



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

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in  
Environmental Engineering  
Montana State University  
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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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**A thesis submitted in partial fulfillment  
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APPROVAL

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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.

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.

## 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  $[\text{Fe}(\text{OH})_2]$ , which may be further oxidized to ferric hydroxide  $[\text{Fe}(\text{OH})_3]$  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 $\text{CaCO}_3$ 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 $\text{CaCO}_3$ on pipe surfaces and favor the deposition of the more soluble aragonite form of $\text{CaCO}_3$ .

### 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<sub>3</sub>] (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

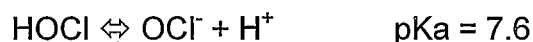
this study, evidence was found that *P. aeruginosa* can adapt to prolonged or repeated treatments. It was theorized that at low doses (<0.5 mg/L) these bacteria produce monochloramine-neutralizing biomass constituents. It was also theorized that cells exposed to monochloramine acquire reduced susceptibility 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:



The hypochlorous acid may further react to form the hypochlorite ion [OCl<sup>-</sup>] by the following reaction:

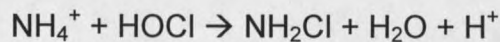


Since hypochlorous acid is a better disinfectant than the hypochlorite ion, the efficacy of free chlorine as a disinfectant is very pH dependent (Haas, 1999). At

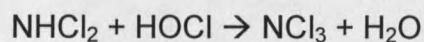
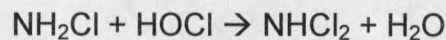
a pH less than 7.6 hypochlorous acid is the dominant species in the water while at a pH greater than 7.6, the hypochlorite ion dominates. This is an important issue if the pH of the finished water is increased for corrosion control. Such an adjustment would require an increase in the application of free chlorine to maintain a consistent level of efficacy.

### Monochloramine Chemistry

The reaction of aqueous ammonia [ $\text{NH}_4^+$ ] with hypochlorous acid [ $\text{HOCl}$ ] produces monochloramine [ $\text{NH}_2\text{Cl}$ ] according to the following reaction:



Monochloramine can further react with hypochlorous acid to produce dichloramine [ $\text{NHCl}_2$ ] and trichloramine [ $\text{NCl}_3$ ].



The efficacy of chloramines is dependent on pH. Of the chloramines, monochloramine has the highest efficacy as a disinfectant, followed by dichloramine then trichloramine (Haas, 1999). At a pH of roughly 8.3 – 8.4, monochloramine is the dominant species whereas dichloramine is favored at lower pHs. Thus, the efficacy of monochloramine is greatest at a pH of 8.3 – 8.4. If pH adjustment is used for corrosion control, monochloramine would be a better choice of disinfectant since it has a high efficacy at a much higher pH than chlorine.

## Natural Organic Matter

Decaying plant materials and animal matter are sources of natural organic matter (NOM). NOM can be sub-classified as dissolved organic matter (DOM), colloidal organic matter (COM), and particulate organic matter (POM). COM and POM can both be removed by filtration with 0.22  $\mu\text{m}$  filters and 1.0  $\mu\text{m}$  filters, respectively. The material remaining after filtration, DOM, can be further classified as humic substances and non-humic substances. Non-humic substances include amino acids, proteins, carboxylic acids, and carbohydrates. These non-humic substances can be differentiated from humic substances through methods such as protein and carbohydrate assays (Owen et al., 1995).

The fraction of dissolved organic matter that can be mineralized by heterotrophic microorganisms is known as biodegradable dissolved organic carbon (BDOC). The fraction of the DOC that is available as a carbon and energy source for microorganisms is known as assimilable organic carbon (AOC) (van der Kooij et. al, 1982). Both BDOC and AOC are of concern to the drinking water industry as they can provide food for the growth of microorganisms in the distribution system if they are not completely removed (Rittman and Snoeyink, 1984).

### Chemistry of Humic Substances

The term humic substances is used to collectively refer to the humic acids and related pigments which are widely distributed in soils, natural waters, marine

and lake sediments, peat, carbonaceous shales, lignites, brown coals, and miscellaneous other deposits. The term "humus" is used to refer to the soil humic isolates which are separated through alkali extraction. The term "humic acid" refers to the fraction that is precipitated through acidification. Although the terms humus and humic acid are often used interchangeably, these two fractions are not the same and should not be confused with each other (Stevenson, 1985).

Although the exact mechanism by which humic substances are formed is not known, it is known that it involves the microbial degradation of plant organic matter, including lignins, cellulose and polypeptides. These products may be modified by polymerization, condensation, and oxidation reactions. Humics in aquatic environments may come from the leaching of terrestrial plant and soil organic matter or they may be formed by bacterial action on phytoplankton (Beckett, 1990). Thus, it is not surprising that there are some inherent differences in the composition of humic substances depending on the type of environment, flora, and fauna through which they form. For example, humic substances from groundwater typically contain greater than 60% carbon and less than 30% oxygen, while humic substances in surface water contain an average of 52% carbon, and 42% oxygen (Thurman, 1985). Groundwater also typically contains less humic substances (generally less than 1 mg/L dissolved organic carbon) than surface waters (generally 2-10 mg/L dissolved organic carbon) (Beckett, 1990).

Humic substances are usually subdivided into three major fractions based on their solubility in alkali and acid. According to Choudhry (1984), humic acid (HA) refers to the fraction that is soluble in dilute alkaline solutions but is precipitated by acidification. Fulvic acid is the fraction that remains in the acidified solution and is soluble in both acid and base. The third fraction, humin, is not soluble in either acid or base and is therefore not extractable from the soil.

There is much discrepancy concerning the molecular structure of humic substances. It has been suggested that in general, humic substances contain roughly 45-55% carbon, 4-5% hydrogen, 35-40% oxygen, 1-2% nitrogen, and less than 1% sulfur and phosphorus (Beckett, 1990). However, the manner in which these molecules combine is open to conjecture. One study suggested that the molecular weight of humic substances ranges from 500 – 200,000 (Thurman, 1985). Another study suggests that the average molecular weight is 800-1500 Daltons for fulvic acids and 1500-4000 Daltons for humic acid (Beckett, 1990). Choudhry (1984) revealed that molecular weights ranging from a few hundred to several million Daltons have been reported for humic substances. He attributed this discrepancy in part to differences in methods of measurements and differences in the origin, extractants, and degree of purification of the humic substances.

The major functional groups in humic substances include carboxyls, phenols, hydroxyls, carbonyls, ether, and esters. In general, fulvic acids are found to be more aliphatic than humic acids. Humic substances in soils are

characterized as being more aromatic than humics derived from terrestrial surface water, and both are more aromatic than marine humics (Beckett, 1990).

The conformational structure of humic substances has also been widely debated. The traditional theory is that humic substances are large polymers and may occur in linear or coiled, cross-linked conformations, depending on the properties of the solution. According to this model, at high concentration, low pH, and high ionic strength these molecules exist in the coiled conformation, while they exist as flexible linear polymers at neutral pH, low ionic strength, and low concentration. Another theory suggests that humic substances in solution are a loosely bound self-association of relatively small molecules, which are dominated by intermolecular hydrophobic interactions as binding forces (Conte and Piccolo, 1999).

### Humics and Iron Oxides

Several mechanisms have been proposed to explain the ready adsorption of natural organic matter to iron oxides. These mechanisms include anion exchange (electrostatic interaction), ligand exchange-surface complexation, hydrophobic interaction, entropic effect, hydrogen bonding, and a cation bridging effect (Gu et al, 1994). The most popular of these theories is the ligand exchange mechanism. Parfitt et al. (1977) used infrared spectroscopy to demonstrate that the adsorption of a fulvic acid on goethite involves the complexation between carboxylate groups of fulvic acid and surface hydroxyl

groups of the goethite. It was also noted that the adsorption of these organic acids is usually accompanied by an increase in pH. This suggests that the carboxylates of fulvic acid replaced the hydroxyls on the oxide surfaces. Another study by Gu et al. (1994) demonstrated the pH dependence of the adsorption of natural organic matter to iron oxide surfaces. At lower pHs the adsorption of the NOM was high, but it decreased rapidly as the pH increased.

Gu et al. (1994) attempted to model the mechanism of desorption of organic matter. In this study, a modified Langmuir model was used to describe different types of adsorption isotherms. The results of the study showed a strong hysteresis in the adsorption and desorption of NOM ( $h = 0.72-0.92$ ), which should be considered for better modeling of NOM transport. This high hysteresis coefficient indicates that NOM that is adsorbed by iron oxides is very difficult to be desorbed at a given pH and ionic composition. However, since the adsorption of NOM on iron oxides is very dependent on pH, decreasing the pH can induce some desorption.

In another study, Gu et al. (1996) demonstrated that the adsorption of NOM on iron oxides is governed by competitive adsorption when surface sites are limited. This study demonstrated competitive adsorption between Suwanee River NOM and several other model organic compounds. This competitive adsorption may help explain the hysteresis observed in the adsorption and desorption of NOM on iron oxides. As iron oxide surfaces are exposed to NOM, the strongly binding components of NOM would competitively adsorb onto the

surfaces, displacing the weakly binding components when adsorption sites are limited.

Previous studies by Gu et al. (1995) showed that different fractions of NOM are adsorbed by iron oxide with different affinities and capacities. It has been shown that on a carbon-weight basis, larger size hydrophobic NOM fractions had higher adsorption affinities and capacities than smaller size fractions. In addition, the larger sized hydrophobic fractions were preferentially adsorbed over the smaller sized hydrophilic fractions. Due to the difficulty in quantitatively identifying NOM fractions from a mixture before and after adsorption, competitive adsorption has not yet been explored (Gu et al., 1996).

The large potential of iron oxides to adsorb NOM has some practical applications for the drinking water industry. Iron oxides can be used in treatment processes to enhance the removal of NOM, which has been implicated as a utilizable food source for the growth of biofilms in distribution systems (Camper et al., 1999). In one study, layered double hydroxide containing iron was used to remove humic substances from waters. The advantage of this technology was the high adsorption capacity of the hydrotalcite and ferric compounds and the low water content of their sludges (Seida and Nakano, 2000). Another study showed that a combined iron oxide and ultrafiltration process can be used to reduce the fouling of ultrafiltration membranes by NOM. NOM deposits formed on the cake layer of heated iron oxide particles before the membrane rather than on the membrane itself. The cake can be removed chemically or physically and

replaced, without disturbing the membrane surface (Chang and Benjamin, 1996). Iron oxid-coated olivine was demonstrated as an effective filter media for NOM removal by Chang et al. (1997). The only drawback to this technology is that repeated backwashing may reduce the sorption capacity of this media.

### Bioavailability of Humics

Abernathy (1998) suggested that the coiled structure of humic substances may decrease the bioavailability of humic substances to suspended bacteria because the enzymatically active sites of the molecules are hidden within the coils. However, adsorption of humic substances to iron oxides has been linked to an increased ability of microorganisms to use the humic substances as a food source (Camper et al., 1999). Qi (1999) demonstrated that sorbed humic substances alone could not support biofilm accumulation, but that they can be used as a supplementary carbon source in biofilm accumulation on iron surfaces when humic substances are supplied in the bulk fluid. In low carbon environments like drinking water distribution systems, this effect may be of greater importance.

It has been hypothesized that humic substances undergo a conformational change in structure as they are absorbed to iron surfaces, which exposes their utilizable attached functional groups. First, the adsorption of humics on iron oxide surfaces forces the humic molecules to collapse, allowing for maximum points of interaction between their oxygen-containing functional groups and iron

oxide surface sites. This may occur through ligand exchange or H-binding mechanisms. (Gu et al., 1994). The collapse of the molecule may cause it to uncoil and expand, exposing the previously hidden usable functional groups to microorganisms (Gu et al., 1994, Qi 1999). Qi (1999) suggested other theories regarding the increased bioavailability of humic substances on iron oxide surfaces. First, he suggested that the adsorption of humic materials on iron oxide surfaces may fix the conformation of the humic molecule so that it is less capable of forming complexes with extracellular enzymes. Another theory proposes that the adsorption of humic molecules to iron oxides makes them easier to locate by biofilm cells since they are in a fixed position. In addition, the adsorption may increase the concentration of humic substances on the surface, making them more available for utilization by microorganisms.

#### Biological Treatment for NOM Removal

The traditional method used by the water treatment industry to reduce microbial counts is the application of antimicrobial chemicals. However, the use of biological pretreatment is gaining increasing popularity for reducing microbial growth. The basic principle of biological pretreatment is that by encouraging bacterial growth in a specific location upstream, the growth can be controlled and optimized. The result of this growth is the depletion of nutrients in the water so that there is a minimum of available nutrients available for the growth of biofilms downstream. Biological treatment uses indigenous organisms adapted to the

types of organic carbon found in the water (Costerton et al., 1999). It has been shown that the effectiveness of dissolved organic carbon removal through biological treatment is dependent on biomass concentration (Carlson and Amy, 1998).

Granular activated carbon (GAC) is a commonly used in biological treatment. GAC in drinking water is generally composed of wood, peat, lignite, subbituminous coal, and bituminous coal, which is converted to char and oxidized to develop the internal pore structure. The shape of GAC particles can be regular (extruded activated carbon) or irregular (crushed activated carbon) and is an important factor affecting the filtration and backwash properties of GAC beds. The pore size distribution and surface area of GAC particles are two of their more important characteristics. These characteristics influence the adsorptive characteristics of the GAC. The large surface area to volume ratio typical of GAC particles and the abundance of pores provide an ideal environment for the accumulation of biofilms. Thus, GAC is commonly used as a filter media in biological treatment. When GAC is colonized with biofilms for biological treatment it is known as biologically activated carbon (BAC). It has been shown that besides removing nutrients from water, BAC can reduce disinfectant residuals without significant harm to the microbial population in the BAC. In an attempt to kill the attached bacteria on GAC, LeChevallier et al. (1992) found that instead, the bacteria significantly reduced the disinfectant residuals and continued to proliferate.

As biological treatment has become increasingly popular, concerns have been raised over the detachment of colonized carbon fines from biological filters and their potential to contribute to growth downstream. However, it has been shown that some of this concern is without due cause. A study by Morin and Camper (1997) showed that when these carbon fines accumulate in biofilms they do not appear to enhance the numbers of bacteria in the biofilm, nor do they act as a focal point for microcolony development. This study also showed that carbon fines do not protect biofilms against disinfection and that disinfection actually causes detachment of the carbon fines. However, this study also showed that the attachment of carbon fines into a biofilm is size-dependent and that larger particles are more likely to persist in biofilms. This phenomenon is of concern because larger particles are more likely to be colonized if they are released from a biological filter and therefore have a greater potential to impact biofilms in the distribution system.

### Summary

Much research has been devoted to the impacts that pipe materials, natural organic matter, and disinfectants have on bacterial growth in aquatic systems. However, these factors have generally been examined on an individual basis and not as a group, giving a limited view of the roles that these factors play. In this study these factors are examined together to give a comprehensive understanding of how they interact in a drinking water distribution system.

Based on previous research it is expected that biofilms grown on iron surfaces in the presence of humic substances and/or disinfectants will be most problematic. Since an increase in the bioavailability of humic substances in the presence of iron and a decreased efficacy of chlorine or monochloramine in the presence of iron has been demonstrated.

Little research has been conducted on the relative tendencies of cement, epoxy, and PVC to support biofilm growth. Since these materials have not been shown to have any significant interactions with humic substances or disinfectants they are not expected to support as much growth as iron. The available literature also suggests that the PVC is likely to support less biofilm growth than the cement.

Previous studies have also shown that in general, monochloramine is better than chlorine at controlling biofilm growth. However, those studies were performed with doses much greater than the 0.2 mg/L used in these experiments. The same literature has shown that at low residuals (<0.5 mg/L) the efficacy of monochloramine is significantly reduced (Sanderson et al., 1997). Thus it is expected that at the low dose (0.2mg/L) used in this experiment there will be little difference in the efficacies of either chlorine or monochloramine on the biofilms.

## CHAPTER 3

## METHODS AND MATERIALS

Annular Reactors

The laboratory setup consisted of four pairs of annular reactors containing coupons coated with four common pipe materials. Each pair of reactors contained coupons of only one of the following materials: epoxy, ductile iron, cement, or PVC. The annular reactors used in this experiment were model 920LJ manufactured by Biosurface Technologies Corporation. These reactors have a variable speed rotating drum, a volume of roughly 1 liter, and a high ratio of surface area for growth to the fluid volume. Each annular reactor drum holds 20 coupons measuring roughly 1 cm in width and 15 cm in length. The rotational speed of the reactors was set at roughly 90 rpm to simulate the shear stress in a four-inch pipe with a fluid velocity of 1 ft/s. This rotational speed was selected based on calculations provided by the manufacturer of the annular reactors.

Bozeman tap water flowed through a granular activated carbon (GAC) column and then through a biologically activated carbon (BAC) column into a 2 liter holding tank. The columns were operated in an up-flow mode. The purpose of using the GAC and BAC columns was to remove chlorine and some organic carbon from the Bozeman tap water and create an influent of consistent biological and chemical quality to the annular reactors. From the holding tank this

water was pumped at a constant flow rate into the annular reactors to achieve a detention time of 2 hours per reactor. This detention time was sufficient to allow biofilm growth while minimizing planktonic growth.

Table 3. The Timing of the Four Feeds to the Annular Reactors.

Phase	GAC/BAC Water	Nitrogen/Phosphorus	Disinfectant	Humics-Derived Carbon
1 (Control)	Yes	Yes	No	No
2	Yes	Yes	4 with Chlorine 4 with Monochloramine	No
3	Yes	Yes	4 with Chlorine 4 with Monochloramine	0.5 mg/L TOC
4	Yes	Yes	4 with Chlorine 4 with Monochloramine	2 mg/L TOC

The laboratory experiment was divided into four phases. Each phase lasted a minimum of 3-4 months to allow the processes in the annular reactors to approach equilibrium. Sampling was initiated approximately one week after the start of each phase. In each phase all reactors were fed with the Bozeman tap water, as described above, and a nitrogen/phosphate solution (Table 3). The nitrogen/phosphate solution was added to ensure that the reactors were carbon limited. In the first control phase nothing else was added to the reactors. In the second phase chlorine was added to one reactor in each pair and monochloramine was added to the other reactor to achieve target effluent concentrations of 0.2 mg/L as free chlorine and total chlorine, respectively. In the third phase humics-derived carbon was added to all reactors at a concentration of 0.5 mg/L TOC. In the fourth phase the humics-derived carbon was increased to 2 mg/L TOC. In phases three and four chlorine and monochloramine were

also added to achieve the target effluent concentration of 0.2 mg/L as free chlorine and total chlorine, respectively.

In phase one fresh coupons were used in all 20 slots on the reactor drum. At the beginning of phase two alternate coupons on the drum were removed and replaced with new coupons. At the beginning of phase three all coupons that were not removed at the beginning of phase two were replaced with fresh coupons. At the beginning of phase four the same coupons that were replaced at the beginning of phase two were again replaced with fresh coupons. Thus in each of the last three runs 10 new coupons were available for examination of initial growth of new biofilms. In addition 10 coupons containing biofilms from the previous phase were available for the examination of the less dramatic changes in older biofilms nearer to equilibrium. New coupons and old coupons were sampled in alternate weeks starting with the new coupons.

#### Chlorine Preparation

The chlorine feed solution was prepared from household bleach (sodium hypochlorite) containing no additives or buffers. A small volume of bleach was injected into a large volume of water. The appropriate ratio of water to bleach for each reactor was determined through trial and error to achieve roughly a 0.2 mg/L residual of free chlorine in the reactors. The ratio changed frequently during the course of the experiment to accommodate fluctuations in the water

quality in the annular reactors. The chlorine feed was always operated at a flow rate of approximately 0.25 mL/min into each treated reactor.

### Monochloramine Preparation

Monochloramine was prepared using techniques developed in previous studies at the Center for Biofilm Engineering. A phosphate buffer was made by adding 0.5 g of dibasic potassium phosphate to 1 L of ultrapure water in a sterile glass bottle. The pH of this buffer was then adjusted to between 8.9 and 9.2 using 0.1 N NaOH. In a separate container, 0.11 g of ammonium chloride was added to 100 mL of the phosphate buffer and mixed. While continuing to mix this solution, 1 mL of a ~4% sodium hypochlorite bleach solution was added at a rate of no more than 20  $\mu$ L per 6 seconds. The solution was allowed to stir for 30 minutes before the chlorine residuals were measured.

The concentration of the monochloramine was checked for consistency with the HACH DR/2000 using DPD powder packets and the HACH method 80. The difference between the total and free chlorine residuals was considered the monochloramine concentration. The method described above for monochloramine preparation produced a concentration of 400-500 mg/L.

The appropriate level of dilution of the monochloramine stock solution for each reactor was determined through trial and error to achieve roughly a 0.2 mg/L residual of total chlorine in the reactors. The level of dilution changed frequently during the course of the experiment to accommodate fluctuations in

the water quality in the annular reactors. The monochloramine feed was always operated at a flow rate of approximately 0.25 mL/min into each treated reactor.

#### Nitrogen/Phosphate Solution Preparation

A molar ratio of 100:10:1 for carbon:nitrogen:phosphorus was used to determine the nitrogen and phosphorus needs in the annular reactors. This calculation was based on the average carbon levels measured in the reactors and was adjusted only when significant fluctuations were observed and at the beginning of each phase when the carbon was deliberately increased. Potassium nitrate was used as the nitrogen source and the phosphorus was derived from equimolar concentrations of dibasic and monobasic potassium phosphate. A stock solution was created such that one mL of stock solution was added per L of autoclaved ultrapure water in the feed solution. This flow rate of this feed solution into each reactor was approximately 0.25 mL/min.

#### Humics Solution Preparation

The humics solution was prepared using Elliot Silt Loam obtained from the International Humic Substances Society and has been used extensively in research projects at the Center for Biofilm Engineering. 100g of this soil was added to 1L of 0.1 N sodium hydroxide in a baked glass bottle to minimize carbon contamination. This soil solution underwent constant mixing for 2 – 4 days after which it was centrifuged at a g factor of 4 for 20 minutes. The

supernatant was then poured into another baked glass bottle and a small sample was removed and diluted with nanopure water for determination of the carbon concentration of this stock solution.

The dissolved organic carbon (DOC) concentration of the solution was measured with the Shimadzu TOC-5000A Total Organic Carbon Analyzer using the methods described below for the annular reactor samples. The final concentration usually measured between 1000 mg/L and 1500 mg/L DOC depending on the length of the mixing time. The volume of humics stock solution to add to the feed jug was calculated using the flow rate and volume in the annular reactors, the volume of autoclaved ultrapure water in the feed jug, and the desired concentration of humics to be added to the reactors. After the humics were added to the ultrapure water in the feed jug, the pH was adjusted with 2N hydrochloric acid (HCl) to match the pH in the influent as closely as possible. The flow rate of the humics feed into the reactors was approximately 0.25 mL/min.

### Biological Analyses

#### Heterotrophic Plate Counts

One coupon was removed for sampling from each reactor roughly once every week during each phase, beginning one week after the initial startup. The surface of the coupon was scraped using a flat-headed spatula into a 100 mL beaker with 10 mL of sterilized water. The contents of the beaker were then

carefully transferred to a sterile test tube for further handling. The influent water was sampled by taking 10 mL from the holding tank and placing it in a sterile test tube. The reactor effluent lines had a tendency to grow biofilms along the sides of the tubing which would detach during sampling so effluent samples of 10 mL were instead drawn directly from the inside of the annular reactors. Since the fluid in the annular reactors is thoroughly mixed the concentrations in the effluents are the same as the concentrations inside the reactors.

R2A plates were used for the heterotrophic plate counts. These plates were prepared using 12.7 g of a pre-mixed powder, which was added to 700 mL of water and autoclaved for a minimum of 30 minutes. The autoclaved media was allowed to cool in a water bath for 45 minutes then poured into petri dishes. These R2A plates were allowed to sit for a minimum of two days before use to allow some hardening and drying to occur and to ensure that the plates were not biologically contaminated during pouring.

Biofilm samples from the reactors were homogenized using a Janke & Kunkel model T 25 S1 homogenizer for 30-60 seconds at  $20,500 \text{ min}^{-1}$  to break up clumps of the biofilms before they were diluted for plating. The homogenizer probe was soaked in alcohol, which was burned off, then rinsed in sterile dilution water between samples to reduce the risk of cross contamination. After homogenization, the biofilm, influent, and effluent samples were diluted one or more times by removing by transferring 1 mL to a sterile test tube containing 9 mL of water using aseptic techniques. This process was repeated for each

sample until an appropriate final dilution was achieved for that sample. Then three 0.1 mL samples were drawn from each dilution and spread on three separate R2A plates using aseptic techniques. All R2A plates containing samples were then incubated at room temperature for 7 days before the colonies on each were counted. Plates with the sterile dilution water and the rinse water from the homogenizer probe were also incubated as controls. After all samples had been plated, 0.2 mL of filter sterilized 33% formaldehyde was added to each sample tube. Tubes were refrigerated for a minimum of 24 hours to preserve the samples for total direct counts.

The average number of colony forming units per mL of sample was calculated based on the average number of colonies per plate, the dilution plated, and the volume plated. For the biofilm samples this number was converted to the average colony forming units per cm<sup>2</sup> of the coupon using the area of the coupon and the volume of sterile dilution water that the biofilms were scraped into.

#### Total Direct Counts

Samples for the total direct counts were vortexed for 1 minute to ensure thorough mixing, then vacuum filtered through 0.22 µm polycarbonate filters. The polycarbonate filters used in this study were black, 25 mm diameter filters manufactured by Osmonics, Inc. (material #1215609). 0.25 to 0.5 mL of 100 mg/L DAPI (4',6-diamidino-2-phenylindole) stain from Sigma was added and

allowed to stand on the filter for 15-20 minutes before being vacuum filtered. The filter was then fixed onto a microscope slide and cells in 20 randomly selected fields from the filter were counted under a UV light on the Nikon Eclipse E800 microscope. The average number of cells per field were calculated and used to calculate the average number of cells per filter based on the area of the counting field and the area through which the sample was filtered. The cells were counted with a 100x objective with a 10x/25 eyepiece.

### Chemical Analyses

#### Total Organic Carbon

Glassware and glass sample vials for the TOC analysis were soaked in a 36 normal sulfuric acid bath for no less than 8 hours, then rinsed repeatedly with ultrapure water and covered with aluminum foil. The glassware and sample vials were then baked for 48 hours at roughly 350°C. This process cleaned and sterilized the glassware and sample vials and ensured minimal carbon contamination. Volumetric flasks and graduated cylinders did not undergo baking as the high temperature tends to slightly warp glassware, which would affect the accuracy of the volume markings.

Samples for the TOC analysis were filtered through Fisherbrand 0.2  $\mu\text{m}$  nylon filters. A cleaning process was devised in prior research projects to minimize carbon contamination from these filters. The filters were rinsed with 30 mL of 0.1 N sodium hydroxide 3 times followed by 30 mL of ultrapure water 3

times. A fourth rinse with ultrapure water immediately preceded sampling and the filtrate was measured to ensure that the background carbon levels on the filters had been reduced to 100 ppb or less.

Samples for the TOC analysis were collected by inserting the tip of a sterile 30 mL syringe directly into the annular reactors or water holding tank. These samples were each filtered through the cleaned filters described above into the acid washed and baked sample vials. The samples were acidified with 0.2 mL of 2 N hydrochloric acid and stored at 4°C until measurement. TOC was measured as Non-Purgeable Organic Carbon on the Shimadzu TOC-5000A Total Organic Carbon Analyzer, using a high temperature catalytic method with a high sensitivity catalyst for low carbon analysis. Samples were sparged for 5 minutes during measurement to remove all inorganic and volatile organic carbon.

Prior to sample measurement of TOC, a standard curve was developed using potassium hydrogen phthalate ( $KC_8H_5O_4$ ), which was baked in an oven for at least one week until dehydration was achieved. 1.0626 g was then added to 1.0 L of ultrapure water in an acid washed and baked volumetric flask to create a stock solution with a concentration of 1000 mg/L C. 25 mL of this stock solution was then added to 1.0 L of ultrapure water to create a second stock solution with a concentration of 25 mg/L. This stock solution was then further diluted in separate volumetric flasks to obtain concentrations of 250, 500, 1000, 2000, and 4000  $\mu\text{g/L}$  C. A four point linear regression was performed using four of these concentrations to calibrate the Shimadzu for sample analysis. This calibration

was performed every time new standard solutions were made or instrument maintenance took place.

### Free/Total Chlorine

Free and total chlorine measurements were made using HACH method 80 with the HACH DR/2000 and DPD powder packets specific to each test. Reactors containing monochloramine as a disinfectant were maintained at 0.2 mg/L as total chlorine. Reactors containing chlorine as a disinfectant were maintained at 0.2 mg/L as free chlorine. Measurements were taken in each reactor every 2-3 days during phases 2, 3, and 4 ensure that these target levels were achieved. When significant deviations from these target levels were noticed, the monochloramine or chlorine concentrations in the feed jugs were increased or decreased accordingly.

### Statistical Analyses

A one-way analysis of variance (ANOVA) was performed using MINITAB™ version 13.2. The logarithms of the cell densities were entered as the response variable. The output from the analysis was a confidence interval for the difference between the actual means of each of the pairs of data sets that were compared. The null hypothesis was that the difference between the actual means was zero. Thus, if the null hypothesis (zero) fell within the confidence interval, the difference between the pair of data sets was not significant.

However, if the confidence interval fell completely above zero or completely below zero, the data sets were statistically significantly different.

All confidence intervals calculated as part of the one-way ANOVA were simultaneously correct with probability 0.95. The associated significance tests have a simultaneous Type I error rate of 0.05. The Bonferroni method was used to determine the individual error rate to be used in the statistical analyses; it divided the desired simultaneous error rate of 0.05 by the number of pairwise comparisons that were considered in each analysis. For example, when the effluents from the four reactors containing different materials were compared to each other in a given phase with a given disinfectant there were a total of six pairwise comparisons (6 equals the number of combinations of 4 things taking 2 at a time or  ${}_4C_2$  on the typical electronic calculator). Thus, the individual error rate was 0.0083 and the simultaneous error rate for the analysis was only 5%.

#### Bozeman Water Quality Data

Table 4 lists the water quality data for the finished water from the Bozeman drinking water treatment plant for the year 2000. Bozeman tap water was passed through GAC and BAC columns and then used as a source water in this study.

Table 4. Bozeman Water Quality Data

	MCL	Units	2000 Range	2000 Average
Alkalinity	N/A	ppm	50 - 103.8	83.73
Chlorine residual, free (>0.2)	4	ppm	1.17 - 1.92	1.54
Flouride (add to = 1.0)	4	ppm	0.00 - 1.38	1.01
Hardness, calcium	N/A	ppm	38 - 73	59.29
Calcium	N/A	ppm	15.2 - 29.2	23.71
Hardness, magnesium	N/A	ppm	17.5 - 42.8	29.45
Magnesium	N/A	ppm	4.27 - 10.45	7.35
Hardness, total	N/A	ppm	56.8 - 110.4	88.74
Hardness, total	N/A	grain/gal	3.32 - 6.46	5.19
pH	6.5-9.3	units	7.41 - 8.97	8.45
Sodium	20	ppm	1.32 - 7.94	3.5
Sulfate	500	ppm	0.00 - 9.30	0.95
Iron	0.3	ppm	0.01 - 0.21	0.04
Total dissolved soils	500	ppm	56.2 - 111.4	92.44
Turbidity (daily average)	0.5	NTU	0.03 - 0.18	0.06
Total coliforms	There were no positive samples in Bozeman's drinking water after treatment.			

## CHAPTER 4

## RESULTS

Total Organic Carbon Analyses

Table 5. Influent and Effluent DOC Results. The average, low, and high DOC concentrations ( $\mu\text{g/L C}$ ) are shown for all reactors in all phases.

Phase		Epoxy Epoxy		Iron		Cement Cement		PVC 1	PVC 2	Influent
		1	2	1	2	1	2			
1	Ave	883	884	800	790	892	915	643	631	912
	Lo	418	411	355	355	432	440	289	260	429
	Hi	1571	1593	1423	1389	1618	1593	1277	1314	1602
2	Ave	745	699	581	565	754	704	712	735	737
	Lo	629	610	426	491	658	616	618	652	645
	Hi	965	835	703	671	878	876	871	841	868
3	Ave	1198	1059	745	638	1010	961	849	873	619
	Lo	572	561	532	514	732	475	539	525	512
	Hi	4245	4114	929	782	1184	2558	1161	1059	784
4	Ave	2573	2556	2080	1793	3119	2553	2840	2711	1573
	Lo	1960	1899	1163	1124	2394	1420	1459	1619	1214
	Hi	3548	3584	3252	2642	4856	3766	4342	4445	2484

Table 5 shows the average, high, and low values for the dissolved organic carbon (DOC) measured in each reactor and the influent in each phase of the experiment. This table shows a visible increase in the DOC in all reactors between phases two and three and phases three and four. This corresponds to the addition of supplementary humic derived carbon in phase three and at a higher dose in phase four. Table 6 shows that this increase was primarily due to the supplementary carbon and not strictly to increases in the Bozeman tap water carbon concentration. In the first two phases, where no supplementary carbon was added, the effluent DOC concentrations are all nearly equal to or below the

level in the influent. In phases three and four all of the effluent DOC concentrations were greater than the influent DOC concentration.

Table 6. Comparison of Effluent DOC to Unsupplemented Influent DOC. The average effluent minus the average influent DOC concentrations ( $\mu\text{g/L C}$ ) are shown for all reactors in all phases.

Phase	Epoxy 1	Epoxy 2	Iron 1	Iron 2	Cement		PVC 1	PVC 2
					1	2		
1	-30	-28	-112	-122	-20	3	-269	-281
2	8	-38	-156	-172	17	-33	-26	-2
3	579	440	127	19	391	343	230	254
4	1001	983	507	220	1546	980	1268	1138

Table 6 also indicates the relative carbon depletion in the reactors. The only carbon entering the reactors came from the influent and the supplementary humic substances feed, which were fed to all reactors at the same mass flow rate. Since biomass was filtered out in the sampling process, differences in the values of the effluent DOC minus the influent DOC indicate differences in carbon depletion within the reactors. Lower values indicate greater carbon depletion in a reactor. In phase one, the greatest carbon utilization occurred in the reactors containing PVC, followed by the reactors containing iron. In phase one there was little difference between the carbon utilization in the epoxy and cement reactors. In all of the remaining phases the iron reactors utilized the most carbon. In phases two through four the order of ascendance varied among reactors containing materials other than iron.

### Reading Boxplots

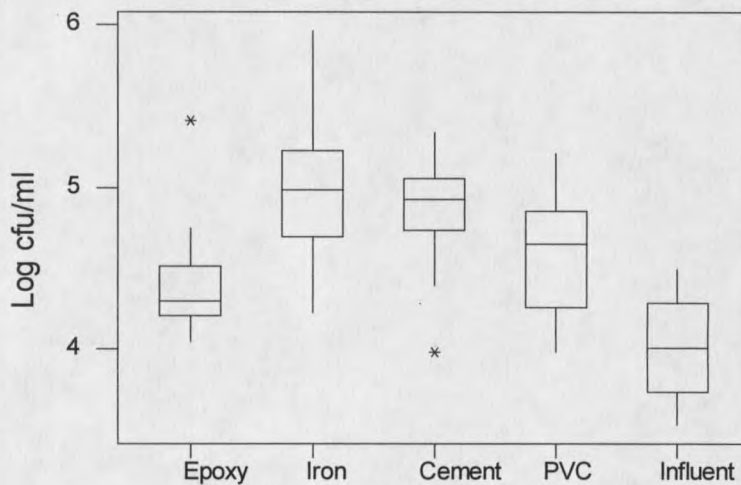
The boxplots used to describe the data that follows consist of three major features; a box, a horizontal line drawn through the box, a pair of vertical lines extending from the upper and lower ends of each box, and asterisks. The box portion of the plot represents the middle 50% of the observations. The line drawn through the box represents the median of the data. The lines extending from the box are called whiskers. These whiskers indicate the lowest and highest values in the data set (excluding outliers). The asterisks represent possible outliers in the data set. A data point is considered an outlier if it is outside of the box by more than 1.5 times the middle 50% of the observations.

### Influent/Effluent Heterotrophic Plate Counts

A statistical analysis of the mean influent heterotrophic plate counts (HPCs) in all of the phases showed that there was no significant difference between the influent HPCs in any of the phases. This means that fluctuations in the influent HPC concentrations were not a major factor influencing changes in the planktonic populations within the reactors between phases in the reactors. Thus, any variation in these populations can be attributed to experimental changes in the disinfectant and/or carbon levels in the reactors. The data from this analysis, and all other statistical analyses that were performed are included in the appendix.

Statistical analyses of the effluent HPCs in the control phase (phase one) showed that there were no significant differences between reactor pairs containing the same materials (data not shown). This indicates that the results from these reactors are repeatable. In addition, it was possible to use the average for each material in further statistical comparisons rather than making comparisons to individual reactors. The influent and effluent HPC data for each reactor type in phase one are illustrated in Figure 1.

Figure 1. HPC Influent and Effluent Densities in Phase 1.



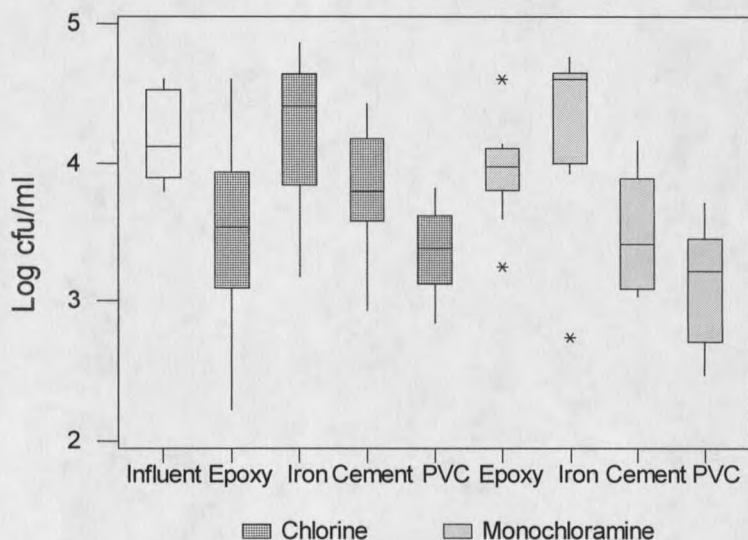
Statistical comparisons of the effluent cell concentrations for different materials in phase one indicated that only the iron and cement reactors did not significantly differ from each other (Figure 2). Also shown in this figure is that the

effluent counts did not differ by more than one logarithm ( $10^4$  cfu/mL). Another analysis showed that all of the effluent concentrations were significantly higher than the influent concentration (average of 4.01 log(cfu/mL)).

Figure 2. Statistical Comparisons of HPCs in Effluents from Reactors in Phase 1. Lines between materials indicate that no significant statistical differences were found.

Material	Epoxy	PVC	Cement	Iron
Ave. log(cfu/ml)	4.37	4.58	4.90	4.99

Figure 3. HPC Influent and Effluent Densities in Phase 2.



The influent and effluent HPC data for phase two are illustrated in Figure 3. In phase two, no significant differences were found between the effluents from reactors containing the same materials that were treated with monochloramine or

chlorine (data not shown). Figure 4 shows the results of statistical analyses of reactors containing different materials but treated with the same disinfectants. Only two similarities were observed between reactors treated with monochloramine and those treated with chlorine. The first was that the reactors containing iron had significantly higher microbial counts in the effluents than those containing PVC, which were approximately one logarithm (log) lower. The second was that the reactors containing cement did not have significantly different effluent counts than those containing PVC.

Figure 4. Statistical Comparisons of HPCs in Effluents from Reactors in Phase 2. Lines between materials indicate that no significant statistical differences were found.

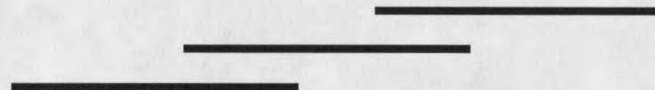
(a) Reactors treated with chlorine.

Material	PVC	Epoxy	Cement	Iron
Ave. log(cfu/ml)	3.37	3.48	3.81	4.23



(b) Reactors treated with monochloramine.

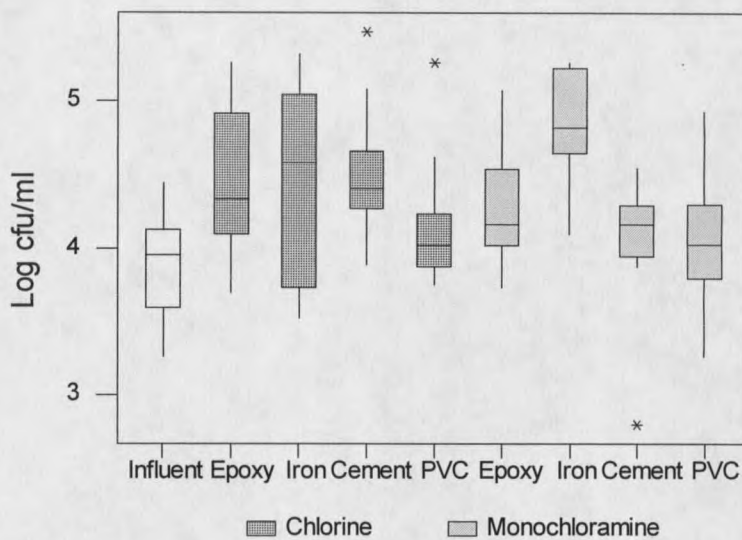
Material	PVC	Cement	Epoxy	Iron
Ave. log(cfu/ml)	3.12	3.51	3.94	4.27



Statistical comparisons of the reactor effluents to the influent in phase two showed that there was no significant difference between the influent (4.18 log(cfu/mL)) and the effluent from the iron reactors treated with either monochloramine or chlorine. The effluents from the cement reactor treated with chlorine and the epoxy reactor treated with monochloramine were also found to

be not statistically different from the influent. However, the effluents from the remaining reactors were found to be significantly lower than the influent with differences ranging from 0.7 to 1.0 logs.

Figure 5. HPC Influent and Effluent Densities in Phase 3.



The influent and effluent HPC data for phase three are illustrated in Figure 5. In phase three, no statistically significant differences were found between any pairs of reactors containing the same materials that were treated with monochloramine or chlorine (data not shown). In addition, no statistically significant differences were found between reactors containing different materials that were treated with free chlorine (Figure 6). Figure 6 also shows the results of statistical analyses of reactors containing different materials that were treated

with monochloramine. Among these reactors, the iron had significantly higher (0.8 logs) effluent counts than the PVC and cement, while all other differences in effluent counts were not statistically different.

Figure 6. Statistical Comparisons of HPCs in Effluents from Reactors in Phase 3. Lines between materials indicate that no significant statistical differences were found.

(a) Reactors treated with chlorine.

Material	PVC	Epoxy	Iron	Cement
Ave. log(cfu/ml)	4.15	4.45	4.46	4.49

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(b) Reactors treated with monochloramine.

Material	PVC	Cement	Epoxy	Iron
Ave. log(cfu/ml)	4.04	4.07	4.26	4.83

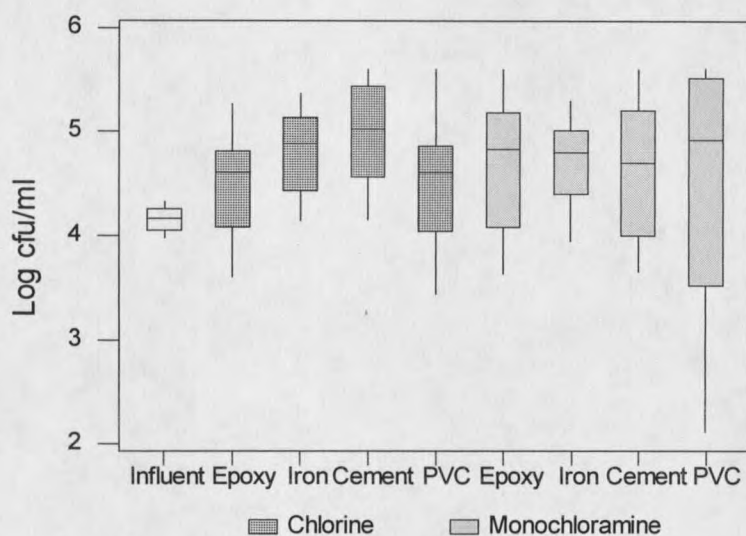
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Statistical comparisons of the reactor effluents to the influent in phase three showed that there was no significant difference between the influent and the effluent from the PVC reactors treated with either monochloramine and chlorine. The remaining reactors treated with chlorine had significantly higher cell counts (0.6 logs) in their effluents than in the influent (3.87 log(cfu/mL)). All of the effluent counts from the remaining reactors treated with monochloramine were not statistically different from the influent except for the effluent from the iron reactor, which was higher than the influent by about 1 log.

The influent and effluent HPC data for phase four are illustrated in Figure 7. In phase four no statistically significant differences were found between the

effluents from reactors containing the same materials that were treated with either chlorine or monochloramine (data not shown). In addition, no statistical differences were reported between the effluents from reactors containing different materials and treated with the same disinfectant, either chlorine or monochloramine (Figure 8).

Figure 7. HPC Influent and Effluent Densities in Phase 4.



When the effluents from each of the reactors in phase four were compared to the influent (4.15 log(cfu/mL)), no significant statistical differences were found except for the iron and cement reactors that were treated with chlorine (data not shown). The effluents from these reactors were both found to contain higher cell concentrations than the influent by about 0.7 and 0.8 logs, respectively.

Figure 8. Statistical Comparisons of HPCs in Effluents from Reactors in Phase 4. Lines between materials indicate that no significant statistical differences were found.

(a) Reactors treated with chlorine.

Material	PVC	Epoxy	Iron	Cement
Ave. log(cfu/ml)	4.49	4.50	4.81	4.99

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(b) Reactors treated with monochloramine.

Material	PVC	Cement	Epoxy	Iron
Ave. log(cfu/ml)	4.50	4.68	4.70	4.70

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Figure 9 illustrates the statistical relationships between the phases for reactors treated with the same disinfectants. For all materials with either monochloramine or chlorine as a disinfectant, statistical analyses showed that there was a significant difference between phase one and phase two, where phase one was greater than phase two. In most cases these analyses also showed that there was a significant difference between phases two and three, where phase three was greater than phase two. The exceptions were the iron reactors and the epoxy reactor treated with monochloramine for which phase two was not significantly different from phase three. On the other hand, the analyses showed that there was no significant difference in the effluent HPCs between phase one and phase four. In addition, in most cases, there was no significant difference between phases three and four. The exception was the cement reactors where phase four was greater than phase three.

Figure 9. Statistical Comparisons of Effluent HPCs Between Phases. Lines between materials indicate that no statistically significant differences were found.

(a) Epoxy reactor treated with chlorine.

Phase	2	1	3	4
Ave. log(cfu/ml)	3.48	4.37	4.45	4.50

---

(b) Epoxy reactor treated with monochloramine.

Phase	2	3	1	4
Ave. log(cfu/ml)	3.94	4.26	4.37	4.70

---

(c) Iron reactor treated with chlorine.

Phase	2	3	4	1
Ave. log(cfu/ml)	4.23	4.46	4.81	4.99

---

(d) Iron reactor treated with monochloramine.

Phase	2	4	3	1
Ave. log(cfu/ml)	4.27	4.70	4.83	4.99

---

(e) Cement reactor treated with chlorine.

Phase	2	3	1	4
Ave. log(cfu/ml)	3.81	4.49	4.90	4.99

---

(f) Cement reactor treated with monochloramine.

Phase	2	3	4	1
Ave. log(cfu/ml)	3.51	4.07	4.68	4.90

---

(g) PVC reactor treated with chlorine.

Phase	2	3	4	1
Ave. log(cfu/ml)	3.37	4.15	4.49	4.58

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(h) PVC reactor treated with monochloramine.

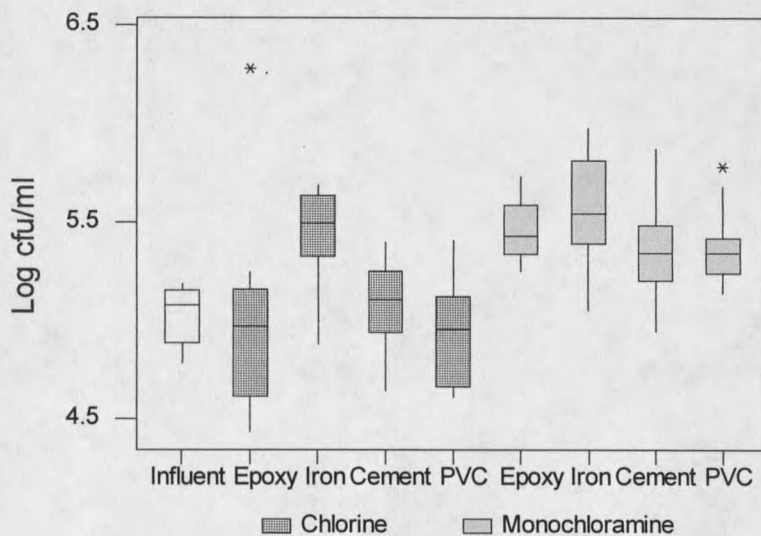
Phase	2	3	4	1
Ave. log(cfu/ml)	3.1215	4.0490	4.5038	4.5839

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### Influent/Effluent Total Direct Counts

A statistical analysis of the total direct counts (TDCs) in the influent showed that only the counts in phases two and three were comparable. This analysis also showed that the influent counts in phase four were significantly greater than those in both phase two and phase three by about 0.3 and 0.4 logs, respectively.

Figure 10. TDC Influent and Effluent Densities in Phase 2.



The influent and effluent TDC data for phase two are illustrated in Figure 10. In phase two, no significant differences were found between the effluents from the iron reactors or the cement reactors when treated with either monochloramine or chlorine. However, the epoxy and PVC reactors treated with

monochloramine had statistically significantly higher effluent counts than their pairs treated with free chlorine by about 0.4 to 0.5 logs.

Figure 11. Statistical Comparisons of TDCs in Effluents from Reactors in Phase 2. Lines between materials indicate that no significant statistical differences were found.

(a) Reactors treated with chlorine.

Material	PVC	Epoxy	Cement	Iron
Ave. log(cfu/ml)	4.93	4.99	5.08	5.45

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(b) Reactors treated with monochloramine.

Material	Cement	PVC	Epoxy	Iron
Ave. log(cfu/ml)	5.37	5.37	5.46	5.55

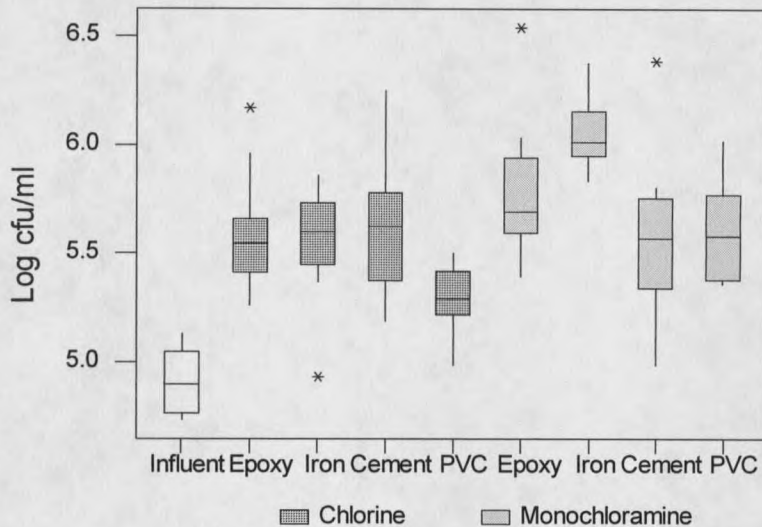
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Statistical analyses comparing reactors of different materials that were treated with the same disinfectant found no significant differences between any of the reactors treated with monochloramine. Figure 11 shows the results of this analysis for reactors containing different materials that were treated with the same disinfectant. These results show that among the reactors treated with chlorine there were no significant differences between the effluent counts from the PVC, cement, and epoxy reactors, but that all three were significantly lower than the iron reactor.

Statistical comparisons of the effluents to the influent (5.04 log(cfu/mL)) in phase two showed that only the epoxy reactor treated with monochloramine (5.46 log(cfu/mL)) and the iron reactors treated with either chlorine (5.45 log(cfu/mL)) or monochloramine (5.55 log(cfu/mL)) were significantly different

from the effluent. The mean effluent counts from all three of these reactors were greater than the mean influent count.

Figure 12. TDC Influent and Effluent Densities in Phase 3.



The influent and effluent TDC data for phase three are illustrated in Figure 12. In phase three, no significant differences between the effluents from the epoxy reactors or the cement reactors when treated with either monochloramine or chlorine were found. However, these analyses showed that the iron and PVC reactors treated with monochloramine had significantly higher effluent counts than their pairs treated with chlorine.

Figure 13 shows the results of statistical analyses comparing reactors of different materials that were treated with the same disinfectant. These results

show that the only significant difference in the effluent counts from reactors treated with free chlorine was between the cement and PVC reactors. In this case the cement reactor had a higher mean effluent count than the PVC reactor. Among the reactors treated with monochloramine, the only significant differences were between the iron and PVC reactors and the cement and iron reactors. Here, the iron reactor had the highest effluent. Statistical comparisons of the effluent counts to the influent count (4.91 log(cfu/mL)) showed that all of the effluents were significantly higher than the influent by about 0.6 to 1.2 logs.

Figure 13. Statistical Comparisons of TDCs in Effluents from Reactors in Phase 3. Lines between materials indicate that no significant statistical differences were found.

(a) Reactors treated with chlorine.

Material	PVC	Iron	Epoxy	Cement
Ave. log(cfu/ml)	5.30	5.56	5.59	5.63



(b) Reactors treated with monochloramine.

Material	Cement	PVC	Epoxy	Iron
Ave. log(cfu/ml)	5.57	5.59	5.77	6.06



The influent and effluent TDC data for phase four are illustrated in Figure 14. In phase four no statistically significant differences were found between the effluents from reactors containing the same materials that were treated with either chlorine or monochloramine. In addition, Figure 15 shows that no statistical differences were reported between the effluents from reactors

containing different materials and treated with the same disinfectant (chlorine or monochloramine). Statistical analyses also showed that the effluent counts from all of the reactors in phase four were significantly higher than the influent (5.28 log(cfu/mL)) by about 0.5 to 0.8 logs.

Figure 14. TDC Influent and Effluent Densities in Phase 4.

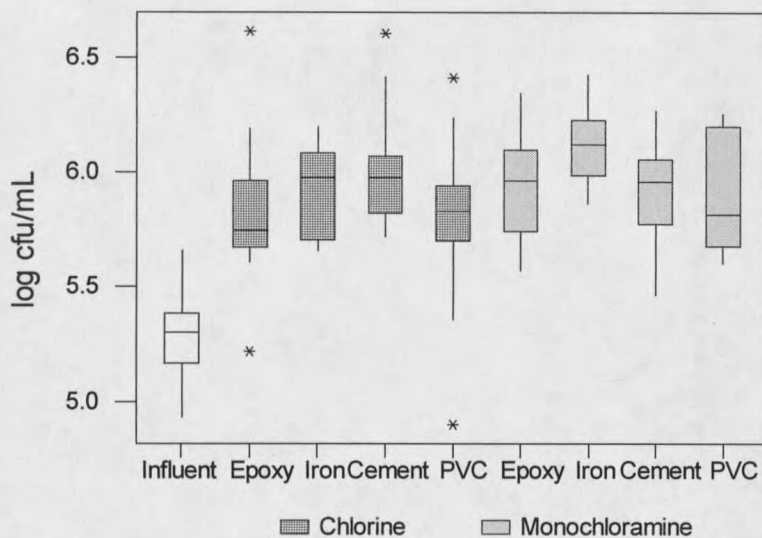


Figure 15. Statistical Comparisons of TDCs in Effluents from Reactors in Phase 4. Lines between materials indicate that no significant statistical differences were found.

(a) Reactors treated with chlorine.

Material	PVC	Epoxy	Iron	Cement
Ave. log(cfu/ml)	5.80	5.82	5.92	6.01

(b) Reactors treated with monochloramine.

Material	PVC	Epoxy	Cement	Iron
Ave. log(cfu/ml)	5.90	5.93	5.92	6.11

Figure 16. Statistical Comparisons of Effluent TDCs Between Phases. Lines between materials indicate that no statistically significant differences were found.

(a) Epoxy reactor treated with chlorine.

Phase	2	3	4
Ave. log(cfu/ml)	4.99	5.59	5.82

---

(b) Epoxy reactor treated with monochloramine.

Phase	2	3	4
Ave. log(cfu/ml)	5.46	5.77	5.93

---

(c) Iron reactor treated with chlorine.

Phase	2	3	4
Ave. log(cfu/ml)	5.45	5.56	5.92

---

(d) Iron reactor treated with monochloramine.

Phase	2	3	4
Ave. log(cfu/ml)	5.55	6.06	6.12

---

(e) Cement reactor treated with chlorine.

Phase	2	3	4
Ave. log(cfu/ml)	5.08	5.53	6.01

(f) Cement reactor treated with monochloramine.

Phase	2	3	4
Ave. log(cfu/ml)	5.37	5.57	5.92

---

(g) PVC reactor treated with chlorine.

Phase	2	3	4
Ave. log(cfu/ml)	4.93	5.30	5.80

(h) PVC reactor treated with monochloramine.

Phase	2	3	4
Ave. log(cfu/ml)	5.37	5.59	5.90

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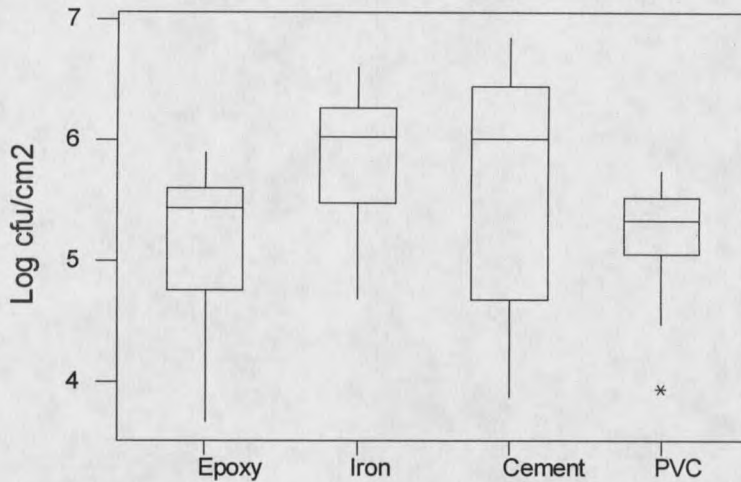
Figure 16 illustrates the statistical relationships between phases of reactors treated with the same disinfectants. The analyses show that for the cement reactors and PVC reactors treated with chlorine, there were significant differences between the effluents in all three phases. The trends in the data for all of the combinations of materials and disinfectants show that, in general, the order of increasing effluent TDCs is as follows: phase two is less than or equal to phase three, which was less than or equal to phase four. For all materials treated with either disinfectant the effluent concentrations in phase four were significantly different from those in phase two.

#### Biofilm Heterotrophic Plate Counts

Statistical analyses of the HPCs for biofilms in phase one showed that there were no significant differences between reactors containing the same materials. This indicates that the results from these reactors are repeatable. In addition, it was possible to use the average for each material in further statistical comparisons rather than making comparisons to individual reactors.

Statistical analyses of the biofilms in all phases showed that there was no significant difference between growth on any of the new slides and the old slides in the same reactors. Thus, in further statistical analyses the results from the new and the old slides were combined for each reactor to simplify the comparisons.

Figure 17. HPC Biofilm Densities in Phase 1.



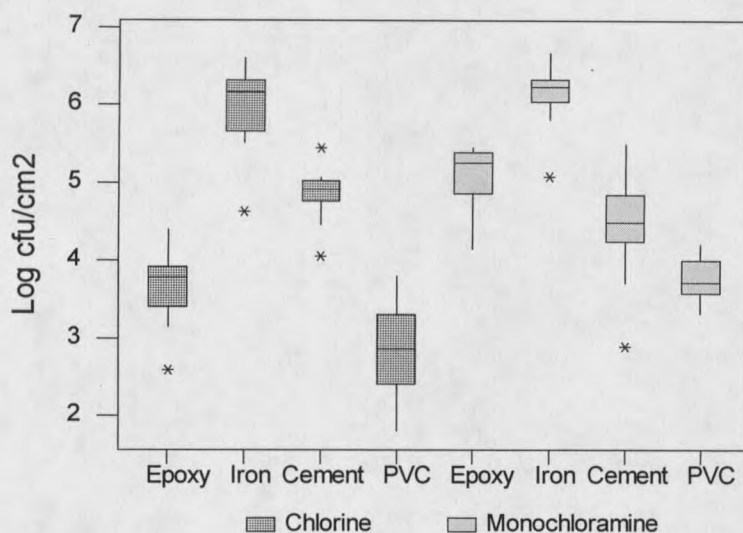
The HPC biofilm data for phase one are illustrated in Figure 17. In phase one, no statistical differences were found between pairs of reactors containing the same materials. The results of statistical comparisons of reactors with different materials are shown in Figure 18. The cement and iron were not significantly different from each other, nor were the PVC and cement, or the epoxy and PVC. Of the remaining comparisons, the iron was greater than the PVC and the epoxy, and the cement was greater than the epoxy.

Figure 18. Statistical Comparisons of Biofilm HPCs in Phase 1. Lines between materials indicate that no statistically significant differences were found.

Material	Epoxy	PVC	Cement	Iron
Ave. log(cfu/cm <sup>2</sup> )	5.14	5.26	5.58	5.90

Horizontal lines below the table indicate no significant differences between Epoxy and PVC, Epoxy and Cement, PVC and Cement, and Epoxy and Iron. A line between Cement and Iron indicates a significant difference.

Figure 19. HPC Biofilm Densities in Phase 2.



The HPC biofilm data for phase two are illustrated in Figure 19. In phase two, no statistically significant differences were found between iron or cement reactors that were treated with the same disinfectant. However, the biofilm densities in the epoxy and PVC reactors treated with monochloramine were greater than biofilm densities in reactors with the same materials that were treated with chlorine. Statistical analyses of the biofilms in reactors with different materials that were treated with the same disinfectant showed that all of the reactors were significantly different from each other (Figure 20). The highest counts were always found in the iron reactors while the lowest counts were always found in the PVC reactors.

Figure 20. Statistical Comparisons of Biofilm HPCs in Phase 2. Lines between materials indicate that no statistically significant differences were found.

(a) Reactors treated with chlorine.

Material	PVC	Epoxy	Cement	Iron
Ave. log(cfu/cm <sup>2</sup> )	2.84	3.66	4.86	5.97

(b) Reactors treated with monochloramine.

Material	PVC	Cement	Epoxy	Iron
Ave. log(cfu/cm <sup>2</sup> )	3.76	4.46	5.11	6.14

The HPC biofilm data for phase three are illustrated in Figure 21. In phase three, statistical analyses of pairs of reactors of the same materials that were treated with different disinfectants showed no significant differences between any of the four pairs.

Figure 21. HPC Biofilm Densities in Phase 3.

