The influence of distribution system infrastructure on bacterial regrowth
by Kristin Van Andel

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
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Abstract:
This study examined the interactions and influences of pipe materials/linings, organic carbon levels, and disinfectants on bacterial regrowth in drinking water distribution systems. The study consisted of laboratory experiments with annular reactors which simulate the conditions in distribution systems. The information from this study can be used by utilities to determine the best way to maintain water quality as distribution systems age and deteriorate.

The laboratory experiments utilized four pairs of annular reactors. Each reactor within a pair contained the same coupon material (ductile iron, PVC, epoxy, or cement). The experiments were conducted in four phases with varying controlled conditions. During the first phase of the laboratory experiments all reactors were treated identically. The reactors were fed biologically treated tap water and amended with nitrogen and phosphorus to maintain a carbon-limited growth condition. In the second phase, one reactor within each pair received free chlorine while the other received monochloramine to maintain a residual of 0.2 mg/L measured as free and total chlorine, respectively; all other conditions remained the same as in the first phase. In the third phase, all of the reactors were supplemented with 0.5 mg/L total carbon derived from humic substances.

All other conditions remained the same as in the second phase. In the fourth phase, conditions were the same as the third phase except that the supplemented carbon level was raised to 2 mg/L total carbon.

The results showed that there was no significant difference in the efficacies of chlorine and monochloramine against planktonic cells or biofilms at a residual of 0.2 mg/L. There was also no significant difference in the impacts of these disinfectants on either planktonic or biofilm cells as a function of material. Increases in organic carbon levels led to general increases in biofilm and planktonic densities. This effect was most pronounced for biofilms in reactors containing iron coupons. Of the reactors containing epoxy, PVC, and cement coupons there was no definite order of ascendance in regard to biofilm or planktonic growth. However, PVC was always the lowest or not significantly different from the lowest. In the presence of disinfectants and supplementary organic carbon, the reactors containing iron coupons had the highest biofilm and planktonic densities of any of the materials.

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APPROVAL

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Kristin Van Andel

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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## ABSTRACT

This study examined the interactions and influences of pipe materials/linings, organic carbon levels, and disinfectants on bacterial regrowth in drinking water distribution systems. The study consisted of laboratory experiments with annular reactors which simulate the conditions in distribution systems. The information from this study can be used by utilities to determine the best way to maintain water quality as distribution systems age and deteriorate.

The laboratory experiments utilized four pairs of annular reactors. Each reactor within a pair contained the same coupon material (ductile iron, PVC, epoxy, or cement). The experiments were conducted in four phases with varying controlled conditions. During the first phase of the laboratory experiments all reactors were treated identically. The reactors were fed biologically treated tap water and amended with nitrogen and phosphorus to maintain a carbon-limited growth condition. In the second phase, one reactor within each pair received free chlorine while the other received monochloramine to maintain a residual of 0.2 mg/L measured as free and total chlorine, respectively; all other conditions remained the same as in the first phase. In the third phase, all of the reactors were supplemented with 0.5 mg/L total carbon derived from humic substances. All other conditions remained the same as in the second phase. In the fourth phase, conditions were the same as the third phase except that the supplemented carbon level was raised to 2 mg/L total carbon.

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# CHAPTER 1

## INTRODUCTION

Bacterial regrowth in drinking water distribution systems is a vital water quality and public health issue to both utilities and consumers. Regrowth, or the growth of microorganisms in a distribution system after treatment and disinfection, has many causes. It can occur as a result of sloughing from biological filters, the revival of injured cells, genetic resistance to treatment, and detachment from biofilms in distribution systems. Biofilms are of special public health concern because they are resistant to disinfection and they may harbor potential pathogens that can later be re-released into the drinking water supply (Warnecke, 1998). Biofilms have also been linked to deteriorating water quality including taste and odor problems, corrosion, and nitrification. This is of particular concern to industrial users that need water of a consistently high or known water quality for industrial processes.

Many factors have been shown to influence the growth of microorganisms attached to surfaces. These factors include the availability of organics (nutrients), temperature, pH, corrosion control techniques, type and dose of disinfectant, and pipe material. Research has demonstrated that distribution system materials are one of the most important factors influencing the growth of biofilms in distribution systems (Camper et al, 1996). Although a reasonable amount of information is available on the relationship between metal pipe surfaces and bacterial regrowth, relatively little research has been devoted to the study of non-corrodible pipe materials such as cement lining, epoxy lining, and plastics. Research in this area is of increasing importance to many of today's drinking water utilities as aging sections of distribution systems must be repaired or replaced and networks must be expanded to meet a growing consumer base.

## Goals

The goal of this project is to examine the interactions between distribution system materials, organics, and disinfectants and their impacts on bacterial regrowth. The relationships revealed in this study can be used to develop recommendations for utilities to reduce regrowth in their distribution systems. These recommendations can be used by utilities undertaking the re-lining and replacement of aging pipe sections or attempting to optimize finished water quality to control distribution system regrowth.

# CHAPTER 2

# LITERATURE REVIEW

## Biofilms in Drinking Water Distribution Systems

The conditions in drinking water distribution systems appear to be hostile to microorganisms. However, in spite of the low organic carbon concentrations, presence of disinfectants, generally low temperatures, and flow regimes, the growth and persistence of bacteria has been widely observed. Bacterial growth in these systems typically occurs in biofilms on pipe surfaces (Camper et al., 1999). The concentration of planktonic cells in distribution systems is increased by erosion and sloughing from these biofilms (van der Wende et al., 1988).

A wide variety of microorganisms can be found in drinking water distribution systems including coliforms, actinomyces, molds, fungi, nitrifying bacteria, iron oxidizing bacteria, and sulfate reducing bacteria. Bacteria, viruses, protozoa, and algae are also present and have all been implicated in waterborne disease (Cohn et al., 1999). Table 1 shows some of the water quality problems associated with these organisms (Abernathy, 1998).

Camper et al. (1994) summarized several mechanisms that have been suggested for the adsorption of bacteria to surfaces, including physical adsorption and chemisorption. Physical adsorption is a reversible or equilibrium adsorption, involving primarily physical factors. Chemisorption, on the other hand, is generally considered irreversible and results from short-range forces, including chemical bonds, dipole interactions, and hydrophobic bonding. It has also been theorized that an adsorbed cell can either inhibit or enhance the adsorption of other nearby cells. The term blocking refers to the inhibition effect and the phrase positive cooperativity refers to the enhancement effect. According to the theory of blocking, the initial colonizing cells would be arranged in a regular pattern with few near neighbors. According to the positive cooperativity theory, the initial cells would instead be arrayed in aggregates with many near neighbors.

Table 1. Problematic Microorganisms in Distribution Systems (Abernathy, 1998)

| Type of Microorganism | Infrastructure or Water Quality Problem |
|---|---|
| Coliforms | Positive samples may be a violation of the Total Coliform Rule. |
| Actinomycetes, Molds, and Fungi | Produce earthy-musty-moldy taste and odor compounds. Commonly found in surface waters. |
| Iron Bacteria | Oxidize soluble iron to precipitate forms increasing the mass of corrosion products on pipe walls and pump casing. Excessive iron deposits cause increased pipe friction and lower pump efficacy. |
| Sulfate Reducing Bacteria (SRBs) | Reduces sulfate to hydrogen sulfide creating rotten egg taste and odor. Increases corrosion rates. |
| Nitrifying Bacteria | Oxidizes ammonia to nitrate. Consumes alkalinity, which may result in pH reduction. |
| Protozoans | Will not reproduce in biofilm, but may reside in biofilm. |

## Pipe Materials

Although extensive research has been conducted regarding the growth of bacteria on iron surfaces (LeChevallier et al., 1990, Abernathy, 1998, Geesey et al, 1989, LeChevallier et al., 1993), very little research has been conducted on inert materials such as epoxy, cement, and polyvinylchloride (PVC). Some characteristics of pipe materials that can influence the proliferation of biofilms include roughness and reactivity. In one study, similar biofilm densities were observed on PVC and polyethylene, suggesting that materials with similar porosity and roughness support similar biofilm densities. The same study showed that plastic based materials such as PVC or polyethylene support less growth than cement-based materials, while iron materials support the most growth (Niquette et al, 2000).

## Corrosion

Corrosion is an oxidative process that occurs at the surface of the metal where it contacts water and its constituents. The pure metal is oxidized into ferrous hydroxide [$Fe(OH)_2$], which may be further oxidized to ferric hydroxide [$Fe(OH)_3$] by reaction with oxygen. Over time the oxidation reaction slows as corrosion products adhere to the iron surface and form a protective layer between the pure iron and the reactants in the bulk fluid (Geesey et al., 2000).

As iron oxide corrosion products form on the surface of the pipe, the texture of the pipe changes from a smooth, homogeneous surface to a rough, heterogeneous surface. This rough surface provides a sheltered habitat for the growth of microorganisms into biofilms. In addition, the corrosion products react with disinfectant residuals, which prevent disinfectants from penetrating these biofilms (LeChevallier et al., 1993). Table 2 shows many of the factors that have been demonstrated to influence corrosion and corrosion control of metal pipe materials.

Table 2. Factors that Influence Corrosion and Corrosion Control (LeChevallier et al., 1993)

| Factor | Effect |
|---|---|
| pH | Low pH may increase corrosion rate; high pH may protect pipes and decrease corrosion rates or could cause dezincification of brasses. |
| Alkalinity | Alkalinity may help form protective coating; helps control pH changes. Low to moderate alkalinity reduces corrosion of most materials. High alkalinities increase corrosion of copper and lead. |
| Dissolved oxygen (DO) | DO increases rate of many corrosion reactions. |
| Chlorine Residual | Chlorine residual increases metallic corrosion, particularly for copper, iron, and steel. |
| Total dissolved solids (TDS) | High TDS increases conductivity and corrosion rate. |
| Hardness (Ca and Mg) | Calcium may precipitate as $CaCO_3$ and thus provide protection and reduced corrosion rates. Ca and Mg may enhance the buffering effect of alkalinity and pH. |
| Chloride, sulfate | High levels of chloride or sulfate increase corrosion of iron, copper, and galvanized steel. |
| Hydrogen sulfide | Hydrogen sulfide increases corrosion rates. |
| Ammonia | Ammonia may increase the solubility of some metals such as copper and lead. |
| Natural color, organic matter | Organic matter may decrease corrosion by coating pipe surfaces. Some organics can complex metals and accelerate corrosion or metal uptake. They may stimulate microbially influenced corrosion. |
| Copper | Copper causes pitting in galvanized pipe. |
| Magnesium (and other trace metals) | Trace metals may inhibit the precipitation of calcite from $CaCO_3$ on pipe surfaces and favor the deposition of the more soluble aragonite form of $CaCO_3$. |

Microbiologically Influenced Corrosion

Bacterial biofilms have also been linked to increased corrosion in iron pipelines. This phenomenon is known as biocorrosion or microbiologically influenced corrosion (MIC). Until recently it was believed that MIC occurs mainly in anaerobic environments in the presence of sulfide-producing bacteria. However, more recent work has shown that several other types of microorganisms, including hydrogen-producing bacteria, iron bacteria, and aerobic bacteria, can play a role in MIC (Geesey, 1991). Iron bacteria have been found on pipe surfaces and in water samples in distribution systems in Southern California (Ridgway et al., 1981). Additionally, sulfate-reducing bacteria were detected in 80% of the tubercles in the Columbus, Ohio distribution system (Tuovinen et al., 1982). This evidence shows that bacteria that have been implicated in MIC can be prevalent in drinking water distribution systems.

Geesey (1991) reviewed several methods by which microorganisms can contribute to corrosion. A differential aeration cell can occur as a result of uneven distribution of bacterial colonies on a metal surface submerged in an aerated fluid. As the bacteria in these microcolonies respire, they create an oxygen gradient near the metal surface. As the oxygen concentration at the surface under the microcolony is reduced, this area becomes anodic to the uncolonized surface area exposed to the bulk fluid, causing corrosion of the surface. Sulfur reducing bacteria, living in the anoxic zones created by other respiring microorganisms, can contribute to corrosion in several ways. Through the use of a hydrogenase these bacteria can impede cathodic polarization by preventing the accumulation of molecular hydrogen at the cathode. The hydrogen sulfide that these bacteria produce through respiration can also contribute to cathodic depolarization.

Iron-reducing bacteria contribute to corrosion through reduction reactions that dissolve the passive oxide/hydroxide layer on iron surfaces. With the destruction of this layer, the iron surface is re-exposed to the bulk fluid, allowing further corrosion to occur (Geesey et al., 2000). The presence of chloride ions can increase the electrochemical potential for corrosion by combining with the ferric ions produced by the iron-reducing bacteria to form ferric chloride [$FeCl_3$] (Geesey, 1991).

The chemical and metabolic differences between different types of bacteria in a biofilm can also contribute to corrosion. The varying exopolymers secreted by bacteria differ in their affinities for and interactions with metal ions. This can lead to the formation of a metal concentration cell in which areas underneath exopolymers with high affinities for the underlying metal are anodic to those underneath exopolymers with low affinities for the metal (Geesey et al., 1989).

Problems Associated With Corrosion

The corrosion of iron has been recognized as being one of the primary factors affecting biofilm growth (Volk, 2000). Studies suggest that biofilms on iron surfaces are protected from chlorine residuals due to the reaction of corrosion products with the free chlorine (LeChevallier et al., 1990). In addition, the corrosion of iron pipe can produce tubercles, which increase the surface area of the pipe. Cracks and crevices can provide protection from hydraulic currents and disinfectants for biofilm growth. Corrosion is also linked to increased precipitation of organic compounds and increases the hydraulic mixing in the bulk fluid, allowing for better transportation of nutrients to the surface (LeChevallier et al., 1996). The combination of these all of these factors creates an ideal situation for increased microbial growth.

Many studies have demonstrated that increasing corrosion control can decrease microbial growth on iron surfaces. One study showed that the addition of zinc-orthophosphate or polyphosphate can reduce biofilm densities in chlorinated iron annular reactors. The addition of zinc-orthophosphate also decreased biofilm densities in iron annular reactors treated with monochloramine (Abernathy, 1998). A survey of 31 North American water systems showed a link between the use of phosphate-based corrosion inhibitors and lower coliform levels (LeChevallier et al., 1996). Several factors have been suggested to play a role in the reduction of biofilms through corrosion control. Some of these factors include changes to the surface chemistry of the pipe surface, reduction in the leaching of ferrous iron from the pipe surface to increase disinfectant efficacy, decreased biofilm habitat, and reduced disinfectant demand of the pipe surface (Abernathy, 1998).

## Disinfectants

Drinking water utilities in the United States are required to maintain a disinfectant residual in drinking water distribution systems. Monochloramine and chlorine are the two most commonly used disinfectants for this purpose and must be maintained at a residual of 0.2 mg/L in the distribution system. The purpose of maintaining this residual is to prevent regrowth in the distribution system and to inactivate any microorganisms that enter the distribution system as a result of contamination. However, the efficacy of this residual in preventing biofilm growth is limited as biofilms are significantly more resistant to disinfection than suspended cells of the same strain (Costerton et al., 1987).

Two main mechanisms of biofilm resistance to disinfection have been proposed. The first is a transport limitation resulting from a reaction-diffusion interaction in a biofilm. The microorganisms, exopolysaccharides, and other reactive biofilm constituents could consume the antimicrobial agent, protecting the biofilm beneath from exposure. Research using alginate gel beads with and without entrapped bacteria has demonstrated the viability of this hypothesis for chlorine (Xu, 1996). Another study of the action of chlorine, glutaraldehyde, an isothiazolone, and a quaternary ammonium compound on bacteria entrapped in alginate beads revealed that the reaction-diffusion phenomenon can occur for both oxidizing and non-oxidizing antimicrobial agents (Stewart, 1998).

Another theory of biofilm resistance to antimicrobial agents deals with the spatial heterogeneity in growth rates within a biofilm. Cells on the interior of a biofilm may be slow growing due to nutrient limitations or other regulatory mechanisms that render the cells dormant. It has been proposed that these slow growing or dormant cells in the biofilm are less susceptible to growth-rate-dependent antimicrobial agents than rapidly growing cells at the surface of the biofilm. In conjunction with this theory it has also been proposed that the more rapidly growing cells on the surface are not destroyed by the antimicrobial, but merely damaged or prevented from reproducing. These cells would thus cease to contribute to the growth of the biofilm, but would continue to consume nutrients for hours or even days and shield interior cells from access to nutrients (Xu et al., 2000).

As discussed above, it has been shown that decreased chlorine efficacy against biofilms is due in part to reaction-diffusion limitation of the chlorine by the biofilm. This effect is enhanced in the presence of iron (LeChevallier et al., 1993). Monochloramine is also believed to have reduced efficacy against biofilms due to a reaction-diffusion limitation (Srinivasan et al., 1995). Like chlorine, the disinfectant efficacy of monochloramine is further reduced against biofilms in the presence of iron, though to a lesser degree than chlorine (LeChevallier et al., 1993).

Although chlorine has been traditionally used as a disinfectant in drinking water treatment, chloramines are becoming increasingly popular (Camper, 1994).

Although free chlorine is cheap, it forms disinfection by-products such as trihalomethanes, which are of increasing health concern. Monochloramine is increasingly popular for it has been shown to be a more slowly reacting disinfectant than free chlorine and is more specific in the types of compounds it will react with (LeChevallier et al., 1996). Since chlorine is more widely reactive, it can be rapidly consumed by system components and materials in the water, lowering its performance as a biocide. In an annular reactor study comparing chlorine and monochloramine efficacies against biofilms, chlorine was found to be highly reactive in the uninoculated system whereas monochloramine did not react (Griebe et al., 1994).

Monochloramine is also gaining popularity for its increased efficacy against biofilms compared to that of chlorine. In one study, monochloramine was shown to be more effective against *Pseudomonas aeruginosa* biofilms than free chlorine (Griebe et al., 1994). Another study of the Greater Vancouver Water District also showed that chloramine is a more effective disinfectant for controlling biofilm growth in distribution systems. This was evidenced by decreased levels of coliform and HPC bacteria. This study also indicated that chloramine, as a secondary disinfectant, produces a more stable residual, less taste and odor, and is significantly less expensive than chlorine (Neden et al., 1992).

One downside to monochloramine as a biofilm disinfectant was shown in a study of monochloramine as a disinfectant against *Pseudomonas aeruginosa*. In this study, evidence was found that *P. aeruginosa* can adapt to prolonged or repeated treatments. It was theorized that at low doses (<0.5 mg/L) these bacteria produce monochloramine-neutralizing biomass constituents. It was also theorized that cells exposed to monochloramine acquire reduced susceptibility to disinfection. This study suggests that it is more effective to deliver monochloramine in a short concentrated dose than in a longer, less concentrated dose (Sanderson et al., 1997). This phenomenon is of importance to drinking water science as *P. aeruginosa* is common in finished waters and distribution system biofilms. Although it is not a frank pathogen, it is an opportunistic pathogen, and can cause severe respiratory and other infections in populations with weakened immune systems such as newborns, the elderly, and AIDS patients (Cohn et al., 1999).

Chlorine Chemistry

Dissolved aqueous chlorine reacts with water to form hypochlorous acid [HOCl] according to the following reaction:

$$Cl_2(aq) + H_2O \Leftrightarrow H^+ + HOCl + Cl^-$$

The hypochlorous acid may further react to form the hypochlorite ion [$OCl^-$] by the following reaction:

$$HOCl \Leftrightarrow OCl^- + H^+ \qquad pKa = 7.6$$

Since hypochlorous acid is a better disinfectant than the hypochlorite ion, the efficacy of free chlorine as a disinfectant is very pH dependent (Haas, 1999). At