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To assess the interactions between pipe materials, organic carbon levels, and disinfectants, studies using annular reactors with ductile-iron, polyvinyl chloride (PVC), epoxy, and cement-lined coupons were carried out in the laboratory and at four field sites. Laboratory studies used biologically treated water with and without 0.2 mg/L residual free chlorine or monochloramine, in the presence or absence of 0.5 or 2.0 mg/L humic substances. In the lab studies, the type of disinfectant did not lead to significant differences in effluent and biofilm counts. Increases in carbon led to greater numbers of biofilm and effluent organisms, with the effect most pronounced on iron. Regardless of carbon level, PVC systems typically had the lowest numbers of bacteria, whereas iron had the highest. Cement and epoxy were intermediate. Depending on the site, field studies showed that iron had the highest number of bacteria or there was no difference in materials.

Effect OF DISTRIBUTION SYSTEM MATERIALS on bacterial regrowth

Solving bacterial regrowth problems (e.g., positive coliforms) has historically been linked to improving source water treatment or optimizing secondary disinfection in the distribution system. Specifically, most water systems have battled their bacterial regrowth problems by increasing disinfectant residual, converting from free chlorine (Cl_2) to chloramine, or installing source water treatment processes to decrease the concentration and/or change the nature of the organic matter entering the distribution system. All of these approaches rely on modifying water quality parameters. Another possible solution may be in the rehabilitation or replacement of distribution system infrastructure.

A growing body of data strongly indicate that the amount of attached bacteria on iron pipe can be several orders of magnitude higher than on other materials, such as cement linings, polyvinyl chlorinde (PVC), or polyethylene (Niquette et al, 2000; Kerr et al, 1999; LeChevallier et al, 1998; Delanou et al, 1997;

TABLE 1 **Water quality conditions for the four phases of the laboratory experiments**

Phase	Granular Activated Carbon/ Biologically Activated Carbon Water	Nitrogen/ Phosphorus	Disinfectant*	Humics-derived Carbon
1 (Control)	Yes	Yes	No	No
2	Yes	Yes	4 reactors with chlorine	No
			4 reactors with monochloramine	
3	Yes	Yes	4 reactors with chlorine	0.5 mg/L DOC†
			4 reactors with monochloramine	
4	Yes	Yes	4 reactors with chlorine	2 mg/L DOC
			4 reactors with monochloramine	

*Target of 0.2 mg/L effluent concentration
†DOC—dissolved organic carbon

LeChevallier, 1997; Neden et al, 1992). These data suggest that the amount of bacterial regrowth depends on the relationship between the type of pipeline materials and the specific water quality in the distribution system—which is a function of source water characteristics and treatment—but there has been no systematic study of these variables. In addition, much attention has been given to the ability of disinfectants (Cl_2 and chloramine) in the distribution system to reduce bacterial regrowth. It is generally believed that increasing the concentration of a disinfectant should control regrowth, but in many instances, the opposite effect has been seen (LeChevallier et al, 1987; Oliveri et al, 1985; Reilly & Kippen, 1984; Hudson et al, 1983; Martin et al, 1982). In addition, this practice typically promotes excessive disinfection by-product formation and offensive tastes and odors.

The goal of this project was to examine the interactions between distribution system materials, organics, and disinfectants, and their effects on bacterial regrowth. The relationships from this study can be used to develop recommendations for utilities to reduce regrowth in their distribution systems by relining and replacing aging pipe sections or attempting to optimize finished water quality. This goal was addressed using annular reactors in controlled laboratory experiments and field study evaluations.

MATERIALS AND METHODS

Laboratory experiments. The laboratory setup consisted of four pairs of annular reactors[1] each containing 20 coupons of one of four common pipe materials (epoxy, ductile iron, cement, or PVC). Details on the reactor can be found in Sharp et al (2001). These reactors have a variable-speed rotating drum, a volume of approximately 1 L, and a high surface area–to-volume ratio. The rotational speed of the reactors was set at approximately 90 rpm to simulate the shear stress in a 4 in. (100 mm) pipe with a fluid velocity of 1 ft/s (0.3 m/s). Flow rates into the reactors were set so that a total hydraulic residence time of 2 h was maintained.

Tap water from Bozeman, Mont., flowed through a granular activated carbon (GAC) column and then through a biologically activated carbon (BAC) column into a 2-L holding tank. The columns were operated in an upflow mode. The GAC and BAC columns removed Cl_2 and some organic carbon (C) from the Bozeman tap water and produced an influent of consistent biological and chemical quality for the annular reactors.

The laboratory work was divided into four phases (Table 1), with each lasting a minimum of three to four months to allow the processes in the annular reactors to approach equilibrium. Sampling was initiated approximately one week after the start of each phase. The annular reactors were fed with the processed Bozeman tap water and a nitrogen/phosphate solution at a ratio of 100:10:1 (C:N:P) to ensure that the reactors were C-limited. Nitrogen and phosphorus (P) levels were calculated based on the background dissolved organic carbon (DOC) as well as the amounts added as humic substances. In the first control phase, nothing else was added to the reactors. In the second, third, and fourth phases, Cl_2 was added to one reactor and monochloramine was added to the other reactor in each coupon material pair to achieve target effluent concentrations of 0.2 mg/L as free Cl_2 and total Cl_2, respectively. In the third phase, humics-derived C was added to all reactors at a concentration of 0.5 mg/L DOC. In the fourth phase, the humics-derived C was increased to 2 mg/L DOC.

In phase 1, fresh coupons were used in all 20 slots on the reactor drum. At the beginning of phase 2, alternate coupons on the drum were removed and replaced with new coupons. At the beginning of phase 3, all coupons that were not removed at the beginning of phase 2 were replaced with fresh coupons. At the beginning of phase 4, the same coupons that were replaced at the beginning of phase 2 were again replaced with fresh coupons. Thus, in each of the last three phases, 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 established biofilms. New and old coupons were sampled in alternate weeks. Statistical

TABLE 2 Influent water quality to field study annular reactors

Parameter	Utility 1		Utility 2		Utility 3		Utility 4
	Unfiltered	Filtered	Treatment Plant	Distribution System	Before DAF*	After DAF	
pH	8.8 (8.2–9.2)	9.0 (8.6–9.2)	8.9 (8.5–9.2)	8.8 (8.3–9.0)	7.6 (6.8–8.3)	7.5 (6.3–9.2)	7.9 (7.1–8.1)
Temperature—*°C*	10.5 (0.5–19.7)	11.2 (1.2–21.5)	16.3 (3–26)	17.5 (5–27)	14.6 (8.0–24.0)	19.0 (10.2–26.7)	18.4 (8.0–26.7)
Alkalinity—*mg/L* $CaCO_3$†	28 (25–31)	37 (34–43)	71.4 (55–86)	69.9 (55–78)	10.8 (9.5–14.5)	10.4 (5.0–16.0)	219 (197–239)
TOC‡—*mg/L*	2.0 (2.0–2.1)	††	0.8 (0.501.3)	0.8 (0.6–1.3)	1.4 (1.3–1.5)	0.9 (0.5–1.3)	3.9 (3.4–4.2)
HPC§—*cfu**/mL*			5.7 (0–160)	47.3 0–1,200)			
Free chlorine—*mg/L*	0.1 (0–0.1)	0.4 (0.3–0.5)					
Total chlorine—*mg/L*	2.1 (1.7–2.5)	1.6 (1.4–1.9)	1.2 (0.4–4.3)	0.7 (0.4–1.2)	2.6 (1.6–3.5)	3.0 (1.5–4.4)	3.4 (2.1–3.8)

*Dissolved-air flotation
†$CaCO_3$—calcium carbonate
‡TOC—total organic carbon
§HPC—heterotrophic plate count
**cfu—colony-forming units
††Data not available

analyses showed that there were no differences in biofilm counts on the new and old coupons (data not shown); therefore, the data were combined.

Cl_2 preparation. The Cl_2 feed solution was prepared from household bleach (sodium hypochlorite [NaOCl]) containing no additives or buffers. The appropriate dilution of bleach for each reactor was determined by trial and error to achieve a 0.2-mg/L residual of free Cl_2 in the reactors, and the Cl_2 feed was operated at a flow rate of approximately 0.25 mL/min into each treated reactor.

Nitrogen/P solution. Potassium nitrate was used as the nitrogen source, and P was derived from equimolar concentrations of dibasic and monobasic potassium phosphate. A stock solution was created so that 1 mL of filter-sterilized stock solution was added per litre of autoclaved high-quality water in the feed solution. The flow rate of this feed solution into each reactor was approximately 0.25 mL/min.

The highest biofilm heterotrophic plate counts were always found in the iron reactors, whereas the lowest were on polyvinyl chloride.

Monochloramine preparation. A phosphate buffer was made by adding 0.5 g of dibasic potassium phosphate to 1 L of high-quality water in a sterile glass bottle. The pH was then adjusted to between 8.9 and 9.2 using 0.1 normal (N) sodium hydroxide (NaOH). In a separate container, 0.11 g of ammonium chloride was added to 100 mL of the phosphate buffer and mixed. While the solution continued to be mixed, 1 mL of a ~4% NaOCl bleach solution was added at a rate of no more than 200 µL/min. The solution was stirred for 30 min before the Cl_2 residuals were measured.

The appropriate dilution of the monochloramine stock solution for each reactor was determined by trial and error to achieve an approximate 0.2-mg/L residual of total Cl_2 in the reactors. The monochloramine feed was operated at a flow rate of approximately 0.25 mL/min into each treated reactor.

Humics solution preparation. The humics solution was prepared using Elliot Silt Loam, obtained from the International Humic Substances Society, that has been used extensively in research projects at the Center for Biofilm Engineering (Butterfield et al, 2002a; Butterfield et al, 2000b; Ellis et al, 2000). Further information on the justification for using humics as a C source for drinking water biofilms can be found in Camper et al (2000). All glassware used in the preparation of the humics solution was baked at 260°C to minimize organic C contamination. One hundred grams of this soil was added to 1 L of 0.1 N NaOH. This soil solution underwent constant mixing for two to four days, after which it was centrifuged at a *g* factor of 4,000 (39,240 m/s^2) for 20 min. The supernatant was then poured into a glass bottle and a small

TABLE 3 **Depletion of DOC across the reactors calculated from the average of all paired influent/effluent DOC readings for the duration of the experiment***

Phase	Epoxy 1	Epoxy 2	Iron 1	Iron 2	Cement 1	Cement 2	PVC 1	PVC 2
1—*no humics*	30	28	112	122	20	-3	269	281
2—*no humics*	–8	38	156	172	-17	33	26	2
3—*0.5 mg/L humics*	–79	60	374	481	109	158	270	246
4—*2 mg/L humics*	1,001	1,017	1,493	1,780	454	1,020	733	862

*Values are in µg/L carbon. Disinfectant was added in phases 2, 3, and 4, with chlorine added to those designated "1" and monochloramine added to those designated "2." DOC—dissolved organic carbon

sample was removed and diluted with high-quality water for determination of the organic C concentration.

After the humics were added to the high-purity water in the feed jug to attain the desired concentration, the pH was adjusted with 2 N hydrochloric acid (HCl) to match that of the influent as closely as possible. The flow rate of the humics feed into the reactors was approximately 0.25 mL/min.

Heterotrophic plate counts (HPCs). One coupon was removed for sampling from each reactor about once each week during each phase. The surface was scraped using a flat-headed spatula into a sterile 100-mL beaker with 10 mL of sterilized water. The contents 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. Effluent samples of 10 mL were drawn directly from the inside of the annular reactors. Because the fluid in the annular reactors is thoroughly mixed, the effluent concentrations are the same as the concentrations inside the reactors.

Biofilm samples were homogenized[2] for 30–60 s at 20,500 rpm to break up biofilm clumps according to method E2196-02 (ASTM, 2002). After homogenization, the biofilm, influent, and effluent samples were diluted as needed and spread on three separate R2A plates using aseptic techniques (*Standard Methods*, 1998). Plates were then incubated at room temperature for seven days.

The average number of colony-forming units per millilitre of sample was calculated. For the biofilm samples, this number was converted to the average colony-forming units per square centimetre of the coupon using the area of the coupon and the volume of sterile dilution water used.

DOC. Samples 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 cleaned filters into the acid-washed and baked sample vials. Glassware and glass sample vials for the DOC analysis were soaked in a 36 N sulfuric acid bath for at least 8 h, then rinsed repeatedly with high-quality water and covered with aluminum foil. The glassware and sample vials were then baked for 48 h at 350°C. Filtration was done through 0.2-µm nylon filters.[3] The filters were rinsed with 30 mL of 0.1 N NaOH three times followed by 30 mL of high-quality water three times. A fourth rinse with high-quality water immediately preceded sampling, and the filtrate was measured to ensure that the background C levels on the filters had been reduced to 100 µg/L or less.

This indicates that the disinfectant level of 0.2 mg/L was sufficient to control the increased growth at the highest dissolved organic carbon level in epoxy, cement, and polyvinyl chloride reactors compared with the control phase but not on iron.

The samples were acidified with 0.2 mL 2 N HCl and stored at 4°C until measurement. DOC was measured as nonpurgeable organic C on a total organic carbon (TOC) analyzer[4] using a high-temperature catalytic method with a high-sensitivity catalyst for low C analysis. Samples were sparged for 5 min during measurement to remove all inorganic and volatile organic C.

Prior to sample measurement of DOC, a standard curve was developed using dehydrated potassium hydrogen phthalate ($KC_8H_5O_4$). A four-point linear regression was performed using four concentrations to calibrate the TOC analyzer for sample analysis. This calibration was performed every time new standard solutions were made or instrument maintenance took place.

Free/total Cl_2. Free and total Cl_2 measurements were made according to photometer method 80 (Hach Co., 1997) using a spectrophotometer[5] and DPD powder packets specific to each test. Measurements were taken in each reactor every two to three days during phases 2, 3, and 4 to ensure that these target levels were achieved. When significant deviations from these target levels were mea-

TABLE 4 ANOVA* pairwise statistical comparisons of HPC† in effluents from reactors‡

Phase 1				
Material	Epoxy	PVC	Cement	Iron
Average log—*cfu§/mL*	4.37	4.58	4.90	4.99
Phase 2				
1. Reactors treated with chlorine				
Material	PVC	Epoxy	Cement	Iron
Average log—*cfu/mL*	3.37	3.48	3.81	4.23
2. Reactors treated with monochloramine				
Material	PVC	Cement	Epoxy	Iron
Average log—*cfu/mL*	3.12	3.51	3.94	4.27
Phase 3				
1. Reactors treated with chlorine				
Material	PVC	Epoxy	Iron	Cement
Average log—*cfu/mL*	4.15	4.45	4.46	4.49
2. Reactors treated with monochloramine				
Material	PVC	Cement	Epoxy	Iron
Average log—*cfu/mL*	4.04	4.07	4.26	4.83
Phase 4				
1. Reactors treated with chlorine				
Material	PVC	Epoxy	Iron	Cement
Average log—*cfu/mL*	4.49	4.50	4.81	4.99
2. Reactors treated with monochloramine				
Material	PVC	Cement	Epoxy	Iron
Average log—*cfu/mL*	4.50	4.68	4.70	4.70

*ANOVA—a one-way analysis of variance
†HPC—heterotrophic plate count
‡Lines between materials indicate that no significant statistical differences were found.
§cfu—colony-forming units

sured, the monochloramine or Cl_2 concentrations in the feed jugs were increased or decreased accordingly.

Statistical analyses. A one-way analysis of variance (ANOVA) was performed using data analysis software.[6] The logarithms of the cell numbers 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 a probability of 0.95. The associated significance tests have a simultaneous type 1 error rate of 0.05. The Bonferroni method (Neter et al, 1996) 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 with one another in a given phase with a given disinfectant, there was a total of six pairwise comparisons (six equals the number of combinations of four things taking two at a time). Thus, the individual error rate was 0.0083, and the simultaneous error rate for the analysis was only 5%.

Pairwise analyses were made within phases and between phases for disinfectants and material type for both effluent and biofilm counts. Selected pairwise comparisons are shown in the tables.

Field experiments. Field studies incorporating annular reactors[7] with three materials (ductile iron, cement lining,

TABLE 5 ANOVA* pairwise statistical comparisons of effluent HPC† between phases‡

1. Epoxy reactor treated with chlorine				
Phase	2	1	3	4
Average log—*cfu§/mL*	3.48	4.37	4.45	4.50
2. Epoxy reactor treated with monochloramine				
Phase	2	3	1	4
Average log—*cfu/mL*	3.94	4.26	4.37	4.70
3. Iron reactor treated with chlorine				
Phase	2	3	4	1
Average log—*cfu/mL*	4.23	4.46	4.81	4.99
4. Iron reactor treated with monochloramine				
Phase	2	4	3	1
Average log—*cfu/mL*	4.27	4.70	4.83	4.99
5. Cement reactor treated with chlorine				
Phase	2	3	1	4
Average log—*cfu/mL*	3.81	4.49	4.90	4.99
6 Cement reactor treated with monochloramine				
Phase	2	3	4	1
Average log—*cfu/mL*	3.51	4.07	4.68	4.90
7. PVC reactor treated with chlorine				
Phase	2	3	4	1
Average log—*cfu/mL*	3.37	4.15	4.49	4.58
8. PVC reactor treated with monochloramine				
Phase	2	3	4	1
Average log—*cfu/mL*	3.12	4.05	4.50	4.58

*ANOVA— a one-way analysis of variance
†HPC—heterotrophic plate count
‡Lines between materials indicate that no significant statistical differences were found.
§cfu—colony-forming unit

and epoxy) were conducted concurrently with the laboratory experiments described previously. The field studies took place at four utility locations over an 18-month period. Table 2 describes the field study reactor setups and the water quality conditions at these utility locations. These utilities were chosen for the following reasons: (1) there were before and after treatment changes (utilities 1 and 3) or travel time through a distribution system (utility 2) that influenced organic C concentrations; (2) there were high (utility 4) and low (utilities 2 and 3) organic C concentrations; and (3) there were differences in secondary disinfection. These variables were analogous to those used in the laboratory studies.

At utility 1, six annular reactors were installed at an existing aboveground valve station located at the head of the distribution system. Two reactors were allocated for each of the three materials. At the beginning of this study, the influent to the reactors was unfiltered water treated with Cl_2 as a primary disinfectant and chloramines as the secondary disinfectant. During the course of the study, a new water treatment plant composed of preozonation, coagulation, dissolved-air flotation (DAF), ozonation, and deep-bed biologically active GAC filtration that includes biologically activated filtration came online. Chloramines were used for disinfection, and the pH was adjusted to 9.0–9.3 with lime for corrosion control.

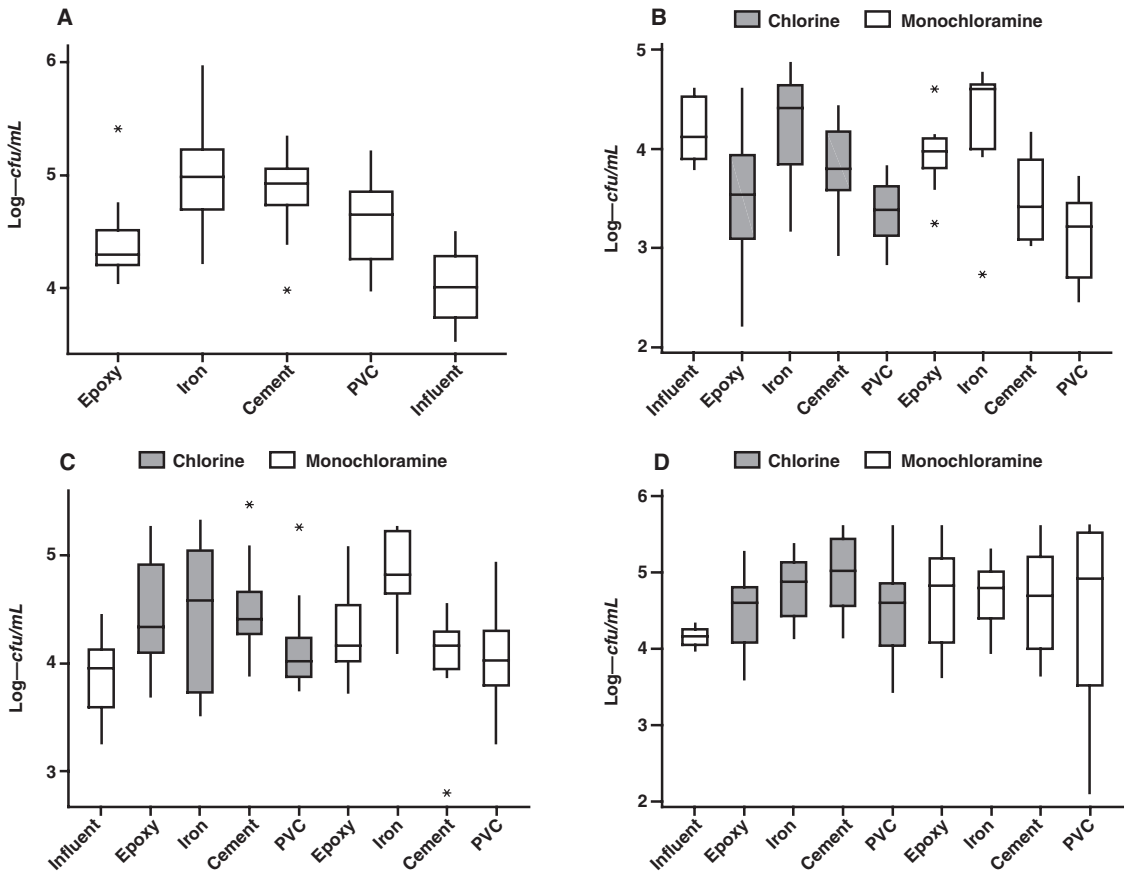
The testing program for utility 2 had two components—testing water at the treatment plant and at a remote location in the distribution system to determine the effect of water age on regrowth. Three annular reactors were installed at the treatment plant, and three annular reactors were installed at a location in the distribution system where the water age was approximately three days. Each testing location contained reactors with each of the materials.

At utility 3, six parallel annular reactors were installed with two reactors allocated for each of the three materials. For the first several months of operation, the water delivered to the annular reactors was unfiltered. During the last year of operation, the water in the system was fed through a new plant, composed of prechlorination, coagulation, DAF, and monomedia anthracite filtration.

Utility 4 is a wholesale water supply utility with a groundwater source exhibiting TOC levels ranging from 3 to 5 mg/L, with typical values of 4–4.5 mg/L. The treatment scheme consists of aeration and chlorination. Three parallel annular reactors, one of each type of material, were installed at the treatment plant.

Water quality samples were collected from the reactor influent for pH, temperature, alkalinity, HPC, TOC, and Cl_2 residual every two weeks and were done within certified laboratories at the utilities (average values shown in Table 2). Coupons were taken from the reactors approximately once every two weeks. For all field studies, the annular reactor coupons were removed from the reactors, replaced with a coupon of similar material, and sealed in sterile, wide-mouth, screw-topped test tubes filled with sterile phosphate-buffered saline or sterile water prior to transport and analysis. The tubes were stored on ice and sent by overnight delivery to Montana State University for analysis of biofilm HPCs. Coupon

FIGURE 1 Heterotrophic plate count influent and effluent values in laboratory experiments—phase 1 (A), phase 2 (B), phase 3 (C), and phase 4 (D)



The box represents the middle 50% of the observations, the horizontal line is the median, the whiskers designate the range, and asterisks denote outliers (1.5 × middle 50% of values). PVC—polyvinyl chloride

biofilm analysis was conducted within 24 h of sampling, and determinations of average colony-forming units per square centimetre of coupon were conducted as described previously.

RESULTS

Laboratory studies. *DOC levels.* Table 3 shows the relative C depletion across the reactors. Carbon entering the reactors came from the influent dilution water and the supplementary humic substances, which were fed to all reactors at the same mass flow rate. In phase 1 (dilution water only, no added humics), the greatest C utilization occurred in the reactors containing PVC, followed by the reactors containing iron, whereas there was little difference between the epoxy and cement reactors. In all of the remaining phases when humics were added, the iron reactors used the most C with no regular order of ascendance in reactors with other materials. These data are useful in determining whether the C removals can be associated with biofilm and effluent HPC values.

Influent and effluent HPC levels. As these data are presented, recall that effluent HPCs are the sum of the influent HPC plus any additional cells arising from detached biofilm. When disinfectants are added (phases 2–4), there is a balance between the production of bacteria in the biofilm and the effect of the disinfectant on both the biofilm and the effluent culturable cell numbers. There is also a difference in the way that the data are shown in the figures and tables. Figure 1 shows the effluent HPC data with the range and median for each type of material in phases 1–4, which are given to show the inherent variability in each of the experiments, as well as to illustrate the general trends in the datasets. Subsequent statistical analyses are provided in the tables.

Before the results for the effluent counts are provided, it is important to state that there was no significant difference between the mean influent HPC in any of the phases (data not shown). These results demonstrate the stability in the number of cells entering the reactors across the entire experimental time period.

TABLE 6 ANOVA* pairwise statistical comparisons of biofilm HPC† for phases 1 through 4‡

Phase 1				
Material	Epoxy	PVC	Cement	Iron
Average log—*cfu§/cm²*	5.14	5.26	5.58	5.90
Phase 2				
1. Reactors treated with chlorine				
Material	PVC	Epoxy	Cement	Iron
Average log—*cfu/cm²*	2.84	3.66	4.86	5.97
2. Reactors treated with monochloramine				
Material	PVC	Cement	Epoxy	Iron
Average log—*cfu/cm²*	3.76	4.46	5.11	6.14
Phase 3				
1. Reactors treated with chlorine				
Material	PVC	Epoxy	Cement	Iron
Average log—*cfu/cm²*	4.17	4.52	4.79	5.76
2. Reactors treated with monochloramine				
Material	PVC	Cement	Epoxy	Iron
Average log—*cfu/cm²*	4.12	4.49	5.12	5.40
Phase 4				
1. Reactors treated with chlorine				
Material	PVC	Epoxy	Cement	Iron
Average log—*cfu/cm²*	4.88	5.15	5.42	6.65
2. Reactors treated with monochloramine				
Material	PVC	Epoxy	Cement	Iron
Average log—*cfu/cm²*	5.14	5.14	5.33	6.45

*A one-way analysis of variance
†HPC—heterotrophic plate count
‡Lines between materials indicate that no significant statistical differences were found.
§cfu—colony-forming units

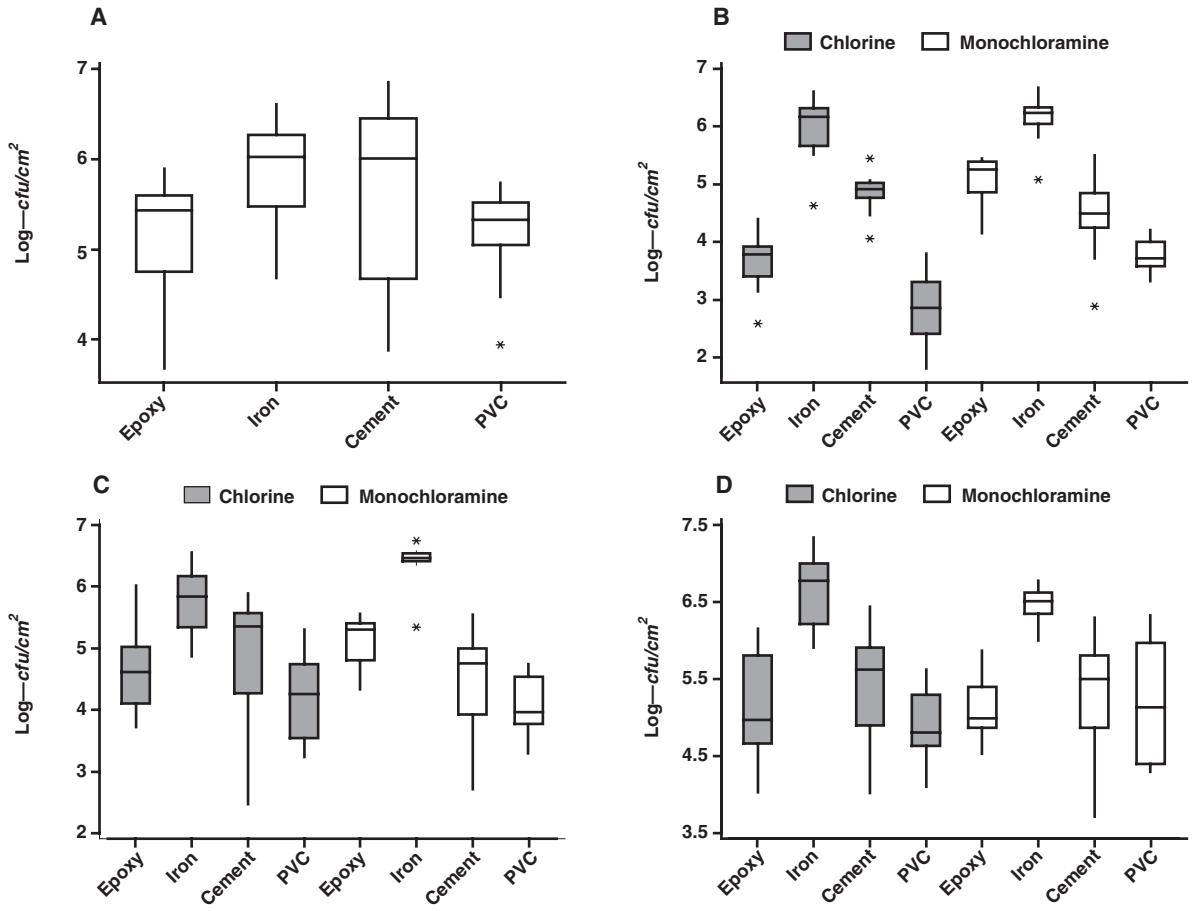
Statistical analyses of the effluent HPC in the control phase (phase 1) showed that there were no significant differences between reactor pairs containing the same materials (data not shown), indicating that the results from these reactors are repeatable. These results made it possible to use the averages for each material in further statistical comparisons rather than making comparisons between individual reactors. In phase 1, only the iron and cement reactors did not significantly differ from each other, and the effluent counts did not differ by more than 1 log (10^4 cfu/mL) (Table 4). All of the effluent concentrations were higher than the influent concentration (average of $10^{4.01}$ cfu/mL).

In phase 2, no significant differences were found between the effluents from reactors containing the same materials that were treated with monochloramine or Cl_2 (data not shown). The reactors containing iron had significantly higher microbial counts in the effluents than those containing PVC, which were approximately 1 log lower (Table 4). For the reactors treated with Cl_2 or monochloramine, there was no difference observable between the influent ($10^{4.18}$ cfu/mL) and the effluent from the iron reactors treated with either monochloramine or Cl_2 (Figure 1), and this was supported by statistical analysis (data not shown). In addition, the effluents from the cement reactor treated with Cl_2 and the epoxy reactor treated with monochloramine were not different from the influent, but others were lower by 0.7–1.0 log.

In phase 3, no statistically significant differences were found between any two reactors containing the same materials that were treated with monochloramine or Cl_2 (data not shown). In addition, no statistically significant differences were found between reactors containing different materials that were treated with free Cl_2 (Table 4). Among reactors containing different materials that were treated with monochloramine, the iron had significantly higher (0.8 log) effluent counts than the PVC and cement, whereas all other effluent counts were not statistically different. Comparisons of the reactor effluents to the influent in phase 3 (0.5 mg/L C + disinfectant) showed that there was no observable difference between the influent and the effluent from the PVC reactors treated with either monochloramine or Cl_2 (Figure 1). The remaining reactors treated with Cl_2 had significantly higher cell counts ($10^{0.6}$ log) in their effluents than in the influent ($10^{3.87}$ log cfu/mL) (Table 4). All of the effluent counts from the remaining reactors treated with monochloramine were not different from the influent except for the effluent from the iron reactor, which was higher than the influent by about 1 log.

In phase 4 (2 mg/L C + disinfectant) no statistically significant differences were found among the effluents from reactors containing the same materials treated with Cl_2 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 Cl_2 or monochloramine. When the effluents from each of the reactors in phase 4 were compared with the influent ($10^{4.15}$ log cfu/mL), no statistically significant differences were found except for the iron and cement reactors that were treated with Cl_2 (data not shown). The effluents from these reactors were

FIGURE 2 Heterotrophic plate count biofilm values in laboratory experiments—phase 1 (A), phase 2 (B), phase 3 (C), and phase 4 (D)



The box represents the middle 50% of the observations, the horizontal line is the median, the whiskers designate the range, and asterisks denote outliers (1.5 × middle 50% of values). PVC—polyvinyl chloride

both found to contain higher cell concentrations than the influent by about 0.7 and 0.8 log, respectively.

Table 5 shows the statistical relationships between the phases for reactors treated with the same disinfectants. For all materials and both disinfectants, phase 1 counts were significantly higher than those in phase 2. Phase 3 counts were significantly higher than those in phase 2 with the exception of the iron and epoxy reactors treated with monochloramine. In most cases, there was no difference in effluent HPCs between phases 3 and 4. There was also no difference in effluent counts between phase 1 (control) and phase 4 (high C + disinfectant).

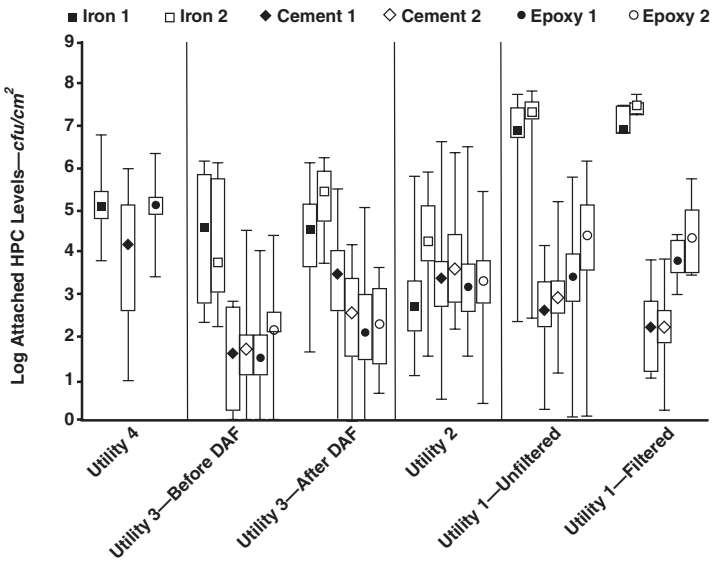
Biofilm HPC levels. The data presentation for the biofilm HPC is similar to that of the effluent results. The HPC biofilm data trends for phases 1–4 are shown in Figure 2. Statistical comparisons among reactors with different materials are shown in Table 6. During the control phase (no added disinfectant), there were no significant differences between pairs of reactors containing the same materials, demonstrating that consistency could be attained between duplicate reactors. In the other phases, one reactor in each pair received Cl_2, while the other was fed monochloramine.

One set of pairwise comparisons was made between reactors in each phase with the same material but different disinfectants. The biofilm HPCs in these pairs were not statistically significant in phases 3 and 4. In phase 2, there was a significant difference between Cl_2 and monochloramine only on epoxy and cement, with monochloramine values being higher (data not shown). These trends can be seen in Figure 2.

Under the low-DOC conditions in the absence of disinfectant in phase 1, biofilm HPCs were generally highest on the iron coupons (Table 6). When conditions were altered by the addition of the disinfectants in phase 2, comparisons made within one disinfectant showed that the materials were all significantly different from one another (Table 6). The highest biofilm HPCs were always found in the iron reactors, whereas the lowest were on PVC.

In phase 3, the biofilm HPCs in the iron reactors were significantly different and higher (1–2.3 log) than the epoxy, PVC, and cement reactors treated with the same

FIGURE 3 Heterotrophic plate count biofilm values from field study utilities



The box represents the middle 50% of the observations, the symbol is the median, and the whiskers designate the range. Iron 1, cement 1, and epoxy 1 for utility 2 are from the treatment plant location; iron 2, cement 2, and epoxy 2 are from the distribution system location. DAF—dissolved-air flotation, HPC—heterotrophic plate count

disinfectants (Table 6). The biofilm HPCs in the PVC and epoxy reactors receiving monochloramine were also significantly different. All remaining differences in biofilm HPC among the different coupon materials for either disinfectant were not statistically significant.

Biofilms in phase 4 were similar to those in phase 3 (Figure 2); average colony-forming units ranged from 4.9 to 6.7 log (cfu/cm^2) (Table 6). In addition, the biofilm densities in the iron reactors were significantly higher than those in other reactors treated with the same disinfectant. Also in phase 4, the PVC, epoxy, and cement reactors were not significantly different from one another with either disinfectant (data not shown).

Table 7 shows the statistical relationships for all phases in reactors treated with the same disinfectants. The iron reactors treated with either disinfectant had higher biofilm densities in phase 4 than in phase 1; no other materials were statistically different. All reactors that were treated with monochloramine had no significant differences between biofilm densities in phases 2 and 3. For all material and disinfectant combinations, the biofilm densities in phase 2 (no humics, added disinfectant) were the lowest or not significantly different from the phase with the lowest densities. In addition, phase 4 (highest DOC, added disinfectant) values were highest or were not significantly different from the phases with the highest counts.

Field study results. Biofilm HPCs at the four utility field-study locations are provided in Figure 3. Table 8 shows the statistical relationship between the biofilm HPC at each utility location for the different coupon materials.

At utility 1, there were no significant differences in biofilm HPC for the duplicate cement and iron annular reactors in which unfiltered water was tested or for the duplicate cement and epoxy annular reactors when filtered water was tested (data not shown). The data from these coupons were grouped for the remainder of the statistical analyses. Data from the duplicate epoxy reactors with unfiltered water and from duplicate ductile-iron reactors with filtered water were evaluated separately. The biofilm HPCs for the cement and epoxy coupons were not statistically different from one another (Table 8), but the biofilm HPCs for the iron coupons were significantly higher than either of these materials for both the unfiltered and filtered water. There was no difference in the biofilm HPC levels measured from any of the materials when the reactors were receiving unfiltered water versus filtered water. The filter plant came online just three months before the end of data collection from the annular reactors, and there may be a lag in observing changes in biofilm growth with changes in treatment.

At utility 2, biofilm HPCs for all coupon materials located at the treatment plant were not statistically different. For the reactors in the distribution system, HPCs on cement were similar to those on both the epoxy and the iron, but the epoxy and iron coupons were statistically different from each other. The iron coupons in the distribution system had the highest biofilm HPC. When the results were compared for coupons of the same material located at the treatment plant to those in the distribution system, there were no differences in the biofilm HPCs on the epoxy coupons. However, the iron and cement coupons had significantly higher biofilm HPCs in the distribution system compared with those from the plant.

At utility 3, the duplicate cement and iron annular reactors did not have statistically similar biofilm HPC values (data not shown); therefore, the results from duplicate reactors were not averaged. The biofilm HPC measured on iron before the DAF treatment was online was significantly higher than that from either cement or epoxy, but there was no statistical difference between the cement and epoxy coupons. All three materials had statistically different biofilm HPCs after DAF treatment was implemented, with iron having the highest biofilm HPC. There was a statistical difference in the biofilm HPC measured on the cement coupons before and after DAF treatment, but no difference with the iron and epoxy coupons.

At utility 4, there was no statistical difference in log-attached HPC levels among the three materials tested.

When biofilm HPC was compared among utilities, the iron coupons were the highest, while the lowest numbers were measured on either the cement or epoxy coupons depending on the specific utility (Table 9). The only exception was utility 2. The highest biofilm HPCs on iron were measured in utility 1, followed by utility 4, whereas the lowest iron HPCs were measured at the treatment plant location at utility 2. Statistically similar biofilm HPCs were measured at utility 2 in the distribution system, utility 3 before DAF treatment was implemented, and at utility 4. Growth on cement and epoxy at all utilities was several logs lower than on the iron coupons, with the exception of utility 2. For cement, utility 4 exhibited the highest biofilm HPC, and utility 3 before DAF treatment was implemented had the lowest. Statistically similar levels were observed at utility 1 with unfiltered water, utility 2 at the treatment plant, and utility 3 after DAF treatment was implemented. For the epoxy coupons, utility 4 exhibited the highest biofilm levels, and utility 3 before DAF treatment exhibited the lowest. Statistically similar biofilm levels were measured in utility 1 with unfiltered water, utility 2 in the distribution system, and utility 2 at the treatment plant.

TABLE 7 ANOVA* pairwise statistical comparisons of biofilm HPC† between phases‡

1. Epoxy reactor treated with chlorine				
Phase	2	3	4	1
Average log—*cfu§/cm²*	3.66	4.63	5.15	5.14
2. Epoxy reactor treated with monochloramine				
Phase	2	3	1	4
Average log—*cfu/cm²*	5.11	5.12	5.14	5.14
3. Iron reactor treated with chlorine				
Phase	3	1	2	4
Average log—*cfu/cm²*	5.76	5.90	5.97	6.65
4. Iron reactor treated with monochloramine				
Phase	1	2	3	4
Average log—*cfu/cm²*	5.90	6.14	6.40	6.45
5. Cement reactor treated with chlorine				
Phase	3	2	4	1
Average log—*cfu/cm²*	4.79	4.86	5.42	5.58
6. Cement reactor treated with monochloramine				
Phase	2	3	4	1
Average log—*cfu/cm²*	4.46	4.49	5.33	5.58
7. PVC reactor treated with chlorine				
Phase	2	3	4	1
Average log—*cfu/cm²*	2.84	4.17	4.88	5.26
8. PVC reactor treated with monochloramine				
Phase	2	3	4	1
Average log—*cfu/cm²*	3.76	4.12	5.14	5.26

*A one-way analysis of variance
†HPC—heterotrophic plate count
‡Lines between materials indicate that no significant statistical differences were found.
§cfu—colony-forming units

DISCUSSION

Laboratory experiments. The 2-h detention time in the annular reactors for the laboratory experiment was sufficient to allow biofilms to accumulate and grow but not to allow significant planktonic growth. Thus, any difference between the influent and effluent HPC can be attributed to detachment from the biofilm. This is important to remember when the influent and effluent data are interpreted.

The low disinfectant residual in phases 2–4 represents the worst-case scenario for distribution systems in which it is near the minimum of 0.2 mg/L. This may occur at the end of the distribution system or in longer sections where residuals have degraded.

Within phases 2–4, there were few statistical differences in effluent HPC between materials for each disinfectant. These differences became less significant as the DOC levels increased. However, these statistical results are based on probabilities, tend to be conservative, and point out major differences. There may also be practical differences that can be found by inspection of the means. These trends show that iron reactors generally had the highest effluent HPC, or the results were not significantly different from reactors with the highest effluent numbers. In addition, PVC always had the lowest HPC, or these values were not significantly different from the reactors with the lowest HPC. The effluents from the cement and epoxy reactors fell between the iron and PVC with no regular pattern with respect to each other.

Another evaluation can be made between phases. For all materials with either monochloramine or Cl_2, there was a significant difference in effluent HPCs between phases 1 (control) and 2 (disinfectant, no added DOC). This shows the efficacies of both Cl_2 and monochloramine against detached biofilms cells. The effluent counts in phase 3, when DOC was added in the presence of disin-

TABLE 8 ANOVA* pairwise statistical comparisons of biofilm HPC† levels for utility field studies‡

Utility 1—*unfiltered water*			
Material	Cement	Epoxy	Iron
Average log—*cfu§/cm²*	2.85	3.3–4.3	6.96
Utility 1—*filtered water*			
Material	Cement	Epoxy	Iron
Average log—*cfu/cm²*	2.2	4.2	7.1–7.4
Utility 2—*treatment plant location*			
Material	Epoxy	Cement	Iron
Average log—*cfu/cm²*	3.2	3.3	2.8
Utility 2—*distribution system location*			
Material	Epoxy	Cement	Iron
Average log—*cfu/cm²*	3.3	3.8	4.2
Utility 3—*before DAF***			
Material	Cement	Epoxy	Iron
Average log—*cfu/cm²*	1.6	2.1	4.3
Utility 3—*after DAF*			
Material	Epoxy	Cement	Iron
Average log—*cfu/cm²*	2.2	2.5–3.2	4.2–5.3
Utility 4—*groundwater*			
Material	Cement	Epoxy	Iron
Average log—*cfu/cm²*	4.5	5.1	5.2

*A one-way analysis of variance
†HPC—heterotrophic plate count
‡Lines between materials indicate that no significant statistical differences were found.
§cfu—colony-forming units
**DAF—dissolved-air flotation

fectants, were always greater than or equal to the effluent counts in phase 2. In addition, in both of these data sets, the effluent HPCs in phase 4, in which DOC levels were higher, were always greater than or equal to the effluent HPCs in phase 3. This shows a general trend that increased DOC concentration is accompanied by higher effluent HPCs even when the same residual is maintained. Thus, Cl_2 and monochloramine at a residual of 0.2 mg/L do not completely control biofilm growth. However, at the DOC levels studied, this residual was sufficient to maintain effluent HPCs at or below the levels in the control (phase 1), in which no DOC or disinfectants were added. The practical implication is that at high-DOC concentrations, disinfectants may reduce the HPC levels to those maintained with no disinfectant at low-DOC levels.

Some interesting effects of disinfection were also seen on the biofilm HPC. One result of note was the influence of disinfection when no humics were added to the reactors (phases 1 and 2, Table 7). Here, differences were not significant for both disinfectants in the presence of iron, although average values were higher in the presence of the disinfectant. In contrast, there were significant changes on the other three materials for one or both disinfectants, with values in the presence of the disinfectants being lower than in the control. This result is consistent with previous studies that have shown reduced efficacy of disinfectants against biofilms in the presence of iron (Camper, 1996; LeChevallier et al, 1993; LeChevallier et al, 1990).

The biofilm HPC data also showed no conclusive statistical evidence that one disinfectant had the ability to reduce biofilm HPC to a greater extent than the other at a residual concentration of 0.2 mg/L. However, the trends showed that PVC always had the lowest HPC or did not differ significantly from the material with the lowest count. The cement and epoxy fell between the PVC and iron with no apparent order of ascendance.

Another comparison that can be made is the response of biofilm HPC to the disinfectants on the materials as the DOC level was varied. HPC in the iron reactors in phase 4 at the highest DOC level in the presence of disinfectants was significantly greater than the biofilm in the same reactors in the control phase (phase 1). However, biofilm HPCs on the remaining materials in phase 4 did not significantly differ from those on the same materials in phase 1. This indicates that the disinfectant level of 0.2 mg/L was sufficient to control the increased growth at the highest DOC level in epoxy, cement, and PVC reactors compared with the control phase but not on iron.

The general lack of efficacy of the disinfectants against biofilms on iron in these experiments may be because of the low residuals that were maintained. LeChevallier et al (1990) showed that there was a minimum threshold level at which monochloramine reduced biofilms in iron pipes. This threshold was determined to be a dose of 2.0 mg/L (residual of 1.1 mg/L) but is likely to vary with water quality and pipe characteristics. The same study found that Cl_2 did not result in a decrease in biofilm HPC at doses of up to 4 mg/L (residual of 2.8 mg/L). Another study (Sanderson & Stewart, 1997) found that *Pseudomonas aeruginosa* showed evidence of adaptation to monochloramine concentrations below 0.5 mg/L. Both of these concentrations are well above the residuals used in this experiment and suggest that the apparent equality of the disinfectants against the biofilms may be related to the low concentration.

Evidence from this laboratory experiment supports the phenomenon of increased adsorption of humic substances and the possibility of greater bioavailability of this C source to biofilms on iron surfaces. In phase 1 (no humics), the biofilm HPCs in the iron reactors were not significantly different from those in the cement reactors, and there was very little difference between HPCs on all four materials. In this same experiment, removals of DOC originating from the dilution water across the reactors (Table 3) were comparable for all materials with the exception of PVC. However, when the reactors were supplemented with humics at 0.5 and 2 mg/L C, the DOC removals were higher on iron, and the biofilm and effluent HPCs were significantly higher than those in reactors containing any of the other materials. This is in spite of the disinfectant residuals maintained at 0.2 mg/L. The increased effluent and biofilm HPC on the iron is likely due to higher C adsorption (Gu et al, 1994) and subsequent utilization and growth by bacteria in the iron reactors.

TABLE 9 ANOVA* pairwise statistical comparison of biofilm HPC† between utilities‡

Utility	Average Log HPC cfu§/cm^2
Ductile-iron coupons	
Utility 2—*treatment plant*	2.8
Utility 2—*distribution*	4.2
Utility 3—*before DAF***	4.3
Utility 3—*after DAF*	4.2–5.3
Utility 4—*groundwater*	5.2
Utility 1—*unfiltered water*	7.0
Utility 1—*filtered water*	7.0–7.5
Cement coupons	
Utility 3—*before DAF*	1.6
Utility 1—*filtered water*	2.2
Utility 1—*unfiltered water*	2.9
Utility 3—*after DAF*	2.5–3.2
Utility 2—*treatment plant*	3.3
Utility 2—*distribution*	3.8
Utility 4—*groundwater*	4.5
Epoxy coupons	
Utility 3—*before DAF*	2.1
Utility 3—*after DAF*	2.5
Utility 2—*treatment plant*	3.2
Utility 2—*distribution*	3.3
Utility 1—*unfiltered*	3.3–4.3
Utility 1—*filtered*	4.2
Utility 4—*groundwater*	5.1

*A one-way analysis of variance
†HPC—heterotrophic plate count
‡Lines between materials indicate that no significant statistical differences were found.
§cfu—colony-forming units
**DAF—dissolved-air flotation

A comparison of the HPC biofilm values to the HPC effluent trends showed similar results between both data sets. For both the effluent and biofilm HPCs, the iron reactors were highest, whereas the PVC reactors were lowest, with the cement and epoxy reactors intermediate with no defined order of ascendance. In addition, both data sets showed a general trend of increasing HPC as the DOC levels increased.

In general, these results support previous studies that have shown that biofilms on iron surfaces are more problematic than those on other types of surfaces (Niquette et al, 2000; Kerr et al, 1999; LeChevallier et al, 1998; Delanou et al, 1997; LeChevallier, 1997; Neden et al, 1992). LeChevallier et al (1996) showed that distribution systems with more miles of unlined cast-iron pipe had much higher coliform occurrences than systems with significantly less unlined cast-iron pipe. Another study showed that the biofilm densities on iron were significantly higher than those on plastic-based materials such as PVC, with densities on cement intermediate to plastic-based materials and iron (Niquette et al, 2000). Neden et al (1992) reported that cast-iron pipes had the highest counts, whereas PVC was the lowest. Camper (1996) showed in laboratory studies that mild steel had tenfold higher HPCs and coliforms than polycarbonate surfaces in reactors operated in an identical fashion. In another laboratory study, Kerr et al (1999) found highest counts and species diversity on cast iron compared with medium-density polyethylene and unplasticized PVC.

Field study. The results from the field study evaluations of biofilm density on various materials complement the laboratory results. Although biofilm HPCs on any of the materials were specific to each utility, some general observations can be made regarding the water quality conditions for these four sites and the resulting biofilm growth. Fluctuations in influent water quality occurred throughout the course of these studies; however, in general, the influent water to the reactors at utility 4 had the highest TOC values (average of 4.0 mg/L C) and the highest free Cl_2 residual of all the studies (average of 3.4 mg/L Cl_2). Utility 2 had the lowest TOC values (average of 0.8 mg/L C) and lower average Cl_2 residuals (1.2 mg/L Cl_2). The TOC levels for utility 3 and utility 1 ranged from 0.9 to 2.0 mg/L C, and both of these utilities used

chloramines with average values ranging from 1.6 to 3.0 mg/L. No differences in biofilm numbers were observed between materials for either utility 4 (higher TOC groundwater) or utility 2 (lower TOC), whereas the iron coupons from the utilities with intermediate TOC values that chloraminate displayed significantly higher biofilm HPCs than the other materials (cement and epoxy). The biofilm HPCs on the cement and epoxy coupons at utility 4 were higher than the HPCs on the cement and epoxy coupons from the other utility locations, indicating that the higher organic loading may have supported more growth. The numbers on the iron coupons from utility 4 were lower than those on the iron coupons at utility 1, even though the influent TOC levels were higher. This may indicate lower biodegradability of the organics from the groundwater source at utility 4.

CONCLUSIONS

The laboratory testing showed that increases in DOC led to general increases in biofilm and effluent HPC, and this effect was most pronounced for reactors that contained iron. In the presence of disinfectants and supplementary organic C, the iron reactors had the highest effluent counts of any of the materials tested. For the other materials (cement, epoxy, and PVC), there was no definite order of ascendance with respect to biofilm or effluent cells, but the PVC reactors always exhibited the lowest levels or were not statistically different from the reactors with the lowest levels. The straightforward effect of these findings is that the combination of higher TOC levels and cast-iron pipe can lead to relatively high numbers of bacteria, while this effect was not as pronounced for the other materials.

The field-testing results indicate that either iron had the highest regrowth or the type of material had no influence on the number of bacteria present. Regrowth appeared to depend on the nature and quantity of the organic material, disinfectant, and type of coupon material. Although iron pipe exhibited the highest biofilm levels, it should not be concluded that this would always be the case. Site-specific testing to determine whether material is a contributing factor in regrowth should be performed. This type of testing will help utilities identify if piping material, in this case iron pipe, is a primary contributor to regrowth.

The field sites in this study used very different levels and types of disinfectant. The strategy that most utilities use to mitigate regrowth is to increase the disinfectant residual or convert from free Cl_2 to chloramine, which is considered to be more effective for controlling regrowth. Despite the very high levels of disinfectant present in this study, the system with the lowest free Cl_2 disinfectant residual had the lowest regrowth on iron coupons. This system also uses GAC treatment and lowers its final distributed water TOC concentration to <0.80 mg/L. This is not to say that the presence of disinfectant residual cannot aid in controlling regrowth, but a disinfectant residual as was present in the systems tested did not lead to lower biofilm numbers on iron pipe. When regrowth is a problem, such as for coliforms in the distribution system, utilities should evaluate the effects of organics and cast-iron pipe.

These conclusions have practical implications for the drinking water industry. This study has shown that higher levels of C support greater biofilm growth and planktonic populations on any pipe material, although the effect is most pronounced on iron. Thus, reducing the organic C in the finished water would be effective for a utility wishing to reduce regrowth problems in its distribution system. This can be accomplished using one of several treatment technologies, including enhanced coagulation, membrane filtration, filtration using iron-oxide-coated media, or biological filtration. However, it is important to recognize that biofilms can still flourish

REFERENCES

ASTM (American Society for Testing and Materials), 2002. E2196-02 Standard Test Method for Quantification of a *Pseudomonas aeruginosa* Biofilm Grown With Shear and Continuous Flow Using a Rotating Disk Reactor, Vol. 11.05. ASTM International, West Conshohocken, Pa.

Butterfield, P.W. et al, 2002a. Minimizing Biofilm in the Presence of Iron Oxides and Humic Substances. *Water Res.,* 36:3893.

Butterfield, P.W. et al, 2002b. Chlorination of Model Distribution System Biofilm: Implications for Growth and Organic Carbon Removal. 36:4391.

Camper, A.J. et al, 2000. *Biological Stability of Water in Treatment Plants and Distribution Systems.* AWWA Res. Fdn., Denver.

Camper, A.J., 1996. *Factors Limiting Microbial Growth in the Distribution System: Laboratory and Pilot-scale Experiments.* AWWA Res. Fdn., Denver.

Delanou, A. et al, 1997. Effect of Pipe Materials on Biofilm Growth and Deposit Formation in Water Distribution Systems. Proc. 1997 AWWA WQTC, Denver.

Ellis, B. et al, 2000. Effects of Carbon Source, Carbon Concentration, and Chlorination on Growth-related Parameters of Heterotrophic Biofilm Bacteria. *Microbiol. Ecol.,* 38:330.

Gu, B. et al, 1994. Adsorption and Desorption of Natural Organic Matter on Iron Oxide: Mechanisms and Models. *Envir. Sci. & Technol.,* 28:38.

Hach Co., 1997 (3rd ed.) *Hack Water Analysis Handbook,* Loveland, Colo.

Hudson, L.D.; Hankins, J.W.; & Battaglia M., 1983. Coliforms in a Water Distribution System: A Remedial Approach. *Jour. AWWA,* 75:11:564.

Kerr, C.J. et al, 1999. The Relationship Between Pipe Material and Biofilm Formation in a Laboratory Model System. *Jour. Applied Microbiol.,* 85:29S.

LeChevallier, M.W., 1997. Disinfection of Biofims in Drinking Water Systems. Proc. 1997 AWWA WQTC, Denver.

even with very low C concentrations. As observed in this study, this is especially true for unlined iron surfaces.

Utilities with significant amounts of iron pipe in their distribution systems are faced with the greatest regrowth potential. For utilities that are also distributing waters high in organic C, this problem is compounded. Thus, the best option for improving water quality is to replace or reline the iron pipe in their distribution systems. This alternative may be more economically feasible for utilities with deteriorating iron pipe that must be replaced or remedied rather than for utilities with newer iron pipes. For utilities with a smaller amount of unlined iron pipe, reducing the amount of organic C entering the distribution system may be a better option. However, it is important to weigh the expense of reducing the C in the distribution system against the actual potential for significantly reducing biofilm growth.

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FOOTNOTES

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LeChevallier, M.W. et al, 1998. *Microbial Impact of Biological Filtration.* AWWA Res. Fdn., Denver.

LeChevallier, M.W.; Welch, N.J.; & Smith, D.B., 1996. Full-scale Studies of Factors Related to Coliform Regrowth in Drinking Water. *Applied & Envir. Microbiol.,* 62:7:2201.

LeChevallier, M.W. et al, 1993. Examining the Relationship Between Iron Corrosion and the Disinfection of Biofilm Bacteria. *Jour. AWWA,* 85:7:111.

LeChevallier, M.W.; Lowry, C.D.; & Lee, R.G., 1990. Disinfecting Biofilms in a Model Distribution System. *Jour. AWWA,* 82:7:87.

LeChevallier M.W.; Babcock T.M.; & Lee R.G., 1987. Examination and Characterization of Distribution System Biofilms. *Applied & Envir. Microbiol.,* 53:2714.

Martin, R.S. et al, 1982. Factors Affecting Coliform Bacterial Growth in Distribution Systems. *Jour. AWWA,* 74:1:34.

Neden, D.G. et al, 1992. Comparing Chlorination and Chloramination for Controlling Bacterial Regrowth. *Jour. AWWA,* 84:7:80.

Neter, J. et al, 1996. Applied Linear Statistical Models, pp. 153–5 and 736–8. Irwin, Chicago.

Niquette, P.; Servais, P.; & Savoir, R., 2000. Impacts of Pipe Materials on Densities of Fixed Bacterial Biomass in a Drinking Water Distribution System. *Water Resources*, 34:6:1952.

Oliveri, V.P. et al, 1985. Recurrent Coliforms in Water Distribution Systems and the Presence of Free Residual Cl_2 (R.L. Jolley et al, editors). Lewis Publs., Boca Raton, Fla.

Reilly, K.J. & Kippen, J.S., 1984. Relationship of Bacterial Counts With Turbidity and Free Cl_2 in Two Distribution Systems. *Jour. AWWA,* 75:6:309.

Sanderson, S.S. & Stewart, P.S., 1997. Evidence of Bacterial Adaptation to Monochloramine in *Pseudomonas aeruginosa* Biofilms and Evaluation of Biocide Action Model. *Biotechnol. Bioengrg.,* 56:2:201.

Sharp, R.R. et al, 2001. Evaluation of Drinking Water Biostability Using Biofilm Methods. *Jour. Envir. Engrg.,* 127:5:403.

Standard Methods for the Examination of Water and Wastewater, 1998 (20th ed.). APHA, AWWA, and WEF, Washington.