stimulation was due to removal of toxic metabolites. Also, heterotrophs can produce extracellular polymers that can improve the aggregation of nitrifiers into biofilms and protect nitrifiers from detachment by pre-dominating in the outer layer of multi-species biofilms (Furumai and Rittmann 1994; Rittmann and Manem 1992; Rittmann et al. 1994).

The production of soluble microbial products (SMP) by nitrifying bacteria can provide a sole or supplemental organic substrate for heterotrophic bacteria. For *Nitrobacter* at stable and complete substrate oxidation, about 0.025 mg COD is produced for every mg N oxidized, and for *Nitrosomonas*, 0.073 mg COD is produced for every mg N oxidized (Rittmann et al. 1994). SMP produced by nitrifiers can therefore enhance heterotroph accumulation and stability, especially when inputs of organic substrates are low (Kindaichi et al. 2004; Rittmann et al. 1994). Heterotrophic growth is sustained by SMP formed by nitrifiers when there is no organic-carbon substrate supplied (Furumai and Rittmann 1994; Gieseke et al. 2005; Kindaichi et al. 2004). When the input ratio of COD to NH$_4^+$-N is 1, more than 40% of the COD utilization by the heterotrophs is nitrifier-generated SMP (Furumai and Rittmann 1992; 1994; Rittmann et al. 1994), and Kindaichi et al. (Kindaichi et al. 2004) noted that an autotrophic nitrifying biofilm fed with only NH$_4^+$ as an energy source and no organic carbon was composed of 50% nitrifiers (AOB and NOB) and 50% heterotrophic bacteria. In a drinking water distribution system, occurrence of AOB and Heterotrophic Plate Count (HPC) can be linearly related (Wolfe et al. 1990). This would obviously be expected if HPC were dependent on AOB for fixed organic carbon.
Confusing matters somewhat, it is known that certain heterotrophic bacteria can carry out nitrification, e.g. *Alcaligenes faecalis*, *Pseudomonas*, *Thiosphaera pantotropha*, fungi and some algae (Bock et al. 1992; Focht and Verstraete 1977; Killham 1986; Verstraete and Alexander 1973; 1986; Watson et al. 1989), although at a slower rate and using different mechanisms than autotrophic nitrifiers. Recently, ammonia oxidizing Archaea have been identified in a marine environment and in wastewater treatment plants, proving that bacteria are not the only organisms that can carry out nitrification (Könneke et al. 2005; Park et al. 2006). Additionally, Archaea are suspected to play a major role in nitrification at low DO.

**Micro-Nutrient Limitation and Inhibition**

All microbial growth depends on the availability of nutrients. Microbial growth can be represented by a simple overall equation:

\[
\begin{align*}
\text{Carbon} + \text{Nitrogen} + \text{Phosphorus} + \text{Trace Nutrients (K, Cu, Fe, etc.)} + \text{O}_2 \\
\rightarrow \text{Aerobic Microbial Growth}
\end{align*}
\]  

Nitrifiers also follow this growth pattern. The nutrients in a potable water system that are present at relatively low levels (and which therefore limit the maximum number of bacteria that can grow) can be limiting (Edwards et al. 2005; Madigan et al. 2000; Morton et al. 2005). In terms of the key nutrients of carbon and ammonia, it is expected that inorganic carbon is rarely limiting (Jun et al. 2000), whereas ammonia is frequently found to be limiting (Leu et al. 1998; Rittmann and Soneyink 1984; Wolfe and Lieu 2001).

Phosphorus in the environment occurs mostly as orthophosphate (PO$_4^{3-}$), but phosphites [hypophosphate PO$_2^{3-}$ and phosphate PO$_3^{3-}$] and phosphides [PH$_3$] are also present at trace levels in water distribution systems (Morton et al. 2003). Much of the total phosphorus in natural waters is not readily available for bacterial metabolism, and phosphorus has been reported to limit heterotrophic bacterial growth in areas of the US, Finland, Japan and China (Marshall and Edwards 2004; Miettinen et al. 1997; Sang et al. 2003; Sathasivan and Ohgaki 1999). Van der Aa et al. (Van der Aa et al. 2002) and Kors et al. (Kors et al. 1998) reported that nitrification processes used to remove ammonia at water treatment plants were
limited when phosphorus was almost completely removed or below 15 μg/L-P at low temperatures. Nitrification rates recovered in both plants after dosing phosphate at 35-50 and 100-150 μg/L-P. Van der Aa et al. (Van der Aa et al. 2002) proposed that at least 10 μg/L-P phosphate is necessary for nitrification to remove 1 mg/L NH₃-N ammonia from the raw water. The reported minimum phosphorus concentration for nitrification in raw water treatment in the literature range from 3 to 20 μg/L-P, and very little nitrite oxidation by *Nitrobacter* occurred when phosphorus was less than 50 μg/L-P (Aleem and Alexander 1960; Van Droogenbroeck and Laudelout 1967). Other studies found that growth of both *Nitrosomonas europaea* and *Nitrobacter winogradskyi* depend on cell phosphate content, which is related to the history of the nitrifier culture (Van Droogenbroeck and Laudelout 1967). Although this does not affect the growth as much as the phosphate concentration in the growth medium, it is predicted that the effect of culture history would be more pronounced if nitrifiers are growing under very low ammonium/nitrite and phosphate concentrations as opposed to the high ammonium/nitrite (> 300 mg/L-N) and phosphate concentration range (> 310 mg/L) used in the study (Van Droogenbroeck and Laudelout 1967).

In addition to the macronutrients of inorganic carbon, ammonia and phosphorus, nitrifier growth also requires trace nutrients such as potassium, calcium, copper, magnesium and other constituents. Trace nutrients have four possible metabolic impacts dependent on concentration (Figure 1.2). In deficiency, bacterial activity can be limited if an essential nutrient concentration is too low. Deficiencies of trace elements like potassium (Fransolet et al. 1988), boron, calcium, chromium, cobalt, copper, iron, magnesium, manganese, molybdenum, nickel, vanadium, and zinc can decrease growth or cause bacterial death (Reeves et al. 1981). As the trace nutrient concentration increases, bacterial activity may be restored and reach an optimum dependent on the circumstance of growth (e.g., fixed film versus batch culture, pH condition). At excess concentrations, however, detrimental effects may result. Many trace nutrients, including metals, can stimulate growth at low concentrations and cause toxicity at high concentrations (Sato et al. 1986).
For nitrifying bacteria, metal inhibition can occur by blocking the enzyme function (Martin and Richard 1982). Active cultures are more sensitive than resting cultures (Ibrahim 1989), and inhibition worsens with decreased detention time and organic matter concentration (Disalvo and Sherrard 1980). *Nitrosomonas* was found to be at least as sensitive as *Nitrobacter* in terms of inhibition by heavy metals (Lee et al. 1997).

A survey was recently completed of 330 raw drinking waters in the U.S. to determine the concentration of various metal and phosphorus constituents (AWWARF 2004). While the concentration of these constituents is sometimes altered somewhat by treatment process, analysis of that data indicates many trace elements occur in ranges that would be limiting in some cases and toxic in others (Table 1.3). Each of the key nutrients that might be limiting are discussed in the sections that follow, since this could be one key to understanding why nitrifier growth is rampant in some potable water systems but not others.

It is also important to note that the concentrations of many nutrients are altered as a result of water treatment and/or distribution, either purposefully or as a result of corrosion processes. It is therefore possible that decisions made about treatment and inhibitor dosing could strongly influence the extent of nitrification occurring in the distribution system.
Table 1.3: Limiting and Inhibiting Range of Various Nutrients and Occurrence in US Raw Drinking Water

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration Range (specific stimulating/inhibiting extent)</th>
<th>Culture purity (Growth Condition)</th>
<th>References</th>
<th>Occurrence in US. Raw Drinking Water ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (II)</td>
<td>Stimulating</td>
<td>0.1 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Tomlinson 1966</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.005-0.03 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 0.1 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05- 0.56 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
</tr>
<tr>
<td></td>
<td>Inhibiting</td>
<td>4 ppm had 75 % inhibition</td>
<td><em>Nitrosomonas europaea</em>, short term exposure</td>
<td>Tomlinson 1966</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01 ppm had 5 % inhibition</td>
<td>Wastewater Treatment Inoculum</td>
<td>Waara and Wilander 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 0.4 ppm</td>
<td>Not specified</td>
<td>Martin and Richard 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 ppm</td>
<td>Activated sludge</td>
<td>Pettet 1956</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 20 ppm</td>
<td>Activated sludge, long term exposure</td>
<td>Tomlinson 1966</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 ppm had 75 % inhibition</td>
<td>Activated sludge, short term exposure</td>
<td>Tomlinson 1966</td>
</tr>
<tr>
<td></td>
<td></td>
<td>640 ppm</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
</tr>
<tr>
<td>Ni (II)</td>
<td>Stimulating</td>
<td>0.5 ppm</td>
<td>Activated sludge</td>
<td>Disalvo &amp; Sherrard 1980</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 0.25 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.8 ppm complete inhibition</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 0.1 ppm</td>
<td>Not specified</td>
<td>Martin and Richard 1982</td>
</tr>
<tr>
<td></td>
<td>Inhibiting</td>
<td>0.7 ppm</td>
<td>Activated sludge</td>
<td>Harper 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 ppm</td>
<td>Activated sludge</td>
<td>Pettet 1956</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 ppm had 20 % inhibition</td>
<td>Activated sludge</td>
<td>Ibrahim 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 ppm had 88 % inhibition</td>
<td>Activated sludge</td>
<td>Martin and Richard 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.6 ppm</td>
<td>Activated sludge, AOB</td>
<td>Tomlinson 1966</td>
</tr>
<tr>
<td></td>
<td></td>
<td>295 ppm</td>
<td>Activated sludge, NOB</td>
<td>Tomlinson 1966</td>
</tr>
<tr>
<td>Mg (II)</td>
<td>Stimulating</td>
<td>12.5-50 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 50 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
</tr>
<tr>
<td></td>
<td>Inhibiting</td>
<td>12 g/L complete inhibition</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
</tr>
<tr>
<td>K (I)</td>
<td>Inhibiting</td>
<td>19.5 g/L complete inhibition</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
</tr>
<tr>
<td>Cr</td>
<td>Inhibiting</td>
<td>&gt; 0.25 ppm Cr (III)</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 ppm Cr (III)</td>
<td>Activated sludge</td>
<td>Harper 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 1 ppm</td>
<td>Not specified</td>
<td>Martin and Richard 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 ppm had 10 % inhibition</td>
<td>Activated sludge</td>
<td>Ibrahim 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 100 ppm Cr (VI) had 75 % inhibition</td>
<td>Activated sludge</td>
<td>Tomlinson 1966</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 0.3 ppm Cr (III)</td>
<td>High ammonia, Low biodegradable organic leachate</td>
<td>Harper 1996</td>
</tr>
</tbody>
</table>

¹ The concentration range and inhibiting extent vary widely depending on the specific conditions and sources.
<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration Range (specific stimulating/inhibiting extent)</th>
<th>Culture purity (Growth Condition)</th>
<th>References</th>
<th>Occurrence in US. Raw Drinking Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (II)</td>
<td>Up to 1 ppm had no effect</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
<td>0-614 ppb</td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.08-0.5 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
<td>0-500 ppm</td>
</tr>
<tr>
<td>Inhibiting</td>
<td>3 ppm had 80% inhibition</td>
<td>Wastewater Treatment Inoculum</td>
<td>Waara and Wilander 1985</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>&gt; 1 ppm</td>
<td>Activated sludge</td>
<td>Pettet 1956</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>11 ppm had 25% inhibition</td>
<td>Activated sludge</td>
<td>Martin and Richard 1982</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>&gt; 10 ppm</td>
<td>Not specified</td>
<td>Martin and Richard 1982</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.65 ppm</td>
<td></td>
<td>Meikleohn 1954</td>
<td></td>
</tr>
<tr>
<td>Na (I)</td>
<td>Stimulating 0.06-0.15% (600-1500 ppm)</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.7% (7000 ppm)</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
<td>0-22 ppm</td>
</tr>
<tr>
<td>Inhibiting</td>
<td>11.5 g/L complete inhibition</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>Stimulating Increasing from 0.5 ppm to 0.6 ppm chelated Fe (II)</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
<td></td>
</tr>
<tr>
<td>Optimal</td>
<td>6 ppm</td>
<td>Pure <em>Nitrosomonas</em> and <em>Nitrobacter</em></td>
<td>Meikleohn 1954</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>560 ppm</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
<td></td>
</tr>
<tr>
<td>Co (II)</td>
<td>Up to 1 ppm had no effect</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.08-0.5 ppm</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.59 ppm</td>
<td>Unspecified <em>Nitrobacter</em> Strain</td>
<td>Finstein and Delwiche 1965</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>59 ppm had 60% inhibition</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
<td></td>
</tr>
<tr>
<td>Pb (II)</td>
<td>0.005-0.5 ppm, no effect</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
<td>0-8.3 ppb, but corrosion of lead pipes could contribute lead to the water</td>
</tr>
<tr>
<td>Inhibiting</td>
<td>&gt; 0.5-1 ppm</td>
<td>Not specified</td>
<td>Martin and Richard 1982</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>&gt; 1 ppm, 1.7 ppm had 90% inhibition</td>
<td>Wastewater Treatment Inoculum</td>
<td>Waara and Wilander 1985</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>2.1 ppm</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.1 ppm had 18% inhibition</td>
<td>Activated sludge</td>
<td>Ibrahim 1989</td>
<td></td>
</tr>
<tr>
<td>Cd (II)</td>
<td>Inhibiting &lt; 75 ppb had &lt; 10% inhibition, 500 ppb had 85% inhibition</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>14.3 ppm had 42% inhibition</td>
<td>Activated sludge</td>
<td>Martin and Richard 1982</td>
<td></td>
</tr>
<tr>
<td>Mo (VI)</td>
<td>Stimulating 0.096-960 ppb</td>
<td>Unspecified <em>Nitrobacter</em> Strain</td>
<td>Finstein and Delwiche 1965</td>
<td></td>
</tr>
<tr>
<td>W (VI)</td>
<td>Stimulating 1.84 ppb</td>
<td>Unspecified <em>Nitrobacter</em> Strain</td>
<td>Zavarzin 1958</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>1.84 ppm</td>
<td>Unspecified <em>Nitrobacter</em> Strain</td>
<td>Finstein and Delwiche 1965</td>
<td></td>
</tr>
<tr>
<td>P (V)</td>
<td>stimulating &gt; 3-20 ppb-P</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Van Droogenbroeck and Laudelout 1967</td>
<td>0-1 ppm</td>
</tr>
<tr>
<td>Inhibiting</td>
<td>&gt; 50 ppb-P</td>
<td><em>Nitrobacter agilis</em></td>
<td>Aleem and Alexander 1960</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.05 ppm is enough</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Skinner and Walker 1961</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.3 ppm is necessary</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Boltjes 1935</td>
<td></td>
</tr>
<tr>
<td>Inhibiting</td>
<td>0.5-20 ppm is enough</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Loveless and Painter 1968</td>
<td></td>
</tr>
<tr>
<td>Ca (II)</td>
<td>Complete inhibition</td>
<td>Pure <em>Nitrosomonas</em></td>
<td>Meikleohn 1954</td>
<td></td>
</tr>
</tbody>
</table>

1: (Parks et al. 2004)
Copper

Copper (II) is believed to be a key component of the ammonia monooxygenase (AMO) enzyme, which is essential for ammonia oxidation (Ensign et al. 1993; Richardson and Watmough 1999) and AOB growth. For a pure culture of *Nitrobacter* (NOB) 48 ppb copper has been recommended (Tang 1992). However, excess copper is known to be toxic to nitrifiers as in the case for heterotrophs (Braam and Klapwijk 1981). Copper levels as low as 2 ppb have been found to be toxic to nitrifying bacteria in waters with low chelating capacity (Waara and Wilander 1985). The inhibition of nitrification with copper is sometimes reversible through addition of copper chelating compounds within 24 hours (Braam and Klapwijk 1981).

The effect of copper on nitrification was also found to be dependent on the free copper Cu$^{2+}$ concentration (Braam and Klapwijk 1981). The free copper concentration increases at lower pH and the dependence of toxicity with pH probably results from this chemical change (Loveless and Painter 1968).

Certain complexes of copper may increase toxicity. For example, the effect of copper on nitrification is dependent on the ammonia concentration, as copper inhibition increases as ammonia concentration increases. The level of copper that caused 50% inhibition of *N. europaea* decreased from 0.5 to 0.01 ppm as the total ammonia concentration rose from 3 to 23 mg/L-N. This was attributed to higher toxicity of copper-ammine complexes [Cu(NH$_3$)$_X$$^{2-}$] (Sato et al. 1988).

The effect of copper also varies based on the purity of the nitrifying culture. Concentrations of copper necessary to inhibit nitrification in activated sludge are much higher than those required to give the same effect in pure culture, possibly because of the biomass level [Mixed Liquor Suspended Solids (MLSS) concentration] and likely associated sorption of free copper (Tomlinson et al. 1966). For example, at pH 7.6, 4 ppm copper had 75% inhibitory effect on ammonia oxidation in pure culture, whereas about 200 ppm copper is required to achieve 75% inhibition in activated sludge having a concentration of 1350 to 1700 mg of dry matter/L (Tomlinson et al. 1966).

The range of copper that stimulates or inhibits nitrification is therefore expected to vary widely based on nitrifier strain and growth conditions (Table 1.3).
Phosphate, pH interaction in relation to copper effect on nitrification

Phosphate and pH interact to control free and total copper (II) concentrations (Edwards et al. 2002; Schock et al. 1995), and would therefore be expected to exert a key control over nitrification in premise plumbing in which copper is the most common pipe material. Copper pipes are covered with thin patina (rust) or scale layers, the identity of which controls the free copper in water during overnight stagnation. The scale layer could be composed of Cu(OH)₂ scale, CuO, malachite (Cu₂CO₃(OH)₂) or other compounds.

About 56% of utilities add phosphorus to water (0.2 to 3 mg/L) to form a low solubility Cu₃PO₄ scale to attempt and reduce copper solubility (McNeill and Edwards 2002). When Cu(OH)₂ is present as in the case of new pipe, free Cu²⁺ released to the water is higher than when phosphorus is added, especially at lower pHs (Figure 1.3). Specifically, free Cu²⁺ is predicted to be 27 mg/L at pH 6 and decrease 10 fold for every 0.5 pH unit increase in pH. Consequently, in this type of pipe and water, nitrification would be inhibited by toxic levels of free copper at lower pH and might be limited by the absence of free copper at higher pH. Using thresholds of 0.1 ppm copper based on the completely mixed suspended growth tests mentioned earlier, it is predicted that copper toxicity is likely below pH 7 and growth might be limited by low free copper above pH 7.5.

When phosphorus is present in water along with a scale-forming, relatively insoluble Cu₃(PO₄)₂ solid, free Cu²⁺ is much lower in water at lower pH (Figure 1.3). Thus, phosphate corrosion inhibitor might be expected to reduce copper-induced toxicity to nitrifiers in copper pipe when pH is below 7, especially at higher phosphorus doses. The potential dual stimulating action of phosphorus as a nutrient and also as an inhibitor of copper-induced toxicity was studied and this complex interaction was confirmed (Zhang et al. 2008a).
Nickel

Addition of low levels of nickel (II) to soil stimulated nitrification either directly or by inhibiting competitors of nitrifiers (de Cantanzaro and Hutchinson 1985). High concentrations of nickel could inhibit nitrifier growth by inducing an extended lag phase. Nickel toxicity is most evident for *N. europaea* at low ammonia concentration and high nickel concentration (Sato et al. 1986). The threshold levels of nickel toxicity also vary largely and are dependent on bacterial strain and growth conditions (Table 1.3). Nickel is a major component of stainless steel (Lula 1986), the corrosion of stainless steel plumbing material might contribute nickel to drinking water and MCL regulates nickel at 0.1 mg/L (USEPA 2001a)

Chromium

Chromium is a commonly studied metal ion for its toxicity in the nitrification process and there are a wide range of impacts on chromium inhibition (Table 1.3). Chromium (III) was used in some studies
(Harper et al. 1996; Skinner and Walker 1961), Chromium (II) in others (Martin and Richard 1982), and chromium (VI) (Tomlinson et al. 1966) in still others. The different oxidation states might partly explain the ranges of effects observed (Table 1.3), although the definitive work on this has not yet been conducted. Like Nickel, chromium is a major component of stainless steel (Lula 1986), and MCL regulates chromium at 0.1 mg/L (USEPA 2001a).

**Zinc**

Zinc (II) can cause toxicity by precipitating phosphorus nutrients (Harper et al. 1996). No stimulatory effect has been found for pure *Nitrosomonas* culture in a range of 0.005-0.5 ppm Zn, while 0.08-0.5 ppm Zn has been noted to inhibit nitrification (Loveless and Painter 1968). For *Nitrobacter*, the threshold inhibitory level is higher than for *Nitrosomonas* culture; up to 1 ppm Zn had no inhibition (Waara and Wilander 1985). Recent lab study on non-purified *Nitrosomonas europaea* also showed clear inhibition of nitrification by 0.15 ppm Zn$^{2+}$ (Zhang and Edwards 2005). However, another study with a full scale drinking water treatment plant concluded that no nitrification would be expected if 0.5 ppm Zn$^{2+}$ were added (Bott 2005). Zinc is often added to water supplies in conjunction with phosphorus corrosion inhibitors and its potential role in inhibition or stimulation of growth is therefore of high interest.

**Iron**

Iron is also considered to comprise an active binding site on AMO (Zahn et al. 1996). Limited growth of nitrifiers was observed without the addition of supplemental iron (Sato et al. 1988). However, Ensign et al., 1993 found that the addition of Fe (II) or Fe (III) had no stimulating effect on AMO activity in a pure *Nitrosomonas europaea* culture, and addition of up to 2 ppm-Fe as ferrous sulfate had no effect on the growth of *Nitrosomonas*. Conversely, addition of chelated iron (Skinner and Walker 1961) or exogenous siderophores (biologically-generated iron chelating agents) (Wei et al. 2006) have been shown to enhance AOB growth. In fact, although *Nitrosomonas europaea* is incapable of producing its own siderophores (Chain et al. 2003), its ability to utilize exogenous siderophores may explain why nitrifiers grow better in the presence of other bacteria if they provide iron chelating agents that can support AOB growth. Finally, the solubility of ferrous and ferric iron is reduced by addition of phosphorus, analogous to the preceding discussion of copper.
Lead

As with the other heavy metals, lead complexation and solubility play a role in the observed effects, and this most likely accounts for the different inhibitory levels of lead reported for various pure culture experiments (Table 1.3). In one study of activated sludge, up to 100 ppm lead (II) had no inhibiting effect on nitrifier growth rate because it was virtually all precipitated or complexed (Martin and Richard 1982). In another study by Ibrahim (Ibrahim 1989), addition of 0.1, 0.5, 1 and 2 ppm Pb caused 18, 29, 53 and 78% inhibition of respiration rate for active nitrifiers grown with adequate substrate and 12, 25, 37 and 50% for resting nitrifiers grown with limited substrate, respectively. The toxicity of lead was higher than that observed for nickel in the same study. As was the case with copper, solubility of free lead can be dramatically reduced by the presence of phosphorus in water.

Molybdenum

Nitrite oxidoreductase (enzyme involved in nitrite oxidation) is a molybdenum-iron-sulphur protein, containing 0.12 or 0.7 molybdenum per 400,000 molecular weight (Ferguson 1998; Prosser 1986). A minimum of 2000 atoms of molybdenum are required for the synthesis of one Nitrobacter cell (Finstein and Delwiche 1965). Zavarzin (Zavarzin 1958) proposed that molybdenum participates directly in the enzymatic oxidation of nitrite, but a later study found no evidence for this (Prosser 1986). Nonetheless, addition of molybdenum (VI) produced an 11-fold increase in both nitrite utilization and cell mass development of Nitrobacter over five days (Finstein and Delwiche 1965). Stimulating effects were found at concentrations as low as $10^{-9}$ M (0.1 ppb) by Finstein and Delwiche (Finstein and Delwiche 1965) and $10^{-7}$ M (10 ppb) by Zavarzin (1958). Zavarzin also found that molybdenum (VI) stimulated nitrification most effectively at pH 7.7 and actually inhibited nitrification at pH 8.8; moreover, the stimulating effect of molybdenum (VI) occurred only in the presence of 5.6 ppm iron as FeSO$_4$. There is currently no drinking water maximum contaminant level for molybdenum, although the irrigation standard is 0.010 ppm Mo (Chakrabarti and Jones 1983).

Tungsten

Tungsten (VI) is reported to stimulate nitrite oxidation at a concentration of $10^{-5}$ mM (1.84 ppb-W); however, there was no stimulation when $10^{-4}$ mM molybdenum and $10^{-5}$ mM tungsten were added together (Zavarzin 1958). Contrary to molybdenum (VI), tungsten (VI) stimulated nitrite oxidation most
effectively at pH 8.8 while it inhibited oxidation at pH 7.7 (Zavarzin 1958). Addition of $10^{-5}$ M (1.84 ppm-W) tungsten slightly inhibited substrate oxidation of *Nitrobacter* pure culture (Finstein and Delwiche 1965).

*Sodium, Calcium, Magnesium, Potassium, Cadmium and Cobalt*

Several studies report the effects of sodium, calcium, magnesium, potassium and cobalt on nitrification (Table 1.3). Sodium in natural water is typically in the range of 3 to 300 ppm. These concentrations allow ready growth of nitrifiers based on the minimum required level, but might be less than optimal. The magnesium levels found in natural water are physiologically relevant to nitrifying bacteria growth as it can limit growth at the lower range and inhibit growth at the higher range. The ability of magnesium to limit growth was more dramatic at lower pH (Loveless and Painter 1968). Cadmium also can inhibit nitrification in a manner similar to copper, but of lesser magnitude (Sato et al. 1986).

The effect of mixed trace elements appears complex. For example, a mixture of copper (decrease growth rate) and nickel (cause extended lag phase) affected both growth rate and lag phase, but the inhibition intensity is decreased compared to copper or nickel alone. A mixture of cadmium (decrease growth rate) and nickel (cause extended lag phase) also affected both growth rate and lag phase, but the growth rate was further reduced compared to cadmium alone while the lag phase was shortened compared to nickel alone (Sato et al. 1986).

**Disinfectant Residual**

Monochloramine disinfectant is formed by combination of free chlorine with ammonia at a mass ratio of approximately 4 mg/L Cl$_2$ per 1 mg/L NH$_3$-N or less. About ¾ of utilities in the U.S. using chloramine target the residual between 1 and 3 mg/L (Wilczak et al. 1996). Monochloramine can be viewed as having two competing effects on AOB: inactivation resulting from the presence of chloramine disinfectant (biocide) and growth from the presence of ammonia (food) (Edwards et al. 2005; Fleming et al. 2005). That is, although AOB are approximately 13 times more resistant to monochloramine than free chlorine disinfectant (Wolfe et al. 1990), monochloramine is nonetheless a disinfectant. However, when monochloramine disappears through a variety of reactions (Table 1.1), free ammonia is often formed. Due to the difference of strain, temperature, chlorine to ammonia ratios, water chemistry,
enumeration method and pH values, reported CT$_{99}$ values in different studies range from 3 to 19,000 mg Cl$_2$-min/L (Cunliffe 1991; Oldenburg et al. 2002; Wolfe et al. 1990).

Fleming et al., 2005 proposed that nitrification occurrence in a distribution system depends on the relative concentration of chlorine (biocide) and free ammonia (food). A nitrification potential curve was constructed for a pilot scale study. Based on this curve, a threshold total chlorine value of 1.6 mg/L was established, above which, nitrification would be prevented regardless of the free ammonia concentration and below which, nitrification occurrence depends on the ratio of chlorine and free ammonia. Based on the slope of the nitrification potential curve, we can further conclude that nitrification is prevented when the biocide to food mass ratio is above 8. Similarly, in a Florida utility, nitrification rarely occurred when chlorine residual was above 1 mg/L and biocide to food ratio was more than 5 (Liu et al. 2005).

Nitrification has been observed to occur more frequently in systems with low chloramine doses (Lieu et al. 1993; Odell et al. 1996; Skadsen 1993; Wilczak et al. 1996). Chloramine levels typically used for potable water disinfection-1 to 2 mg/L according to (Wolfe et al. 1990) should be sufficient to eliminate nitrifiers.

On the other hand, nitrification occurred in the Ann Arbor, Michigan system which had an average of 5 to 6 mg/L monochloramine and an upper dose up to 8 mg/L (Skadsen 1993). In a study in Australia, 20.7 % of samples with monochloramine concentrations greater than 5 mg/L had nitrifying bacteria detected (Cunliffe 1991). These observations are not contradictory to the theory of Fleming et al. (Fleming et al. 2005; Fleming et al. 2008). The high dose of chloramines in Ann Arbor reservoir was applied after nitrification started, so chloramine decay was accelerated by the nitrite produced and the disinfectant concentration in the distribution system was much lower than expected (Fleming et al. 2005; Odell et al. 1996). Nitrifiers might stay viable under high disinfectant dose for a period of time as proposed in Cunliffe’s study (Cunliffe 1991). Also, the nitrification potential curve and the necessary chloramine level to prevent nitrification vary depending on the specific water quality and treatment practices in different systems (Fleming et al. 2005; Lieu et al. 1993). Although the Fleming study (Fleming et al. 2005) used real water, nitrifier growth in a pilot system is still vastly different from a real
system where biofilm could be better established and different pipe materials could exert a protective effect by reacting with and destroying the chloramine disinfectant (Table 1.1).

**Distribution Materials and Corrosion Control Strategies**

Different pipe materials can be expected to strongly influence occurrence of nitrification since the pipe can serve as a source of trace nutrients, toxic metals, attached growth and disinfectant destruction. Heterotrophic bacteria exhibit the best growth on reactive surfaces such as iron pipes, whereas PVC pipes typically have lower levels of bacterial growth (Camper et al. 2003). Cement and epoxy were intermediate in terms of support for attached heterotrophs (Camper et al. 2003). Iron pipes are problematic, not only because of the beneficial surface, but also because it destroys chlorine disinfectant (Camper et al. 2003). Likewise, for nitrifying bacteria, it was speculated that iron tubercles in distribution pipes may exert chlorine demand and facilitate nitrifier growth (Odell et al. 1996). Iron corrosion by-products can accelerate chloramine decay and release ammonia for nitrifying bacteria growth:

\[
\frac{1}{2}NH_2Cl + H^+ + Fe^{2+} \rightarrow Fe^{3+} + \frac{1}{2}NH_4^+ + \frac{1}{2}Cl^- \]  

(7)

Fe\(^0\) from iron pipe can also recycle ammonia from nitrate:

\[
4 \text{Fe}^0 + \text{NO}_3^- + 10 \text{H}^+ = 4 \text{Fe}^{2+} + \text{NH}_4^+ + 3\text{H}_2\text{O} \]  

(4)

The ammonia thus generated could be used by nitrifiers or lost through breakpoint chlorination when chlorine is present (Figure 1.4). Under some circumstances, iron corrosion could provide essential micronutrients necessary for nitrifying bacteria growth, such as phosphorus impurities in the metal or iron dissolution from scale (Morton et al. 2005).

Concrete materials can leach lime and therefore have a higher surface pH (> pH 12 for fresh concrete) than bulk water (AWWARF and DVGW-TZW 1996). Leaching of lime and other trace nutrients from concrete is very dependent on water chemistry (Guo et al. 1998). Concrete lined pipes had the lowest AOB and HPC in a study at two California utilities using chloramines. The authors suggested it was because of the high pH due to concrete leaching (Steward and Lieu 1997). But cement lined ductile iron pipe supported a higher heterotrophic biomass than did unlined iron in Pinellas county, Florida (LePuil
It has been speculated that concrete might accelerate bacterial growth by facilitating chloramine autodecomposition by surface catalysis (Woolschlager and Soucie 2003), and previous modeling work did suggest that cement lined pipes exerted a substantial and surprising chloramine demand (Woolschlager et al. 2001). While the results are seemingly contradictory in the two studies, they might be attributed to fresher concrete or lower alkalinity in the earlier study which caused a high pH rise and inhibited nitrifier growth.

**Figure 1.4: Disinfectant, nitrifiers interaction and nutrient release on iron pipe surface** (Note: Numbers marked in red represent reactions listed in Table 1)

For lead pipes and materials containing lead such as brass, two contrary effects on nitrifying bacteria growth could exist. As in the case of iron, corrosion reactions between lead and nitrate could recycle ammonia from nitrate and support a large nitrifier population (Reaction 10, Table 1.1). On the other hand, soluble lead leached during corrosion might be able to inhibit nitrification, although lead
inhibition on nitrification was only observed in soil and not in aquatic environments (Loveless and Painter 1968; Shkelqim and Malcolm 2002).

Zinc which leaches from galvanized iron pipes and copper leaching from copper pipes could both serve as a trace nutrient source at low levels and toxic elements at high levels for nitrifiers. The net effect of a pipe material depends on the corrosion intensity and water quality. Nitrification has been reportedly stimulated in copper pipes in Willmar, Minnesota (Murphy et al. 1997).

**Filtration Treatment Strategies**

Granular activated carbon (GAC) and powdered activated carbon (PAC) are applied for enhanced removal of organic matter and taste and odor problems. As early as 1935, Feben recognized that filter beds receiving water with ammonia offer a nearly ideal environment for nitrifying bacteria. GAC has high porosity and can increase nutrient adsorption and bacterial attachment (Rollinger and Dott 1987).

Activated carbon can also accelerate chloramine decay through the following reactions:

\[
\text{NH}_2\text{Cl} + \text{H}_2\text{O} + \text{C}^* \rightarrow \text{NH}_3 + \text{H}^+ + \text{Cl}^- + \text{CO}^* \\
2\text{NH}_2\text{Cl} + \text{CO}^* \rightarrow \text{N}_2 + \text{H}_2\text{O} + 2\text{H}^+ + 2\text{Cl}^- + \text{C}^* 
\]

Note: C* and CO* indicate active carbon and surface oxide on the carbon.

Growth of nitrifying bacteria was easily initiated in GAC beds when chloramine concentrations were less than 1 mg/L-Cl₂, and the established nitrifying bacteria could easily tolerate higher chloramine concentrations of 4 mg/L-Cl₂ (Fairey et al. 2004). Nitrification was also reported to occur immediately after a change in treatment from sand to GAC in Ann Arbor, Michigan (Skadsen 1993). It was suspected that GAC helped to convert chloramines to excess free ammonia and enabled nitrifying bacteria to proliferate (Tokuno 1997; Vahala 2002). This was confirmed in a bench scale study (Zhang et al. 2008b). Higher rates of nitrification also occurred after installing GAC in a full scale distribution system in Finland due to decreased competition for space between nitrifying and heterotrophic bacteria, the introduction of a shelter inside the carbon fines and a slightly higher excess ammonia concentration (Vahala 2002; Vahala and Laukkanen 1998).
EFFECTS OF NITRIFICATION

Increased Nitrite and Nitrate

Nitrification converts ammonia to nitrite and nitrate. Nitrite and nitrate are regulated by the USEPA and have primary Maximum Contaminant Levels (MCLs) of 1 and 10 mg/L as N, respectively. The concentration of nitrite in typical surface and groundwater is far below 0.1 mg/L. Theoretically, the ammonia from 4 mg/L chloramines (chlorine to ammonia ratio is 4:1) could be converted to 1.1 mg/L-N nitrite (USEPA 2005). Thus, the US EPA MCL of 4 mg/L chloramine also serves to prevent exceedence of the drinking water MCL for nitrite even under the worst case in which all chloramine ammonia is converted to nitrite. In drinking water systems, increases in nitrite and nitrate are usually on the order of 0.05 – 0.5 mg/L-N, but increases above 1 mg/L-N have been noted in stagnant parts of some distribution systems (USEPA 2006; Wilczak et al. 1996). Nitrite accumulation can possibly cause the potentially fatal condition of methemoglobinemia, which is also called “blue baby syndrome” (Peavy et al. 1985). Nitrite has also been proven to accelerate chloramine decay (Margerum et al. 1994; Valentine 1984). Stoichiometrically, 1 mg/L-N nitrite could consume 5 mg/L-Cl₂ monochloramine (Table 1.1, Reaction 6).

In most systems, 50 μg/L nitrite-nitrogen is set as a criterion indication of nitrification (Kirmeyer 1995; Wilczak et al. 1996). Breakpoint chlorination is applied in the Metropolitan Water District of Southern California when nitrite exceeds this level (Wolfe et al. 1988), but a lower warning level of 25 μg/L was adopted for the Ann Arbor system because nitrification had become a significant problem at this level (Skadsen 1993).

pH and alkalinity

The oxidation of ammonia to nitrite and then to nitrate by nitrifying bacteria reduces pH and consumes large amounts of alkalinity. Reduction of pH and alkalinity can lead to violation of USEPA Lead and Copper Rule either through failure to maintain designated optimal water quality parameters, or through an action level exceedence at the tap (Odell et al. 1996). The extent of pH and alkalinity changes caused by nitrification will depend on the amount of water buffering capacity and nitrifying bacteria activity. In most drinking water systems, significant pH and alkalinity changes were not observed (pH changes
mostly ranged within ± 0.3) (Wilczak et al. 1996). Reduction of alkalinity by precipitation of CaCO₃ on pipes and increasing of alkalinity by dissolution of cement further complicate matters.

**Heterotrophic bacterial growth and Coliform Growth**

Heterotrophic bacterial growth is generally monitored by Heterotrophic Plate Count (HPC) in drinking water systems (Clesceri et al. 1998). Increase in HPC has always been noted to accompany nitrification occurrence (Powell 2004; Skadsen 1993; Wilczak et al. 1996; Wolfe et al. 1990). The HPC increase could be the result of the depletion of chloramines or supported growth by organic carbon released from nitrifying bacteria (Wilczak et al. 1996). High HPC may be associated with a variety of problems including proliferation of undesirable organisms and aesthetic-compromised water quality (Yang et al. 2004). Systems experiencing nitrification might not be able to meet the total coliform rule (TCR) (USEPA 2001b). Disinfectant residual decrease during nitrification can lead to increased growth of coliform. Also, when certain control practices are implemented, they may attack biofilm in the distribution system and lead to positive total coliform samples. Two Florida utilities reported periodic violations of the TCR when free chlorine was used for nitrification control after extended periods of chloramination (Wilczak et al. 1996).

**Rapid Decay of Chloramines**

The first indication of nitrification is often a difficulty in maintaining a constant chloramine residual (Cunliffe 1991; Odell et al. 1996; Skadsen 1993; Wilczak et al. 1996; Wolfe et al. 1988). A possible explanation for the accelerated chloramine decay could be: first, nitrite can accelerate chloramines decay; second, ammonia oxidation can shift the equilibrium of monochloramine formation so that chloramine is hydrolyzed as free ammonia (Cunliffe 1991).

The disappearance of the chloramine residual may cause a violation of disinfectant residual standards in the Surface Water Treatment Rule. This rule requires the detection of a disinfectant residual in at least 95% of monthly distribution system samples (Yang et al. 2004). Compliance with the D/DBP rule may also be affected when utilities change their chloramine chemistry by increasing the chloramine dose or increasing the chlorine to ammonia ratio. Systems using chloramines at levels as high as 7 or 8 mg/L are at a high risk of violating D/DBP rules (Harms and Owen 2004; Skadsen 1993).
Dissolved Oxygen (DO)

DO has been noted to decrease in distribution systems when nitrification is occurring (Odell et al. 1996; Wilczak et al. 1996), sometimes by as much as 7 mg DO/mg NH₃-N. This significantly exceeds the stoichiometric level of 4.33 mg DO/mg NH₃-N (Grady et al. 1999). Although other reactions such as corrosion might remove oxygen, this high depletion indicated a probability of recycled ammonia due to reactions with pipe materials (Table 1.1). This recycling reaction could regenerate ammonia and cause excessive nitrification and oxygen consumption. Another explanation might be due to increased aerobic respiration by increased HPC.

Corrosion

Corrosion could be affected by nitrification through decreased pH and alkalinity (Table 1.4), and increases in bacteria growth are generally believed to increase corrosion (McNeill and Edwards 2001). Nitrite levels produced by nitrification in drinking water system might stimulate corrosion rate by shifting the redox potential (Rozenfeld 1981) Increased corrosion can result in violation of USEPA Lead and Copper Rule (LCR), increased customer complaints about red water (high level of iron release in drinking water due to iron pipe corrosion), and taste and odor problems (Seidel et al. 2005).

<table>
<thead>
<tr>
<th>Change due to nitrification</th>
<th>Possible direct/indirect effect on water quality</th>
<th>Possible effect on corrosion-materials degradation</th>
<th>Other Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite production</td>
<td>Nitrite MCL, increased lead from brass, disinfectant loss</td>
<td>Increased microbial corrosion, nitrite catalyzed stress corrosion failures and attack on grain boundaries (brass)*</td>
<td>Samples for nitrite MCL not collected in premise plumbing, so potential problem can be missed.</td>
</tr>
<tr>
<td>Lower pH, alkalinity and DIC</td>
<td>Taste &amp; Odor from corrosion, red and blue water complaints.</td>
<td>Divergence from targeted optimal corrosion control relative to finished water</td>
<td>Lead and Copper Rule, Toxicity from Blue Water typically occurs in new homes not tested in LCR</td>
</tr>
<tr>
<td>Higher HPC</td>
<td>Concern over pathogen re-growth and loss of disinfection</td>
<td>More microbial corrosion, likely link to some cases of pinhole leaks in copper tube</td>
<td>Total coliform rule, HPC action levels, Legionella and Mycobacterium as emerging issues</td>
</tr>
<tr>
<td>Rapid decay of chloramine</td>
<td>Failure to maintain residual at distant parts of distribution system</td>
<td>Effect on corrosion rates dependent on relative corrosivity of chloramine vs. decay products</td>
<td>Chlorine residual not routinely monitored in premise plumbing, where it controls opportunistic pathogens</td>
</tr>
<tr>
<td>Decreased DO</td>
<td>Low redox in iron pipe associated with more red water</td>
<td>Highly corrosive sulfate reducing bacteria are anaerobic</td>
<td>Lead and Copper Rule, color, taste and odor complaints</td>
</tr>
</tbody>
</table>

Profound adverse impacts of nitrification on corrosion of lead pipe in drinking water were first noted more than 100 years ago (Garret 1891) and the issue of nitrification in chloraminated water supplies was reasonably described 70 years ago (Feben 1935; Hulbert 1933; Larson 1939). Recently, work performed in Pinellas County, Florida (Powell 2004) highlighted some concerns related to iron corrosion control and red water. Furthermore, elevated copper levels at the tap were clearly tied to activity of nitrifying bacteria in Willmar homes (Murphy et al. 1997), and nitrification was implicated in higher lead leaching in Ottawa (Douglas et al. 2004) and to some extent in Washington D.C. (Edwards and Dudi 2004). The severe degradation of concrete by nitrification is well-established in other industries (Bastidas and Sánchez-Silva 2006; Kaltwasser 1976). The destruction of concrete materials by nitrification is mainly from a loss of binding material by acidification (Meincke et al. 1989).

**Nitrification Monitoring in Drinking Water Systems**

**Biological Monitoring**

*MPN*

Relatively little is known about the occurrence of nitrifying bacteria in drinking water due to the difficulty in isolating and enumerating these organisms in environmental samples (Wolfe et al. 1988). Nitrifiers are most frequently enumerated by a most-probable-number (MPN) technique, using a media selective for ammonia or nitrite oxidizers. The MPN tubes are generally incubated for at least three weeks for ammonia oxidizers and up to 15 weeks for nitrite oxidizers. Unfortunately, low recovery efficiencies, ranging from 0.1 to 5 % have been obtained with the MPN technique (Wolfe et al. 1988). Despite this limitation, this technique is still the most common method used by many researchers for nitrifier quantification (Fleming et al. 2005; Fleming et al. 2008; Wolfe et al. 1990).

*Molecular Techniques*

Because of limitations in culturing, the use of nucleic acids to detect and identify AOB has increased in the past few years (Regan 2001). For identification of nitrifiers, 16S rRNA (or 16S rDNA), which is present in all bacteria but has regions of highly variable nucleotide sequences, is commonly targeted. *amoA* is a gene that is only present in AOB, and it has been used increasingly for AOB detection and characterization since 1995 (Regan 2001; Wagner et al. 1998; Wagner et al. 1995). Fluorescent *in situ*
hybridization (FISH), typically targeting 16S rRNA involves fixation of cells, hybridization with fluorescent-tagged complementary probes and analysis of fluorescence signal (Regan 2001; Wagner et al. 1998; Wagner et al. 1995). Although FISH has been found to be an effective tool for determining cell activity for some species, for many AOB, the rRNA and mRNA content under starved conditions (low nitrifying activity) was not lower than that under nutrient abundant conditions (high nitrifying activity), therefore, FISH, which detects nitrifier activity by detecting 16S rRNA, is not particularly effective for determining AOB activity (Bollmann et al. 2005; Wagner et al. 1995). Also, false positives might result due to lack of specificity and false negatives may result due to the stringency issues with probes and low fluorescent responses (Yilmaz and Noguera 2004). The method is also limited due to the limitation of existing databases (Regan 2001).

Polymerase Chain Reaction (PCR) is also used to identify and quantify nitrifiers, targeting either 16S rDNA or \textit{amoA} gene sequences (Baribeau 2006; Purkhold et al. 2003; Regan et al. 2007). PCR involves DNA extraction, amplification and sequencing or quantification of target gene products (Purkhold et al. 2003; Regan et al. 2007). Erroneous information might also result in PCR analysis during sample collection, cell lysis, DNA extraction and amplification (Wintzingerode et al. 1997).

If one is interested in determining the diversity of nitrifiers, denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), cloning and sequence analysis can be used (Baribeau 2006; Qin et al. 2007; Regan et al. 2002).

\textit{Fluorescent antibodies}

Fluorescent-antibody (FA) technique can also be used to detect and enumerate nitrifier strains (Belser and Schmidt 1978; Volsch et al. 1990). This approach does not require lengthy incubation periods like in culturing techniques, but it requires different FAs for different nitrifier strains and analysis of a single sample could require excessive work (Baribeau 2006; Belser and Schmidt 1978), also it can have non-specific binding of FA to extracellular polymeric substances (Szwerinski et al. 1985).
**Cell mass**

Nitrifier concentration can be determined directly by counting cell numbers under a microscope using a counting chamber (Keen and Prosser 1987; Murphy et al. 1997; Regan et al. 2007). This method however only counts total cell number and it is difficult to distinguish nitrifiers in a mixed culture.

Optical density (turbidity) could also be used to measure biomass concentration. A good linear relationship was found between dry matter concentration and optical density (Groeneweg et al. 1994). In the Ann Arbor, Michigan system, a small negative correlation between turbidity and monochloramine (monochloramine decreased during nitrification) was observed (Skadsen 1993). Turbidity increased or remained the same in most of the systems investigated in a 1991 survey (Wilczak et al. 1996).

**Nitrification Indicator**

Ideally, utilities would have a method of detecting nitrification early to allow the implementation of control strategies before water quality degrades significantly. However, traditional MPN methods are imperfect and require lengthy incubation times. The water industry therefore relies on surrogate water quality parameters as early warning indicators (Feben 1935; Regan 2001). Every indicator has a rationale for its use in nitrification detection (Table 1.5), but may not be applicable in certain systems (Table 1.6). So, no single water quality parameter by itself is a perfect indicator of nitrification, and several important factors need to be tracked including nitrite, nitrate, chloramine dosage and residual, ammonia concentration, pH, HPC, and dissolved oxygen (Wilczak et al. 1996).
Table 1.5: Nitrification Indicator and Rationale for Its Use

<table>
<thead>
<tr>
<th>Nitrification Indicator</th>
<th>Rationale</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite</td>
<td>Nitrite production corresponds with the viable cell count of <em>Nitrosomonas europaea</em>, but may lag behind MPN method</td>
<td>Engel and Alexander 1958; Lieu et al. 1993; Wilczak et al. 1996</td>
</tr>
<tr>
<td>Nitrogen species and balance</td>
<td>Decreased total ammonia concentrations accompanies nitrite and nitrate increase during nitrification</td>
<td>Wilczak et al. 1996</td>
</tr>
<tr>
<td>Difficulty in maintaining disinfectant residual</td>
<td>nitrification causes rapid loss of chloramine residual</td>
<td>Cunliffe 1991; Odell et al. 1996; Skadsen 1993; Wilczak et al. 1996; Wolfe et al. 1988</td>
</tr>
<tr>
<td>pH and alkalinity</td>
<td>Nitrification reduces pH and consume large amount of alkalinity</td>
<td>Hill 1946; Larson 1939</td>
</tr>
<tr>
<td>HPC</td>
<td>HPC increase accompany nitrification and a linear relationship exists between AOB and HPC when above 350 cfu/ml</td>
<td>Wolfe et al. 1990; Skadsen 1993; Odell et al 1996</td>
</tr>
<tr>
<td>DO</td>
<td>4.33 mg oxygen is consumed per mg ammonia oxidized during complete nitrification;</td>
<td>Grady et al. 1999; Wilczak et al. 1996; Odell et al. 1996</td>
</tr>
</tbody>
</table>

Table 1.6: Situations where Nitrification Indicators Do Not Always Work

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Pitfalls of Nitrification Indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disinfectant residual</td>
<td>Copper pipes-chloramines decay fast with/without nitrification; dead ends-chloramines are too low to compare decay with/without nitrification</td>
</tr>
<tr>
<td>pH</td>
<td>Concrete pipes-release high alkalinity; Iron pipe-consume H$^+$ through equation (4); high alkalinity water</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Concrete pipes-release high alkalinity; Iron and other pipes- nitrification effect is trivial compared to scale deposition during corrosion; high alkalinity water</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Iron/lead pipes-recycle nitrification product back to ammonia; ammonia loss through nitrification is insignificant compared to other pathways</td>
</tr>
<tr>
<td>Nitrite/Nitrate</td>
<td>Iron/lead pipes-recycle nitrite/nitrate back to ammonia; GAC-removes Nitrite/Nitrate ions</td>
</tr>
<tr>
<td>Nitrogen balance</td>
<td>Situations where organic nitrogen is present are hard to quantify</td>
</tr>
<tr>
<td>HPC</td>
<td>Dead ends-cannot tell if HPC increase is caused by chloramines depletion or nitrification; HPC distribution between water and biofilms on pipes varies due to water quality, flushing program, etc</td>
</tr>
<tr>
<td>DO</td>
<td>Dead ends-DO is affected more by the water flowing frequency; situations where pipe corrosion affects DO more than nitrification</td>
</tr>
</tbody>
</table>
CONTROL AND PREVENTION OF NITRIFICATION
The first and most important step in addressing nitrification is to conduct sufficient monitoring to
document normal fluctuations and identify abnormal values as early as possible and then initiate control
methods before severe nitrification problems occur (Harms and Owen 2004). Different control methods
might work differently in a specific environment (Table 1.7).

Optimize Chloramine Dose
The easiest and most cost-effective means of nitrification control is to optimize the chloramine dosage at
the treatment plant (Lieu et al. 1993). This includes optimizing the ratio of chlorine to ammonia or
increasing chloramine dose. Both of these methods are not effective control methods once nitrification
has begun, but they can be used as preventative measures.

Increasing the ratio of chlorine to ammonia can reduce the amount of free ammonia available as an
energy source for nitrifiers. Free ammonia is almost completely eliminated at a 5:1 weight ratio of Cl₂:
NH₃-N (Kirmeyer et al. 1993). Ratios of 4 to 4.5: 1 or 5: 1 have been suggested to control nitrification
(Harms and Owen 2004). Optimizing chlorine to ammonia ratio (68%) is the most common reported
nitrification control strategy in a recent survey (Seidel et al. 2005). Increasing the ratio of chlorine to
ammonia may have precluded nitrification in Garvey Reservoir in 1986 (Wolfe et al. 1988) but evidence
suggests that nitrification can take place even when only small amounts of ammonia are available (Odell
et al. 1996). No trend was found between the ratio of chlorine to ammonia and nitrification incidence in
an industry survey (Wilczak et al. 1996) , suggesting that this strategy is not always successful. This is
further illustrated by the experience of Ann Arbor, where a ratio of 4.75:1 was unsuccessful due to poor
control of ammonia (Skadsen 1993).
Table 1.7: Nitrification Control Methods: Effectiveness and Rationale

<table>
<thead>
<tr>
<th>Control Method</th>
<th>Likely effect in premise plumbing</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase chlorine to ammonia ratio</td>
<td>Sometimes effective</td>
<td>Reduces ammonia concentration, but nitrifiers could grow even with small amount of ammonia</td>
</tr>
<tr>
<td>Increase chloramine dose</td>
<td>Sometimes effective</td>
<td>Effective at preventing onset of nitrification, but not effective at controlling it once it has begun since nitrite increases chloramine decay rate</td>
</tr>
<tr>
<td>Breakpoint chlorination</td>
<td>Sometimes effective</td>
<td>Chlorine can inactivate nitrifiers, and no ammonia provided compared to chloramine, but sometimes chlorine completely decays before it gets to premise plumbing</td>
</tr>
<tr>
<td>Decrease water age (flushing)</td>
<td>Always effective, but for short term</td>
<td>Nitrifiers can be flushed from system, but could reestablish between flushing intervals</td>
</tr>
<tr>
<td>Enhance facility property</td>
<td>Always effective</td>
<td>Better design will mean fewer stagnation and dead end areas</td>
</tr>
<tr>
<td>(better equipment design)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using chlorite</td>
<td>Effective but use with caution</td>
<td>Chlorite can control nitrification, but can be toxic in high levels</td>
</tr>
<tr>
<td>Remove organic matter</td>
<td>Sometimes effective</td>
<td>Reduces chloramine decay and heterotrophic growth</td>
</tr>
<tr>
<td>Adjust pH</td>
<td>Sometimes effective</td>
<td>Competing effects of increasing pH: 1. reducing nitrifier growth, reducing chloramine decay; 2. reducing chloramine inactivation effect</td>
</tr>
<tr>
<td>Control nutrient level</td>
<td>Sometimes effective</td>
<td>Some nutrient levels may be in a range that is impractical to control</td>
</tr>
</tbody>
</table>

Nitrification can theoretically be prevented if the total chlorine concentration in a specific location within the distribution system is greater than the value predicted when AOB growth and inactivation rates are equal (Fleming et al. 2005; Fleming et al. 2008). This method can only prevent the onset of nitrification and the suggested value is between 2-4 mg/L (Harms and Owen 2004). However, it is not effective when nitrification has begun, even with a high dose up to 8 mg/L (Fleming et al. 2005; Odell et al. 1996; Skadsen 1993). Also, long-term operation of increasing chloramine dose is not feasible under the Stage 1 DBPR which sets a Maximum Residual Disinfectant Level (MRDL) of 4 mg/L for chloramine (Harms and Owen 2004).

Adding booster stations to increase chloramine residual can be another control method (Wolfe and Lieu 2001). Uncontrolled blending of chlorinated and chloraminated water could occur near the booster station, and in some cases, cause breakpoint chlorination, increases in DBP levels, or decreases in disinfectant residuals (USEPA 2005).
Regardless of chlorine dose and chlorine: ammonia ratio in the main distribution system, problems can still occur in buildings, since chloramine can rapidly decay through reactions with copper pipe (Nguyen and Edwards. 2005).

**Breakpoint Chlorination**

Breakpoint chlorination is the process of switching from chloramines to free chlorine within the distribution system for a period of time (Harms and Owen 2004). Also known as “chlorine burn,” breakpoint chlorination is probably the most effective control measure for nitrification once an episode is under way (Odell et al. 1996). About ¼ of utilities surveyed periodically switched to free chlorine to control nitrification; mostly once per year with the duration ranging from less than a week to more than a month (Seidel et al. 2005). Breakpoint chlorination has been found effective in different studies (Odell et al. 1996; Skadsen 1993) but the switch to free chlorine can increase HPC or coliform growth, either due to free chlorine’s poor ability to disinfect particle-associated bacteria from GAC (Skadsen 1993) or biofilm sloughed off by the change (Odell et al. 1996). Other disadvantages to prolonged use of free chlorine are the formation of disinfection by-products and consumer complaints about chlorinous taste (Ferguson et al. 2005; Harms and Owen 2004).

**Decrease Water Age**

Operational practices that reduce water age can minimize nitrification. Increased turnover is practiced in water reservoirs and flushing is practiced in distribution systems to reduce water age (Odell et al. 1996; Wolfe et al. 1988). Flushing is the second most common practice (54%) for nitrification control (Seidel et al. 2005). Flushing can also remove tubercles and sediments and eliminate dead-ends, thus allowing the disinfectant better access to biofilms containing nitrifiers (Harms and Owen 2004). Flushing has been proved effective for short term nitrification control (Odell et al. 1996; Skadsen 1993), and it has to be implemented frequently and regularly to be effective (Wolfe and Lieu 2001). However, it is not possible for water utilities to control water age in buildings, since flush times are under the control of residents (Edwards et al. 2005).

**Upgrading Properties**
Utilities can employ a number of operational and design measures including installing recirculation facilities on standpipes and elevated storage, designing new reservoirs with inlet and outlet pipes to prevent short-circuiting, retrofitting reservoirs with baffles to improve circulation, looping dead-end mains and implementing regular flushing programs in problem areas (Odell et al. 1996).

**Chlorite Ion (ClO\textsubscript{2}⁻)**

Oxidation of ammonia by *Nitrosomonas europaea* is strongly inhibited by NaClO\textsubscript{2} (Ki, 2 μM). McGuire et al. (McGuire et al. 1999) proposed the addition of chlorite or use of chlorine dioxide (ClO\textsubscript{2}) to produce chlorite to inhibit the oxidation of ammonia and nitrite. Results in full scale distribution systems proved that chlorite ion is likely to suppress nitrification. However, the most recent studies by Passantino (Passantino 2003) and Karim & LeChevallier (Karim and LeChevallier 2006) showed that nitrification was not controlled by 0.5 mg/L chlorite especially after long-term application. Chlorite and chlorine dioxide are also known to cause respiratory problems and irritation when present at high concentrations and they are also regulated compounds (ATSDR 2005). The MCL for chlorite in drinking water is 1 mg/L and the Maximum Residual Disinfectant Level (MRDL) for chlorine dioxide is 0.8 mg/L (USEPA 2001a). Philosophically, it is difficult to justify addition of one disinfection by-product (chlorite) to water in order to allow use of a disinfection strategy designed to remove other disinfection by-products. Doing so implies a high degree of confidence in the lower potential health detriments of chlorite versus detriments from the disinfection by-products formed from free chlorine.

**Removing Organic Matter**

Organic matter can react with chloramines through oxidation reactions (Reaction 4, Table 1.1) and different studies have proven the role of Natural Organic Matter (NOM) in accelerating chloramine decay (Margerum et al. 1994; Song et al. 1999; Tomas 1987). Measures designed to slow the decay of chloramine and its associated release of ammonia should theoretically reduce the occurrence of AOB growth (Harrington et al. 2002). From this aspect, removing organic compounds at the treatment plant has the potential to be effective for long-term improvement of distribution system nitrification. In the Harrington et al. (2002) pilot scale study, removal of NOM by enhanced coagulation delayed the onset of nitrification compared to conventional coagulation. From another perspective, heterotrophic growth is suppressed by removing organic matter, and nitrification might be promoted considering the competition
between nitrifiers and heterotrophs. Removal of organic matter to low levels is not emphasized in the U.S. to the same extent as in some European countries, and further investigation is needed (Odell et al. 1996).

**Adjusting pH**

Water with a high pH may reduce nitrification by creating suboptimal growth conditions for AOB (Skadsen 2002). High pH can also reduce the rate of chloramine decay and formation of free ammonia. Tomas (Thomas 1987) stated that the rate of chloramine decay approximately doubles for a drop of 0.7 pH units. Elevating the pH of the finished water to greater than 9.3 reduced the frequency of nitrification in Ann Arbor (Skadsen 2002). Other water utilities also reported success in controlling nitrification by increasing water pH to 9 (Gates and Lavinder 1997; Kirmeyer 1995). Raising the pH can also help control corrosion and reduce lead and copper leaching to the water. It also controlled taste and odor problems by promoting monochloramine versus dichloramine formation (Skadsen 2002). On the other hand, high pH has been shown to decrease the effectiveness of chloramine for inactivating AOB (Harrington et al. 2002; Oldenburg et al. 2002), so the effect of pH in controlling nitrification is site specific (Oldenburg et al. 2002). In a pilot scale study, a rough rank of pH onset time of nitrification at three different pHs is pH 8.5, 8.9 and 7.9 from earliest to latest (Harrington et al. 2002).

**Control Nutrient Level**

Theoretically, nitrifying bacteria growth could be limited when a specific nutrient is less than optimal or inhibited when a nutrient is in excess. The levels of some nutrients present in U.S. drinking waters are in a range relevant to nitrifying bacteria growth (Table 1.3), so reducing a nutrient level to limit nitrifying bacteria growth or increasing nutrient level (for nutrients not toxic to human health) to inhibit nitrifying bacteria growth can be a possible approach to control nitrification. This method has not been considered in drinking water systems and more research should be conducted. Previous discussion in this paper also highlighted the potential importance of this mode of control in copper pipe and galvanized plumbing often found in buildings.
REGULATIONS REGARDING CONTROL OF NITRIFICATION
The state of Florida has required utilities to monitor for nitrite in distribution systems since the 1980s (Wolfe et al. 1988). EPA Phase II inorganic contaminant regulations require water systems to sample for nitrite and nitrate at each entry point to the distribution system on at least an annual basis. Additional monitoring is required on a quarterly basis for at least one year following any one routine sample in which the measured concentration is greater than 50% of the MCL (USEPA 2001a). Some consideration has been given to require more nitrite monitoring within chloraminated distribution systems (USEPA 2003). Chlorination is required annually by North Carolina. The duration is from a few days to a few months (Harms and Owen 2004).

CONCLUSIONS
More water utilities are switching to chloramine as a secondary disinfectant to comply with disinfectant rules and nitrification is a major concern in this implementation. Drinking water systems provide a favorable environment for the growth of nitrifying bacteria. Nitrification occurring in drinking water systems can cause water quality deterioration, corrosion, and difficulty maintaining disinfectant residuals. Monitoring for nitrification could be conducted through a culturing method, molecular method or from water quality indicators, but every method has limitations. This poses a significant challenge to understand and control nitrification in drinking water systems. Until now, optimizing disinfectant dosing, controlling water quality and improving water facilities are the three major considerations for nitrification control; but these methods are all site-specific and no consistently efficient methods have been proposed.

ACKNOWLEDGEMENT
This work was supported by National Science Foundation (NSF) under Grant No. DMI-0329474, American Water Works Association Research Foundation (AWWARF) and the United States Environmental Protection Agency (USEPA). Matching funds were also provided by the Copper Development Association (CDA). The opinions, findings, conclusions, or recommendations are those of the authors and do not necessarily reflect the views of AWWARF, USEPA, or the CDA.
1. Conceptual model based on Verhagen and Laanbroek’s theory that the existence of nitrifiers depends on whether heterotrophic bacteria are limited by carbon or nitrogen. If heterotrophic growth is limited by carbon, nitrifiers can coexist with heterotrophs and the heterotrophic growth rate is:

\[ \mu_1 = \mu_{\text{max}} \cdot \frac{C}{K_s + C}, \]

where \( \mu_{\text{max}} \) is the maximum specific growth rate of heterotrophs, \( K_s \) is the half saturation constant for carbon, \( C \) is the carbon concentration.

If heterotrophic growth rate is limited by ammonia nitrogen, no ammonia is available for nitrifiers and therefore nitrifier growth is negligible. In this case the heterotrophic growth rate is:

\[ \mu_2 = \mu_{\text{max}} \cdot \frac{N}{K_N + N}, \]

where \( \mu_{\text{max}} \) is the maximum specific growth rate of heterotrophs, \( K_N \) is the half saturation constant for ammonia nitrogen, \( N \) is the ammonia concentration.

\[ \mu_1 = \mu_2 \] when the carbon-to-nitrogen ratio is at a critical value:

\[ \frac{C}{N} = \frac{K_s}{K_N} \]

By applying known kinetic parameters to a system, the critical C/N ratio can be calculated, and nitrification occurrence in a system can be predicted, specifically, nitrification is not likely to occur if C/N is above the critical value and nitrification is possible if C/N is below the critical value. Verhagen and Laanbroek, 1991 measured a critical C/N ratio of about 10. Plot this line for carbon and nitrogen levels in drinking water (Figure 1), and if the C and N concentrations are above this line, nitrifiers are negligible in the system. If C and N concentrations are below this line, both nitrifying bacteria and heterotrophic bacteria grow in the system, and which one dominates can be defined by the following equation:

2. Compare relative growth rate of nitrifiers and heterotrophs-Dominancy of growth depends on the relative growth rate of heterotrophic bacteria and nitrifiers.
At 20°C, nitrifier growth is limited by ammonia, assuming $\mu_{\text{max}} = 0.034 \, \text{hr}^{-1}$, $K_s = 1.3 \, \text{mg/L}$ (Grady et al., 1999),

$$\mu = 0.034 \cdot \frac{N}{1.3 + N},$$

where $\mu_{\text{max}}$ is the maximum specific growth rate for nitrifiers, $K_s$ is the half saturation constant, $N$ is the ammonia concentration.

Heterotrophic growth is limited by carbon, assuming $\mu_{\text{max}} = 0.62 \, \text{hr}^{-1}$, $K_s = 5 \, \text{mg/L}$ as COD (Grady et al., 1999),

$$\mu = 0.62 \cdot \frac{C}{5 + C},$$

where $C$ is the organic carbon concentration.

Let nitrifier growth rate equal heterotroph growth rate:

$$C = \frac{0.17N}{0.806 + 0.586N}$$

Plot this line for carbon and nitrogen levels in drinking water (Figure 1), and if $C$ and $N$ concentrations are above this line, heterotrophic bacteria will overgrow nitrifiers and dominate the system. If $C$ and $N$ concentrations are below this line, nitrifying bacteria will overgrow heterotrophs and dominate the system.
REFERENCES


CHAPTER II: EFFECT OF AMBIENT NUTRIENTS AND TOXINS ON
NITRIFICATION IN PREMISE PLUMBING

Yan Zhang and Marc Edwards

ABSTRACT

Growth of nitrifying bacteria in potable water systems was strongly impacted by nutrient levels and the presence of certain metals. Copper levels above 100 ppb were toxic to nitrifiers. Low levels of phosphate (< 5 ppb) inhibited nitrification, an effect that could be exacerbated by competition from heterotrophic bacteria. Different pipe materials can release nutrients or metals to water, and can either inhibit or enhance the growth of nitrifier biofilms. Alkalinity has important direct and indirect impacts on nitrification, since at least a trace amount of alkalinity (2-3 ppm) or inorganic carbon is required for autotrophic nitrifier growth, and higher alkalinitities can reduce the toxic effect of copper and zinc to nitrifying bacteria through complexation/precipitation. Nitrifiers stimulated growth of heterotrophic bacteria by production of organic carbon and by accelerating the rate of chloramine disinfectant decay.
INTRODUCTION

The conversion of ammonia to nitrate by nitrifying bacteria (Wolfe and Lieu 2001; Zhang et al. 2009b) poses an indirect health concern in drinking water systems. This is because it can accelerate loss of disinfectant residuals, enhance corrosion of water distribution system materials, or increase lead/copper contamination of water under at least some circumstances (Douglas et al. 2004; Murphy et al. 1997; Odell et al. 1996; Skadsen 1993; Wilczak et al. 1996). Nitrification is usually controlled by optimizing chloramine, adjusting pH, adding chlorite or flushing (Harrington et al. 2002; McGuire et al. 1999; Skadsen 1993; Zhang et al. 2009b). But recent laboratory and field work has highlighted the potential importance of nutrients and pipe materials in controlling the growth of nitrifying bacteria in potable water systems (Zhang et al. 2008). If these factors proved to be important, they could help explain why nitrifiers proliferate in some systems and not others, and could therefore be exploited to control nitrification via nutrient limitations in some situations.

Limiting nutrients have been of interest for heterotrophic bacteria (Srinivasan et al. 2008), and it could be anticipated that the availability of nutrients and potential microbial toxins in a potable water supply could affect the occurrence of nitrification as well. This dependency has been successfully addressed for chloramine disinfectant and free ammonia substrates in prior work (Fleming et al. 2005; Fleming et al. 2008). For example, a nitrification potential curve was proposed based on the equation:

\[
[\text{Total chlorine}] = \frac{R_{gi} \times [\text{free ammonia}]}{Ks + [\text{free ammonia}]},
\]

Where

[Total chlorine]: the sum of free chlorine, monochloramine and dichloramine concentrations, ppm-Cl₂

\(R_{gi}\): the minimum total chlorine concentrations needed to prevent nitrification for any free ammonia concentration, ppm-Cl₂

Ks: half saturation constant for ammonia oxidizing bacteria, ppm-N

[free ammonia]: the sum of ammonia (NH₃) and ammonium (NH₄⁺) concentrations, ppm-N

In essence, the equation illustrates that if the death rate of nitrifying bacteria via disinfection exceeds the growth rate from ammonia consumption, then nitrifying bacteria have difficulty in becoming
established. In contrast, if the nitrifier growth rate exceeds the death rate, then serious nitrification problems can occur even under continuous flow conditions present in water distribution systems (Fleming et al. 2005; Fleming et al. 2008).

While this work (Fleming et al. 2005; Fleming et al. 2008) was a critical first step, there are many other nutrients and toxins present in a water distribution system that can also play controlling roles in nitrification. For example, nitrifiers need nutrients other than ammonia to grow, as represented by an overall equation (Zhang et al. 2009b):

\[ C + \text{NH}_3 + P + \text{Other Nutrients (i.e., K, Mg, Ca, Co, Mo)} + O_2 \rightarrow \text{Nitrifier biomass} \]

The inorganic carbon, ammonia, phosphorus and oxygen are termed macro nutrients in this work, because they are utilized by nitrifiers in significant quantities, whereas other nutrients are only required at trace levels. Each of these nutrients has four possible metabolic impacts on nitrification, depending on its concentration. If the nutrient concentration is zero or too low, the deficiency can decrease the growth rate or completely prevent microbial growth (Reeves et al., 1981; Fransolet et al., 1988). As the nutrient concentration increases, bacterial activity may be restored, and can reach an optimum concentration that is dependent on the circumstance of growth. At very high concentrations, some constituents may inflict detrimental effects to nitrifier growth (Zhang et al. 2009b).

In a recent survey of 330 raw drinking waters in the U.S. (i.e., waters prior to treatment), many elements important to nitrifier growth were found to occur in concentrations of likely biological significance relative to nitrifiers nutrient requirements (Parks et al. 2004; Zhang and Edwards 2005). For example, based on the study of Loveless and Painter 1968 (Loveless and Painter 1968), it is estimated that in about 40% of U.S. waters magnesium is below optimal levels for nitrifiers, whereas about 10% of waters have magnesium levels high enough to inhibit growth of nitrifying bacteria (Zhang and Edwards 2005).
In addition to consideration of nutrients in the raw water supply, pipe materials can also release or modify levels of nutrients available to nitrifiers (Zhang et al. 2009b). For example, earlier studies demonstrated that iron pipe can release bioavailable phosphorus for bacterial growth (Morton et al. 2005), and similar reactions might be important for nitrifiers. Iron and lead pipe might also form ammonia from nitrate (Huang and Zhang 2005; McIntyre and Mercer 1993; Zhang and Edwards 2007), thereby allowing a relatively small amount of free ammonia to sustain large populations of nitrifying bacteria. These reactions could require modification of the governing nitrification potential curve prediction by Fleming et al. 2005 and 2008 in some waters, and might even completely control nitrification occurrence in others.

The interplay between organic carbon, and the potential competition between heterotrophic bacteria and nitrifying bacteria is also a worthy research concern. Work in wastewater systems has demonstrated that nitrifiers and heterotrophs compete for surfaces, dissolved oxygen, ammonium and other nutrients (Rittmann and Manem 1992). Nitrification is an energy intensive process and nitrifiers have lower growth rates compared to heterotrophs (Rittmann and Manem 1992), so nitrifying biofilms can be reduced due to competition for ammonia and other substrates (Ohashi et al. 1995; Verhagen and Laanbroek 1991). On the other hand, when no dissolved organic carbon is present in the water, heterotrophs can be completely dependent on the lysis and extracellular products of nitrifiers as their source of organic carbon (Rittmann et al. 1994).

The overall objective of this study was to investigate the impact of nutrient levels and pipe materials on nitrification activity, under conditions found in premise plumbing systems. Several different experiments were undertaken, each designed to address specific research objectives.
MATERIALS AND METHODS

Nutrient Effect Study in PVC Pipes

PVC pipes were used as a control material throughout this experiment. Nitrifiers were established in PVC pipes (30 cm length × 1.9 cm diameter) using Blacksburg tap water, which was dechlorinated as described in Chapter III. Water in the pipes was changed twice a week, using a “dump and fill” protocol, to simulate infrequent water use in buildings and to replenish nutrients for nitrifier growth. These pipes were maintained at room temperature and were covered with black plastic throughout the experiment, to prevent possible light inhibition to nitrification (Wolfe and Lieu 2001). After 3 months of inoculation, complete nitrification (100% ammonia loss) was confirmed to occur in PVC pipes for less than 24 hours of stagnation time.

The water fed into the PVC pipes was then changed from Blacksburg tap water to synthesized potable water. This synthesized water contained (NH₄)₂SO₄ (1 ppm N), MgSO₄ (0.5 ppm Mg), CaCl₂ (1 ppm Ca), KCl (10 ppm K), Na₂HPO₄ (10 ppb P), NaHCO₃ (500 ppm) and other trace nutrients (5 ppb Cu²⁺, 1.7 ppb Mo⁶⁺, 0.1 ppb Co²⁺, 5.6 ppb Mn²⁺, 2.6 ppb Zn²⁺ and 0.1 ppm Fe²⁺). The pH of the water was adjusted to 8 before it was poured into the pipes. Complete nitrification continued in all PVC pipes after the switch to synthesized water. After two months exposure, the water was systematically modified to introduce possible nutrient limitations (Table 2.1), and the resulting impacts on nitrifier activity were assessed. At another point in this experiment, the alkalinity of the synthesized water was reduced to 10 ppm as CaCO₃. The role of trace nutrients was also studied by completely eliminating a given trace nutrient (no Mo⁶⁺, no Co²⁺, no Mn²⁺, no Zn²⁺ or no Fe²⁺).
Table 2.1: Nutrient levels investigated via modification of control water.

<table>
<thead>
<tr>
<th>Copper (ppb)</th>
<th>Zinc (ppb)</th>
<th>Phosphorus (ppb)</th>
<th>Magnesium (ppm)</th>
<th>Calcium (ppb)</th>
<th>Potassium (ppb)</th>
<th>Alkalinity (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>5</td>
<td>0.05</td>
<td>50</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>10</td>
<td>0.5</td>
<td>300</td>
<td>1000</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>50</td>
<td>5</td>
<td>1000</td>
<td>10000</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td>500</td>
<td>100</td>
<td>25</td>
<td>4900</td>
<td></td>
<td>50</td>
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<tr>
<td>500</td>
<td>1000</td>
<td>1000</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: for each nutrient level tested, there are 5 replicate pipes, giving $38 \times 5 = 210$ pipes total.

Effect of Inorganic Carbon and Phosphate Nutrients in Lead Pipes

Specifics of this experiment are described elsewhere (Zhang et al. 2009a). Nitrification was established in lead pipes at 5, 60 and 1000 ppb orthophosphate (4 pipes at each phosphate level). The alkalinity of the synthesized water was then dropped stepwise from 100 ppm down to 30, 15 and then 0 ppm as CaCO$_3$, allowing sufficient time at each alkalinity for relatively stable levels of nitrification, pH and lead leaching to be achieved. Water in the pipes was changed twice a week.

Nutrient Leaching from Copper, Concrete and PVC pipes

In this experiment new pipes (PVC, soft copper and hard copper pipes) were purchased from a hardware store to test for leaching of possible nutrients from these materials to potable water. The pipes were 30 cm length $\times$ 1.9 cm diameter. Synthesized phosphate-free control water was added to the pipes for a 96 hour holding time, after which water samples were collected for analysis. A small piece of soft and hard copper was also dissolved in 5% nitric acid and tested to determine pipe composition.

To test leaching of nutrients from concrete materials and its effect on nitrifier growth, glass pipes (30 cm length $\times$ 1.9 cm diameter), containing small mounted coupons of aged concrete, were used (Concrete SA:Volume = 0.6 cm$^{-1}$). Parallel tests were conducted using glass pipes without concrete. Nitrifier
growth was established in both sets (w/ and w/o concrete) by inoculating pure culture *Nitrosomonas europaea* ATCC 19718 and no effort was made to sustain the pure culture over a period of three months. After stable nitrification was established in the glass pipes, both with and without concrete, inoculation was stopped and water with different nutrient levels was introduced (Table 2.1). Water was held stagnant for 96 hours between water changes and tested for nutrient changes and nitrification activity.

**Recycling of ammonia from nitrite and nitrate by iron, copper, lead and zinc**

This experiment examined possible cycling of ammonia, from nitrite and nitrate, by reactions with iron, lead and zinc metals. Amber glass TOC test vials (25 mm × 95 mm) with Mininert valves that allowed for headspace sampling were used (Lee 2004). Pure metal wires (20 mm length × 1 mm diameter) (Sigma Aldrich) were placed into vials containing 30 ml of simulated Potomac River water (Chapter V). In some cases the water was spiked with either 10 ppm NO$_2^-$ or 10 ppm NO$_3^-$. Water in the vials was changed twice a week and was analyzed for changes in ammonia, nitrite and nitrate. A total of 36 tests were conducted (= 3 metals (iron, lead or zinc) × 2 oxygen conditions (aerobic or anaerobic) × 2 pHs (6 or 8) × 3 (triplicate samples))

**Effect of Organic Carbon in PVC Pipes**

PVC pipes of 30 cm length × 1.9 cm diameter were used. The PVC pipes were exposed to a synthesized water for one year without nitrification, and then exposed to water with ammonia (and resulting nitrification) for 15 months, as described elsewhere (Zhang et al. 2008). No disinfectant was ever added to these pipes. Thirty pipes were exposed at 5, 60 and 1000 ppb orthophosphate-P (10 pipes at each phosphate level). The ten replicate pipes were separated into three groups. The first group (4 pipes) was a control which was unchanged. The second group (3 pipes) was modified by incrementally adding organic carbon at 20, 100, 500 and 1000 ppb TOC. The organic carbon used initially was ozonated natural organic matter (for preparation method, see (Zhang and Edwards 2007)). Thereafter, glucose was used as per other studies (Lechevallier et al. 1990). The remaining group (3 pipes) was tested in parallel with organic carbon, but without any added ammonia.
Analytical Methods

Nutrient Effect Study in PVC and Lead Pipes
A composite sample from the five duplicate pipes at each nutrient condition was collected after 7 hours stagnation for routine quantification of nitrification activity (measurement of ammonia loss, production of nitrite and nitrate). NH$_4$-N was measured via salicylate method using a HACH DR/2400 spectrophotometer, according to Standard Method 4500 NH$_3$ (Clesceri et al. 1998). NO$_2$-N and NO$_3$-N were measured using DIONEX, DX-120 ion chromatography (IC), according to Standard Method 4110 (Clesceri et al. 1998). At the end of the experiment, nitrifier density was also monitored by a five-tube Most Probable Number (MPN) procedure (Wolfe et al. 1990b), as detailed elsewhere (Zhang et al. 2008).

Nutrient Release from Copper, Concrete and PVC Pipes
Total metal and phosphorus release was quantified after digestion in 2% nitric acid for 24 hours in a 90°C oven. Metal concentrations were quantified using an Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS), according to Standard Method 3125-B (Clesceri et al. 1998).

Recycling of Ammonia from Nitrite and Nitrate by Iron, Copper, Lead and Zinc
Samples of water before and after stagnation in the pipes (composite sample from triplicate samples) were analyzed routinely for pH, NH$_4^+$, NO$_2^-$ and NO$_3^-$.

Effect of Organic Carbon in PVC Pipes
Nitrifier activity was monitored by ammonia loss. Nitrifier density was monitored by the five-tube Most Probable Number (MPN) procedure (Wolfe et al. 1990a; Zhang et al. 2008). Heterotrophic bacteria were monitored by Heterotrophic Plate Count (HPC) according to Standard Method 9215 (Clesceri et al. 1998) using the spread plate method with R2A medium.
RESULTS AND DISCUSSION

Experiments were conducted that included: 1) survey of possible nutrient effects on nitrifier activity, 2) nutrient release and ammonia cycling by different pipe materials, and 3) interplay between nitrifiers and heterotrophs.

Effect of Nutrient Level on Nitrifier Growth and Activity

**Copper.** Although copper (II) is a key component of the ammonia monooxygenase (AMO) enzyme that is essential for nitrifier growth (Ensign et al. 1993; Richardson and Watmough 1999), high copper levels are known to be toxic to nitrifiers (Braam and Klapwijk 1981). At 10 ppm as CaCO$_3$ alkalinity and initial pH 8, the percentage of ammonia oxidized increased from 50% to 70% when the copper level was increased from 0 to 20 ppb. Increases of the copper concentration to 100 ppb decreased the percentage of ammonia oxidation to 28%, and further increases in copper nearly eliminated nitrifier activity (Figure 2.1). The corresponding nitrifier MPN increased by an order of magnitude when 5 ppb copper was added versus 0 ppb, but when the copper concentration was increased to above 100 ppb (from 20 ppb), the MPN decreased by two orders of magnitude. While the MPN results were not significant at > 95% confidence, the overall trend confirms the full range of impacts on nitrifier activity, and the role of copper in preventing colonization of brass and copper surfaces by nitrifiers (Zhang et al. 2008).
Figure 2.1: Average ammonia loss (%) and nitrifier MPN at different copper concentrations after 7 hour stagnation. Ammonia loss data represent the average of 8 measurements on water collected after 7 hours stagnation. MPN measurement was on water collected at the end of the test. Error bars in all figures indicate 95% confidence intervals.

**Phosphorus.** Phosphorus is an essential nutrient for all microbial growth, including nitrifying bacteria (Zhang et al. 2009b). Previous studies have reported that at least 3-20 ppb phosphate is necessary for effective nitrification for ammonia removal in drinking water treatment (Van der Aa et al. 2002; Van Droogenbroeck and Laudelout 1967). In this study, for the first two weeks, ammonia oxidation (30-60%) was observed even in the pipes with no phosphate added (Figure 2.2). It is speculated that this is because 10 ppb phosphorus was previously dosed in all pipes before the phosphorus levels were varied, and some of this phosphate might have been stored in biofilm or pipe surface deposits (Van Droogenbroeck and Laudelout 1967).

After two weeks, the effect of phosphorus on nitrification activity became very clear (Figure 2.2). Specifically, only about 10% of the ammonia was oxidized when no phosphorus was dosed. At phosphate levels of 5 and 10 ppb, the percentage of ammonia oxidized increased to 40% and 50%,
respectively (Figure 2.2). The highest percentage of ammonia oxidation was observed at 1000 ppb phosphate. The corresponding data for nitrifier MPN indicated two orders of magnitude more nitrifiers at 1000 ppb-P (100000 MPN/100 ml) versus no and 5 ppb phosphate (1000 MPN/100 ml).

![Figure 2.2: Average ammonia loss (%) at different phosphorus concentrations after 7 hour stagnation.](image)

**Figure 2.2: Average ammonia loss (%) at different phosphorus concentrations after 7 hour stagnation.** Note: Alkalinity = 10 ppm. Data reported are the average of five duplicates measured after 7 hours.

**Zinc.** Previous research indicated that zinc can inhibit nitrification, possibly by precipitating phosphorus nutrients (Harper et al. 1996), and that 0.5 ppm Zn$^{2+}$ significantly reduced nitrification (Bott 2005). While the effect was weak in this study, dosing of 1000 ppb zinc in PVC pipes significantly decreased ammonia loss (%) and nitrifier MPN, versus lower zinc levels (Figure 2.3).

**Other Trace Nutrients Tested.** For all other elements investigated in this study (Table 2.1) including magnesium, potassium and calcium, no significant ammonia loss or nitrifier MPN difference was observed at different levels, although high levels of magnesium (50 ppm) did slightly decrease nitrification compared to lower levels ($p = 0.006$). Eliminating the trace nutrients from the synthesized water also had no effect on ammonia loss in a nine week experiment.
Figure 2.3: Average ammonia loss % and nitrifier MPN at different zinc concentrations after 7 hour stagnation. Note: Alkalinity = 10 ppm. For Ammonia loss %, data reported were the average of 8 measurements; each measurement was taken 4 hours after water change. MPN measurement was done at the end of the test.

Direct and Indirect Effects of Alkalinity

**Direct Effect.** A large amount of alkalinity is consumed during the oxidation of ammonia to nitrate. Specifically, 8.62 mg $\text{HCO}_3^-$ (14 ppm as CaCO$_3$) is consumed for every mg $\text{NH}_4^+$-N oxidized (Grady et al. 1999). If alkalinity is consumed in wastewater applications, which are open systems, nitrification activity can be severely impacted. It was previously believed that this limitation arose due to acidic pHs ($\text{pH} < 6$) inhibiting nitrification, but it was recently suggested that the loss of alkalinity corresponded with a virtual complete loss of inorganic carbon in the open systems under investigation due to stripping. That is, nitrifiers were demonstrated to remain active even at pH 3.2 in a closed system (Tarre and Green 2004).

In this study of PVC pipes that were closed to the atmosphere, higher alkalinity was clearly beneficial to nitrifier growth, as indicated by higher MPN and ammonia loss (Figure 2.4). But nitrification activity
was not completely stopped when no alkalinity was added, and the only alkalinity source was 2-3 ppm as CaCO₃ attributed to CO₂ contamination in NaOH used to adjust pH. In later experiments with lead pipes (Zhang et al. 2009a), in which fresh caustic was used without any significant CO₂ contamination, nitrification activity was immediately stopped when inorganic carbon was reduced to 0 ppm (Figure 2.7). The key point is that nitrifiers in closed potable water systems only require very low levels of alkalinity (< 5 ppm as CaCO₃) to fulfill their need for inorganic carbon.

![Graph](image)

**Figure 2.4: Average ammonia loss % and MPN values at different alkalinity added.** Note: For Ammonia loss %, data reported were the average of 9 measurements; each measurement was taken 4 hours after water change. MPN measurement was done at the end of the test. “0” added alkalinity actually had a trace 2-3 ppm inorganic carbon from contamination of NaOH used to adjust pH.

**Indirect Effect of Alkalinity on Copper and Zinc Toxicity.** Alkalinity did have an important indirect effect on the levels of copper and zinc that were toxic to nitrifiers. For example, at 10 ppm alkalinity, ammonia loss was significantly lower when copper levels were >100 ppb, but no such inhibiting effect was observed for the same level of copper at 500 ppm as CaCO₃ alkalinity (Figure 2.5). This might be
expected, given that the level of free Cu\textsuperscript{2+} can be reduced by complexation or precipitation with carbonate species (Braam and Klapwijk 1981). Indeed, solubility models (AWWARF and DVGW-TZW 1996; Edwards et al. 1996) predict less free Cu\textsuperscript{2+} at 500 ppm alkalinity than at 10 ppm alkalinity (Figure 2.6). It is quite possible, then, that the high alkalinity (400-450 ppm) in Willmar, MN (Murphy et al. 1997) was a key reason that nitrification could occur in copper pipes, whereas in our research at 100 ppm alkalinity or lower, no nitrifier activity could be established in copper pipe even after 2.5 years. Similar mechanisms can explain the mitigating effect of alkalinity on zinc toxicity to nitrifiers.

Figure 2.5: Effect of copper and zinc concentrations on ammonia loss at two different alkalinitities.

Note: Data reported for each alkalinity is the average of at least four measurements. At very high alkalinity zinc and copper toxicity was eliminated.
Figure 2.6: Prediction of % free Cu$^{2+}$ at two different alkalinity levels for a range of pH levels.

Note: Error bars in this figure illustrate the upper and lower range of the prediction when added cupric concentrations vary from 5-500 ppb.

**Indirect Effect of Alkalinity on Phosphate Limitations.** Alkalinity had no effect on phosphorus limitations in PVC pipes. That is, phosphate limitation was observed at both low (10 ppm) and high (500 ppm) alkalinity. But in lead pipes, at 100 ppm alkalinity, complete nitrification (100% ammonia loss) occurred at all three phosphate levels (5, 60, and 1000 ppb—Figure 2.5), indicating that nitrification was not limited by the low levels of phosphate. When alkalinity was reduced to 30 and 15 ppm, ammonia loss in the pipes with 5 and 60 ppb phosphate was reduced to <60%, while 100% ammonia loss was still occurring in the pipes with 1000 ppb-P.

There are two possible explanations for this effect. First, it is important to note that there was substantial uptake (>90%) of the phosphorus by the lead material, and as a result the available phosphorus concentration in the bulk water was much lower. Phosphate limitation was obvious in lead pipes with 60 ppb-P added (Figure 2.7), but these levels of phosphate do not apply to pipe systems such as PVC, where no P is removed from the water. Besides, higher alkalinity might tend to reduce the uptake of phosphate from the water by lead corrosion because carbonate and phosphate are both anions that could adsorb to lead surface or form precipitates with lead ions; consequently, phosphate limitation is less obvious at higher alkalinity levels (Figure 2.7). Second, lower alkalinity and lower phosphate might
induce a “dual limitation” to growth rates. Further research is needed to explicitly examine these hypotheses.

![Graph showing ammonia loss % at different alkalinity and phosphate levels in lead pipes.](image)

**Figure 2.7: Average ammonia loss % at different alkalinity and phosphate levels in lead pipes.**

Note: data reported were the average of three replicates; each measurement was taken 3.5 days after water change.

### Nutrient Release and Ammonia Cycling from Different Pipe Materials

**P Release from Soft Copper.** Pipe materials can directly release or modify the nutrient levels available to nitrifiers (Zhang et al. 2009b). For example, earlier studies demonstrated that P-contaminants in iron metal can be released to water and increase bioavailable phosphorus for bacterial growth (Morton et al. 2005). In this study, soft copper pipe was confirmed to contain 0.05% (weight %) phosphorus, and 20-40 ppb phosphorus was detected in water held in soft copper pipe after 96 hours stagnation. Phosphorus released from these pipes is therefore sufficient to support nitrifier growth, even if no phosphorus is available from the drinking water. In contrast, no phosphorus was detected from hard copper or PVC pipes, although earlier studies have indicated that reduced phosphorus (i.e., phosphite PO$_2^-$) can be released from polyethylene pipes (Lehtola et al. 2004).

**Concrete.** The leaching of lime from concrete resulted in an initial increase in pH to 11, which inhibited nitrifier growth until the pH dropped back below 9.3. But on the other hand, concrete materials
leached out essential nutrients like phosphorus, magnesium, calcium and potassium (Table 2.2) and removed a substantial fraction of copper and zinc from the water, thereby reducing their toxicity (Table 2.2). Indeed, in this study, after lower pHs and stable nitrification were established on concrete, no nutrient limitation or toxicity was observed, whereas a control experiment with glass pipes resulted in severe nutrient limitations (e.g. low PO$_4^{3-}$ and K$^+$) and inhibitions (e.g. high Zn$^{2+}$ and Cu$^{2+}$). These observations can help explain why some studies found lower nitrifiers/HPC were associated with concrete pipe materials (Steward and Lieu 1997) versus others, where more heterotrophic bacterial counts have been observed with concrete lined pipes than other materials (Le Puil et al. 2003). It is possible that higher pHs inhibited nitrifier growth in the former study, whereas the availability of nutrients from concrete caused higher microbial growth in the latter study.

Table 2.2: Change in nutrient level by leaching from concrete materials to water. Concrete added essential trace nutrients such as P, Mg, Ca and K, and removed toxic constituents including zinc and copper.

<table>
<thead>
<tr>
<th></th>
<th>Concentration added, ppb</th>
<th>Final concentration with concrete, ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0</td>
<td>230</td>
</tr>
<tr>
<td>Mg</td>
<td>0</td>
<td>1910</td>
</tr>
<tr>
<td>Ca</td>
<td>0</td>
<td>9850</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
<td>2590</td>
</tr>
<tr>
<td>Cu</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>66</td>
</tr>
<tr>
<td>Zn</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>52</td>
</tr>
</tbody>
</table>

**Ammonia Cycling.** Earlier studies reported that iron and lead materials abiotically converted nitrate to ammonia (McIntyre and Mercer 1993; Uchida and Okuwaki 1998; Zhang and Edwards 2007). In this study, at all pH and oxygen conditions, pure iron consistently produced 0.5-1.2 ppm NH$_3$-N from 10 ppm NO$_2$-N and NO$_3$-N. The small fractional conversion is not surprising considering the very small
metal surface area to volume ratio (0.02 cm⁻¹) used (Kielemoes et al. 2000), which would be equivalent to water being held in a 200 cm diameter pipe. Greater fractional conversion would be expected in smaller diameter pipes.

For lead and zinc, significant ammonia production was only observed at the pH 6 aerobic condition, for which up to 1.3 ppm ammonia was converted by lead and up to 0.5 ppm ammonia was converted by zinc. Overall, the results confirm that under some circumstances these metals (iron, lead and zinc) can generate ammonia from nitrite and nitrate. The implication is that very significant nitrification and pipe corrosion might occur even at relatively low levels of free ammonia (Edwards and Dudi 2004).

In part because of cycling and an overall lack of toxicity effects from Fe⁺² or Pb⁺² species, iron and lead pipes proved to be beneficial to nitrification versus inert materials such as PVC or glass, as evidenced by higher levels of MPN bacteria and more rapid colonization (Zhang et al. 2008). In contrast, copper pipe can completely stop nitrification due to the disinfection properties of copper. But it is speculated that in some unusual situations, such as soft copper at very high alkalinities, copper surfaces might be beneficial due to released phosphorus and benefits from low levels of cupric ion in the water (Table 2.3). Concrete materials can also be either beneficial or detrimental to nitrification occurrence, depending on the circumstances (Table 2.3).
### Table 2.3: Predicted and observed effects of different pipe materials on nitrification

<table>
<thead>
<tr>
<th>Water Quality Change</th>
<th>Predicted Impact on Nitrification</th>
<th>Water Quality Change</th>
<th>Confirmed in this Project Impact on Nitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Serve as source of Fe, P; convert nitrate to ammonia; destruct disinfectant; good surface for attachment</td>
<td>Beneficial to nitrification</td>
<td>Consume disinfectant fast, convert nitrate and nitrate to ammonia</td>
</tr>
<tr>
<td>Concrete</td>
<td>Nutrients released, destroys chloramine and creates free ammonia, higher pH and alkalinity</td>
<td>High pH inhibit nitrification</td>
<td>Release necessary nutrient (P, Mg, Ca and K), absorb toxic nutrient (Cu and Zn), increase pH (up to 11) and alkalinity</td>
</tr>
<tr>
<td>Copper</td>
<td>Release copper</td>
<td>copper can be toxic to nitrifiers</td>
<td>Release copper (&gt;200 ppb) and phosphorus (20-40 ppb)</td>
</tr>
<tr>
<td>Lead</td>
<td>Convert nitrate to ammonia, release Pb</td>
<td>If convert nitrate to ammoia, beneficial to nitrification; but lead might be toxic to nitrifiers</td>
<td>Convert nitrate to ammonia under some circumstances, up to 8000 ppb lead released</td>
</tr>
</tbody>
</table>

### Effect of Organic Carbon

Nitrifiers and heterotrophs compete for ammonia, oxygen, space and other nutrients, and nitrifiers are usually at a competitive disadvantage to heterotrophs due to their slow growth (Furumai and Rittmann 1994; Jansson 1958; Ohashi et al. 1995; Verhagen and Laanbroek 1991). Regarding the competition for ammonia between nitrifiers and heterotrophs, previous modeling efforts (Zhang et al. 2009b) predicted that at 2 ppm ammonia, organic carbon levels need to be very high (> 10 ppm) in order for heterotrophs to significantly reduce nitrification. Experiments confirmed the expectation that organic carbon levels typically present in drinking water (up to 1 ppm) would not affect nitrification when phosphorus was 60 and 1000 ppb (data not shown, p ≥ 0.23).

Earlier studies indicated that nitrifiers and heterotrophs are both expected to be limited at low
phosphorus levels (P < 10 ppb) (Lehtola et al. 2004; Sathasivan and Ohgaki 1999; Van der Aa et al. 2002; Van Droogenbroeck and Laudelout 1967), and a competition for phosphorus can exist between the two groups. In this study, at 5 ppb-P, nitrifier MPN values in water with added TOC were lower (Figure 2.8) than without TOC (p = 0.03). This result indicates that while heterotrophs are unlikely to out-compete nitrifiers for ammonia, they might out-compete nitrifiers for phosphorus. As would be expected, at all three phosphate levels, nitrifier MPN values without added ammonia were significantly lower than those with ammonia (Figure 2.8 for 5 ppb-P).

In terms of heterotrophic bacterial growth, on average, there were $10^5$ cfu/ml heterotrophic bacterial counts even without added dissolved organic carbon to the water (organic free water was used in the study). It is therefore clear that heterotrophs can grow very satisfactorily on organic products of autotrophic nitrification, consistent with expectations based on earlier studies in wastewater systems (Rittmann et al. 1994). Compared to the pipes without added organic carbon, the addition of organic carbon (as glucose) up to 1 ppm either slightly increased HPC or had no effect (data not shown).

![Figure 2.8: Nitrifier MPN at 5 ppb phosphorus](image-url)
CONCLUSIONS

Nitrifying bacterial growth was demonstrated to be affected by different nutrient levels and pipe materials, specifically:

• Copper > 100 ppb and zinc at 1000 ppb significantly reduced nitrifier activity, but this effect was ameliorated at very high alkalinity. This can affect nitrification in galvanized iron (zinc coated iron), copper and brass plumbing materials.

• Without some inorganic carbon, nitrifiers cannot grow, although even 2-3 ppm DIC is sufficient for autotrophic growth. High alkalinity can stimulate nitrification due to high buffering capacity, reduced toxicity of copper and zinc (Figure 2.9). The higher buffering capacity keeps pH higher and the carbonate complexes/precipitates toxic Cu$^{+2}$ and Zn$^{+2}$, reducing their toxicity to nitrifiers. Alkalinity can also be influential in pipes such as lead, in which carbonate might decrease phosphate uptake by the pipe and allow more growth of nitrifiers.

• Nitrification activity can be limited if phosphate levels are very low. Availability of phosphorus to nitrifiers is a complex function of bulk water phosphate levels, phosphate that may be released from pipe material, phosphate uptake by pipe materials, and potential competition from heterotrophic bacteria (Figure 2.10). For example, in iron, plastic, concrete or copper pipes that can release phosphorus compounds, phosphate was less likely to be a limiting nutrient. For lead pipe, where phosphate was removed from the water by corrosion, phosphate can become a limiting nutrient especially at low alkalinity. Adding organic carbon might stimulate the competition for phosphate from heterotrophic bacteria and limit nitrification.
Different pipe materials can release nutrients or metals to water. These released constituents can either inhibit or enhance the growth of nitrifier biofilms (Table 2.3).

**Figure 2.9: Summary of alkalinity impacts on nitrification**

Inorganic Carbon Concentration, ppm-CaCO$_3$

Enhanced nitrification due to:
1. Buffering capacity increases
2. Toxicity from Cu$^{2+}$, etc decreases
3. PO$_3^{3-}$ availability increases

**Figure 2.10: Summary of phosphate impacts on nitrification**

(*see Chapter III-Figure 3.5, # see chapter I-Table 1.1)
REFERENCES


CHAPTER III: NITRIFICATION IN PREMISE PLUMBING: ROLE OF
PHOSPHATE, PH, AND PIPE CORROSION

Yan Zhang, Allian Griffin, Marc Edwards

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ABSTRACT

Nitrification in PVC premise plumbing is a weak function of pH over the range 6.5-8.5 and is insensitive to phosphate concentrations 5-1000 ppb. Lead pipe enhanced nitrification relative to PVC, consistent with expectations that nitrifiers could benefit from ammonia recycled from nitrate via lead corrosion. Relatively new copper pipe (< 1.5 years old) did not allow nitrifiers to establish, but nitrifiers gradually colonized over a period of months in brass pipes, when copper concentrations were reduced by pH adjustment or orthophosphate. Nitrifiers were inhibited by trace copper, but not by lead levels up to 8,000 ppb. In some systems using chloramines, brass in plastic plumbing systems might be more susceptible to lead/copper leaching, and accelerated dezincification, due to lower pH values resulting from nitrification.
INTRODUCTION

Preventing water quality degradation in potable water premise plumbing systems is an underappreciated challenge and a high priority for future research (Edwards et al. 2003; Snoeyink et al. 2006). Maintenance of safe water stored in buildings is of world-wide concern in situations where potable water is distributed to homes from central treatment plants or even produced on-site (Snoeyink et al. 2006; Sobsey et al. 2003). The type of degradation to water quality that can occur during storage and its ultimate significance to public health is controlled by a complex interplay of materials, corrosion, microbiology, aquatic chemistry and other factors.

In the United States utilities are increasingly using chloramine to comply with regulations for disinfection by-products (Seidel et al. 2005). The ammonia formed via chloramine decay can support autotrophic microbial nitrification. Organic carbon and acid produced via nitrification can stimulate growth of heterotrophic bacteria, contribute to loss of disinfectant, and also create problems with lead and copper contamination from corrosion of premise plumbing systems (Edwards and Dudi 2004; Harrington 2002; Murphy et al. 1997b; Powell 2004; Skadsen 1993; Wilczak et al. 1996; Wolfe et al. 1990b; Zhang et al. 2007). Nitrification also creates nitrite which has a relatively low maximum contaminant level (MCL) of 1 mg/L-N. Prevention of nitrification is therefore deemed to have desirable public health consequences.

With the exception of the Lead and Copper Rule (LCR) (USEPA 1991), routine distribution system monitoring for nitrification products and other contaminants in the United States stipulates extensive pre-flushing of water from premise plumbing lines before sample collection. As a result, even if water samples are collected from building taps, they rarely quantify the extent of water quality degradation occurring during storage. Only a few reports have discussed occurrence of nitrification during storage in premise plumbing (Murphy et al. 1997a), and neither the prevalence of the problem or key factors that contribute to its occurrence are understood.
A recent literature review predicted that the incidence of nitrification in premise plumbing systems would be at least partly controlled by water chemistry and materials usage (Zhang et al. 2007). Copper and brass plumbing materials invariably leach copper to water supplies at concentrations dependent on numerous factors including pipe age and water chemistry. In the range of copper known to occur in water of premise plumbing systems (0-5000 ppb), slight increases in copper (≈ 1-10 ppb) from corrosion could stimulate nitrification, whereas higher levels of copper (above about 100 ppb) could inhibit nitrification (Zhang et al. 2007). Corrosion of copper and brass plumbing used in many buildings may therefore be preventing degradation of water quality from nitrification in at least some instances, and well-intentioned actions to minimize copper leaching to water (increasing pH, adding orthophosphate corrosion inhibitors or using only plastic pipes) might increase the likelihood of establishing nitrifiers.

Lead materials, in contrast, are capable of converting products of nitrification (nitrite and nitrate) back to ammonia via anodic corrosion reactions (Pb\(^0\) \(\rightarrow\) Pb\(^{2+}\) + 2e\(^-\)) (Uchida and Okuwaki 1998). Zinc in galvanized pipe has also been reported to convert nitrite to ammonia under drinking water conditions (Kunzler and Schwenk 1983). It is therefore possible that nitrifier growth on lead and zinc alloy surfaces would be favored relative to more inert surfaces such as polyvinyl chloride (PVC), exacerbating nitrification occurrence and undermining efforts to mitigate lead contamination of water supplies from pH adjustment (Edwards and Dudi 2004; Zhang et al. 2007).

The goal of this work is to investigate the interplay between materials selection, pH, and orthophosphate corrosion inhibitor dosing on nitrification occurrence as it occurs in home plumbing systems.

**EXPERIMENTAL SECTION**

**Pipe materials**
Pipe materials included PVC (Polyvinyl chloride), copper, lead and non-leaded brass. PVC, copper and lead pipe sections were 30 cm length × 1.9 cm diameter and brass pipe sections were 30 cm in length × 1.3 cm diameter. PVC and copper pipes were purchased from a local hardware store, and pure lead and brass pipes were specially fabricated. Water in the pipes was changed every 3.5 days (twice a week) using a “dump and fill” protocol to simulate typical water use and replenish nutrients for microbial nitrifier growth. These pipes were maintained at room temperature throughout the entire experiment.

**Water Chemistry**

Pipes were first conditioned using synthesized potable water. This synthesized water contained MgSO$_4$ (1 ppm-Mg), CaCl$_2$ (4.9 ppm-Ca), KCl (10 ppm-K), Na$_2$HPO$_4$ (1 ppm-P), NaHCO$_3$ (2 mM) and other trace nutrients (5 ppb Cu$^{2+}$, 1.7 ppb Mo$^{6+}$, 0.1 ppb Co$^{2+}$, 5.6 ppb Mn$^{2+}$, 2.6 ppb Zn$^{2+}$ and 0.1 ppm Fe$^{2+}$). The pH was adjusted to 8.6 before filling up the pipes. This water simulates typical drinking water conditions and provides essential nutrients for nitrifying bacteria. After a year exposure and conditioning of the pipes, no nitrification/nitrifier activity could be detected, as would be expected given that nitrifiers were never inoculated to the rig and no ammonia was in the water. Pipes were then exposed to the same water with addition of 2 ppm-N (NH$_4$)$_2$SO$_4$ and 10 % dechlorinated Blacksburg, VA tap water to provide opportunity for nitrifiers to establish (Supporting Information-Figure S-3.1).

After complete nitrification was established in PVC, brass and lead pipes (as measured by 100 % conversion of ammonia to nitrite or nitrate), the inoculation with Blacksburg, VA water was stopped and only the synthesized water was used thereafter. Use of this water was continued for three additional weeks at initial pH 8.6. Thereafter, pipes were divided into three groups destined to receive either 5, 60 or 1000 ppb PO$_4$-P. These levels are representative of phosphate occurrence in potable water supplies considering use of orthophosphate as a corrosion inhibitor. A total of 60 pipes were used (4 materials × 3 phosphate concentrations × 5 replicates). After exposing the pipes to water with an initial pH of 8.6 for one month, pH was decreased stepwise in 0.5 unit increments (from 8.6 to 6.5) with a one-month exposure at each pH level.
Analytic Methods

Water samples were collected before and after introduction to the pipes each week so that nitrification activity and associated water quality changes could be quantified. Nitrifier activity was tracked by measuring loss of ammonia, production of nitrite and nitrate and reduction of pH. pH was monitored by using pH electrode according to Standard Method 4500 H⁺ B (Clesceri et al. 1998). NH₄-N was measured with salicylate method using a HACH DR/2400 spectrophotometer, according to Standard Method 4500 NH₃ (Clesceri et al. 1998). NO₂-N and NO₃-N were measured using DIONEX, DX-120 ion chromatography (IC), according to Standard Method 4110 (Clesceri et al. 1998). Nitrifier density was monitored once at the third week of each pH level (no monitoring was conducted at pH 8.6) by a five-tube Most Probable Number (MPN) procedure (Wolfe et al. 1990b). Five tenfold serial dilutions were used, resulting in concentrations of 10⁻¹ to 10⁻⁵. Each tube contained 9 ml medium inoculated with 1 ml of sample or 1 ml from the preceding dilution (Supporting Information Figure S-3.2). The medium and dilution water used was modified from (Wolfe et al. 1990b) by adding 0.084 g NaHCO₃, 0.0001 g Na₂MoO₄•2H₂O, 0.000172 g MnSO₄•2H₂O, 0.000004 g CoCl₂•6H₂O, 0.0001 g ZnSO₄•7H₂O and 0.0000133 g CuCl₂•2H₂O; and no phenol red solution was added. The medium pH was adjusted to 8 and was sterilized by autoclaving before use. The tubes were incubated at 30 °C in the dark for three weeks and then tested for nitrite/nitrate using sulfanilic acid and N,N-dimethyl-α-naphthylamine, and zinc dust (Mac Faddin 2000). MPN index was calculated according to standard method 9221 C (Clesceri et al. 1998), which gives most probable nitrifier numbers and corresponding 95 % confidence limits. The lowest reportable MPN index was 20/100 mL (Regan et al. 2007).

Bulk water heterotrophic bacteria were monitored with Heterotrophic Plate Count (HPC) according to Standard Method 9215 (Clesceri et al. 1998) using the spread plate method with R₂A medium. Total Organic Carbon (TOC) was analyzed using a SIEVERS 800 Total Organic Analyzer according to Standard Method 5310C (Clesceri et al. 1998). Dissolved oxygen was quantified at each pH level according to Standard Method 4500 O G (Clesceri et al. 1998) using a Dissolved Oxygen Meter YSI Model 58. Soluble and total metal release was also quantified. Soluble metal concentration was operationally defined by filtration though a 0.45 µm pore size syringe filter. Total metal release was
quantified by digesting samples with 2% nitric acid in a 90 °C oven. Metal concentrations (Cu, Zn, Pb) and phosphorus levels were quantified using an Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) according to Standard Method 3125-B (Clesceri et al. 1998).

**RESULTS AND DISCUSSIONS**

**Nitrification in Different Pipe Materials**

*Nitrifier Establishment*

At pH 8.6, nitrifier activity (as measured by ammonia loss during 3.5 day stagnation) was established first in lead pipes, followed by PVC and then brass (Figure 3.1). Ammonia loss was 100% after just 10 days in lead pipes and 38 days in PVC pipes (Figure 3.1). Ammonia loss in brass pipes was non-detectable initially, but markedly increased after 122 days and eventually rose to 100% after 143 days. In copper pipes, ammonia loss was never above 30% throughout the 164 days’ inoculation period, consistent with expectations of copper toxicity to nitrifiers (Zhang et al. 2007).

The increase of nitrifier activity with time in brass pipes occurred only after levels of copper leaching to water dropped below about 0.1 ppm as the pipe metal aged (Figure 3.2). Interestingly, total lead release up to 8000 ppb and soluble lead release up to 220 ppb in the lead pipes seemed to have no inhibitory effect on nitrification. It is unclear whether the enhanced nitrification observed in the early stages of the experiment on lead pipe versus PVC was attributable to benefits of nutrient cycling via lead corrosion or other possible factors such as increased surface roughness that aid colonization of the pipe surface.
Changes in Water Chemistry

The occurrence of nitrification significantly impacted water chemistry during stagnation (Supporting Information-Table S-3.1). For example, when the initial pH of water was 8.0, final pH decreased by 0.75 unit in PVC and 0.5 unit in bulk water from lead pipes. Consideration of nitrification stoichiometry, acid production and water chemistry (Zhang et al. 2007) predicts a pH drop of up to 0.9 unit in this water from complete nitrification, so observed drops in pH are consistent with expectations, although other chemical reactions impacting pH are also occurring. Dissolved oxygen (DO) decreased by up to 2.5
mg/L in PVC, lead and brass pipes, while there was very little change in DO in copper pipes. This study did not find higher bulk water HPC associated with nitrification (Supporting Information-Table S-3.1), contrary to some earlier studies (Powell 2004; Skadsen 1993; Wilczak et al. 1996; Wolfe et al. 1990b). But the morphology of bulk water bacterial colonies plated from water in copper pipes, where no nitrification was observed, was clearly different from those plated from the lead, PVC and brass pipes (Supporting Information-Figure S-3.3).

TOC was present in the initial water at 8-60 ppb and in some situations increased more than ten-fold after 3.5 days of stagnation (Figure 3.3). Amongst the four pipe materials, brass had the largest increase in TOC, followed by lead and then copper and PVC (Figure 3.3). The TOC levels detected in brass and lead pipes far exceeds any reasonable expectation of TOC production due to nitrification alone even with cycling of nutrients, and suggests that other forms of autotrophic growth or TOC creation may be occurring. That might include evolution of H₂ substrate from metal corrosion or other pathways. The higher levels of TOC could reflect a short-term sloughing of biofilm on the days of sampling.

It has previously been observed that distribution system materials with higher surface area and higher corrosion rates support more biofilm growth and biofilm sloughing (Camper et al. 2003). Likewise, in this work for lead and brass, it is possible that higher rates of metal corrosion could translate to more cycling of nitrate and more biofilm. Consistent with that idea, TOC tended to be lower in the presence of more phosphate corrosion inhibitor for lead and brass (Edwards et al. 2002; McNeill and Edwards 2002; Schock et al. 1995). If lower phosphate acted by inducing a nutrient limitation to nitrification (Van Droogenbroeck and Laudelout 1967) the opposite trend should have been observed (Figure 3.3).
Figure 3.3: TOC levels after 3.5 day stagnation.

Note: Each data point is the average of five replicate samples. Error Bars indicate 95 % confidence interval.

Effect of pH and Phosphate Levels on Metal Release

pH and phosphate can directly impact nitrifier growth, since the optimum pH for nitrification is generally believed to be 7.5-8 and phosphate is an essential nutrient (Zhang et al. 2007). But pH and phosphate also influence total metal leaching to water via corrosion of lead, copper and brass, as well as the percentage of metal that is present as the free ion (e.g., Cu$^{2+}$, Pb$^{2+}$ and Zn$^{2+}$) in water (Edwards et al. 2002; Schock et al. 1995). Thus, phosphate and pH were expected to influence nitrification occurrence in premise plumbing where copper is the most common pipe material. Currently, about 50% of U.S. utilities use some form of phosphate corrosion inhibitor (McNeill and Edwards 2002).

Models were recently used to predict the role of phosphate and pH in controlling free [Cu$^{2+}$] levels in water and their likelihood to impact nitrification (Zhang et al. 2007). As pH decreases, free Cu$^{2+}$ deemed especially toxic to nitrifiers increases. But at a given pH, phosphate dosing to water can decrease the concentration of total copper and Cu$^{2+}$ by reducing the solubility product of corrosion products on the pipe surface. Zhang et al, 2007 used solubility models (Zhang et al. 2007) and
predicted that pH levels below about 7.3 would be highly toxic for nitrifiers in copper pipe, but the presence of high orthophosphate (e.g., 1 ppm at PO4-P) nitrification could allow nitrification at much lower pH levels.

Copper Pipes

At all phosphorus levels tested, total copper release increased as pH decreased (Figure 3.4), consistent with expectations from earlier research (AWWARF and DVGW-TZW 1996). Soluble copper was always between 48 % - 95 % of the total copper (data not shown). At a given pH, total and soluble copper release was lower at the condition with 1000 ppb-P phosphate (Figure 3.4), also consistent with expectations from earlier work (AWWARF and DVGW-TZW 1996; Edwards et al. 2002; Schock et al. 1995).

![Figure 3.4: Copper release in copper pipes](image)

Note: Each data point is the average of at least four weeks testing. Error Bars indicate 95 % confidence interval.

Brass Pipes

Total copper release from brass pipes in this research was not strongly affected by either pH or phosphorus levels (Table 3.1). Although average soluble copper release from brass tended to decrease
slightly at higher pH or if 1000 ppb-P was dosed, this trend was not statistically significant at greater than 95% confidence (Supporting Information-S-3.2). In other studies, such as that of Lytle and Schock, total copper release from brass at pH 7 was significantly higher than that at pH 8.5 (Lytle and Schock 1996). They also found orthophosphate either decreased or had no effect on total copper leaching depending on the brass types (Lytle and Schock 1996).

Zinc toxicity to nitrifiers has been reported (Zhang et al. 2007). Total and soluble zinc (61 %- 98 % of total zinc) release in brass pipes was significantly increased (up to 9 times) when pH was decreased from 8.6 to 6.5; however, zinc release was not affected by different phosphorus levels (Table 3.1), consistent with Lytle and Schock’s results (Lytle and Schock 1996). The trends in zinc leaching were statistically significant at > 95 % confidence.

<table>
<thead>
<tr>
<th>Phosphorus-ppb</th>
<th>Total Cu, ppb</th>
<th>Soluble Cu, ppb</th>
<th>Total Zn, ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6.5</td>
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<td>91</td>
<td>74</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
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<tr>
<td>8.6</td>
<td>92</td>
<td>80</td>
<td>69</td>
</tr>
</tbody>
</table>

Note: each data reported was the average of at least four weeks testing

**Lead Pipes**

In lead pipes, phosphorus levels had a more dramatic effect on lead release than did pH changes. Regardless of inhibitor dose, total and soluble lead release did not increase when the initial pH was decreased from 8.5 to 7.5, but soluble lead release increased markedly (Supporting Information-Table S-3.3) when initial pH was reduced to 6.5-7.0. The effects of pH and orthophosphate on soluble lead were roughly consistent with solubility model predictions (AWWARF and DVGW-TZW 1996; Lytle and Schock 1996; Schock 1989). Soluble and total lead release were reduced by addition of 1000 ppb
orthophosphate (Supporting Information-Table S-3.3), but was less significantly impacted by 60 ppb orthophosphate compared to the lowest dose of 5 ppb, consistent with earlier work and solubility models (AWWARF and DVGW-TZW 1996; Lytle and Schock 1996; Schock 1989).

**Combined Effect of pH and Phosphate Levels on Nitrifier Activity**

*PVC Pipes*

Reported optimal pH for nitrifier growth generally falls between 7.5-8 (Wolfe and Lieu 2001). In PVC pipes average nitrifier MPN was 0.5 – 1 log lower at pH 6.5 than at pH 8 (Table 3.2), however, this decrease was not statistically different at 95 % confidence (Supporting Information-Table S-3.3). The decrease in pH did not reduce ammonia loss in PVC pipes, since complete ammonia conversion to nitrite and nitrate occurred even at pH 6.5. Thus, nitrifiers can remain active in PVC premise plumbing at much lower pH ranges than have been previously reported (Wolfe and Lieu 2001).

At phosphorus levels below about 50 ppb-P, it has been reported that nitrification was inhibited via nutrient limitation (Van Droogenbroeck and Laudelout 1967). However, in this study using PVC pipe, nitrifier MPN (Table 3.2) and nitrifier activity (indicated by ammonia loss) was very high even at 5 and 60 ppb-P levels. This might be due to the higher levels of phosphorus used (1000 ppp-P) in the six month inoculation period before the phosphorus level in the medium was reduced to 5 ppb or 60 ppb. The high cell phosphate content in nitrifier biofilm (Van Droogenbroeck and Laudelout 1967) or pipe surface deposits containing phosphate might serve as reservoirs for bioavailable phosphorus after the level introduced to the pipe was reduced. The effects of high cell phosphate content has also been reported to be more significant at lower ammonia/nitrite levels (Van Droogenbroeck and Laudelout 1967).
Table 3.2: Logarithm of nitrifier MPN at different phosphorus and pH levels in different pipe materials

<table>
<thead>
<tr>
<th></th>
<th>P-ppb</th>
<th>pH</th>
<th></th>
<th></th>
<th></th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>8</td>
<td>7.5</td>
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<td>6.5</td>
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<td>5</td>
<td>5.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.5</td>
<td>5.2</td>
<td>4.8</td>
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</tr>
<tr>
<td></td>
<td>1000</td>
<td>5.7</td>
<td>5.1</td>
<td>4.5</td>
<td>4.7</td>
</tr>
<tr>
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<td>5</td>
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<td>&lt;1.3</td>
<td>&lt;1.3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.2</td>
<td>&lt;1.3</td>
<td>&lt;1.3</td>
<td>&lt;1.3</td>
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<td>&lt;1.3</td>
<td>&lt;1.3</td>
<td>&lt;1.3</td>
</tr>
<tr>
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<td>4.4</td>
<td>4.5</td>
<td>2.5</td>
</tr>
<tr>
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<td>5.4</td>
<td>4.3</td>
<td>3.2</td>
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<tr>
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<td>4.7</td>
<td>5.4</td>
<td>4.7</td>
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<tr>
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<td>6.2</td>
<td>5.4</td>
<td>5.4</td>
<td>5.2</td>
</tr>
</tbody>
</table>

**Copper Pipes**

In copper pipes, nitrifiers were only detected at pH 8 (Table 3.2) with 60 or 1000 ppb phosphate. MPN indexes were two orders of magnitude higher at 1000 ppb versus 60 ppb phosphate (Table 3.2). Both trends were consistent with expectations for lower copper toxicity to nitrifiers in the presence of phosphate and higher pH. Average ammonia loss was less than 10 % at all pH and phosphorus levels, illustrating the role of copper pipe in preventing nitrification in premise plumbing relative to PCV or lead.

**Brass Pipes**

In brass pipes, average nitrifier MPN was 1 – 2.9 log lower at pH 6.5 than at pH 8 (Table 3.2) and this trend was significant at > 95% confidence (Supporting Information-Table S-3.4). The more dramatic effect of lower pH in brass relative to PVC was possibly due to the increased concentration of Cu^{2+}. As was the case with copper, nitrifier MPN was lower at 5 and 60 ppb-P than at 1000 ppb-P. Corresponding to the lower MPN, as pH decreased, ammonia loss also decreased at lower pH and phosphate levels (Figure 3.5).
Figure 3.5: Average ammonia loss % versus pH at different Phosphorus levels in brass pipes.

Note: Data reported was the average of at least four weeks testing. Error Bars indicate 95 % confidence interval of these testings.

**Lead Pipes**

Nitrifier MPN from lead pipes either stayed the same or decreased slightly as pH decreased, and no significant difference was observed among the three phosphorus levels (Table 3.2). Similar to PVC, complete ammonia conversion to nitrite and nitrate occurred even at pH 6.5. Average nitrifier MPN in lead pipes was slightly higher than that in PVC pipes (Table 3.2); however, this trend was not statistically different at 95 % confidence (Supporting Information-Table S-3.4).

**Interplay of Phosphorus, pH and Pipe Materials on Nitrification**

Appropriate levels of phosphorus and pH are necessary for nitrifier growth, but phosphorus and pH also influence nitrifiers indirectly by controlling corrosion rates and metal release. Dependent on the pipe material and specific circumstance, metal leaching can be observed to completely inhibit nitrification (e.g., newer copper or brass) or possibly stimulate nitrification (e.g., lead pipe) in at least some cases.

Attempts were made to universally correlate predicted free copper, soluble copper or total copper to nitrifier activity observed in the brass and copper pipe during stagnation, over the range of conditions...
tested in this study. The best overall relationship was observed for nitrifier activity versus soluble copper in brass pipes (Figure 3.6). Consistent with prior batch studies (Zhang and Edwards 2005), nitrifier activity was markedly inhibited above about 80 ppb soluble copper. Attempts were also made to develop correlations based on predicted Cu$^{2+}$ using the approach in Zhang et al., 2007 (Zhang et al. 2007), but this approach did not provide improved predictive ability. This is likely due to uncertainties in the type of solids that are present which actually control Cu$^{2+}$ solubility, differences between the pH at the pipe surface versus that measured for the bulk water, and other limitations.

Overall, the indirect effect of pH and phosphate on nitrifiers as exerted through control of corrosion and metal toxicity, was more important than the direct effects. That is, levels of phosphate and pH tested had stronger impacts on nitrifier activity in copper and brass pipes than in PVC and lead pipes where no metal toxicity was observed.

![Figure 3.6: Correlation of ammonia loss % vs. soluble Cu in copper and brass pipes.](image)

**IMPLICATIONS FOR DRINKING WATER SYSTEMS**

The interplay between water chemistry and microbial growth in premise plumbing is obviously very complex and system dependent. Consequently, many presumptions about “worst case” locations for sampling of water quality degradation in water distribution/premise plumbing systems should be examined carefully. For example, based on the analysis presented in this paper, first
draw lead leaching in systems using chloramine can be expected to be much worse in some new homes plumbed completely with PVC or plastic pipe, rather than with copper. This is because the lower pH levels resulting from enhanced nitrifier colonization of the PVC pipe systems might adversely impact lead and copper leaching from brass used in consumer faucets, when compared with lead leaching in identical new homes plumbed with copper pipe. Indeed, recent field data collected by the authors and others (Kimbrough 2007) has demonstrated that first draw lead, nickel and zinc can be higher in homes Plumbed completely with plastic pipe (and leaded brass fixtures) relative to homes plumbed with copper pipe. All these systems used chloramine, and therefore, enhanced nitrification and larger pH drops potentially occurring from nitrification in the mainly plastic versus copper pipe systems could be partly responsible. It is particularly ironic that copper levels in first draw water collected from homes plumbed with PVC and brass faucets, can often exceed those observed in similar homes plumbed with copper pipe/brass faucets (Kimbrough 2007). The pH drop occurring during overnight stagnation should be examined as a key contributing factor to this phenomena in future research.

Likewise, in homes plumbed with PVC and other plastics, brass is still frequently used to connect pipe fittings and in valves. The lower pH levels resulting from nitrification could contribute to premature failure of this brass via dezincification and other corrosion reactions, relative to what is commonly observed when the same fittings are used with copper pipe. There have recently been very high rates of brass fitting failures occurring in plastic premise plumbing systems in certain U.S. localities (Kimbrough 2007).

ACKNOWLEDGEMENT

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USEPA or the CDA. The authors would also like to thank Meredith Raetz for help in maintaining the experiment.

**SUPPORTING INFORMATION**

**Figure S-3.1: Nitrifier Inoculation using Blacksburg Tap Water**

Description: The nitrifier growth in Blacksburg, VA tap water was stimulated and maintained in a plastic container by adding dechlorinated tap water and ammonia and adjusting pH to 8 twice a week; a portion of this water was used to inoculate pipes while the rest was kept stagnant to mix with the next batch of dechlorinated tap water.
Figure S-3.2: Serial dilution used for Nitrifier MPN procedure (Adapted from (Vikesland et al. 2006)).

Figure S-3.3: Bulk Water Heterotrophic Plate Colonies from the Four Pipe Materials
### Table S-3.1: Typical Water Chemistry

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>DO, mg/L-O₂</th>
<th>HPC, cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial</strong></td>
<td>8.00 ± 0.10</td>
<td>8.30 ± 0.20</td>
<td>140000</td>
</tr>
<tr>
<td>PVC</td>
<td>7.25 ± 0.28</td>
<td>6.75 ± 0.60</td>
<td>120000</td>
</tr>
<tr>
<td>Copper</td>
<td>8.20 ± 0.34</td>
<td>8.16 ± 0.19</td>
<td>130000</td>
</tr>
<tr>
<td>Brass</td>
<td>7.77 ± 0.23</td>
<td>5.94 ± 1.12</td>
<td>120000</td>
</tr>
<tr>
<td>Lead</td>
<td>7.51 ± 0.19</td>
<td>6.58 ± 0.57</td>
<td>110000</td>
</tr>
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### Table S-3.2: 95 % Confidence Interval for Metal Release in Brass Pipes

<table>
<thead>
<tr>
<th>Phosphorus-ppb</th>
<th>5</th>
<th>60</th>
<th>1000</th>
<th>5</th>
<th>60</th>
<th>1000</th>
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<th>60</th>
<th>1000</th>
</tr>
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<tbody>
<tr>
<td>pH</td>
<td>Total Cu, ppb</td>
<td>Soluble Cu, ppb</td>
<td>Total Zn, ppb</td>
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<td>22</td>
<td>48</td>
<td>24</td>
<td>12</td>
<td>35</td>
<td>2876</td>
<td>2003</td>
<td>2267</td>
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<tr>
<td>7</td>
<td>11</td>
<td>51</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>1988</td>
<td>1928</td>
<td>1552</td>
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<td>17</td>
<td>16</td>
<td>14</td>
<td>17</td>
<td>667</td>
<td>554</td>
<td>526</td>
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### Table S-3.3: Total and Soluble Lead Release in Lead Pipes

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<th>Phosphorus-ppb</th>
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<th>8</th>
<th>7.5</th>
<th>7</th>
<th>6.5</th>
<th>Average total Pb release, ppb</th>
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<tbody>
<tr>
<td>pH</td>
<td>Average soluble Pb release, ppb</td>
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<td></td>
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<tr>
<td>5</td>
<td>2516 ± 1166</td>
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<td>1193 ± 1371</td>
<td>913 ± 353</td>
<td>1155 ± 973</td>
<td>1611 ± 2076</td>
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<tr>
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<td>1751 ± 1731</td>
<td>1545 ± 1279</td>
<td>2637 ± 5675</td>
<td>2255 ± 3228</td>
<td>928 ± 606</td>
<td>1233 ± 740</td>
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<tr>
<td>1000</td>
<td>1528 ± 777</td>
<td>643 ± 149</td>
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<td>292 ± 126</td>
<td>326 ± 261</td>
<td>398 ± 173</td>
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<td>95 ± 9</td>
<td>88</td>
<td>138 ± 35</td>
<td>194 ± 50</td>
<td>207 ± 45</td>
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<td>60</td>
<td>83 ± 3</td>
<td>73 ± 10</td>
<td>80</td>
<td>98 ± 20</td>
<td>152 ± 48</td>
<td>162 ± 30</td>
</tr>
<tr>
<td>1000</td>
<td>72 ± 16</td>
<td>66 ± 4</td>
<td>66</td>
<td>78 ± 11</td>
<td>110 ± 25</td>
<td>122 ± 15</td>
</tr>
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</table>

Note: each data reported was the average of at least four weeks testing, ± indicate 95 % confidence interval
Table S-3.4: Logarithm of 95 % Confidence Limit of Nitrifier MPN at Different Phosphorus and pH Levels in Different Pipe Materials

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<tr>
<th></th>
<th>P-ppb</th>
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REFERENCES


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CHAPTER IV: LEAD CONTAMINATION OF POTABLE WATER DUE TO NITRIFICATION

Yan Zhang, Allian Griffin, Mohammad Rahman, Ann Camper, Helene Baribeau and Marc Edwards

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ABSTRACT

Nitrification can increase levels of soluble lead in potable water by reducing pH. The magnitude of the pH effect depends on the initial alkalinity, extent of nitrification and associated acid production. At 100 mg/L alkalinity as CaCO$_3$, complete nitrification did not significantly decrease pH (pH stayed > 7.5) or increase lead contamination from lead pipe, but at 15 mg/L alkalinity, nitrification decreased the pH by 1.5 units (pH reduced to < 6.5) and increased soluble lead contamination by 65 times. Lower pH values from nitrification also leached 45% more lead and 81% more zinc from leaded brass connected to PVC pipes versus copper pipes. Particulate lead leaching was high, but did not seem to vary dependent on nitrification. Production of nitrite and nitrate, or reductions in inorganic carbon or dissolved oxygen via nitrification, did not significantly influence lead leaching.
INTRODUCTION

As more utilities in the United States switch to chloramines for residual disinfection of potable water (Seidel et al. 2005; Zhang et al. 2009), there is increasing concern about potential costs and health implications of corrosion induced by nitrification (Zhang et al. 2009). Nitrification, the conversion of ammonia to nitrite ($\text{NO}_2^-$) and then nitrate ($\text{NO}_3^-$) by nitrifying bacteria, can impact corrosion by decreasing pH, alkalinity and dissolved oxygen (Zhang et al. 2009). Production of organic carbon, and accelerated disinfectant decay, might also stimulate growth of corrosion-influencing microbes which could also affect corrosion (Wilczak et al. 1996; Zhang et al. 2009). A 1991 survey indicated that two thirds of the medium and large utilities that use chloramines report nitrification problems in water mains (Wilczak et al. 1996), and it is very likely that even a greater percentage have nitrification issues if premise plumbing is considered (Zhang et al. 2008a; Zhang et al. 2009).

There is some anecdotal evidence of corrosion problems triggered by chloramines and nitrification in at least some circumstances. For example, recent work in Pinellas County, Florida highlighted some concerns related to iron corrosion control and red water (Powell 2004). Likewise, elevated copper levels at the tap were suspected to be linked to action of nitrifying bacteria in Willmar, Minnesota homes (Murphy et al. 1997). Nitrification also co-occurred with higher lead leaching in Ottawa (Douglas et al. 2004), Washington D.C., Durham and Greenville, NC homes (Edwards and Dudi 2004; Edwards and Triantafyllidou 2007; Triantafyllidou et al. 2007). However, in most studies, the link between nitrification and corrosion is not confirmed, and the mechanism by which nitrification affects corrosion was not postulated. Only in the Ottawa study (Douglas et al. 2004), the higher lead was suspected to be
the result of nitrification causing a pH decrease, but whether this pH decrease occurred upstream or in the lead pipe was not clear.

Given the high costs and health implications of corrosion to utilities and consumers (Edwards and Dudi 2004; Triantafyllidou et al. 2007), and further considering that prior research emphasized nitrification problems occurring in the main distribution system whereas virtually all lead and copper plumbing materials are located after the property line (Committee on Public Water Supply Distribution Systems: Assessing and Reducing Risks 2006), it is important to better understand effects of nitrification on corrosion and metal release under conditions found in premise plumbing. Moreover, the work attempts to scientifically verify anecdotal links established between nitrification and increased lead leaching through a well-controlled laboratory study.

**EXPERIMENTAL SECTION**

**Water Chemistry**

Lead pipes (1.9 cm × 30 cm) were aged by exposure to a synthesized water for one year without nitrification, and then exposed to water with ammonia (and resulting nitrification) for 15 months as described elsewhere (Zhang et al. 2008a). No disinfectant had ever been added to the pipes. Thirty pipes were exposed at 5, 60 and 1000 ppb orthophosphate-P (10 at each phosphate level). The ten replicate pipes were separated into three groups. The first group continued as a control (4 pipes), and the second group was modified by addition of free chlorine to a final concentration of 10 mg/L total chlorine (3
pipes). The added chlorine reacted with the existing ammonia to form monochloramine. The third group of pipes was modified by addition of 1 mg/L chlorite (3 pipes). The high chloramine (Fleming et al. 2005) and chlorite (McGuire et al. 1999; McGuire et al. 2006) levels were used to attempt to inhibit nitrification that was allowed to proceed unimpeded in the control. The pH of each type of water was adjusted to 8 before filling up the pipe. The alkalinity of the water was dropped stepwise from 100 mg/L down to 30, 15 and then 0 mg/L alkalinity by decreasing the amount of NaHCO₃ added. Each alkalinity level was maintained for sufficient time for nitrification, pH and lead leaching to stabilize. Water in the pipes was changed twice a week and pipes were maintained at room temperature.

**Analytical Methods**

Nitrifier activity was measured by loss of ammonia, production of nitrite and nitrate and reduction of pH. pH was monitored by using pH electrode according to Standard Method 4500-H⁺ B (Clesceri et al. 1998). Total ammonia (= NH₃ + NH₄⁺) was measured using a salicylate method with a HACH DR/2400 spectrophotometer, according to Standard Method 4500-NH₃ (Clesceri et al. 1998). NO₂⁻-N and NO₃⁻-N were measured using DIONEX, DX-120 ion chromatography, according to Standard Method 4110 (Clesceri et al. 1998). Soluble metal was operationally defined as the portion of metal passing through a 0.45 µm pore size syringe filter. Total metal release was quantified by digesting samples with 2% nitric acid for 24 hours in a 90 °C oven. Metal concentrations were quantified using an Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) according to Standard Method 3125-B (Clesceri et al. 1998).

**Case Studies**
Two types of case studies were conducted:

Water Utility studies. Five participating utilities coordinated the collection of samples at three consumer homes before and after stagnation. Samples were analyzed for ammonia, nitrite, nitrate, lead and copper release and other basic water quality parameters (pH, chlorine, alkalinity, temperature, HPC, etc). More details of the sampling procedures are included in the Supporting Information.

Montana Bench Test. A plumbing rig was constructed to directly test the effect of pipe material (PVC versus Copper) on nitrification and resulting lead contamination of water by leaded brass. A brass rod (0.64 cm diameter × 10 cm length, C35300 alloy with 2% lead) was machined and placed inside a PVC or copper pipe (1.3 cm diameter × 61 cm length) to simulate the situation in homes with PVC/copper plumbing and leaded brass faucets. The rod was not in electrical contact with the PVC or copper pipe. Each experiment was run in triplicate using synthesized potable water containing nitrifying bacteria. The synthesized water contained (NH₄)₂SO₄ (2.13 mg/L-N), initial pH of 8.15, Na₂HPO₄ (1 mg/L-P), NaHCO₃ (35 mg/L as CaCO₃), Elliot Humics (4 mg/L) and other salts described elsewhere (Rahman 2008). Water in the pipes was changed every Monday, Wednesday and Friday and samples were analyzed for ammonia, pH, lead and zinc release as described above.

RESULTS AND DISCUSSIONS

Inhibition of nitrification with monochloramine/chlorite vs. control condition
Addition of either chloramine or chlorite effectively inhibited nitrification in the lead pipes. Ammonia conversion to nitrate and nitrite (i.e., ammonia loss) decreased from > 80% to < 40% upon addition of chloramine or chlorite (Figure 4.1).

Based on nitrification stoichiometry, 14 mg/L as CaCO$_3$ alkalinity is consumed for every mg NH$_4^+$-N oxidized (Grady et al. 1999), so it was expected that nitrification could be somewhat limited by a lack of alkalinity (usually carbonate species) at the 6% of U.S. utilities with water containing less than 15 mg/L alkalinity as CaCO$_3$ (AWWA. 1996). But in this study, for the control pipes, virtually complete nitrification occurred even when the added carbonate alkalinity was 15 mg/L (Figure 4.1). This is possibly because lead corrosion can increase pH and alkalinity, so the actual alkalinity is more than just carbonate species. But at least some inorganic carbon is required for incorporation into cells: when no carbonate was added, nitrification was abruptly halted even in the control (Figure 4.1). Thus, it is unlikely that nitrification will be inorganic carbon limited at water utilities, unless alkalinity is well below 15 mg/L as CaCO$_3$. 

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Figure 4.1: Ammonia loss % and final pH, versus time when initial alkalinity was reduced in stages from 100 to 0 mg/L. All pipes were dosed with 1 mg/L as P.

All other factors being equal, a lesser amount of nitrification translates to a higher final pH in the pipe, because less acid is produced via nitrification (Zhang et al. 2009). However, due to the relatively high buffering of the water at 100 mg/L alkalinity, pH values in the pipes with monochloramine/chlorite were only slightly higher \((p \geq 0.04)\) relative to the control condition, even though there was less nitrification (Figure 4.1). But when alkalinity (and buffering) was decreased to 30 mg/L and then 15 mg/L, the final pH values with monochloramine/chlorite were much higher \((p < 0.001)\) than in the control (Figure 4.1). Thus, the extent of pH reduction by nitrification depends not only on the extent of nitrification, but also the initial alkalinity level. At 0 alkalinity, where nitrification stopped in the controls, pH values in the
pipes with monochloramine/chlorite were slightly lower relative to that without inhibitors \((p \geq 0.005)\) (Figure 4.1).

The above results were examined relative to predicted trends using solubility and chemical reaction models. Predictions of final pH based only on bulk water chemistry and the expected acid production by nitrification, confirms that the extent of the pH drop should increase with decreasing alkalinity (Supporting Information-Figure S-4.1). In fact, the predicted pH drops correlate very well with actual monitored pH drops \((R^2 = 0.81-1)\), although the actual pH drop is typically only 60-90% of the predicted pH drop. It is not uncommon to see differences between the predicted and actual pH drops due to other reactions (i.e., scale dissolution and corrosion) that tend to increase the pH (Zhang et al. 2008b).

**Effect of nitrification on lead release**

Reductions in pH due to nitrification have been hypothesized to increase lead release (Garret 1891; Odell et al. 1996; Zhang et al. 2009), although there has been no research that directly confirmed this hypothesis (Zhang et al. 2008b). In this study, a head to head comparison of pipes with nitrification inhibitors to those without confirmed that nitrification increased lead release, but the extent of the effect is highly dependent on the initial alkalinity level (Figure 4.2). Specifically, at 100 mg/L alkalinity, lead release was not increased by nitrification, as indicated by similar or even higher total and soluble lead release in the pipes with monochloramine/chlorite versus the condition without nitrification inhibitors (Table S-4.1, Figure 4.2). At 30 mg/L alkalinity, nitrification increased total lead release up to 5 times and soluble lead release up to 21 times \((p \leq 0.002\)-Table S-4.1) (Figure 4.2). At 15 mg/L alkalinity, total
lead release was increased up to 5.5 times and soluble lead release up to 65 times ($p \leq 0.00008$-Table S-4.1) (Figure 4.2). These trends are in agreement with the fact that the pH was reduced most significantly by nitrification at the lower alkalinity levels (Figure 4.1). When alkalinity dropped to 0, lead release in the control pipes dropped to levels similar (or even slightly lower) than those observed with monochloramine/chlorite (Figure 4.2, Table S-4.1), proving that the nitrification inhibitors themselves were not decreasing soluble or total lead.

It was also considered possible that soluble microbial product (SMP) produced by nitrification might be increasing lead solubility. However, based on data considered from earlier studies (Rittmann et al. 1994), nitrification of 2 mg/L ammonia would produce 20 ppb-C SMP from autotrophic growth, which is not sufficient to increase soluble lead by 1000 ppb due to complexation.
Lead in drinking water can be present as truly dissolved soluble species (e.g., $\text{Pb}^{+2}$, $\text{Pb(CO}_3\text{)}^{2-}$), or as colloids/particulates that can include detached solder, brass or lead scale (McNeill and Edwards 2004; Triantafyllidou et al. 2007). In the pipes with high nitrification (no inhibitor), average soluble lead was gradually increased from 60, 220 to 850 ppb as alkalinity levels were dropped from 100, 30 to 15 mg/L, respectively (Figure 4.3). When alkalinity was dropped from 15 to 0 mg/L as CaCO$_3$, soluble lead decreased to 160 ppb, because the final pH was much higher (Figure 4.1). Particulate lead stayed constant at around 200 ppb (Figure 4.3). In the pipes with low nitrification (monochloramine/chlorite), neither soluble nor particulate lead was significantly changed (Figure 4.3). Thus, nitrification increased the soluble lead release, but had no detrimental impact on the particulate lead release (Figure 4.3).
Solubility models were used as described elsewhere to better understand the interplay between pH drops, alkalinity, buffering, DIC and lead solubility (AWWARF and DVGW-TZW 1996; Schock 1989). Percent changes in soluble lead in response to various 0.5 unit pH drops were calculated as a function of initial alkalinity and pH change (Figure 4.4). In the absence of lead phosphate solids, soluble lead is predicted to markedly increase when pH drops, and the percentage increase is very significant at lower alkalinities (Figure 4.4). For example, predicted soluble lead levels increase by 120% (from 470 to 1035 ppb) when pH drops from 7 to 6.5 at 15 mg/L alkalinity. But if the water has 100 mg/L alkalinity, the pH drop from 7 to 6.5 only increases predicted soluble lead by 40% (from 320 to 447 ppb) (Figure 4.4).

The solubility model trends and observed impacts of lower pH on lead release are also consistent with utility experience and other recent data (Zhang et al. 2008a). That is, the effects of pH on lead
contamination and lead solubility are relatively weak at the higher alkalinites. For example, the 90 percentile lead levels reported by utilities are not a strong function of pH if alkalinity is > 30 mg/L, but lower pH markedly increases 90%’ile lead if alkalinity is < 30 mg/L (Dodrill and Edwards 1995).

In situations where a Pb₃(PO₄)₂ solid controls solubility, a pH drop from 8 down to 7.5 or even 7.0 has relatively little effect on soluble lead, especially at higher alkalinites (Figure 4.4). In fact, the lower pH can even decrease lead solubility in some cases. In such systems, the final pH must drop down to about pH 6.5 before solubility is markedly increased (Figure 4.4), and even so, the changes in absolute lead concentrations are low relative to the 15 ppb action level. For example, at 15 mg/L alkalinity, soluble lead levels are predicted to be 9 and 16 ppb at pH 8 and 6.5, respectively. The key conclusion is that pH drops from nitrification might not increase lead solubility at utilities dosing orthophosphate corrosion inhibitors, if the dose is sufficient to form Pb₃(PO₄)₂ or similar solids on the pipe surfaces.

The models were also used to examine the possible role of decreased Dissolved Inorganic Carbon (DIC) due to autotrophic growth. Consideration of this factor had only slight predicted impacts on soluble lead (Figure 4.4), supporting the idea that the primary effect of nitrification is exerted through its impact on pH.

*Direct Comparison of Model Results to Experimental Data*

For the experiments conducted herein, the initial phosphate in the water was virtually all removed during stagnation in the lead pipe. Even when the initial water contained 1000 ppb phosphate, only 10 ppb
phosphate was present in the water after stagnation (i.e., > 99% had been removed by reactions with the lead pipe wall). Assuming even a very low corrosion rate of 0.1 uA/cm², the Pb⁺² formed per unit volume of water in the pipe exceeds the phosphate that is present by more than an order of magnitude. Hence, the extreme loss of phosphate from the water, by processes such as sorption to newly forming lead hydroxyl-carbonates scale is not unexpected. This explains why the data in Figure 4.2, behave more in keeping with trends expected for a system without phosphate inhibitor, because the added phosphate was effectively removed and Pb₃(PO₄)₂ was under-saturated in these tests. In a practical situation with more frequent water changes and flow, it is highly likely that Pb₃(PO₄)₂ solid would have formed. Thus, all results in our tests are representative of expectations at utilities that do not dose a high amount of orthophosphate to the water.

The solubility model predictions (based on measured final pH, final total lead, total phosphate and final alkalinity) are in good agreement with the actual data on soluble lead for situations with high nitrification (R² = 0.76) (Figure 4.5). The conclusion is that the complex response of soluble lead to initial alkalinity (Figure 4.2) is completely consistent with solubility model predictions that consider lead-carbonate solid formation, lead carbonate complexation and other factors (AWWARF and DVGW-TZW 1996; Dodrill and Edwards 1995; Edwards et al. 1999; Schock 1989). This good correlation also suggests that the complexation by SMP, is not the major contributor to the increased soluble lead after nitrification.
It is important to note that higher alkalinity has a dual benefit in preventing problems with soluble lead due to nitrification. First, as a buffer, the extent of the pH drop due to a given amount of ammonia conversion is reduced. This is obvious based on the average pH (-log of average [H⁺]) of 7.7, 6.92 and 6.19 at 100, 30 and 15 mg/L alkalinity, respectively (Figure 4.1). Second, a given pH drop of 0.5 units also has a much lesser impact on soluble lead at higher alkalinity (Figure 4.4).

Figure 4.4: Predicted % soluble lead increase due to pH decrease (Assuming 2000 ppb lead). Lower graph assumes solubility control by Pb₃(PO₄)₂ solids.
Figure 4.5: Actual soluble lead vs. predicted soluble lead in lead pipes at the control condition with 1 mg/L-P. Pb₃(PO₄)₂ or similar solids were not predicted to form due to high removal of phosphate during stagnation.

**Lead Solubility with Nitrification Inhibitors or at Lower Phosphate Levels**

In nearly all tests with lower levels of phosphate, or with nitrification inhibitors, relatively little nitrification occurred. As a result, the final pH was higher, and soluble lead levels were lower versus the control condition dosed at 1 mg/L-P. In these cases the solubility model did not predict trends quite as well as for cases where extensive nitrification occurred (e.g., Supporting Information Figure S-4.2). This is probably because the soluble lead was a relatively small fraction (10-42%) of the total lead present, and soluble lead data therefore has higher error due to significant interference from colloids less than 0.45 um in size (McNeill and Edwards 2004) when the fraction of soluble lead is low relative to total lead.

**Case Studies**
Water Utility studies. Samples collected from homes at five utilities using chloramines indicated extensive nitrification during stagnation via a decrease of ammonia (Table 4.1) and increase of nitrite/nitrate species. Similar to our earlier field studies (Zhang et al. 2008b), significant pH drops (0.34-2) were also observed (Table 4.1). When the actual pH drop is compared to the pH drop predicted based on ammonia consumption, the actual pH drop could either be higher or lower than the predicted pH drop (Table 4.1). This was not surprising considering the complexity of the reactions due to other bacteria that can occur in pipes during stagnation (Figure 4.2). However, there are also cases where the predicted pH drop was very close to the actual pH drop; for example, site #1 in the anonymous and Portland utility. For samples collected at a given utility, the magnitude of the pH drop amongst different sampling sites was generally consistent with the ranking of ammonia consumption (Table 4.1). An exception was site #3 in Portland, OR, where the ammonia consumption was high, but pH was observed to increase rather than decrease. This was possibly due to the presence of concrete lined pipes in the system, which can leach lime to water, as is seemingly confirmed by higher calcium levels in water samples from this site compared to the other two (5 ppm versus 2 ppm).

For all utilities investigated, the sampling sites with the smallest ammonia consumption and pH drop usually had the least lead, copper or zinc contamination (#3 site within each utility). Overall, the level of lead, copper and zinc contamination was somewhat controlled by the magnitude of the pH drop caused by nitrification. Because the plumbing materials present in each home will vary markedly in terms of lead content and other important issues, perfect correlations between pH drop and lead leaching are not expected.
Table 4.1: Water Utility Studies Results

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<td>81</td>
<td>12</td>
</tr>
<tr>
<td># 2</td>
<td>0.44</td>
<td>25</td>
<td>0.2</td>
<td>1.99</td>
<td></td>
<td>658</td>
<td>444</td>
<td>168</td>
</tr>
<tr>
<td># 3</td>
<td>0.08</td>
<td>25</td>
<td>0.03</td>
<td>0.15</td>
<td></td>
<td>12</td>
<td>187</td>
<td>24</td>
</tr>
</tbody>
</table>

Note: Data reported represents the 1\textsuperscript{st} draw sample after overnight stagnation. Calculations are made by comparing water quality to those collected at the POE.

**Montana Bench Test.** Consistent with expectations based on prior research (Zhang et al. 2008a), complete nitrification occurred in PVC pipes after two months, but never occurred in copper pipes even after 9 months due to copper toxicity (Table 4.2). In PVC pipes, nitrification decreased the final pH to 6.77, while in copper pipes, final pH was increased to 9.96, possibly due to copper corrosion (Zhang et al. 2008b) (Table 4.2). Not surprisingly, the average lead and zinc released from the leaded brass in the PVC pipes were 45% and 81% higher, respectively, than was observed in copper pipes (Table 4.2).

This type of effect also may have been observed at one of the utilities sampled (Table 4.1- Hampden, MN). Higher copper, lead and zinc were observed in homes with plastic rather than copper service.
plumbing. Overall, these data offer clear proof that in situations where nitrification is occurred and leded brass faucets are present, the first draw lead might be higher in homes plumbed with PVC/plastic pipe versus homes plumbed with copper pipe (Zhang et al. 2008a).

**Table 4.2: Montana Bench Test Results**

<table>
<thead>
<tr>
<th>Pipe Material</th>
<th>Ammonia loss, %</th>
<th>Final pH</th>
<th>Total Pb, ppb</th>
<th>Total Zn, ppb</th>
<th>Total Cu, ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC</td>
<td>71±3</td>
<td>6.77 ± 0.06</td>
<td>67 ± 3</td>
<td>763 ± 33</td>
<td>1500 ± 128</td>
</tr>
<tr>
<td>Copper</td>
<td>6±5</td>
<td>9.77 ± 0.13</td>
<td>46 ± 4</td>
<td>421 ± 30</td>
<td>75 ± 5</td>
</tr>
</tbody>
</table>

Note: Data reported were the average of 36 measurements over four weeks time. ± indicates 95% confidence interval

**IMPLICATIONS FOR DRINKING WATER SYSTEMS**

Nitrification can sometimes increase soluble lead contamination of potable water by reducing pH. In considering the possible effects of nitrification on lead solubility for a given circumstance, the “worst case” pH drop from nitrification (assuming 100% conversion of total ammonia) for a utility (Figure 4.6 and Figure S-4.3) can be calculated. For example, at a utility with initial pH of 8.5 and 40 mg/L initial alkalinity and 1 mg/L NH₃-N, pH is expected to drop up to 1.5 unit due to nitrification (Figure 4.6). The lead solubility models (i.e., Figure 4.4) can then be used to predict that the 1.5 unit drop in pH from 8.5 to 7 could increase soluble lead by 50%.

This analysis indicates that the initial pH also plays an important dual role. First, systems with pH between 7.5 and 8 are more likely to have active nitrification (Wolfe and Lieu 2001). Second, a given amount of nitrification activity would induce a much larger pH drop in systems with initial pH of 8-8.5,
since buffer intensity is at minimum at pH = 8.3 in carbonate systems (Snoeyink and Jenkins 1980). For example, comparing site #1 at St Paul, MN and the anonymous utility, with similar initial alkalinites (42 and 44 mg/L) and ammonia loss (0.4 and 0.41 mg/L), the predicted pH drop due to nitrification is 0.5 unit in St Paul, MN (initial pH of 9) and 0.61 unit in the anonymous utility (initial pH of 8.3) (Table 4.1). The actual pH drop in St. Paul, MN was even smaller-0.12 pH unit (Table 4.1).

The overall evaluation indicates that serious problems with lead leaching from nitrification are not expected at the alkalinities and initial pHs encountered at many water utilities. Hence, use of Figure 4.6 and Figure 4.4 can serve as an important screening tool, to consider a utility’s susceptibility to problems with higher soluble lead as a result of nitrification. Certainly, utilities like Ottawa, Canada (initial pH 8.5 and initial alkalinity 35 mg/L) (Douglas et al. 2004) are predicted to be very susceptible to elevated soluble lead from nitrification. Similar approach can be taken for evaluating copper leaching problems due to nitrification, but contrary to lead release, copper solubility is most strongly affected by pH changes in higher alkalinity waters, like Willmar, Minnesota (Murphy et al. 1997).
Figure 4.6: Resulting pH drop based on ammonia oxidized at different initial alkalinity (initial pH = 7.5 and 8.5).

ACKNOWLEDGMENTS

This work was supported by American Water Works Association Research Foundation (AWWARF) and the United States Environmental Protection Agency (USEPA). Matching funds to that grant were also provided by the Copper Development Association (CDA), Bangor (ME), Hampden (ME), Irvine (CA), St Paul (MN) and Portland (OR) water utilities. The opinions, findings, conclusions, or recommendations are those of the authors and do not necessarily reflect the views of AWWARF, USEPA, the CDA, or the water utilities.
Figure S-4.1: Actual pH drops versus predicted pH drops at 1 mg/L-P.

Note: Predicted pH drops were modeled using MINIQL software. Acid produced from nitrification in each case is based on measured ammonia loss, nitrate/nitrite production and the following equations (Equation S1 and Equation S2). This produced acid is added to the initial acid (acid needed to adjust the initial pH to 8), and the result is used to calculate the final pH.
\[ \text{NH}_4^+ + 1.9 \text{ O}_2 + 0.069 \text{ CO}_2 + 0.0172 \text{ HCO}_3^- \rightarrow 0.0172 \text{ C}_5\text{H}_7\text{O}_2\text{N} + 0.983 \text{ NO}_2^- + 0.966 \text{ H}_2\text{O} + 1.97 \text{ H}^+ \]

Equation S1.

\[ \text{NO}_2^- + 0.00875 \text{ NH}_4^+ + 0.035\text{CO}_2 + 0.00875 \text{ HCO}_3^- + 0.456 \text{ O}_2 + 0.00875 \text{ H}_2\text{O} \rightarrow 0.00875 \text{ C}_5\text{H}_7\text{O}_2\text{N} + 1.0 \text{ NO}_3^- \]

Equation S2.
Figure S-4.2: Actual soluble lead versus predicted soluble lead at 1 mg/L-P.

Figure S-4.3: Resulting pH drop based on ammonia oxidized at different initial alkalinity and pH (initial pH = 8 and 9)
Table S-4.1: p-value for the paired t-test on total and soluble lead levels (1 mg/L-P)

<table>
<thead>
<tr>
<th>Soluble Lead</th>
<th>Alkalinity</th>
<th>100</th>
<th>30</th>
<th>15</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorine vs. no inhibitor</td>
<td>0.45</td>
<td>0.0002</td>
<td>0.00006</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>chlorite vs. no inhibitor</td>
<td>0.2</td>
<td>0.002</td>
<td>0.00008</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Lead</th>
<th>Alkalinity</th>
<th>100</th>
<th>30</th>
<th>15</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorine vs. no inhibitor</td>
<td>0.46</td>
<td>0.34</td>
<td>0.007</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>chlorite vs. no inhibitor</td>
<td>0.04</td>
<td>0.0003</td>
<td>0.0002</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

S-4.1 Water Utility Study Details

Samples were collected from a selection of six participating utilities. At each utility, samples were collected at the POE(s) to the distribution system, as well as at two to three homes throughout the chloraminated section of the distribution system. First-draw and flushed samples were collected from homes presenting higher risks of lead and copper contamination, and/or homes near areas where nitrification has been experienced. Samples were collected from faucets within homes (not from outside hose bibs), and from homes without after-market home water treatment devices. Residents were asked to let the water stagnate in the pipe for at least 8 hours prior to sample collection, i.e., they could not use the sampled faucet. Five samples are collected at each location: 1st draw bacterial test sample, 1st Draw Unfiltered, 1st Draw Filtered, Flush Unfiltered, Flush Filtered. The first sample collected was for the bacteriological tests (50ml for HPC and MPN test). First-draw samples (250 ml) for the physical and chemical parameters were collected immediately after. After collecting the first-draw samples, the flushed samples (250 ml) were collected after at least 5 minutes of flushing. Sixty ml of both first draw and flush samples were filtered through 0.45-µm to produce filtered samples. Preferably, samples were collected by utility staff; alternatively, homeowners were asked to collect the samples. In either case, clear instructions were provided.

REFERENCES


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CHAPTER V: ACCELERATED CHLORAMINE DECAY AND MICROBIAL GROWTH

RESULTING FROM NITRIFICATION IN PREMISE PLUMBING

Yan Zhang and Marc Edwards

ABSTRACT

Chloramine decay rates are accelerated by nitrifier colonization of premise plumbing, thereby compromising the effectiveness of secondary disinfection. Without nitrification, chloramine was more stable than chlorine as expected, but with nitrification the relative stability (and efficacy) of the two disinfectants could be reversed. The net result can be higher levels of heterotrophic bacteria in the bulk water and pipe biofilms, of water disinfected with chloramine than for free chlorine. These experimental trends were confirmed using field data collected from homes in systems with active nitrification.

Key Words

Chloramine decay, nitrification, bacterial growth, PVC, iron, premise plumbing
INTRODUCTION

The increased use of combined chlorine (chloramines) versus free chlorine is due to its reduced formation of many regulated Disinfectant By-Products (DBP) and greater persistence relative to free chlorine (Neden et al. 1992; Norton and LeChevallier 1997). Chloramines were noted to be more effective than chlorine in reducing heterotrophic plate counts (HPC) (Neden et al. 1992).

In comparing the relative effectiveness of chlorine and chloramine for disinfection of biofilms on pipe walls under continuous flow conditions found in distribution systems, LeChevallier et., al. 1990 determined that both free chlorine and monochloramine were effective for PVC, copper, and galvanized iron pipes (Lechevallier et al. 1990). However, biofilm growth on iron pipe was better controlled by monochloramine than by the same dose of free chlorine (Lechevallier et al. 1990). This was attributed to a very high reaction rate between free chlorine and iron corrosion products on the pipe surface, essentially rendering the free chlorine disinfectant unavailable for controlling biofilm bacteria. In contrast, monochloramine was less affected by reactions with iron scale, thereby manifesting greater “penetrating power” for microbial control (Lechevallier et al. 1990; Lechevallier et al. 1993). In essence, the greater persistence of chloramine outweighs its reduced efficacy as a disinfectant versus the same dose (as Cl₂) of free chlorine (Wolfe et al. 1984).

However, if situations existed in which monochloramine was consumed at a faster rate than chlorine, it would be expected that chlorine would outperform chloramine in control of biofilms and bacteria in the bulk water. Anecdotally, at least one utility that converted to chloramine suspected that chlorine was a more persistent disinfectant than chloramine in their main distribution system in warm summer months (Powell 2004).

Nitrification, which occurs due to free ammonia produced during chloramine decay, might be expected to contribute to this type of situation (Norton and LeChevallier 1997; Sathasivan et al. 2005; Wilczak et al. 1996; Wolfe and Lieu 2001; Zhang et al. 2009), since the nitrite produced from nitrification accelerates decay of chloramine residuals (Valentine 1984). Accelerated HPC growth may then occur.
for chloramine versus chlorine. Indeed, a loss of chloramine disinfectant during nitrification has frequently been associated with relatively high levels of HPCs in main water distribution systems (Powell 2004; Skadsen 1993; Wilczak et al. 1996; Wolfe and Lieu 2001; Zhang et al. 2009).

To oversimplify the situation on iron pipe surfaces with nitrification, chlorine decay rate is controlled mainly by the reactions with bulk water species and iron corrosion products (Equation 1), whereas chloramine decay is controlled by reactions with bulk water species and nitrite produced by nitrifying biofilms (Equation 2):

\[
\frac{d\text{Cl}_2}{dt} = \left(\frac{d\text{Cl}_2}{dt}\right)_{\text{bulk}} + \left(\frac{d\text{Fe}}{dt}\right)_{\text{iron corrosion}} \quad \text{Equation 1}
\]

\[
\frac{d\text{NH}_2\text{Cl}}{dt} = \left(\frac{d\text{NH}_3}{dt}\right)_{\text{autodecomposition}} + \left(\frac{d\text{NH}_3}{dt}\right)_{\text{nitrification}} + \left(\frac{d\text{Fe}}{dt}\right)_{\text{iron corrosion}} \quad \text{Equation 2}
\]

Using the above equations to consider extremes in relative rates of chloramine (as Cl\(_2\)) and chlorine (as Cl\(_2\)) residual decay is instructive. In a pipe without significant nitrification, chloramine is always expected to be more persistent than chlorine as a disinfectant, because autodecomposition and reaction rates in bulk water for chloramine are relatively low compared to corresponding reactions for free chlorine (Neden et al. 1992; Norton and LeChevallier 1997; Vikesland et al. 2001). Chloramine has even greater advantages relative to free chlorine if iron corrosion is significant, due to the higher reactivity between chlorine and iron scales, as proven by other researchers (Lechevallier et al. 1990).

Conversely, if significant nitrification is occurring and controls the chloramine decay rate, chloramine might be less persistent than chlorine when iron corrosion rates are low. For example, assuming initial total chlorine levels of 2 mg/L and a typical first order decay constant of 1.16 d\(^{-1}\) (Vasconcelos et al. 1997), only 0.6 mg/L chlorine (as Cl\(_2\)) would disappear after an 8 hour stagnation time without considering demand from iron corrosion. However, microbial conversion of 1 mg/L ammonia to nitrite over an 8 hour time period would be expected to cause complete consumption of a 5 mg/L chloramine residual, based on the stoichiometry and decay models presented elsewhere (Margerum et al. 1994; Vikesland et al. 2001).
In situations where chlorine decay is controlled by reactions with iron corrosion products and chloramine decay is controlled by reactions with nitrite, the relative persistence of the two disinfectants will be a function of the relative rates of nitrification and iron corrosion. Isopleths of equal persistence could be roughly defined in some situations as:

\[
\frac{dFe}{dt}_{\text{for free chlorine}} = \frac{dNH_3}{dt}_{\text{nitrification for chloramine}} \quad \text{Equation 3}
\]

Assuming that all the iron corrodes to form Fe^{+2} which destroys free chlorine (See details in Appendix), an iron corrosion rate of 0.8 uA/cm^2 in a 3/4 inch pipe would produce equal persistence (for free chlorine) as a nitrification rate of 0.2 mg NH_3/L-h (for chloramine). At lower iron corrosion rates or higher nitrification rates, chlorine would be more persistent than chloramine (Figure 5.1). If only 10% of the iron corrosion led to formation of scales or corrosion products that reacted with free chlorine (i.e., 90% of the corroded iron was non-reactive), then an iron corrosion rate of 8 uA/cm^2 would produce equal persistence (for free chlorine) as a nitrification rate of 0.2 mg NH_3/L-h (for chloramine).

![Figure 5.1: Rough conceptualization of relative secondary disinfectant advantages, if chlorine decay is controlled by reactions with iron corrosion products and chloramine decay is controlled by reaction with nitrite from nitrification. Calculations assume a pipe size of 3/4”](image)

All of these issues including nitrification, chloramine decay rates and increased HPC are expected to be especially significant under the frequent stagnation conditions found in premise plumbing (Edwards et
al. 2005; Zhang et al. 2009). Once established, nitrification in such situations might not be controlled even by 8 mg/L chloramine-Cl₂ (Fairey et al. 2004; Skadsen 1993).

The loss of chloramine is a possible concern in premise plumbing, since it might allow for re-growth of Legionella in water tanks, an opportunistic pathogen which has recently been identified as a major cause of waterborne disease outbreaks (CDC 2008; Edwards et al. 2005; Strickhouser 2007). Limited data collected to date strongly indicate that chloramine is usually far superior to free chlorine in terms of Legionella control, due to greater persistence of the chloramine disinfectant without nitrification (DeGraca 2005; Flannery et al. 2006; Kool et al. 1999a; Kool et al. 1999b; Strickhouser 2007). Conversion to chloramine is further considered a key community-based intervention associated with reduced risk of Legionnaire’s disease (Flannery et al. 2006).

The objective of this study is to rigorously test the conceptual idea that chloramine can be less persistent than free chlorine in certain situations encountered in premise plumbing. This is important, if only to provide an understanding of possible “exceptions to the rule” regarding improved control of pathogens that are attributed to chloramine use. It also sheds light on potential indirect implications of nitrification as a public health concern.

**MATERIALS AND METHODS**

**Pipe Rig Setup**

A large scale pipe rig was designed to simulate operating conditions in a premise plumbing system (Figure 5.2-a). The rig utilized a large reservoir to feed 8 parallel pipe sections, each plumbed with a different type of material commonly used in home plumbing. Three rigs were used to explore extremes of disinfection encountered throughout water distribution systems (Figure 5.2-a). Water flowed through the pipe loops on a regular schedule of two minutes hydraulic flushing (flow rate—900 ml/min) after every 8 hours’ stagnation. The three pipe rigs were maintained at room temperature throughout the test.
Initially, the 8 pipe materials used in each rig were: glass tubes covered with foil as a control, cross linked polyethylene (PEX), polyethylene (PE), chlorinated polyvinyl chloride (CPVC), copper, copper with epoxy coating (CuE), galvanized iron and stainless steel. CPVC, PEX, copper, PE, galvanized iron and stainless steel pipes were purchased from local hardware store. Copper pipes lined with epoxy were acquired from ACE Duraflow®.

Figure 5.2: Pipe rig setup. Note: there are three water scenarios × eight pipe materials

Later in the testing, PEX, glass and PE pipes were replaced with iron, new lead and old lead pipes. Since unlined iron pipe could not be obtained, cylindrical cast iron coupons of size 1 inch (diameter) × 3/4 inch (length) (Grammer,Dempsey& Hudson, Inc,Newark, NJ) were placed inside Tygon tubing (Figure 5.2-c). Sixteen new iron and sixteen old iron coupons, derived from experiments described elsewhere (Zhang and Edwards 2007), were used. The iron coupons were coated with epoxy on all except one side. New pure lead pipes were obtained, and “old” lead pipes were created by subjecting new lead pipes to accelerated aging via continuous exposure to 10 mg/L free chlorine for 3 months. The latter condition reproducibly creates a thick PbO2 scale, thought to be analogous to that produced naturally in many distribution systems, such as in Washington D.C. This approach was selected over use of harvested lead.
pipe in this work, because the method is scientifically reproducible and also has less variability than is observed for harvested lead pipe.

All pipe materials except for the iron coupons were tubular with a ¾ inch internal diameter. Each material had a continuous pipe section 5 ft long, as well as thirty-two 2 inch long coupons connected by Teflon/Tygon tube, which allowed occasional sampling for biofilm and weight loss (Figure 5.2-b).

**Water Chemistry and Nitrifier Inoculation**

Synthesized Potomac River water was used in this study, containing MgSO₄ (39.3 mg/L), CaCl₂•2H₂O (20.5 mg/L), KNO₃ (9.7 mg/L), Na₂HPO₄ (0.046 mg/L), NaHCO₃ (56.8 mg/L), NaSiO₃ (26.3 mg/L), CaSO₄•2H₂O, Al₂(SO₄)₃•18H₂O and ozonated Natural Organic Matter (NOM) (0.18 mg/L TOC) with an initial pH of 8.

Three phases of work were performed on this test rig. During the first phase, three disinfectant regimes were investigated in the three rigs: no disinfectant as a control, chlorine and chloramine. The initial target doses for chlorine and chloramine pipe rigs were 2 and 4 mg/L (Cl₂) respectively for 2.5 months; the doses were then dropped to 0.5 and 1 mg/L (Cl₂) respectively for 15 months. The chloramine was prepared with chlorine to ammonia ratio of 4: 1. The 200% higher dose of chloramine versus chlorine residual was maintained in all experiments. This chloramine level is consistent with established U.S. residual disinfectant practices, and was not an attempt to bias results in favor of chloramine. No detectable signs of nitrification or nitrite production occurred in any of the plumbing rigs during this first phase of testing.

A second phase of testing established nitrification in the system that was dosed with chloramine. The disinfectant doses in both chlorine and chloramine pipe loops were stopped and 0.5% dechlorinated Blacksburg, VA tap water containing nitrifying bacteria (Zhang et al. 2008) and 2 mg/L-N (NH₄)₂SO₄ was introduced into the pipe loop that had been chloraminated during phase one. Trace nutrients (Zhang et al. 2008) were also added and the level of ozonated NOM was reduced to 0.02 mg/L TOC. The
inoculation was continued for a year. During this time iron coupons and lead pipes were placed into the rig. The pipe loops were then operated for another 4.5 months with stable nitrification occurring in all pipe materials except copper, as confirmed by ammonia conversion to nitrite and nitrate.

For the third phase of work, the disinfectant doses for the chlorine and chloramine rigs were incrementally increased (i.e. for chlorinated pipe, to 0.125, 0.25, 0.5, 1, and 2 mg/L-Cl2 and for chloraminated pipe, to 0.25, 0.5, 1, 2 and 4 mg/L-Cl2), with four weeks’ exposure at each disinfectant level. The goal was to examine the possible inactivation of nitrifiers by secondary disinfectants once they were established in the chloramine rig, and to also quantify the relative rates of chloramine and chorine decay.

**Analytical Methods**

Changes in water quality were monitored by collection of water samples before and after 6 hours stagnation in the pipes. Water samples were collected by draining all the water from the continuous pipe sections after 6 hour stagnation period. Nitrifier activity was tracked by measuring loss of ammonia, production of nitrite and nitrate and reduction of pH. pH was monitored by using a pH electrode according to Standard Method 4500-H+ B (Clesceri et al. 1998). NH4+-N was measured with salicylate method using a HACH DR/2400 spectrophotometer, according to Standard Method 4500-NH3 (Clesceri et al. 1998). NO2–-N and NO3–-N were measured using DIONEX, DX-120 ion chromatography according to Standard Method 4110 (Clesceri et al. 1998).

Chlorine and chloramine levels were measured with a DPD colorimetric method using the HACH DR/2400 spectrophotometer, according to Standard Method 4500-Cl (Clesceri et al. 1998). Bulk water and biofilm heterotrophic bacteria were monitored with Heterotrophic Plate Count (HPC) according to Standard Method 9215 (Clesceri et al. 1998) using the spread plate method with R2A medium. Biofilm analysis was performed on the two inch coupon at the end of each phase and before the replacing of pipe materials. The procedure of biofilm collection was modified from work of others (Silhan et al. 2006), in that the inner surface of the coupons was swabbed with two sterile cotton sticks which were then
transferred to 20 mL of sterilized 25 mM phosphate solution (pH = 7.2) and vortexed vigorously for 1 min to release the bacteria from the sticks. Two coupons were sampled for each pipe. The biofilm solutions from the two coupons were then combined together and vortexed for 20 s. The combined biofilm solution from each pipe was analyzed by HPC similar to bulk water HPC. Nitrifier density was monitored by the five-tube Most Probable Number (MPN) procedure (Wolfe et al. 1990a; Zhang et al. 2008). Standardized colorimetric kits BART™ tests were used to examine other bacterial types and concentration (Droycon Inc.), including sulfate reducing bacteria (SRB) and iron reducing bacteria (IRB).

RESULTS AND DISCUSSIONS

Disinfectant Decay

Free chlorine Decay in Different Pipe Materials
The rate of free chlorine decay was strongly affected by the type of pipe material. During both phase I and phase III, a high free chlorine decay rate was observed in metallic pipes that corrode at high rates including cast iron, galvanized, copper and old lead pipes (Figure 5.3). This is attributed to consumption of the free chlorine by corrosion reactions. Over 80% of the chlorine disappeared in these metal pipes within 6 hours of stagnation (Figure 5.3). In contrast, only 10 – 60% of the chlorine decayed in plastic (PVC, PEX, PE, CuE), glass and stainless steel pipes that undergo very low or insignificant metallic corrosion (Figure 5.3). The average chlorine decay rate in new lead pipes was less than in the other metal pipes, as only about 50% of the free chlorine decayed after 6 hours (Figure 5.3). The overall dependency of free chlorine decay rates on corrosion is consistent with other research, which determined that pipes like iron exerted a high chlorine demand whereas plastic pipes like PVC were classified as unreactive (Hallama et al. 2002).

Chloramine (combined chlorine) decay in different Pipe Materials
Before nitrification was established (Phase I), after 6 hours stagnation time, less than 30% of the chloramine residual decayed in the pipes. The only exception was copper pipes, in which about 60% of
the chloramine decayed in 6 hours (Figure 5.3). The higher chloramine demand in copper pipes is consistent with earlier findings, attributed to a rapid abiotic corrosion reaction (Nguyen 2005).

Overall, before nitrification was established in these systems, free chlorine decay rates were always higher than chloramine decay rates in all materials (for paired Student t-test, $p \leq 0.006$), consistent with the conventional wisdom and Equations 1 and 2 (Neden et al. 1992; Norton and LeChevallier 1997).

Figure 5.3: Total chlorine decay in different pipe materials. Note: For phase I, data reported are the typical decay rate at 5 months pipe age, with initial chlorine of 0.6 mg/L and chloramine of 1.1 mg/L. For Phase III, data reported are the average of three weeks’ measurements. Error bars indicate a 95% confidence interval on triplicate samples. Initial chlorine was 1.1 ±0.2 mg/L and chloramine was 2.0 ±0.2 mg/L.

During the third phase of the study, when nitrification had been established in the pipe rig with chloramine, the situation was markedly changed. In relatively inert pipe materials like PVC, CuE, new lead and stainless steel pipes, much more chloramine (as Cl$_2$) decayed than free chlorine ($p \leq 0.0006$) (Figure 5.4). For example, in PVC pipes, at all levels of applied disinfectant, at least 40% more
chloramine disappeared during the 6 hour stagnation than was the case with free chlorine disinfectant (Figure 5.4). A lower final chloramine residual (in mg/L) was even observed, despite the fact that the initial chloramine dose (as Cl₂) was twice as high as the chlorine dose in this work.

In situations where free chlorine decay is controlled by corrosion reactions with pipe materials like iron, copper, galvanized and old lead pipes, and chloramine decay is controlled by nitrification and corrosion, roughly similar decay rates were observed for chlorine and chloramine (Figure 5.4). Even so, greater total chlorine residual was always present when free chlorine was used, compared to the same situation with chloramine (p ≤ 0.03). The only exception was copper pipes, where residual chlorine and chloramine were similar (p = 0.41), perhaps due to the fact that very little nitrification occurred in copper pipes (ammonia loss < 40%) (Zhang et al. 2008).

These results confirm the key hypothesis regarding the relative stability of chloramine and chlorine in premise plumbing systems.
Figure 5.4: Chlorine and chloramine decay after 6 hours stagnation (Phase III) as total chlorine doses were ramped up. Note: the initial chlorine dose is half of the indicated chloramine dose at any time.

Comparing the situations with and without nitrification (Phase I vs. Phase III), the chloramine decay rate (% decayed) with nitrification was 4-40 times faster than without nitrification in the same pipe materials (Figure 5.3). In all pipe systems except copper, when the chloramine dose was \( \leq 2 \) mg/L as Cl\(_2\), 0.8-1.7 mg/L nitrite was detected after 6 hours stagnation. This level of nitrite can stoichiometrically consume 4-8.5 mg/L monochloramine, implicating nitrification as the key cause for the rapid disappearance of the disinfectant. pH was also decreased by 0.6-1.1 unit, which can also partly contribute to the higher decay, since chloramine decay rates have been reported to double for each 0.7 unit decrease in pH (Thomas 1987).
Inactivation of Nitrification

Earlier studies (Wolfe et al. 1990b) indicated that chloramine levels typically used for potable water disinfection (1 to 2 mg/L) should be sufficient to eliminate nitrifiers in water main distribution systems, but there have also been cases where nitrification was not controlled by up to 8 mg/L chloramine Cl₂ (Fairey et al. 2004; Skadsen 1993). In this study using long stagnation times found in many premise plumbing systems, for most pipe materials nitrification activity did not decrease until the chloramine level was increased to 4 mg/L and was maintained at that level for several weeks (Figure 5.5). This is higher than was reported by others (Fleming et al. 2005; Fleming et al. 2008; Wolfe et al. 1990a),

The two exceptions were PVC pipe, in which nitrification activity decreased at only 2 mg/L chloramine residual, and the rig with iron coupons, in which nitrification activity did not decrease even at 4 mg/L chloramine (Figure 5.5). Nitrifying bacteria were also quantified with an MPN technique, different from ammonia loss %. In both PVC pipe and iron coupons, nitrifier MPN only decreased markedly when chloramine was increased to 4 mg/L (Figure 5.5). However, it has to be noted that nitrifier MPN is not representative of the actual nitrifying bacteria, since it can only selectively detect 0.1 to 5 % of the total nitrifiers (Wolfe et al. 1988; Zhang et al. 2009).

In most pipe materials the rate of chloramine decay was highly correlated to changes in nitrifier activity, with R² of 0.7-0.95 for linear correlations between the chloramine decay percentage and ammonia loss percentage. However, in iron, copper and galvanized pipes, ammonia loss % and chloramine decay were not highly correlated, as indicated by R² of less than 0.35. This is possible because ammonia loss does not perfectly represent nitrifier activity with cast iron and galvanized pipes due to nutrient cycling (see detailed explanation in Chapter VI). In addition abiotic corrosion reactions, rather than nitrification, can control chloramine decay rates in copper, cast iron and galvanized iron pipes.
Figure 5.5: Ammonia loss % and MPN with PVC, iron and new lead pipes. Note: the trend of ammonia loss with CuE, old lead, galvanized and stainless steel was similar to that observed for new lead pipe. Ammonia loss % with Cu was below 40% throughout the test.

**Heterotrophic Bacterial Growth**

In drinking water systems, heterotrophic plate counts (HPC) are usually monitored to assess the microbiological quality (Hammes et al. 2008), but this method is culture dependent and detects a small fraction of the total bacterial cells (Allen et al. 2004; Hammes et al. 2008). Recent advances have enabled the quantification of total cells in drinking water. Even so, most of these methods are microscopy-based and are very time and labor consuming, so HPC remains as the primary monitored microbiological quality parameter in drinking water systems (Hammes et al. 2008).

**Effect of Nitrification on HPC without Disinfectant.** An increase in HPC has frequently been noted to accompany nitrification occurrence (Powell 2004; Skadsen 1993; Wilczak et al. 1996; Wolfe et al. 1990). In the absence of disinfectant, it might be expected that nitrification would increase HPC by
releasing organic carbon for heterotrophic bacterial growth (Wilczak et al. 1996; Zhang et al. 2009). In this study, during the second phase when there was no disinfectant, nitrification did not increase HPC as indicated by similar bulk water HPC in rigs with nitrification compared to rigs without nitrification (Figure 5.6-Phase II). Similar trends were observed for biofilm HPC densities, in that the difference of HPC with or without nitrification was less than an order of magnitude (data not shown). Based on earlier studies (Rittmann et al. 1994), nitrification of 2 mg/L-N ammonia can produce 20 ppb-C soluble organic carbon. In this work, this small amount of organic carbon production might not significantly increase HPC, because the de-ionized water used for making up the synthesized water contained \( \approx 100-300 \) ppb total organic carbon as a trace contaminant.

**Effect of Nitrification on HPC with Disinfectants**

Bulk water HPC. During the first phase of the study, chloramine was more persistent than chlorine without nitrification (Figure 5.3), but chloramine was generally not better in controlling bulk water HPC at \( > 95\% \) confidence (\( p > 0.07 \)) (Figure 5.6). But in galvanized iron pipe, chloramine consistently reduced HPC to a greater extent than did chlorine, confirming the results of Lechevallier et al. 1990 (Figure 5.7).

During the third phase of the study with nitrification, chloramine was less persistent than chlorine (Figure 5.3), and was far less effective in reducing bulk water HPC (Figure 5.6). Compared to the control, bulk water HPC was decreased by ten to a hundredfold with free chlorine, while it was not significantly affected by chloramine (Figure 5.6). Therefore, nitrification effectively interfered with secondary disinfection by chloramine.
Figure 5.6: Bulk water HPC in PVC pipes. Note: chlorine and chloramine doses were stopped during Phase II. Nitrification was occurring in the chloraminated rigs during Phase II and Phase III.

Biofilm HPC. Trends in biofilm HPC were similar to bulk water HPC, in that more biofilm bacteria were quantified for chloramine systems when nitrification was causing rapid loss of the disinfectant residual (Figure 5.8). For iron coupons, neither chlorine nor chloramine reduced biofilm HPC, even at chloramine dose up to 4 mg/L when nitrification was occurring (Figure 5.8). Thus, for iron neither chlorine nor chloramine was an effective secondary disinfectant, because the high corrosion rate for iron caused rapid chlorine decay and the persistence of nitrifying bacteria caused rapid chloramine decay.
An exception to these trends occurred in galvanized pipe, for which chloramine decayed faster than chlorine (Figure 5.4), but lower HPCs were still observed with chloramine (Figure 5.8). It is speculated that this discrepancy could be due to the much lower levels of dissolved oxygen in the galvanized pipes with nitrification. Specifically, after stagnation, bulk water DO decreased to 2.8 mg/L in the pipes with nitrification, versus the 5.7-6.2 mg/L DO observed in the same pipes without nitrification (using no disinfectant or chlorine). The lower DO can favor the growth of anaerobic bacteria like sulfate reducing bacteria (SRB), methanogens or iron reducing bacteria (IRB) versus HPCs (Lovley and Phillips 1987). In this work a low level of SRB (200 cfu/ml by BART test) and a very high level of IRB was detected (>140000 cfu/ml) in the bulk water of the chloraminated galvanized pipe. In the same pipes with no disinfectant and with chlorine, the SRB (< 200 cfu/ml) and IRB (< 9000 cfu/ml) were undetectable. Therefore, for galvanized iron, the lower HPC counts do not correspond to lower overall levels of total bacteria in the system. If a wider range of bacteria are considered, including IRB and SRB, more microbes were quantified in the nitrifying rig with chloramine.
Figure 5.8: Biofilm HPC with and without nitrification. Note: Data reported for phase I was the average result of five sampling events over the first year and error bars indicate 95% confidence interval; Data reported for phase III was the result of one time sampling event at the end after applying the highest disinfectant levels for a month (No data available for copper pipes with chloramine).

Field Data

In parallel field work, samples were collected from homes at chloraminated utilities and analyzed for possible changes in the water due to nitrification. In many of the utilities, the occurrence of nitrification varied markedly from building to building (Table 5.1). This might be expected given differences in water usage rates, flow patterns, plumbing materials and influent disinfectant residuals from home to home. For example, at anonymous utility A, site 1 had a larger ammonia loss (up to 20% higher) and more nitrite and nitrate than did site 2 and site 3, indicating higher nitrification activity. Correspondingly, the water at site 1 had the largest pH decrease, fastest chloramine decay rate and highest HPC (up to 0.7 pH decrease, 95% chlorine decay and $10^5$ cfu/ml) (Table 5.1). The general trend
of higher nitrification rates associated with higher chloramine decay rates and HPC counts were observed at all utilities studied (Table 5.1).

**Possible Implications for the Control of *Legionella* and Other Opportunistic Pathogens**

While data regarding benefits of chloramine in control of *Legionella* are strong (DeGraca 2005; Flannery et al. 2006; Kool et al. 1999a; Kool et al. 1999b; Strickhouser 2007), this benefit can only apply to situations in which chloramine residuals are present. Since chloramine residuals can be eliminated by persistent and rapid nitrification in premise plumbing, better understanding of the interplay between premise plumbing, flow, nitrification and chloramine levels in control of *Legionella* would be desirable. On the basis of results presented herein, it is reasonable to expect that nitrification can create circumstances in which free chlorine would outperform chloramine in control of opportunistic pathogens.
Table 5.1: Field study results

<table>
<thead>
<tr>
<th></th>
<th>Ammonia decrease, mg/L</th>
<th>Nitrite + Nitrate increase, mg/L</th>
<th>pH decrease</th>
<th>Cl₂ decay %</th>
<th>HPC, cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anonymous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>#1</td>
<td>0.41</td>
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<tr>
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<td>0.72</td>
<td>94</td>
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<tr>
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<td>0.12</td>
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</tr>
<tr>
<td>#3</td>
<td>0.18</td>
<td>0.02</td>
<td>-0.18</td>
<td>9</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
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<td>0.28</td>
<td>0.71</td>
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<tr>
<td>#2</td>
<td>0.62</td>
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<td>0.58</td>
<td>87</td>
<td>16500</td>
</tr>
<tr>
<td><strong>Hampden, ME</strong></td>
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</tr>
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</tr>
<tr>
<td>#2</td>
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<td>0.53</td>
<td>1.99</td>
<td>98</td>
<td>138000</td>
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<tr>
<td>#3</td>
<td>-0.39</td>
<td>0.02</td>
<td>0.15</td>
<td>27</td>
<td>21000</td>
</tr>
</tbody>
</table>

Note: Data reported are for the 1st draw samples. Differences in ammonia, nitrite, nitrate, pH and chloramine (as Cl₂) were calculated against the flush sample at the entry point of the distribution system.

**CONCLUSIONS**

The relative efficacy of chlorine versus chloramine is a complex function of autodecomposition, reactions with corrosion by-products, nitrification rates and the pipe materials themselves. The following conclusions can be drawn:

- Without nitrification, chloramine was always more persistent than chlorine, confirming expectations based on previous research.
- With rapid nitrification, in relatively inert pipe materials (i.e., PVC, CuE, Stainless Steel pipes), chloramine was less persistent than chlorine. In such situations chloramine was less effective than free chlorine in reducing bulk and biofilm HPC.
• In reactive pipe materials such as cast iron, galvanized iron, old lead pipe, the relative disinfectant decay rate and ability to control bacterial growth, is strongly dependent on the relative rates of corrosion and nitrification. If nitrification rates are high and corrosion rates are low, chlorine would tend to be better at controlling microbial regrowth.

ACKNOWLEDGEMENT

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APPENDIX

Modeling Approach for Figure 5.1:

Chlorine decay with iron corrosion products:

\[
1 \frac{\mu A}{cm^2} = 1 \times 10^{-6} \frac{columb}{s \cdot cm^2} \times \frac{6.24 \times 10^{18} e^-}{columb} \times \frac{1mol - e^-}{6.02 \times 10^{23} e^-} = 1.04 \times 10^{-11} \frac{mol - e^-}{s \cdot cm^2}
\]

Iron Corrosion: \( Fe \rightarrow Fe^{2+} + 2e^- \)

Chlorine reacts with \( Fe^{2+} \): \( 2Fe^{2+} + Cl_2 \rightarrow 2Fe^{3+} + 2Cl^- \)

so \( \frac{Cl_2}{e^-} = \frac{1}{4} \)

\[
1 \frac{\mu A}{cm^2} = 1.04 \times 10^{-11} \frac{mol - e^-}{s \cdot cm^2} \times \frac{Cl_2}{4e^-} \times \frac{71000mg}{1mol - Cl_2} \times \frac{3600s}{1h} = 6.62 \times 10^{-14} \frac{mg - Cl_2}{cm^2 \cdot h}
\]
Chloramine decay with nitrite:

Nitrification: \(2\text{NH}_4^+ + 3\text{O}_2 \rightarrow 2\text{H}_2\text{O} + 2\text{NO}_2^- + 4\text{H}^+\)

Chloramine reacting with nitrite: \(\text{NH}_2\text{Cl} + \text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{NO}_3^- + \text{HCl}\)

So \(
\frac{\text{NH}_2\text{Cl}}{\text{NH}_4^+} \times \frac{1\text{mol}}{1\text{mol}} = \frac{71\text{mg} - \text{Cl}_2}{14\text{mg} - \text{N}}
\)

If ammonia consumption rate is \(\frac{\text{mg} - \text{N}}{\text{L} - \text{h}}\), then chloramine decay rate would be \(5\frac{\text{mg} - \text{Cl}_2}{\text{L} - \text{h}}\), in a 3/4 inch diameter size pipe, this would translate to

\[
5\frac{\text{mg} - \text{Cl}_2}{\text{L} \cdot \text{h}} \times \frac{\text{L}}{10^3 \text{cm}^3} \times \frac{\pi / 4 \times 1.9^2 \times \text{length} \text{ cm}^3}{\pi \times 1.9 \times \text{length} \text{ cm}^2} = 2.4 \times 10^{-3} \frac{\text{mg} - \text{Cl}_2}{\text{cm}^2 \cdot \text{h}}
\]

So at equal decay rate of chlorine and chloramine,

\[
1 \frac{\mu\text{A}}{\text{cm}^2} = 6.62 \times 10^{-4} \frac{\text{mg} - \text{Cl}_2}{\text{cm}^2 \cdot \text{h}} \times \frac{1\text{mg} - \text{N}}{\text{L} \cdot \text{h}} \times \frac{\text{cm}^2 \cdot \text{h}}{2.4 \times 10^{-3} \text{mg} - \text{Cl}_2} = 0.276 \frac{\text{mg} - \text{N}}{\text{L} \cdot \text{h}}
\]

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CHAPTER VI: NITRIFICATION EFFECT ON CORROSION OF GALVANIZED IRON, COPPER AND CONCRETE

Yan Zhang, Allian Griffin, Marc Edwards

ABSTRACT

Nitrifying bacteria rapidly colonized iron, plastic and lead materials under conditions found in premise plumbing, became less readily established in galvanized, stainless steel, and had difficulty surviving in relatively new copper pipes. Nitrification significantly decreased pH and dissolved oxygen (DO) in all pipe materials, and sometimes led to sporadic sloughing of very high TOC from cast iron coupons. The release of zinc from galvanized iron was reduced 6 times due to lower DO resulting from nitrification. In a low alkalinity water, lower pH from nitrification had no effect on copper release from copper pipe. Nitrification did not significantly increase weight loss due to corrosion amongst the three different disinfectant conditions (no disinfectant, chlorine, chloramine + nitrification) for cast iron, new lead, old lead, copper, galvanized iron, stainless steel or concrete. Nitrification did not cause copper pitting corrosion in this study.
Nitrification in potable water systems is a concern (Wolfe and Lieu 2001; Zhang et al. 2009b) due to the increased use of chloramine disinfectant. Nitrification is expected to be strongly influenced by different pipe materials, since the pipe can serve as a source of trace nutrients, toxic metals and destroy disinfectants (Zhang et al. 2009b). For example, earlier research demonstrated that phosphorus can be released from iron pipe (Morton et al. 2005), polyethylene pipes (Lehtola et al. 2004) and soft copper pipes (Chapter II). Concrete pipe materials have been observed to either inhibit nitrifier growth due to increased pH or to stimulate nitrifier growth due to leaching of essential nutrients and detoxifying effects on metals such as copper (Chapter II). Iron and lead were also confirmed to generate ammonia from nitrite and nitrate (Chapter II), which was hypothesized to stimulate nitrification by forming a cycling reaction with nitrification (Edwards and Dudi 2004). These disparate effects can help explain why PVC and lead pipes are more prone to inducing nitrification than copper or brass pipes (Zhang et al. 2008a), and why some studies find lower nitrifiers and HPC associated with concrete pipe materials (Steward and Lieu 1997) but in other studies the contrary trend is observed (LePuil et al. 2003).

Prior research has also reported possible links between certain corrosion problems and nitrification (Douglas et al. 2004; Edwards and Dudi 2004; Edwards and Triantafyllidou 2007; Powell 2004; Triantafyllidou et al. 2007). The reduction of pH due to nitrification has been suspected to increase lead leaching (Douglas et al. 2004; Odell et al. 1996) and this was recently confirmed mechanistically (Zhang et al. 2009a). Elevated copper levels at the tap were also linked to nitrifying bacteria in Willmar, Minnesota homes (Murphy et al. 1997a), and the lower pH due to nitrification could be a contributing factor.

Nitrification is also suspected to influence corrosion through factors other than pH reduction. For
example, accelerated disinfectant decay via nitrification could increase the growth of bacteria that might stimulate microbial induced corrosion (MIC). This has been suspected to contribute to certain cases of copper pitting (Cantor et al. 2006). The production of soluble microbial product (SMP) from nitrifier biomass might increase metal leaching from lead and copper and other pipe materials via complexation with metal ions (AWWARF and DVGW-TZW 1996). The production of nitrite and nitrate has been suspected to increase the corrosion of iron and lead by chemical reactions (Kielemoes et al. 2000). Finally, the reduction in dissolved oxygen might also affect corrosion, since oxygen can control corrosion rates and certain manifestations of non-uniform corrosion via creation of “concentration cells”.

The objective of this study was to examine nitrification in a wide range of pipe materials, with particular attention focused on the possible consequences in relation to weight loss, metals release and type of corrosion.

MATERIALS AND METHODS

A large scale pipe rig setup was designed to roughly simulate operating conditions in a premise plumbing system (see Figure 5.2). The rig utilized a large reservoir to feed 8 parallel pipe sections, each plumbed with a different type of material commonly used in home plumbing. The eight pipe materials used were: glass tubes covered with foil as a control, cross linked polyethylene (PEX), polyethylene (PE), chlorinated polyvinyl chloride (CPVC), copper, copper with epoxy coating (CuE), galvanized iron and stainless steel. Later in the test, PEX, glass and PE pipes were replaced with iron, new lead and old lead pipes. More specifics of the large scale pipe rig testing were described in Chapter V.

The test water used in this study was synthesized Potomac River water, as described in Chapter V. Three rigs were used to explore extremes of disinfection encountered throughout water distribution systems.
Three phases of work were performed on the three test rigs. During the first phase three different disinfectant regimes were investigated including: no disinfectant (i.e., control), chlorine and chloramine. No detectable signs of nitrification or nitrite production occurred during this first phase of testing in any pipe rigs. This is likely because no nitrifiers were introduced, and levels of free ammonia were either 0 or very low in all test rigs (Fleming et al. 2005; Fleming et al. 2008). A second phase of testing purposefully established nitrification in the system that had been dosed with chloramine. No chlorine was added to the rigs previously dosed with chlorine and chloramine, and 0.5% dechlorinated Blacksburg, VA tap water containing nitrifying bacteria (Zhang et al. 2008a) and 2 ppm-N \((\text{NH}_4)_2\text{SO}_4\) was introduced into the rig that had been previously chloraminated. The inoculation was continued for a year. During this time iron coupons (replacing PEX) and lead pipes (replacing glass and PE pipes) were also placed into the rig. The pipe loops were then operated for another 4.5 months with stable nitrification occurring in all pipe materials (as measured by ammonia conversion to nitrite and nitrate), except in copper pipes which are toxic to nitrifiers (Zhang et al., 2008a). For the third phase of work, the disinfectant doses for the chlorine and chloramine rigs were incrementally increased (i.e. for chlorinated pipe, to 0.125, 0.25, 0.5, 1, and 2 ppm-Cl\textsubscript{2} and for chloraminated pipe, to 0.25, 0.5, 1, 2 and 4 ppm-Cl\textsubscript{2}). Four weeks' exposure was allowed at each disinfectant level.

**Mechanistic Investigation of Zinc and Iron Release**

Galvanized pipes were newly purchased with a size of 30 cm (length) × 1.9 cm (diameter) and tested in triplicate. Pipes were maintained in stagnant conditions and water in the pipes was changed every 3.5 days using a “dump and fill” protocol. The control water was the same water used in the large scale test without any ammonia, nitrite or nitrate added, and with an initial pH of 7. The effect of different initial pH (pH 6 or 8), addition of different nitrogen species (2 ppm ammonia, 2 ppm nitrite or 4 ppm nitrate) were studied by modifications to the control water. Anaerobic conditions were investigated by purging the control water with nitrogen and maintaining the test in an anaerobic glove box. Zinc and iron release was measured at each water change.
A similar study was also conducted for pure iron and zinc metals. Pure iron and zinc wires (20 mm length × 1 mm diameter) were placed inside amber glass TOC test vials (25 mm × 95 mm) with 30 ml solution. For each metal, fifteen conditions were tested: 3 waters (control, control + 10 ppm nitrite, control + 10 ppm nitrate) × 2 oxygen conditions (aerobic and anaerobic) × 2 pHs (6 and 8) + pH 8, aerobic, 5 ppm ammonia = 13 × 3 duplicates = 39 vials. The control water was simulated Potomac water (Chapter V) without any nitrogen species added. Water in the vials was changed twice a week and metal release was quantified each week.

**Effect of Nitrification on Concrete Corrosion**

Reactors used were 25 mm × 95 mm Amber Glass TOC Test Vials (Fisher Brand) with 30 ml solution. Four pieces of Portland cement (37 mm × 5 mm × 4 mm) were added into each reactor. Synthesized test water was used, which contained MgSO₄ (1 ppm-Mg), CaCl₂ (4.9 ppm-Ca), KCl (10 ppm-K), Na₂HPO₄ (1 ppm-P), NaHCO₃ (500 ppm-CaCO₃), and other trace nutrients (5 ppb Cu²⁺, 1.7 ppb Mo⁶⁺, 0.1 ppb Co²⁺, 5.6 ppb Mn²⁺, 2.6 ppb Zn²⁺, and 0.1 ppm Fe²⁺) and 2 ppm-N ammonia. There were twelve reactors totally, each inoculated with Blacksburg, VA tap water containing nitrifiers for two weeks. Then, in order to demonstrate the effect of nitrification, for six of the reactors, nitrification was stopped by adding chlorine (up to 1 ppm), and then these reactors were used as a control without nitrification. Nitrification was not re-established. As specified in later text, the target pH was 7 or 8 and initial alkalinity was either 10 or 75 ppm as CaCO₃. Water was changed every weekday using a dump and fill protocol.

**Analytical Methods**

Water samples were collected before and after introduction to the pipe/reactors, so that nitrification activity and associated water quality changes could be quantified. Nitrifier activity was tracked by measurements of ammonia loss, production of nitrite and nitrate, and reduction of pH. pH was monitored by using pH electrode according to Standard Method 4500 H+ B. NH₄-N was measured with salicylate method using a HACH DR/2400 spectrophotometer, according to Standard Method 4500NH₃ (Clesceri et al. 1998). NO₂-N and NO₃-N were measured using DIONEX, DX-120 ion chromatography
(IC), according to Standard Method 4110 (Clesceri et al. 1998). Nitrifier most probable number (MPN) was evaluated using the same approach as described in the bench scale study (Zhang et al. 2008a). Total organic carbon (TOC) was analyzed using a SIEVERS 800 Total Organic Analyzer according to Standard Method 5310C (Clesceri et al. 1998). Dissolved oxygen was quantified according to Standard Method 4500 O G (Clesceri et al. 1998) using a dissolved oxygen meter YSI Model 58.

Soluble and total metal release was also quantified. Soluble metal concentration was operationally defined by filtration though a 0.45 μm pore size syringe filter. Total metal release was quantified in the unfiltered water samples, after heated digestion with 2% nitric acid in a 80 °C oven. Metal concentrations (Fe, Cu, Zn, Pb) were quantified using an inductively coupled plasma mass spectrophotometer (ICP-MS) according to Standard Method 3125-B (Clesceri et al. 1998). Weight loss was determined at the end of the test after light polishing to remove loose scale using a Dremel tool.

RESULTS AND DISCUSSIONS

Nitrification Activity in Different Pipe Materials

Earlier bench scale studies (conducted under mostly stagnant conditions) demonstrated that nitrification established readily in PVC and lead pipes, but less readily in copper or brass pipes due to copper toxicity (Zhang et al. 2008a). In this larger-scale test using a wider range of materials and more realistic flow regimes with water changes three times per day, nitrification activity was rapidly established in iron, plastic and lead materials (Table 6.1). In these materials, significant nitrification was established almost immediately after inoculation, and when the inoculation was stopped about 70% ammonia loss was consistently observed after 6 hours stagnation (Table 6.1).

Although less ammonia loss (and more ammonia residual) was observed in the plumbing line with cast iron coupons (Table 6.1), bulk water nitrifier MPN with iron coupons was similar or even higher than PVC (Figure 5.5). In fact, the low surface area/volume for iron relative to the other materials (Table 6.1)
indicates extremely high rates of nitrification. It is also possible that the ability of cast iron to reduce nitrate/nitrite back to ammonia would reduce the measured extent of ammonia loss while contributing to very high levels of nitrifiers (Westerhoff and James 2003; Zhang and Edwards 2007).

In stainless steel and galvanized pipes, nitrification did not start until one month after inoculation. Lower ammonia loss was also observed in galvanized pipes, possibly due to the toxicity from Zn$^{2+}$ which can inhibit nitrification under at least some circumstances (Bott 2005; Zhang and Edwards 2005). It is also possible that nitrate is converted to ammonia by galvanized pipes as was reported elsewhere (Wagner 1993).

The average ammonia loss of about 40% in the copper pipe (Table 6.1) is not attributed to nitrifier biofilm in copper pipes, but rather to nitrifiers in water from the reservoir tank (Figure 5.2). The interior of the reservoir tank was made of glass and some nitrification was occurring in the tank. In fact, if water was taken from the tank and held in a glass container, ammonia loss was $76 \pm 5\%$ after 6 hours stagnation, versus only 38% for water held in copper pipes. Thus, the copper was strongly interfering with nitrification. Additionally, nitrifier MPN in copper pipes was actually reduced compared to levels in the reservoir tank (500,000 versus 160,000 MPN/100 ml) consistent with toxicity of Cu$^{+2}$ to nitrifiers, but it stayed the same or increased in other pipe materials.
Table 6.1: Nitrification in different pipe materials

<table>
<thead>
<tr>
<th>Rank</th>
<th>Pipe Materials</th>
<th>Time when nitrification started</th>
<th>Ammonia loss % after 6 hours</th>
<th>Surface Area/Volume, cm(^{-1})</th>
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<tr>
<td>1</td>
<td>Cast Iron</td>
<td>&lt; 1 week of inoculation</td>
<td>57 ± 3</td>
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<tr>
<td></td>
<td>PVC</td>
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<td>72 ± 21</td>
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<td></td>
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<td>Epoxy Coated Copper</td>
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<td>68 ± 5</td>
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</tr>
<tr>
<td></td>
<td>Old Lead</td>
<td></td>
<td>76 ± 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>New Lead</td>
<td></td>
<td>71 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glass</td>
<td></td>
<td>52 ± 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Old Concrete (^1)</td>
<td>3 months</td>
<td>100 (^2)</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>Stainless Steel</td>
<td>&lt; 1 month of inoculation</td>
<td>67 ± 14</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Galvanized</td>
<td></td>
<td>57 ± 11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>New Concrete (^1)</td>
<td></td>
<td>60 (^3)</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>Copper</td>
<td>N/A</td>
<td>38 ± 9</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Note: Reported ammonia loss was the average of at least three measurements. ± indicate 95% confidence interval. 1: concrete was tested in glass pipes with small concrete pieces (30 cm × 2.5 cm) placed inside; pure culture of *Nitrosomonas europaea* was used, but effort was not made to maintain the purity. Old concrete pieces have been exposed to other water for a year (Parks et al. 2008). 2: Ammonia loss tested after 72 hours; 3: Ammonia loss tested after 24 hours.

**Nitrification Effects on Water Chemistry**

Nitrification significantly decreased the pH and dissolved oxygen (DO) within a 6 hour stagnation event (Figure 6.1). pH decreased by 0.6 to 1 unit in all pipe materials and DO also decreased to as low as < 3 ppm (Figure 6.1). Nitrification stoichiometry (Zhang et al. 2009a) predicts a pH drop of up to 0.9 unit and a DO drop of 10 ppm if complete nitrification (100% ammonia conversion to nitrate) occurs, so the observed drop in pH and DO was in the range of expectations.
In the pipes without nitrification, pH and DO did not significantly change during stagnation, except for the rig with iron coupons in which pH and DO also decreased (Figure 6.1) due to iron corrosion. In copper pipes, average ammonia loss of 40% (Table 6.1) can only account for a pH drop of 0.5 units. The higher than expected pH and DO drop (Figure 6.1) might be due to corrosion reactions (Zhang et al. 2008b) and the growth of other bacteria. Total Organic Carbon (TOC) was not significantly increased by nitrification in most pipe materials, but with cast iron coupons very high TOC was occasionally observed (Figure 6.1), which was correlated to be higher level of nitrification and possible sloughing of autotrophic bacteria biofilms.

Effect of Nitrification on Pipe Corrosion

Galvanized Pipe

Before nitrification occurred in the galvanized iron pipes (Phase I), total zinc release from galvanized pipes was similar at all three disinfectant conditions ($p \geq 0.13$) (Figure 6.2). After nitrification was established (Phase II), zinc release from the nitrifying rig was significantly lower compared to the rigs without nitrification (6 times lower on average and $p \leq 0.002$) (Figure 6.2). The difference in zinc levels with/without nitrification had very dramatic visual impacts (Figure 6.3). Water samples from the pipes without nitrification were white and turbid; in contrast, water samples from pipes undergoing nitrification were very clear (Figure 6.3).

This effect was further confirmed during phase III. When disinfectants were re-dosed to the chlorine and chloramine rigs, as nitrification activity decreased in the presence of chloramine, the levels of zinc release in the nitrifying pipe gradually increased. Eventually, the zinc release in the presence of chloramine was not significantly different from the rigs with chlorine or with no disinfectant ($p \geq 0.08$ at 4 ppm chloramine) (Figure 6.2).

Iron release from the nitrifying rig was also lower compared to the rig without nitrification ($p = 0.002$), although the trend was not as dramatic as observed for zinc release. The surface of the galvanized pipe
in which nitrification occurred had a yellowish dense scale, whereas the pipes without nitrification had a light grey loose scale (Figure 6.4).

**Figure 6.1: Nitrification Effect on Water Chemistry**

Note: Data reported were the average of four measurements for TOC, and two measurements for pH and DO, ±indicate standard deviation. w/o nitrification data is the rig with no disinfectant added throughout the test. Data for other pipe materials are similar to PVC but not presented.
Figure 6.2: **Total zinc release versus time in galvanized pipes.** Note: chlorine and chloramine doses were stopped during Phase II, nitrification was occurring in chloraminated pipe in Phase II and Phase III.

Figure 6.3: **Water sample from galvanized pipes.**
Mechanistic Investigation of Galvanized Iron Corrosion

The surprising reduction of metal release from galvanized iron pipe by nitrification was contrary to the general expectation that nitrification would invariably increase corrosion and metal release (Zhang et al. 2009b). Since nitrification converted ammonia to nitrite/nitrate and significantly reduced pH and DO in the pipes (Figure 6.1), a mechanistic investigation was designed to evaluate which factors caused the reduction in zinc release from galvanized pipes.

Among the conditions investigated, lower DO reduced zinc release from galvanized pipe most significantly (p = 0.003), in that average zinc release under anaerobic conditions was only 20% of that for aerobic conditions (Figure 6.5). The addition of ammonia also reduced average zinc release by 40% (p = 0.03). Average zinc release at pH 6 and 7 was 60% of that at pH 8, although this was not statistically significant at > 95% confidence (p = 0.08 comparing pH 6 and 8, and p= 0.1 comparing pH 7 and 8). Adding nitrite or nitrate did not effect zinc release at > 95% confidence versus control conditions (p = 0.16 for nitrite, and p = 0.06 for nitrate) (Figure 6.5).
In conclusion, while lower DO, the presence of free ammonia, and lower pH all contribute to the reduced zinc leaching from the galvanized pipe undergoing nitrification, lower DO is the most significant single factor. This is not surprising given that dissolved oxygen can accelerate zinc metal corrosion (Jones 1996).

**Figure 6.5: Zn release in galvanized pipe.** Data reported are the averages of three week testing. The error bars represent 95% confidence intervals.

In the tests with pure iron and zinc metal, the imposition of anaerobic conditions also reduced metal release most significantly. Specifically, at pH 8, over the first four weeks of testing, average iron released without O₂ was 23% of the iron released with O₂ (for student paired t-test, p = 0.002) and average zinc released without O₂ was 14% of the zinc released with O₂ (paired t-test, p = 0.07). Lower pH did not have a consistent effect on metal release with pure iron and zinc. For nitrogen species, over the two months of testing, addition of ammonia and nitrate did not affect the metal release from iron or zinc, but 10 ppm nitrite reduced iron release by 50% (paired t-test, p = 5 × 10⁻⁷) and zinc release by 30% (paired t-test, p = 0.015). The beneficial effect of nitrite on iron and zinc release was contradictory to the expectation that chemical reaction between nitrite and iron/zinc would increase corrosion. Although earlier studies have concluded that nitrite inhibits iron corrosion, the concentrations were a lot higher (> 100 ppm-N) (Hubert et al. 2005; Kielemoes et al. 2000) than the level used in this study.

*Copper Pipe*
In copper pipes, the reduction of pH and DO did not significantly affect copper release (copper release with or without nitrification was similar for student paired t-test, $p = 0.34$). This observation was consistent with solubility modeling (AWWARF and DVGW-TZW 1996; Zhang et al. 2009a). At low alkalinity (30 ppm), when copper pipe is old and the controlling solid is CuO (tenorite), copper solubility is only a very weak function of pH (Figure 6.6). When copper pipe is relatively new and the controlling solid is Cu(OH)$_2$, soluble copper was more significantly affected by decreased pH (Figure 6.6), as observed in Chapter VII.

At high alkalinity copper solubility is predicted to be more strongly affected by pH (Dodrill and Edwards 1995; Edwards et al. 1996), particularly when the controlling solid is Cu(OH)$_2$ (Figure 6.6). A small pH drop of 0.2 unit would increase soluble copper by 30%, and a pH drop of 0.5 unit would double the soluble copper levels (Figure 6.6). In Willmar, MN with high alkalinity water (400-450 ppm), a measured pH drop of 0.5 units in premise plumbing (Murphy et al. 1997a) would be predicted to increase copper levels from 2.5 ppm in main distribution system to 6.6 ppm in premise plumbing (Table 6.2). Although pH and copper contamination were not monitored simultaneously at one site (Murphy et al. 1997a; Murphy et al. 1997b), it is very possible that even a nearly negligible pH drop (i.e., detection limit for pH ± 0.15 units) could markedly increase copper levels.

On the other hand, nitrification significantly decreased DO down to nearly undetectable levels in some premise plumbing samples at Willmar (Table 6.2). At low DO levels, cuprite (Cu$_2$O) is believed to be the controlling solid (AWWARF and DVGW-TZW 1996) and copper (Cu$^+$) release is predicted to be as low as 0.54 ppm (Table 6.2). Considering the detrimental effect of lower pH and beneficial effect of lower DO on copper release, it is predicted that the net overall effect of nitrification on copper release will vary dependent on the circumstance even in the same water system.

Earlier studies have suggested that microbiologically influenced corrosion (MIC) caused copper pipe failures due to pitting corrosion (Cantor et al. 2006). The copper pipes failed by pinhole leaks developed as pits from the interior of the pipe and eventually broke through the exterior of the pipe (Cantor et al.
In this study, no copper pinhole leaks or copper pits were detected even in the pipes without disinfectant where heterotrophic bacterial growth was most significant, or in situations with nitrifiers present (Chapter V). In the pipe with nitrification, since nitrification did not increase heterotrophic bacterial growth (Chapter V), MIC was not increased and no pits or leaks were detected either.

![Graph showing predicted soluble copper at different pH and alkalinities](image)

**Figure 6.6:** Predicted soluble copper at different pH and alkalinities (Assuming 50000 ppb total copper).

<table>
<thead>
<tr>
<th></th>
<th>Measured</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>DO, mg/L</td>
</tr>
<tr>
<td>Treatment Plant</td>
<td>8.1</td>
<td>11</td>
</tr>
<tr>
<td>Main Distribution System</td>
<td>7.5</td>
<td>3</td>
</tr>
<tr>
<td>Premise Plumbing</td>
<td>7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Note: Measured data from (Murphy et al. 1997a) figure 3, Calculated ammonia consumed and pH were based on DO data. *Calculated Cu\(^{2+}\) or Cu\(^+\) was based on the measured pH and controlling solid Cu(OH)\(_2\) or Cu\(_2\)O.
**Metal Release from other pipe materials and weight loss**

In new lead pipes, total lead release was generally increased by nitrification (for student paired t-test, \( p \leq 0.02 \)), consistent with bench scale results at low alkalinites (Zhang et al. 2008b). However, no significant metal release differences were observed in old lead coated with Pb (IV) scale by pre-exposing to high chlorine levels, cast iron and stainless steel pipes between the rig with nitrification and the rigs without nitrification (for student paired t-test, \( p \geq 0.09 \)).

Weight loss is generally considered to involve less error in evaluating corrosion rate compared to electrochemical measurements (AWWARF and DVGW-TZW 1996). In this study, for all the pipe materials, no significant weight loss differences were observed among the three disinfectant conditions (Table 6.3).

**Table 6.3: Weight loss for different pipe materials**

<table>
<thead>
<tr>
<th></th>
<th>no disinfectant</th>
<th>chlorine</th>
<th>chloramine + nitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>cast iron</td>
<td>35 ± 14</td>
<td>41 ± 16</td>
<td>41 ± 15</td>
</tr>
<tr>
<td>Copper</td>
<td>25 ± 13</td>
<td>13 ± 10</td>
<td>21 ± 6.5</td>
</tr>
<tr>
<td>New lead</td>
<td>55 ± 8</td>
<td>54 ± 11</td>
<td>60 ± 8.7</td>
</tr>
<tr>
<td>Old lead</td>
<td>97 ± 55</td>
<td>85 ± 41</td>
<td>91 ± 19</td>
</tr>
<tr>
<td>Galvanized</td>
<td>94 ± 23</td>
<td>117 ± 30</td>
<td>127 ± 17</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>-11 ± 7</td>
<td>-3.4 ± 1.6</td>
<td>-0.3 ± 5.8</td>
</tr>
</tbody>
</table>

Note: ± indicate 95% confidence error. Note: the exposure time is four years for copper and stainless steel pipes and 11 months for cast iron, new and old lead pipes.

**Effect of Nitrification on Concrete Corrosion**

Concrete materials can leach lime and increase bulk water pH (AWWARF and DVGW-TZW 1996). The effect of nitrification on the bulk water pH in the presence of concrete was affected by the initial pH (Figure 6.7). Specifically, at an initial pH 8, nitrification had no effect on pH, but when initial pH was reduced to 7, lower final pH was observed with nitrification (Figure 6.7). Initial alkalinity had no effect on bulk water pH change (Figure 6.7).
Although nitrification lowered the final pH by 0.3-1.1 unit, this pH reduction did not significantly increase concrete corrosion (Figure 6.7). As indicated by calcium leaching from the concrete corrosion, similar calcium leaching was observed with or without nitrification (Figure 6.7).

Figure 6.7: pH change and calcium leaching from concrete w/ and w/o nitrification at different initial pH and alkalinites.

Overall, nitrification can have a range of impacts on the corrosion of different pipe materials (Table 6.4). The reduction of pH due to nitrification was confirmed to increase lead release in low alkalinity water (Chapter IV). However, a reduction in pH had no impact on concrete corrosion and actually decreased zinc release from galvanized pipe in this work. Copper release was also not affected by the pH decrease in low alkalinity waters, but it is predicted to be strongly impacted by even slight reduction in pH for higher alkalinity water. Aside from pH reduction, the removal of dissolved oxygen by nitrification can decrease zinc (and iron) release from galvanized iron pipe, and a review of the literature suggests that copper leaching might be reduced at very low DO as well. The production of nitrite and nitrate from nitrification has been predicted to increase the corrosion of iron and lead (Kielemoes et al. 2000), but no
effect was observed except that high nitrite levels decreased iron and zinc release from pure metals (Table 6.4).

<table>
<thead>
<tr>
<th>Nitrification Effect on Water Quality</th>
<th>Predicted Effect on Corrosion</th>
<th>Confirmed Effect in this Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase nitrite and nitrate</td>
<td>Increase corrosion by chemical reaction with metal</td>
<td>High level nitrite (10 mg/L) reduce iron and zinc release from pure iron and zinc, no effect observed for other metals</td>
</tr>
<tr>
<td>Decreased pH and alkalinity</td>
<td>Increase corrosion of copper and lead, have varying effect on iron corrosion</td>
<td>Increase lead release from lead pipe and brass¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrease zinc release from galvanized pipe</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No effect on concrete</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No effect on copper release with low alkalinity, but increase copper release with high alkalinity</td>
</tr>
<tr>
<td>Increase in HPC</td>
<td>Increase microbiologically influenced corrosion</td>
<td>N/A</td>
</tr>
<tr>
<td>Rapid decay of chloramine</td>
<td>Increase/decrease corrosion depends on specific system</td>
<td>N/A</td>
</tr>
<tr>
<td>Decreased dissolved oxygen</td>
<td>Change redox potential of pipe surface, might increase or decrease corrosion</td>
<td>Decrease zinc release from galvanized pipe, iron and zinc release from pure iron and zinc</td>
</tr>
</tbody>
</table>

¹: (Zhang et al. 2009a)

**CONCLUSIONS**

Nitrification was studied in a wide range of different plumbing materials in a pipe rig system with flow patterns simulating premise plumbing use. The following conclusions were drawn:

- Nitrification activity was rapidly established in iron, plastic, lead and old concrete materials, and less readily in galvanized, stainless steel, and new concrete. Copper pipes were relatively toxic to nitrifiers.
- Nitrification decreased pH by 0.6 to 1 unit and reduced DO down to 2.4 ppm.
- Nitrification caused occasional sloughing of high organic carbon (TOC) from cast iron coupons.
- Nitrification decreased zinc release from galvanized pipe.
- Total lead release was generally increased by nitrification in new lead pipes.
- Nitrification did not significantly impact corrosion rates for copper, old lead, cast iron, concrete and
stainless steel pipes.

ACKNOWLEDGMENTS

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REFERENCES


CHAPTER VII: EFFECT OF NITRIFICATION AND GAC FILTRATION ON COPPER AND LEAD LEACHING IN HOME PLUMBING SYSTEMS

Yan Zhang, Simoni Triantafyllidou and Marc Edwards

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ABSTRACT

Nitrification and Granular Activated Carbon (GAC) filtration impact leaching of lead/copper to potable water under typical home plumbing configurations. GAC filters removed the disinfectant and caused rapid establishment of nitrification in chloraminated systems. The potential adverse consequences of whole house GAC filters deserve increased scrutiny in chloraminated systems. The lower pH values from nitrification and other microbes during overnight stagnation in pipes can markedly increase (up to 800%) lead and copper contamination of water.

Keywords: Activated carbon, Corrosion, Copper, Lead, Nitrification, Plumbing
INTRODUCTION

Elevated lead and copper levels in drinking water have recently received increased scrutiny due to cases of childhood lead poisoning tied to potable water in Durham, NC; Greenville, NC; and Washington DC (Edwards and Triantafyllidou 2007b; Triantafyllidou et al. 2007b). Potable water leaving treatment plants almost invariably has low copper and lead levels, but lead and copper can increase in water as it sits stagnant within home plumbing (Federal-Register 1991; Schock 1989). Copper pipe is the dominant material used to plumb buildings; lead pipe and leaded solder could be used until officially banned in about 1986, and leaded brass (up to 8% lead by weight) are still routinely installed in homes. The most common use of lead pipe is the service line connection between the water main and home plumbing.

Lead can leach from brass, solder or lead pipe by itself, or lead corrosion can be driven by direct galvanic connections with copper (Dudi 2004; Reiber 1991). In such situations, the lead bearing material generally serves as the anode and is sacrificed by the copper:

\[ \text{Pb} \rightarrow \text{Pb}^{+2} + 2e^- \quad \text{Equation 1} \]

and the cathodic reaction (e.g., O₂ reduction) occurs on the copper surface:

\[ \text{O}_2 + 4e^- + 2\text{H}_2\text{O} \rightarrow 4\text{OH}^- \quad \text{Equation 2} \]

The rate of attack on the lead material alone or via galvanic corrosion is influenced by numerous factors including water chemistry, disinfectant types (Dudi 2004) and bacterial growth (Douglas et al. 2004; Reiber and Edwards 1997).

Effect of Disinfectant

Copper and lead release in water can be strongly influenced by disinfectant types (AWWARF and DVGW-TZW 1996; Edwards and Dudi 2004) Theoretically, chlorine can increase lead levels in drinking water by accelerating the corrosion of metallic lead to Pb (II) or Pb (IV), but it was also predicted (AWWARF and DVGW-TZW 1996; Schock et al. 2001) to greatly reduce lead leaching by converting more soluble Pb (II) to less soluble Pb (IV). In contrast, due to its lower oxidation-reduction potential, chloramine does not have this ability to maintain the less soluble Pb (IV) oxides (AWWARF
and DVGW-TZW 1996), so it can sometimes worsen lead levels in drinking water compared to chlorine. The loss of chlorine’s inhibiting effect on lead leaching from service line pipes is now understood to be a major factor in a recent serious problem with lead in Washington D.C. lead problem (Edwards and Dudi 2004; Lytle and Schock 2005), although lead hazards also arose from corrosion of lead solder and brass due to reactions with chloramine. Similar trends have been observed in other field and laboratory studies (Cantor et al. 2003; Vasquez et al. 2006).

The effect of chlorine on copper release varies dependent on circumstance. For example, chlorine has been observed to increase copper leaching at lower pH values (pH < 7) (Atlas et al. 1982; Boulay and Edwards 2001; Hong and MacCauley 1998) but decrease copper corrosion and copper release when there are problems with copper particulates at higher pH (e.g., “blue water”) (Boulay and Edwards 2001; Edwards and Ferguson 1993). Some practical experience has indicated that at some utilities copper solubility increased during periods of chloramination and decreased with breakpoint chlorination (AWWARF and DVGW-TZW 1996). However, other studies did not detect differences in copper corrosion rates or copper release when free chlorine or combined chlorine was used (AWWARF and DVGW-TZW 1996).

Another underappreciated difference between the two disinfectants is the final decay product: both produce chloride during disinfectant decay but chloramine also forms free ammonia. In dead ends or in home plumbing systems with reactive materials, the free ammonia from chloramine might have different impacts on corrosion and water quality relative to experience with chlorine, decayed chlorine or even chloramine. It is therefore desirable to study the possible effects of chlorine and chloramine on lead and copper corrosion which includes conditions with free ammonia and no disinfectant.

**Effect of Nitrification**

On the basis of the above discussion, nitrification is another possible explanation for divergent impacts of chlorine versus chloramine on lead and copper corrosion. Free ammonia remaining after chloramine
decay can be converted to nitrite and then nitrate by nitrifying bacteria (USEPA 2005) with production of acid (Grady et al. 1999):

\[
\text{NH}_4^+ + 1.9 \text{O}_2 + 0.069 \text{CO}_2 + 0.0172 \text{HCO}_3^- \rightarrow 0.0172 \text{C}_5\text{H}_7\text{O}_2\text{N} + 0.983 \text{NO}_2^- + 0.966 \text{H}_2\text{O} + 1.97 \text{H}^+
\]

Equation 3

\[
\text{NO}_2^- + 0.00875 \text{NH}_4^+ + 0.035 \text{CO}_2 + 0.00875 \text{HCO}_3^- + 0.456 \text{O}_2 + 0.00875 \text{H}_2\text{O} \rightarrow 0.00875 \text{C}_5\text{H}_7\text{O}_2\text{N} + 1.0 \text{NO}_3^- - 
\]

Equation 4

Nitrification can decrease pH and alkalinity (Equation 3 and 4) and produce nitrite at levels of concern relative to drinking water guidelines, and create organic carbon that can serve as a substrate for other bacteria.

This increasing concern over lead leaching due to nitrification arose from cases of lead poisoning from water that occurred in chloraminated systems (Edwards and Triantafyllidou 2007b; Triantafyllidou et al. 2007b), although it remains uncertain whether nitrification was a major contributor to the problem. The adverse effects of nitrification on lead leaching to water were first noted more than 100 years ago (Garret 1891) and virtually no research on the subject has been conducted since then. However, one study tied elevated copper levels at the tap to activity of nitrifying bacteria in homes of Willmar, Minnesota (Murphy et al. 1997a).

Corrosion and metal leaching could be affected by nitrification through reduced pH and alkalinity, and perhaps, through other indirect effects on bacterial growth and microbially induced corrosion (McNeill and Edwards 2001; Schock 1989). In some cases microbially influenced corrosion (e.g. blue water) and associated metal leaching is readily controlled by increased disinfection (Cantor et al. 2006).

**Effect of Granular Activated Carbon (GAC) filters**

Home treatment devices that filter water at the Point-of-Use and Point-of-Entry were used in about 37% of consumer homes in 2003 (USEPA 2003a). These devices are installed by consumers to improve taste, color, and odor problems (McFeters 1990), as well as to possibly increase the safety of their water. Devices employing activated carbon are widely used due to their low cost and ability to remove
chlorine. Activated carbon can also remove a wide range of contaminants including organic compounds that are suspected carcinogens and that cause taste and odor problems (Lykins et al. 1992). Elevated total bacterial growth and opportunistic pathogens have sometimes been associated with Granular Activated Carbon (GAC) filters in homes (Lykins et al. 1992), possibly due to the removal of disinfectant residual and the large surface area for bacterial attachment in GAC. In chloraminated water distribution systems, installation of GAC filters has been associated with nitrification problems in the U.S. (Feben 1935; Skadsen 1993) and Europe (Vahala 2002; Vahala and Laukkanen 1998).

If GAC filters were installed in a home served chloraminated water, and nitrifier growth were to occur and lower pH in the premise plumbing system, corrosion of copper and lead pipes downstream of the filter could be adversely impacted. GAC filters also remove chlorine and chloramine disinfectant residuals and significantly reduce organic levels, which can also affect corrosion of copper and lead (Boulay and Edwards 2001; Korshin et al. 1994; Rehring and Edwards 1996).

In light of the above discussion, this study is aimed at a broad preliminary investigation into effects of disinfectant type, GAC filters and nitrification on leaching of metals from home plumbing.

**MATERIALS AND METHODS**

**Pipe rig setup**

The basic experimental rig simulates a connection between copper pipe and a lead bearing material such as brass, lead solder or lead pipe (Figure 7.1). All pipe materials were new. Copper pipes and brass fixtures were purchased new from local hardware store; pure lead pipes were specially ordered; and solder pipes were made by covering new copper pipes with melted solder (50:50 lead: tin). The copper pipe is a 91.4 cm (3 ft - length) × 1.9 cm (¾ in. – diameter) section that is electrically connected to lead-based material (lead, tin solder or brass fixture) via an external grounding wire. The surface area ratio of the copper pipe to the leaded material was approximately 12:1. A plastic ball valve is also placed
between the two materials. By closing the ball valve before collecting a sample, it is possible to collect samples of the water from the lead anode and copper cathode sides of the pipe rig, which is advantageous in determining differences in pH and nitrification in each segment. As a control and basis for comparison, pipe rigs were also constructed using PVC pipes.

![Pipe Rig Setup](image)

**Figure 7.1: Pipe Rig Setup**

**Water Chemistry**

*Phase I-Investigation of Disinfectant Effects on Different Pipe Materials*

Blacksburg, VA tap water was used as the base water for this study. The chloramine residual in this tap water was first removed by adding extra chlorine to achieve breakpoint chlorination (Snoeyink and Jenkins 1980). The dose of Cl₂ required to achieve the breakpoint was estimated based on measurement of total chlorine (in chloramine) and ammonia in the water:

\[
Cl_2 \text{ Dose (mg/L)} = 0.5 \times \text{Measured } Cl_2 \text{ (mg/L)} + NH_3 \text{ (mg/L)} \times 3.6 \quad \text{Equation 5}
\]

After allowing the water to sit for three days to allow disappearance of residual chlorine, it was then modified to achieve four extremes of disinfection that might be encountered in potable water systems including: no disinfectant, free chlorine, chloramine and free ammonia. Water with free chlorine and chloramine represent water quality in homes located nearer the treatment plant where disinfectant decay is negligible. The conditions with no disinfectant and free ammonia represent conditions that might be encountered at distal portions of the distributions system.
Free chlorine and chloramine were targeted at 1.5 mg/L and 4 mg/L, respectively. Chloramine was prepared with 4:1 chlorine to ammonia mass ratio. One mg/L NH₃-N was added to simulate a condition when chloramine was completely decayed. The final pH of all test waters was adjusted to 7.8 ± 0.1, which is the target pH of finished water for Blacksburg water. Each water quality was tested in duplicate, so there were 4 pipe materials (copper-lead, copper-solder, copper-brass, and PVC) × 4 disinfectant types × 2 duplicates = 32 tests.

Water was changed in the pipes every 3.5 days (twice a week) using a “dump and fill” protocol for 11 months duration. This was done in order to replenish nutrients and disinfectant residual. The experiment results therefore simulate corrosion that might occur in sections of infrequently used home plumbing.

**Phase II and Phase III: Investigation of GAC and Nitrification Effect**

After 11 months baseline behavior was established, duplicates for each condition were treated differently, in that one pipe was fed with water pre-treated by a GAC filter whereas the second was fed the same water not treated by GAC (Figure 7.2). The home GAC filters impregnated with carbon used in this study were purchased off the shelf from a local hardware store. The filters were conditioned with Blacksburg tap water with indications of nitrifying activity (up to 0.03 mg/L-N NO₂⁻ detected) for two weeks prior to their use. By adding 1 mg/L-N NH₃ into the conditioning water, Nitrifiers rapidly colonized these filters.

During these two phases of the study, the water conditions tested included 4 pipe materials (copper-lead, copper-solder, copper-brass, and PVC) × 4 disinfectant conditions × 2 treatment conditions (GAC treated and non-GAC treated) = 32 tests. Water changes were made every day during the work week (Monday-Friday) for better nutrient replenishment.

In order to determine the effect of GAC treatment on corrosion, each of the four waters (4 disinfectant types) were divided into two portions (Figure 7.2). One portion was passed through a GAC filter (Figure 7.2), during which time the new water mixed with 500 mL old water retained in the filter from the last
water change. To simulate the same effect in water not treated by GAC, 500 ml water from the previous water change was left in a plastic container and mixed with freshly prepared water before changing water in the rigs (Figure 7.2).

Two phases of experiments (Phase II and Phase III) were designed to examine effects of GAC treatment on metals leaching. In Phase II, there was no pH adjustment before water was filled into the pipes, as would occur in normal installation. But in phase III, to isolate the effect of pH changes occurred due to GAC filtration/mixing, the pH was adjusted back to the target value of 7.8 before water was filled into pipes (Figure 7.2). To investigate the effect of nitrification simultaneously, chronically, six months of Phase III work was conducted before the three day duration study of Phase II.

**Figure 7.2: Water Preparation for Phase II and Phase III study.**

**Experimental Monitoring and Analysis**

The extent of lead, copper leaching was quantified by measuring metal concentrations in the water after sitting stagnant inside the pipes. Lead, copper, phosphorus, iron, aluminum were measured using
Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) according to standard method 3125-B (Clesceri et al. 1998)

Nitrification and nitrifier bacteria activity was tracked by measuring loss of ammonia, production of nitrite and nitrate and reduction of pH. NH\(_4\)-N was measured with a colorimetric method using a HACH DR/2400 spectrophotometer. NO\(_3\)-N was measured using DIONEX, DX-120 ion chromatography (IC). NO\(_2\)-N was measured by both Hach and IC methods. Alkalinity was monitored using HACH alkalinity test kit (model # AL-DT). Total Organic Carbon (TOC) was monitored by SIEVERS 800 Total Organic Analyzer according to standard method 5310C (Clesceri et al. 1998). In addition, the absorbance at wavelength UV\(_{254}\), which is often associated with humic substances (or natural organic matter-NOM), was monitored on BECKMAN DU640 Spectrophotometer. Heterotrophic bacteria growth was monitored during the experiment by Heterotrophic Plate Counting (HPC) method according to Standard Method 9215 C (Clesceri et al. 1998). R\(_2\)A agar was used as the media and plates were incubated in a 20 °C constant temperature room for 7 days prior to counting. The final HPC values were derived from the mean of two duplicate plates at the appropriate dilution.

RESULTS AND DISCUSSION

Experimental findings are organized into four sections including 1) Effect of Disinfectants, 2) Effect of GAC, 3) Nitrification impacts, and 4) Field Studies.

Disinfectant Effect in Blacksburg Water without GAC-Phase I

For the first 11 months the study examined the effects of the four different disinfectant conditions on copper and lead leaching. Nitrification did not occur in any of the rigs to a large extent during this phase of testing as indicated by measurements of ammonia (< 20% loss), low nitrite production (< 0.03 mg/L-N) and lack of a pH change in PVC pipes. This might indicate that a long time is required to establish nitrification, or because the makeup water was completely changed on a regular basis. Later
experiments (Figure 7.2) retained some “old” water during water changes which seemed to markedly enhance nitrifier activity.

Lead Release

Under these conditions without significant detectable nitrification, for a given type of lead bearing material, average lead release was not significantly affected by disinfectant types (Figure 7.3). Lead release from copper-lead and copper-solder rig was above the 15 ppb lead action limit over the whole exposure time. For copper-lead rig, average lead release increased: monochloramine < no disinfect < chlorine < ammonia. And for copper-solder rig, average lead release follow the order of monochloramine < ammonia < chlorine < no disinfectant. Because total lead release was gradually decreasing from 1000 ppb to about 100 ppb in copper-Lead and copper-solder rigs, no confidence interval analysis was conducted for the average; rather, a paired t-test was conducted to compare the head to head results of lead release with time. The difference was rarely significant at 95% confidence (p > 0.05). The only exception for copper-lead rig was that lead release with ammonia was 60% higher than that with monochloramine at 99% confidence (p = 0.01). The exception for copper-solder rig was: lead release with monochloramine is lower than that with chlorine and no disinfectant significantly, with p values of 0.03 and 0.01 respectively. Levels of lead leached from the brass containing rig and PVC were well below the 15 ppb lead action limit in this water.
Figure 7.3: Average lead release after a stagnation period of 3.5 days in the pipes. Data was the average of 16 measurements over the 11 months exposure time.

*Copper Release*

Copper release was much lower than the LCR Action Level in all waters tested, even during the first month of exposure. For copper-lead rig, average copper release follows the order: ammonia < no disinfectant < chlorine < monochloramine. For copper-solder rig and copper brass rig, average copper release follows the order of no disinfectant < ammonia < chlorine < monochloramine. Because total copper release didn’t change as significant as lead release over time, 95% confidence interval was applied to the pooled data (Figure 7.4). Copper release with monochloramine was always higher than with other disinfectants, but only in copper-brass rigs was this trend greater than 95% confidence.

Paired t-tests were also conducted to compare the head to head results of copper release. In copper-lead rigs, the copper release with free ammonia was lower than with other disinfectants > 99% confidence (p < 0.01). In copper-solder rigs, copper release with ammonia was lower than with monochloramine at 99% confidence (p = 0.01) and copper release with no disinfectant was smaller than with monochloramine and chlorine at 99.4% (p = 0.006) and 98.5% (p = 0.015) confidence. For copper-brass rig, the trend of no disinfectant < ammonia < chlorine < monochloramine was confident at > 99%. As would be expected, there was no effect of disinfectant type on copper release from PVC.
Figure 7.4: Average copper release after a stagnation period of 3.5 days in the pipes. Data was the average of 14 measurements over the 11 months exposure time and error bars indicates 95% confidence interval of these measurements.

HPC
The HPC levels in water collected from the pipes after stagnation were ranked monochloramine < chlorine < no disinfectant < ammonia. This result confirms the effectiveness of the disinfectants, and the detriments of ammonia as a nutrient for re-growth in home plumbing, as described by (Edwards et al. 2005). But the HPC levels were not correlated to the extent of lead or copper leaching in this water.

Overall Impacts of GAC Treatment on Water Chemistry-Phase II and Phase III

Impact of GAC on Water Chemistry

Treatment of water by GAC produced a number of changes in water chemistry that can directly and indirectly affect corrosivity (Table 7.1). pH was decreased by about 1 pH unit after the water passed through the GAC filter (Figure 7.5). While microorganisms can produce acidic metabolites and decrease the pH (Bremer et al. 2001; Davidson 1996), since alkalinity did not change significantly, the reduced pH is attributed mostly to increased CO₂ arising from microbe respiration. Parameters associated with humic substances including Total Organic Carbon (TOC) and UV₂₅₄ absorbance were decreased by 80-95%.
Table 7.1: Water quality comparison before and after filtration through GAC

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>pH before</th>
<th>alkalinity, mg/L-CaCO₃</th>
<th>TOC, ppb</th>
<th>UV₂₅₄,a bs</th>
<th>HPC, cfu/ml</th>
<th>Disinfectant residual, mg/L-Cl₂</th>
<th>Ammonia, mg/L-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>ammonia</td>
<td>7.94</td>
<td>31</td>
<td>1412</td>
<td>0.030</td>
<td>43600</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>after</td>
<td>7.03</td>
<td>28</td>
<td>161</td>
<td>0.004</td>
<td>39000</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Mono-chloramine</td>
<td>7.91</td>
<td>29</td>
<td>1307</td>
<td>0.052</td>
<td>7425</td>
<td>4.00</td>
<td>0.80</td>
</tr>
<tr>
<td>before</td>
<td>6.88</td>
<td>25</td>
<td>137</td>
<td>0.005</td>
<td>14900</td>
<td>0.09</td>
<td>0.80</td>
</tr>
<tr>
<td>after</td>
<td>6.88</td>
<td>25</td>
<td>137</td>
<td>0.005</td>
<td>14900</td>
<td>0.09</td>
<td>0.80</td>
</tr>
<tr>
<td>Chlorine</td>
<td>7.86</td>
<td>28</td>
<td>1410</td>
<td>0.030</td>
<td>125</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>7.10</td>
<td>26</td>
<td>63</td>
<td>0.005</td>
<td>3950</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>after</td>
<td>7.10</td>
<td>26</td>
<td>63</td>
<td>0.005</td>
<td>3950</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>No disinfectant</td>
<td>7.94</td>
<td>32</td>
<td>1408</td>
<td>0.031</td>
<td>104750</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>after</td>
<td>6.97</td>
<td>29</td>
<td>275</td>
<td>0.009</td>
<td>58900</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7.5: pH values after water passing through GAC filtration or after mixing fresh water with aged water. Initial pH = 7.8. Data was the average of three measurements and error bars indicates 90% confidence interval of these measurements.

Heterotrophic bacterial counts were also increased by GAC treatment, especially when free chlorine was the disinfectant where a 40X increase was observed relative to the same water without a filter. This is most likely due to the complete removal of chlorine residual by GAC filtration (Table 7.1). Although increased heterotrophic bacterial growth on GAC filters has shown no direct adverse health effects (Wqa 2006), it can still indirectly affect water quality because increased bacterial growth might increase pipe corrosion and metals leaching (McNeill and Edwards 2001). Chloramine residual was also removed by GAC, and this doubled HPC counts (Table 7.1). GAC treatment reduced HPC counts in water without disinfectant, perhaps due to the effective removal of organic carbon (Edwards et al. 2005).
Of these changes lower pH is generally expected to increase corrosivity and metals leaching, but removal of organic matter can also be highly influential. Organic matter has been reported to increase copper release in some cases (Korshin et al. 1994; Kristiansen 1982; Rehring and Edwards 1996) but decrease copper release in others (Rehring and Edwards 1996). Edwards and Boulay demonstrated organic matter can increase copper release by complexation or colloid mobilization/dispersion, but it can also decrease copper release by fueling microbial removal of oxygen or via adsorption to the pipe surface and inhibition of corrosion (Edwards and Boulay 2001). Other researchers also suggest that if the organic matter is readily assimilable, then microbially influenced corrosion might occur (Cantor et al. 2006).

**GAC effect on nitrification occurrence**

When chloramine disinfectant was used, GAC treatment dramatically increased nitrifying bacterial growth. Specifically, in the water without GAC treatment with a high chloramine residual, ammonia loss attributed to nitrification was only 19 % on average, whereas in the water with GAC treatment and where chloramine had been removed, ammonia loss was 90 % on average (Figure 7.6).

![Figure 7.6: Nitrification in PVC pipes with chloramine disinfectant. Y axis indicates ammonia loss after 24 hours stagnation time in the pipe. Higher ammonia loss indicates more nitrifier activity.](image-url)
In waters with free ammonia, GAC treatment produced waters in which ammonia was converted completely to nitrate. In contrast, higher levels of nitrite and some nitrate were present in water not treated by GAC (Figure 7.7). We speculate that the incomplete conversion of nitrite to nitrate in non-GAC treated water versus the complete conversion to nitrate in GAC treated water was because GAC filtration removed the inhibition of chlorate on nitrite oxidizing bacteria (NOB) activity. Chlorate has been noted to partially inhibit the activity of nitrite oxidizing bacteria (NOB) at levels below 1 mg/L (Belser and Mays 1980). Chlorate dropped from 0.43 ppm to 0.12 ppm after GAC treatment. This result illustrates that nitrification can be occurring without detectable nitrite, thus hinder the detection of nitrification in home plumbing systems since nitrite is often used as an indicator of nitrification in drinking water systems (Wilczak et al. 1996).

![Figure 7.7: Representative nitrogen balance 24 hours after water was fed into PVC pipes.](image)

Ammonia consumption is 1 mg/L-N with non-GAC treated water, and 0.9 mg/L-N with GAC treated water.

**GAC effect on copper and lead release-Phase II**

The relative effect of GAC treatment was calculated using a ratio of metal leaching (e.g., lead) as follows:

\[
\text{Ratio} = \frac{\text{Pb concentration (ppb) using GAC treated water}}{\text{Pb concentration (ppb) using non-GAC treated water}}
\]

Equation 6
If the ratio < 1, GAC treatment reduced lead leaching whereas a ratio > 1 indicates that GAC treatment increased lead leaching.

During Phase II work, no pH adjustment was made after GAC treatment in homes to simulate actual installed conditions. The pH depression by GAC treatment would be expected to adversely affect lead corrosion in downstream pipes. However, the qualitative effects of GAC treatment for lead corrosion in Blacksburg water dependent on the plumbing material examined, suggesting that pH depression was not always the dominant factor controlling corrosion.

GAC treatment did increase lead release from copper-lead pipe rigs when the water contained monochloramine, chlorine and no disinfectant (Figure 7.8). In the case without disinfectant lead leaching was increased by 316% after GAC treatment. The lack of effect in water with free ammonia is to be expected, given the similarity in pH value of the water with and without GAC treatment in this case (Figure 7.5).

![Figure 7.8: Effect of GAC treatment on lead release (Phase II).](image)

The qualitative effect of GAC treatment for copper-solder rigs was the same as for copper-lead pipe rigs, as GAC treatment had no effect on lead release with ammonia and increased lead release when there is
no disinfectant. But when monochloramine and chlorine were used, lead release was decreased by GAC treatment in copper-solder rigs. It is possible that the benefits of organic matter removal by GAC filtration overcame the detriments of the lower pH values for copper-solder rigs. Earlier research demonstrated that higher organic matter increased lead leaching from lead-tin solder (Korshin et al. 2005).

For copper-brass rigs, the final average lead concentration was always reduced by GAC pre-treatment (from 4.7 ppb to 1.4 ppb on average). Part of the reduced lead in GAC treated water was due to removal of trace lead from the water even before contacting the pipes (initial water lead decreased from 2.6 ppb in non-GAC treated water to 0.8 ppb in GAC treated water), consistent with the general observation that GAC can remove heavy metals including copper, lead, cadmium, etc (Reed et al. 1993; Scholz and Xu 2002). Another part of the reduced lead in GAC water was due to lower lead release from brass side. It is possible that the benefits of NOM removal outweighed the detriments of lower pH values after GAC treatment, since removal of NOM would be expected to decrease lead leaching from brass (Korshin et al. 2000),

GAC treatment increased copper leaching for all conditions. The largest increase was approximately 800% in copper-brass rigs with chlorine disinfectant. The increase in copper leaching was not as significant when ammonia was used compared to other disinfectants (Figure 7.9). Clearly, copper release was more consistently impacted by the lower pH values after GAC treatment (Figure 7.5).
GAC effect on copper and lead release-Phase III

The relative role of pH depression versus other impacts of GAC treatment was obviously complex and dependent on the plumbing material. The Phase III experiments attempted to examine this in greater detail by re-adjusting the pH of the water back to its initial value (pH 7.8) before filling the pipes.

In the copper-lead rigs, lead leaching was no longer worsened by GAC treatment if pH depression was countered. In fact, when free ammonia was present, lead release was decreased by GAC treatment, perhaps due to slightly lower pH in non-GAC treated water (pH = 7.39) than GAC treated water (pH = 7.57) after stagnation (Table 7.3).

In copper-solder rigs, even without the lower pH values, GAC treatment still increased lead release by three times and 50% when no disinfectant and ammonia were used. However, GAC treatment didn’t have an effect when chlorine or monochloramine disinfectants were used (data not shown). In copper-
brass rigs, similar to phase II work, the final average lead concentration was reduced by 34-89% after GAC treatment.

In copper-lead and copper-solder rigs, GAC treatment increased copper release only when using chlorine as the disinfectant (data not shown). This might be because chlorine helps to control copper release either by control microbially influenced corrosion (Cantor et al. 2006) or by decreasing corrosion rate directly (Edwards and Ferguson 1993) and GAC removed the chlorine. However, in copper-brass rigs, GAC treatment still increased copper release for all conditions (up to 500% when chlorine was used). In the copper brass rigs it was consistently observed that the copper side of the galvanic connection had higher pH values than the brass side in non-GAC treated water. We define this pH difference (pH of copper side – pH of brass side) as ΔpH. The ΔpH of GAC treated water is much smaller than for non-GAC treated water. Galvanic current and voltage were plotted against ΔpH and no correlation was found, indicating that change in galvanic corrosion was not the major mechanism affecting the difference in ΔpH and copper release between non-GAC treated and GAC treated waters. (Rehring and Edwards 1996) also found increases in copper leaching and corrosion rate after waters were treated by GAC.

**Nitrification Effects-Phase III**

*Nitrification occurrence*

Factors that trigger nitrification in potable water systems are poorly understood. A number of observations were made during the course of this study which gave insight to factors which might be important contributors to nitrification in premise plumbing systems. During the first 11 months of the study when no GAC treatment was applied, nitrification was insignificant as indicated by low ammonia consumption (< 20%), low nitrite production (< 0.03 mg/L-N) and no pH change even in PVC pipes. Three months after starting to use GAC, nitrification was detected in the water with free ammonia in both GAC treated and non-GAC treated waters. For the non-GAC water, to isolate the effect of GAC treatment, 500 mL of water sat stagnant between water changes, and was mixed with new water each
time (Figure 7.2). This mixing of aged water with freshly prepared water, instead of using 100% freshly prepared water, is believed responsible for initiation of nitrification that had not been observed at all during 11 months of previous study. The stagnant water used for mixing initially had 1 mg/L NH$_3$-N present. This ammonia was completely converted to nitrite within 24 hours, and the pH was decreased to below 7. So the final pH of non-GAC treated water was similar to the pH observed after GAC treatment (Figure 7.5). For monochloramine, chlorine, and no disinfectant conditions, the pH of water not treated by GAC stayed higher (Figure 7.5).

*Nitrification occurrence and pH change in pipes*

Some of the nitrification was occurring in the containers and GAC before the pipes, whereas in other cases the nitrification and pH changes were occurring in the pipes. For example, in the phase III work where pH had been re-adjusted to 7.8 before water was fed into pipes, in waters containing free ammonia at least some nitrification occurred in all pipes in both non-GAC and GAC treated water. PVC had much higher nitrification activity than lead, solder and brass containing rigs (Table 7.2). This is reasonable considering that > 200 ppb copper was present in copper containing rigs, which is adequate to inhibit nitrification (Loveless and Painter 1968; Skinner and Walker 1961; Zhang et al. 2007). In addition, ammonia consumption and pH were found to be highly correlated in PVC pipes ($R^2 = 0.91$), indicating nitrification was a major cause of pH drop (Figure 7.10). But in lead, solder and brass containing rigs, besides nitrification, there are many other factors that can contribute to pH change in the rigs. For example, the increase in pH due to copper corrosion might counter the pH decrease by nitrification (Equation 7).

$$2Cu + 4H^+ + O_2 \rightarrow 2Cu^{2+} + 2H_2O$$  \hspace{1cm} \text{Equation 7}

So the extent of nitrification and pH change during stagnation were therefore less strongly correlated in these pipe rigs ($R^2 = 0.28, 0.32$ and 0.52 in copper-lead, copper solder and copper-brass containing rigs, respectively). Indeed, amongst all the different plumbing materials, only the pH in PVC and copper-lead rigs consistently dropped (Table 7.3).
For waters containing monochloramine, ammonia consumption was also observed in every pipe (Table 7.2). But due to the low levels of conversion and the reaction between nitrite and chloramine, it is not possible to conclude whether nitrification actually occurred in the pipes with chloramine. On one hand, nitrification may have occurred, because ammonia was consumed, and nitrite/nitrate produced could be removed by reacting with monochloramine or the pipe surface. But on the other hand, it is possible the low levels of ammonia removal could be due to natural loss of ammonia to the air or uptake by heterotrophic bacteria. In any case, nitrification in the presence of chloramine was much lower than was observed in corresponding pipes with ammonia.

**Table 7.2: Average ammonia consumption % after 24 hours stagnation in the indicated pipe material.**

<table>
<thead>
<tr>
<th></th>
<th>non-GAC</th>
<th>GAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PVC</td>
<td>Copper-Lead</td>
</tr>
<tr>
<td>ammonia</td>
<td>97.0</td>
<td>29.9</td>
</tr>
<tr>
<td>monochloramine</td>
<td>16.8</td>
<td>26.1</td>
</tr>
</tbody>
</table>

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Table 7.3: Final pH after 24 hours stagnation in the indicated pipe material. Initial pH = 7.8.

<table>
<thead>
<tr>
<th></th>
<th>non-GAC</th>
<th>GAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PVC</td>
<td>Copper-</td>
</tr>
<tr>
<td>ammonia</td>
<td>7.24</td>
<td>Lead Solder</td>
</tr>
<tr>
<td>monochloramine</td>
<td>7.58</td>
<td>7.68</td>
</tr>
<tr>
<td>chlorine</td>
<td>7.55</td>
<td>7.63</td>
</tr>
<tr>
<td>no disinfectant</td>
<td>7.79</td>
<td>7.56</td>
</tr>
<tr>
<td>ammonia</td>
<td>7.18</td>
<td>7.57</td>
</tr>
<tr>
<td>monochloramine</td>
<td>7.41</td>
<td>7.64</td>
</tr>
<tr>
<td>chlorine</td>
<td>7.59</td>
<td>7.70</td>
</tr>
<tr>
<td>no disinfectant</td>
<td>7.84</td>
<td>7.74</td>
</tr>
</tbody>
</table>

Nitrification Associated Effect on Lead Release

The higher nitrification extent in the pipe with ammonia tended to cause relatively high lead leaching versus all other conditions in copper-lead rigs, since pH was dropped by nitrification in these pipes (Figure 7.11).

![Figure 7.11: Lead release in copper-lead rig, non-GAC.](image)

Nitrification Associated Effect on Copper Release

During phase I study, copper release in the water with free ammonia was lower than that with other disinfectants > 99% confidence (p < 0.01); but in phase III study, after nitrification occurred, copper release with ammonia was increased to levels similar to the other disinfectant conditions (Figure 7.12). Again, this effect was correlated to the lower pH in the water with ammonia, which was partly caused by...
nitrification. Copper release trends in copper-solder and copper-brass rigs did not change significantly after nitrification occurrence.

![Graph showing copper release over time with nitrification started at point B.](image)

**Figure 7.12: Copper release in copper-lead rig, non-GAC.**

**Field Studies**

In parallel field work, samples were collected from homes with chronic lead and copper corrosion problems occurring at the ends of distribution systems. All of the homes were in systems using chloramine disinfectants. Measurements were made of water chemistry after overnight stagnation for comparison to that obtained after extensive flushing.

<table>
<thead>
<tr>
<th></th>
<th>Initial pH</th>
<th>pH after stagnation</th>
<th>Nitrite Increase, mg/L-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indiana</td>
<td>7.90</td>
<td>7.20</td>
<td>0.25</td>
</tr>
<tr>
<td>Hawaii</td>
<td>7.89</td>
<td>6.77</td>
<td>0.00</td>
</tr>
<tr>
<td>Washington D.C.</td>
<td>7.90</td>
<td>6.70</td>
<td>0.50</td>
</tr>
</tbody>
</table>

**Table 7.4: Field Studies at Three Utilities**

As was observed in the laboratory work, large pH drops were occurring while the water sat stagnant in the pipes during overnight stagnation (Table 7.4), contributing to localized lead and copper corrosion problems. In Hawaii homes, detection of acid producing bacteria and high HPC levels were associated with these pH drops (Marshall and Edwards 2004a), whereas in Indiana and Washington D.C., nitrification (indicated by increased nitrite) was detected.
Some of the largest pH drops and highest lead values observed in the field occurred in homes using GAC whole house filters. Ironically, these homes are not sampled during EPA Lead and Copper Rule Monitoring, due to concerns that the filters might produce water with atypically low lead.

In the Indiana study, the consumers had significant blue staining and frequently observed “blue water.” A follow-up bench scale test was conducted to examine the effects of countering the pH drop that was occurring in the home plumbing during overnight stagnation by addition of caustic (sufficient to keep final pH at 7.8). After a period of several weeks, copper release after 24 hours stagnation had stabilized at 6 mg/L in the water without the pH adjustment versus 1 mg/L in the water with the pH adjustment.

While other researchers have made similar observations regarding the potential adverse consequences of pH depression during stagnation for copper leaching (Webster et al. 2000), the increasing use of chloramination and in-house filters can be expected to make such situations more prevalent. Clearly, corrosion control strategies at utilities need to consider this potential complication when determining target pH values for optimal corrosion control.

**SUMMARY AND CONCLUSIONS**

Bio-chemical reactions can drop pH 1 or 2 units during stagnation in premise plumbing systems, undermining control of lead and copper corrosion of drinking water. Carbon dioxide produced by biological activity in the pipes and on GAC filters, or acidity production by nitrification were dominant contributors to pH depression. The largest pH drops occurred in PVC pipes, because overall corrosion and buffering for copper and other metallic plumbing tended to raise pH of the bulk water.

GAC filtration significantly decreased the pH, removed organic matter and disinfectant residuals. GAC stimulated both heterotrophic bacterial growth and nitrification when monochloramine was used.
Complete conversion of ammonia to nitrate occurred in waters pre-treated by GAC, such that nitrite production was undetectable and could not be used to indicate nitrification.

The pH decrease by GAC treatment and nitrification increased copper release in all conditions. But for lead release, the net result of GAC treatment was highly dependent on the lead-bearing pipe material. Specifically, GAC treatment markedly increased lead release in copper-lead rigs; however, in copper-solder and copper-brass rigs the beneficial effect of other changes by GAC can sometimes outweigh the detrimental effect of decreased pH, thereby decreasing lead release.

In Blacksburg water, occurrence of nitrification in pipes during overnight stagnation had no discernable direct adverse impacts on metal leaching, other than those attributable to pH depression. Nitrification was not significant in premise plumbing during the first 11 months of testing when water in the pipe rigs was completely changed twice a week. Nitrification was rapidly established if water was pre-treated using a GAC filter, or if a portion of the water was allowed to sit stagnant for one day before mixing with freshly prepared water. In the time period before nitrification became established, average lead release was not significantly affected by disinfectant types. Average copper release was lowest with free ammonia in copper-lead rigs relative to those exposed to no disinfectant, chlorine or chloramine. In copper brass rigs, copper release followed the order of no disinfectant < ammonia < chlorine < monochloramine.

At three different utilities using chloraminated water, homes with lead and copper leaching problems had significant pH depression during overnight stagnation. Though an exhaustive study was not done, the problematic situations tended to occur in situations with infrequent water use, GAC filters or at the end of the distribution system.
ACKNOWLEDGEMENT

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REFERENCES


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CHAPTER VIII: OVERVIEW, PRACTICAL SYNTHESIS AND KEY CONCLUSIONS

OVERVIEW

This dissertation provides the first comprehensive study of nitrification, as it occurs in premise plumbing potable water systems. The key hypotheses generated via a comprehensive critical literature review (Chapter I) were as follows: 1) premise plumbing would exert a profound influence on occurrence of nitrification in potable water systems, and 2) nitrification would control aspects of water quality and premise plumbing corrosion. It was further believed that dependent on specific circumstances (i.e., water chemistry, nutrient levels and type of plumbing material), the observed interactions between premise plumbing and nitrification could be synergistic, antagonistic or neutral. Subsequent experiments utilizing bench scale rigs, large pilot scale studies, and intensive sampling at consumers’ homes confirmed these hypotheses and revealed the complexity of the interactions.

This chapter provides a practical synthesis of the research for regulators and water industry personnel, and can also serve to quickly guide the reader to key sections of the dissertation that may be of particular interest. Chapter II describes the effects of nutrients, metals and different pipe materials on growth of nitrifiers. Chapter III extends that work to examine the interplay between pH and orthophosphate corrosion inhibitors on nitrification occurrence. In combination, the findings of Chapter II and Chapter III explain why nitrification incidence varies dramatically from home to home in chloraminated systems. They also identify some promising candidate strategies for control of nitrification by limiting nutrients, or through use of plumbing materials such as copper, that may be useful in at least some circumstances.

Chapter IV is the first work to demonstrate that nitrification could markedly increase lead leaching to potable water through lab experiments, solubility modeling and field studies. For utilities considering whether to switch from free chlorine to chloramine, Chapter V provides a head to head comparison of free chlorine and chloramine efficacy as secondary disinfectants. These considerations are extended to other pipe materials including galvanized iron, copper and concrete in Chapter VI. Effects of granular activated carbon (GAC) filtration on nitrification occurrence, and some of its resulting impacts on water quality, are examined in Chapter VII.

PRACTICAL SYNTHESIS

Many of the key findings of the research are captured in a decision tree that can be used by utilities, regulators and scientists (Figure 8.1). If there is nitrification in the main water distribution system or in the premise plumbing sampled for lead and copper under the EPA Lead and Copper Rule (LCR), under certain circumstances significant effects on lead corrosion (i.e., low alkalinity water) and copper
corrosion (i.e., high alkalinity) can be anticipated. It may be necessary to implement corrosion control or nitrification control to stop these problems.

Even when problems are not apparent in the pre-1986 homes of the LCR sampling pool, “hotspots” can occur in other “worst case” consumers’ homes or in schools. Specifically, buildings with galvanized iron, iron or plastic plumbing materials can sometimes develop rampant nitrification problems which can be self-perpetuating due to rapid loss of disinfect (Figure 8.1). Other worst case situations can occur for homes near the end of the distribution system, or which utilize GAC treatment devices. These hotspots could be sites where secondary disinfection is ineffective, or where lead, copper and other corrosion problems are exacerbated. Depending on the seriousness of the problems, corrosion or nitrification control could be implemented to improve water quality.

**KEY CONCLUSIONS**

The overall conclusions of this research are as follows:

Growth of nitrifying bacteria in drinking water systems was strongly affected by nutrient levels and by the presence of certain metals (Chapter II). For example, copper levels > 100 ppb, zinc levels >1000 ppb and low phosphate levels (< 5 ppb) sometimes reduced nitrifier activity (Figure 2.1-2.3). These effects were most noticeable in low alkalinity waters (Figure 2.5). Toxicity from metals inhibited nitrification in galvanized iron (zinc coated iron), copper and brass at many situations (i.e., lower pH water, newer plumbing, no orthophosphate corrosion inhibitor, lower alkalinity water) (Chapter II and III).

Aside from toxic metals, different pipe materials could also release nutrients to water. Therefore, the released constituents could either inhibit or enhance the growth of nitrifier biofilms (Table 2.3).

Phosphate was documented to have numerous impacts on the incidence of nitrification in premise plumbing. Specifically, nitrification activity could be limited at very low phosphate levels (Figure 2.1). Availability of phosphorus to nitrifiers was a complex function of bulk water phosphate levels, phosphate release from pipe materials, phosphate uptake by pipe materials, and potential competition from heterotrophic bacteria (Figure 8.2). For example, in iron, plastic, concrete or copper pipes that can release phosphorus compounds, phosphate was less likely to be a limiting nutrient (Chapter II). For lead pipe, where phosphate was removed from the water by corrosion, phosphate could become a limiting nutrient especially at low alkalinity (Figure 2.7). For copper and brass pipes, phosphate could reduce the concentration of Cu$^{2+}$ which is toxic to nitrifiers (Figure 3.5). Adding organic carbon might stimulate the competition of phosphate from heterotrophic bacteria and limit nitrification (Figure 2.8). Finally, phosphate can reduce corrosion, which might reduce the rate of chloramine decay (Table 1.1) and in turn control nitrification by improved disinfection.
High alkalinity stimulated nitrification due to high buffering capacity, reduced toxicity of copper and zinc (Figure 8.3). Without some inorganic carbon, nitrifiers could not grow; although even 2-3 mg/L dissolved inorganic carbon (DIC) was sufficient for autotrophic growth (Figure 2.4). At much higher levels of inorganic carbon numerous beneficial impacts were documented for nitrification occurrence. The higher buffering capacity keeps pH higher and allows the carbonate to complex/precipitate Cu$^{2+}$ and Zn$^{2+}$, reducing their toxicity to nitrifiers. Alkalinity was also influential in pipes such as lead, in which carbonate might decrease phosphate uptake by the pipe and allow more growth of nitrifiers (Figure 2.7).

Lead pipes enhanced nitrification relative to PVC pipes, but nitrification was difficult to establish in brass and copper pipes due to copper toxicity (Figure 3.1). In brass pipes, pH and phosphate could control nitrification, since these factors control the concentration and toxicity of Cu$^{2+}$ ions (Figure 3.5).

Nitrification could increase levels of soluble lead in potable water by reducing pH. The magnitude of the pH drop depended on the initial alkalinity and extent of nitrification (Figure 4.2). The “worst case” lead contamination due to nitrification can be predicted using solubility modeling (Figure 4.4 and Figure 4.6).

In situations where nitrification is occurring and leaded brass faucets are present, the first draw lead and zinc might be higher in homes plumbed with PVC/plastic pipe versus homes plumbed with copper pipe (Table 4.2). In general, nitrification induced a lower pH after stagnation in PVC pipes than in copper pipes (Table S-3.1, Table 4.2, and Figure 6.1).

Without nitrification, chloramine was always more persistent than chlorine (Figure 5.3). With rapid nitrification, in relatively inert pipe materials such as PVC, CuE, stainless steel, chloramine was less persistent than chlorine (Figure 5.3 and Figure 5.4). Nitrification caused chloramine to be less effective than free chlorine in reducing bulk (Figure 5.6) and biofilm HPC (Figure 5.8).

In plumbing materials that are reactive with both chloramine and chlorine such as cast iron, galvanized iron and old lead pipes, the relative disinfectant decay rate and ability to control bacterial growth is strongly dependent on the relative rates of corrosion and nitrification (Figure 5.1). If corrosion rates are high and nitrification rates are low, chloramine tends to be more effective than free chlorine. In contrast, in situations where nitrification rates are high and corrosion rates are low, free chlorine will tend to be more effective.

The release of zinc from galvanized iron was reduced 6 times by nitrification (Figure 6.2). This was primarily due to lower dissolved oxygen after nitrification (Figure 6.5). Nitrification did not affect copper solubility in low alkalinity water, but was hypothesized to significantly increase copper solubility in higher alkalinity waters (Figure 6.7). Field data from Willmar, MN collected in prior research are consistent with this expectation.

GAC filters could remove chloramine disinfectant, significantly decreasing water pH, and inducing nitrification in situations where it otherwise would not occur (Chapter VII). Whole house GAC filters
might significantly degrade water quality in buildings if the water utility is using chloramine disinfectant.
**Figure 8.1: Practical guide for water utilities to use this dissertation for nitrification diagnosis and mitigation**

- **Nitrification in main distribution system**
  - No → No concern
  - Yes → Nitrification in buildings (PVC/copper and hot/cold water lines)? (Chapter III)
    - No → No concern
    - Yes → Consider lead contamination (Chapter IV)
      - Yes → Pb or Cu elevated in large numbers of homes?
        - Yes → Consider conventional corrosion control: pH adjustment, corrosion inhibitor
        - No → Consider copper contamination (Chapter VI)
          - Yes → Consider microbial growth in situations with rapid chloramine loss (Chapter V)
            - Yes → Pb or Cu elevated? Excessive Bacteria?
              - Yes → Consider lead, copper, microbial growth concerns in such systems (Chapter VII)
              - No → Use of GAC or whole house filters?
                - Yes → Consider nitrification in worst case
                - No → Rapid disinfectant loss in pipes?
                  - Yes → Pb or Cu elevated in large numbers of homes?
                    - Yes → Consider nitrification control (Chapter I), and possible nutrient adjustments (Chapter II and III)
                    - No → Consider nitrification in worst case
                  - No → Alkalinity < 30-50 mg/L as CaCO₃?
                    - Yes → Consider lead contamination (Chapter IV)
                      - No → Consider copper contamination (Chapter VI)
                        - Yes → Consider microbial growth in situations with rapid chloramine loss (Chapter V)
                          - Yes → Pb or Cu elevated? Excessive Bacteria?
                            - Yes → Consider lead, copper, microbial growth concerns in such systems (Chapter VII)
                            - No → Use of GAC or whole house filters?
                              - Yes → Consider nitrification in worst case
                              - No → Rapid disinfectant loss in pipes?
                                - Yes → Pb or Cu elevated in large numbers of homes?
                                  - Yes → Consider nitrification control (Chapter I), and possible nutrient adjustments (Chapter II and III)
                                  - No → Consider nitrification in worst case
                                - No → Alkalinity > 200 mg/L as CaCO₃, and pH drop > 0.2 unit?
                                  - Yes → Consider copper contamination (Chapter VI)
                                    - No → Consider nitrification in worst case
                                  - No → Consider nitrification in worst case

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Figure 8.2: Summary impacts of phosphorus on nitrification

Figure 8.3: Summary impacts of alkalinity on nitrification