Evaluation of Corrosion Inhibitors, Residual, Temperature, and Pipe Material on Bulk and Biofilm Heterotrophic Plate Counts Densities

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The impact of corrosion inhibitors on biofilm and bulk heterotrophic plate counts (HPC) has been studied by a number of investigators and a review of the literature indicates a wide variety of seemingly contradictory results. Hozalski et al. (2005) observed an increase in bulk HPC levels when using phosphate based inhibitors. Miettinen et al. (1997) and Sathasivan et al. (1997) also observed full-scale systems where phosphate was a limiting nutrient and addition of it resulted in stimulation of bacterial growth. Appenzeller et al. (2001) observed a significant decrease in planktonic HPC when phosphate was added, but only when a highly corroded reactor was used. Evaluation of bacterial adhesion led Appenzeller et al. (2002) to conclude that the lower HPC were probably due to phosphate decreasing the ability of bacterial to adhere to corroded pipes due to phosphate sorption onto iron oxyhydroxides, which reversed their surface charge from positive to negative. In non- or lightly-corroded reactors, the phosphate had no measurable impact on bacterial growth. Abernathy and Camper (1998) and Rompre et al. (1999) also observed that phosphate-based inhibitors resulted in lower bacterial densities on corrosion deposits.

In contrast, there are a number of studies where biofilm densities did not change significantly in the presence of phosphate corrosion inhibitors. Batte et al. (2003) observed that phosphate did not increase or decrease biofilm densities. This was also the case for Butterfield et al. (2002), when phosphate alone was added, but there was weak evidence that phosphate in combination with free chlorine reduced biofilm densities more than free chlorine alone. Lyons et al. (1995) also observed no significant change in bacterial densities due to phosphorus based corrosion inhibitors. This was also observed for sodium silicate corrosion inhibitors by Rompre et al. (2000). Batte et al. (2003) speculated that the seemingly contradictory results in the literature might be due to variations in the ratios of carbon:nitrogen:phosphorus (C:N:P ratio) for the waters studied, biomass measurement technique, scale factors, and/or synergistic effects with disinfectants. Butterfield et al. (2002) also note the importance of humic substances that can adsorb to corrosion products and provide substrate for bacterial growth. As a result, many factors (e.g., the degree of corrosion, the quantity of humic substances, the phosphate and ammonia concentrations, and the type and concentration of the disinfectant residual) may all have an impact. In addition, the pipe material has a major impact on the degree of corrosion and the biofilm density and also may impact consumption of the residual. Water age and water quality (e.g., alkalinity, pH, concentrations of sulfate, chloride, etc.), also impact both corrosion and microbial water quality. As a result, the impact of corrosion inhibitors on either biofilms or bulk HPC concentrations is complex. In addition, as the literature is compared, there are often analytical differences that exist (e.g., some investigators used R2A agar for HPC while some did not, incubation periods and temperatures varied, etc.).

In this study, the authors look at the impact of adding phosphate-based corrosion inhibitors on planktonic HPC as a general indicator of microbial water quality. A silicabased inhibitor and the use of pH control were also studied for suppressing corrosion and microbial water quality impacts, and biofilms were also quantified. Four different pipe materials—polyvinyl chloride (PVC), lined cast iron (LCI), unlined cast iron (UCI) and galvanized steel (G)—were used in the

Table 1. Blend ratios of GW, SW, and RO waters used during project

Phase	Time period	% GW	% SW	% RO
Ι	February-May 2006	62	27	11
Π	May–August 2006	27	62	11
Ш	August–November 2006	62	27	11
IV	November2006–February 2007	40	40	20

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pilot distribution systems (PDS) that were studied. All of the metal pipes had significant corrosion, having been removed from fullscale distribution systems. This research focuses on the biological impacts resulting from corrosion inhibitor addition to yield data that can be factored into the decision to use corrosion inhibitors and how to manage the transition from a system without inhibitors to a system with inhibitors.

Methods and Materials

Pilot Distribution Systems

The experimental system for the project consisted of fourteen pilot distribution systems (PDS) being fed a blend of treated groundwater (GW), surface water (SW), and reverse osmosis (RO) permeate from desalination. This blend for each of the four phases of the study were chosen in consultation with the utilities involved in order to make them representative of the blends anticipated in the near future (Table 1). The full-scale systems have been close to 100 percent groundwater for many decades, but are now beginning to receive surface water and RO permeate since the groundwater in central Florida is being utilized at a rate greater than the recharge rate, while water demand continues to increase. Currently, the utility expects that the new blend will be 62 percent groundwater, with 27 percent surface water, and 11 percent RO permeate as the expanded surface water treatment and the new desalination plant comes on line. As a result, during the yearlong study, two of the four experimental phases (each lasting three months) used this blend of water, and there were also two phases that explored other possible blends.

In all four phases, corrosion inhibitors were evaluated at three different inhibitor

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doses each (Table 2; see PDS 1 through 12), with the dosages covering the approximate range anticipated in the full-scale distribution systems. The inhibitors tested were blended ortho and polyphosphates (BOP), orthophosphates (OP), silicates, and zinc orthophosphate (ZOP). In addition, there were two PDS that received no inhibitor but were pH-controlled (PDS 13 and 14). The PDS 13 pH was maintained at pH_s (the pH at equilibrium with respect to CaCO₃), and PDS 14 was 0.3 pH units higher than pH_s. PDS 13 and 14 are referred to as the Control PDS.

The PDS consisted of four segments of pipe joined in series (see bullet list below). The PDS were identical and consisted of sections of PVC, lined cast iron (LCI), unlined cast iron (CI) and galvanized steel (G) pipes connected in series. These pipe segments were taken out of the full-scale systems of the utilities involved in the study and showed signs of pipe age such as inner surface corrosion.

- Approximately 20 ft (6.1 m) of 6-in. (0.15m)-diameter PVC pipe,
- Approximately 20 ft (6.1 m) of 6-in. (0.15m)-diameter LCI pipe,
- Approximately 12 ft (3.7 m) of 6-in. (0.15m)-diameter UCI pipe, and
- Approximately 40 ft (12.2 m) of 2-in. (0.05m)-diameter G pipe.

Seasonal effects (e.g., temperature) were evaluated by repeating the blend scenarios for the first three-month phase (Phase I) during the third three-month phase (Phase III) six months later (Table 3). Combined chlorine was used as the secondary disinfectant. Residual demands were sensitive to high temperatures, but chloramine concentrations were maintained from 1.8 to 2.6 mg/L throughout the study.

Biofilm and Water Quality Analyses

Planktonic (i.e., bulk water) HPC was measured with spread plates on R2A agar. The plates were labeled with sample number and location. Using a 10-100µL pipette, 0.1 mL sample was placed onto the R2A surface of pre-dried agar plates. Using a sterile bent glass rod as a spreader, the inoculum was spread over the R2A surface by rotating the dish on a turntable. After completion of the final plate, the plates were inverted for 15 minutes. Incubation was at 25 degrees Celsius for at least 48 hours, with triplicate plates for each dilution of a sample. The plate counts were conducted following incubation. There were two dilutions for each sample, including blind duplicates. Four dilutions were used for lab replicate quality assurance (QA) samples.

Biofilm HPC was measured in the same way as bulk HPC except that first the biofilm had to be detached, suspended, and then ho-

Table 2 Inhibitor type and dosage in fourteen PDS

PDS	Inhibitor	Dosage
1	BOP	0.5 mg/L as P
2	BOP	1.0 mg/L as P
3	BOP	2.0 mg/L as P
4	OP	0.5 mg/L as P
5	OP	1.0 mg/L as P
6	OP	2.0 mg/L as P
7	ZOP	0.5 mg/L as P
8	ZOP	1.0 mg/L as P
9	ZOP	2.0 mg/L as P
10	Silica	3 mg/L as SiO ₂
11	Silica	6 mg/L as SiO ₂
12	Silica	12 mg/L as SiO ₂
13	pHs	None
14	pHs+0.3	None

Table 3. Average PDS temperature and chloramine residuals

Phase	Time period	Temp	Residual
Ι	February–May 2006	19.9	2.2
Π	May–August 2006	25.1	1.8
III	August–November 2006	24.2	2.6
IV	November 2006–February 2007	21.6	2.6

mogenized prior to spread-plating on R2A agar. Pipe material coupons (pre-cut from the aged pipes) that were colonized by biofilm were removed from the PDS and then rinsed carefully with phosphate buffer solution (PBS) twice. The biofilm was manually detached from the coupon using a sterile cell scraper (sterilized with 70 percent ethanol and flamed) into 10 mL of sterile PBS. Samples were homogenized by using a tissue blender at 5000 rpm for two minutes. The tissue homogenizer probe was cleaned in 10 percent bleach solution for 15 seconds and then in DI water for 15 seconds between samples. The sample was then diluted and spread on R2A agar plate as described in the preceding paragraph on bulk HPC measurement. Identical quality assurance samples were also used. Biofilm coupons were incubated in a PVC cradle receiving the same influent as the PDS.

Residual levels were measured in the field using HACH kits and a spectrophotometer. Both free and total chlorine were measured. Temperature was measured with a probe.

Statistical Analysis of Data

Statistical analysis of data was conducted using technical graphing and statistical analysis software. The data was evaluated and did not conform to a normal distribution. The Mann-Whitney test was used for hypothesis testing to determine when there was enough evidence to determine that the Inhibitor PDS were significantly different from the Control PDS(i.e., the pH-controlled PDS).

Model Development

Bulk HPC Model Development. An empirical model was developed using the entire dataset (all phases and all PDS). The objective of the model was to quantify the impact of water quality on the effluent HPC in the distribution system. Dummy variables (BOP, OP, ZOP, Si and pH control) for each inhibitor and control lines were incorporated into the model. The use of dummy variables allowed estimation of a single parameter that is associated with each of the four corrosion inhibitors. Initial model development segregated the data based on inhibitor type. Once it was determined that inhibitor dosages did not result in significantly different HPC values, all of the data using phosphate-based inhibitors (PDS 1 to 9) were combined for analysis. Similarly, the SI data (PDS 10 to 12) were evaluated as a group. The pH control data (PDS 13 and 14) were also evaluated as a separate data set. In addition, the pooled data from all PDS was evaluated.

Biofilm HPC Model Development. In developing an empirical model, the BF HPC data presented a significant challenge because the model would ideally address both pipe material and corrosion inhibitor effects. However, it was not

possible to use two sets of dummy variables, as a total of 20 such dummy variables would have been required (four pipe materials and five inhibitor treatments). Segregation of the data into 20 subsets would produce undesirably small datasets to support parameter estimation procedures. The models adopted have dummy variables for material, but water quality parameters (i.e., Silica, TP, Zn, pH) were used in the model to quantify the impact of the inhibitors instead of dummy variables. In most cases, however, these water quality parameters were not significant and fell out of the model. The biofilm HPC (BF HPC) model also differed from that used to obtain bulk HPC models since replicate experiments (i.e., duplicate coupons incubated in parallel) were available for biofilm samples. This allowed direct estimation of the experimental error (also referred to as the error mean square) for use in the analysis of variance (ANOVA).

The empirical models presented are intended to provide information regarding the impact of inhibitor addition on the bulk and biofilm HPC. The best models for the two data sets (i.e., bulk and biofilm) were obtained in an iterative procedure. The model was set up first with all the dummy variables and all the independent water quality parameters. The water quality parameter that failed the hypothesis test (F statistic lower than the calculated or tabular F value) by the greatest amount was eliminated from the model in the first iteration. This procedure continued until all the parameters remaining in the model passed the hypothesis test. Two different assemblies of data were used for model development: segregated PDS data sets (PDS 1-9, PDS 10-12 and PDS 13-14) and a pooled data set with data from the PDS. These groupings are subsequently described as segregated and pooled data. All remaining independent variables (i.e., material, temperature, residual and water quality parameters), were statistically significant at a 95 percent level of confidence in the reported models.

Results and Discussion

Impact of Corrosion Inhibitors on Bulk and Biofilm HPC

During the one-year study, 168 HPC duplicates (including many blind duplicates) were run and the average range of the planktonic (bulk liquid) HPC test was equal to 0.32 log. Table 4 shows that, during Phase I and II, the average log HPC values were 6.7 to 4.1 times (0.82 to 0.61 log) greater than the control PDS. Then, in Phase III and IV, HPC values decreased in the Inhibitor PDS such that the differences between them were roughly equal to or lower than the variability of the HPC test (i.e., 0.28 to 0.04 log versus the 0.32 log analytical variability). The data shows that the difference between the Inhibitor and the

Table 4.	Average Plan	ktonic Effluent HPC V	alues of	Inhibitor	PDS (1-12)
	ver	rsus the Control PDS	(13&14)	

	Inhib	Cntrl	Delta		Inhib	Cntrl	Delta
	PDS	PDS	HPC	Factor	PDS	PDS	Log
	(cfu/mL)	(cfu/mL)	(cfu/mL)		(log)	(log)	(log)
P1 avg	4061	610	3451	6.7	3.6	2.8	0.82
P2 avg	5321	1309	4012	4.1	3.7	3.1	0.61
P3 avg	1489	778	711	1.9	3.2	2.9	0.28
P4 avg	1128	1020	108	1.1	3.1	3.0	0.04
All Phases avg	3056	921	2135	3.3	3.5	3.0	0.52

Table 5 Effluent HPC data statistical comparison by Phase (Mann-Whitney Test, p =<0.001)

	Inhib			Cntrl			Sig Diff?
	PDS	25%	75%	PDS	25%	75%	(P≤0.001)
	(cfu/mL)	(cfu/mL)	(cfu/mL)	(cfu/mL)	(cfu/mL)	(cfu/mL)	
P1 median	1177	418	3967	325	141	675	Yes
P2 median	3517	1880	67500	1127	700	1640	Yes
P3 median	1105	620	1893	487	210	987	Yes
P4 median	640	337	1585	802	184	1667	No P=0.744



Figure 1. Effluent HPC Levels in Inhibitor PDS versus Control PDS 13.

Control PDS was transitory and after nine months (3 phases), it had become negligible.

Statistical analysis showed that the data in each phase had a non-normal distribution. A Mann-Whitney Rank Sum Test was conducted for each of the four phases to determine if the differences observed in the inhibitor versus control lines were significant (Table 5). No statistically significant effect on planktonic HPC was observed with changes in dose or type of inhibitor (data not shown), which is why the Inhibitor PDS are treated as a single group. The data in Table 5 showed that the Inhibitor PDS had significantly greater effluent HPC values during the first three phases of the study, but by Phase IV, no significant difference existed. This statistical analysis confirms the trend seen in Table 4.

The difference between the Inhibitor and Control PDS effluent HPC values was transitory and indicated a re-equilibration period was necessary for the HPC values to decrease back down to the same levels as the system without corrosion inhibitors. The data also showed that, in the long run, effluent HPC val-*Continued on page 20*



Figure 2. PDS 4-6 Effluent Soluble and Total Copper

Table 6 Biofilm HPC data statistical comparison by Phas	e
(Mann-Whitney Rank Sum Test)	

	Labib						C:a
	Innio						Sig
	PDS	25%	75%	Cntrl PDS	25%	75%	Diff?
	(cfu/cm ²)	(cfu/cm ²)	(cfu/cm^2)	(cfu/cm^2)	(cfu/cm ²)	(cfu/cm ²)	
							Yes
P1 median	320,761	97,410	538,058	28,795	6,829	98,527	P=0.003
							No
P2 median	346,760	67,144	802,632	116,741 ^a	24,955	664,428	P=0.536
							No
P3 median	109,643	35,055	322,569	82,553	37,121	109,282	P=0.210
							No
P4 median	115,456	11,963	234,787	100,626	27,867	180,575	P=0.861
^a 1 outlier re	emoved						

tlier removed



Figure 3. PDS 10 Biofilm HPC Data

Continued from page 19

ues were unaffected by the presence of inhibitor. This phenomena is analogous to the "conditioning period" sometimes reported when changes in water quality (via blending) and corrosion inhibitors results in a transition period to reach a new equilibrium with respect to the pipe inner surface. In the case reported by Price and Jefferson (1997), transitory metal release was observed when blending surface water into what had been a groundwater distribution system. Corrosion inhibitors and pH control were also evaluated; however, any change in HPC levels were not reported.

Figure 1 shows that in Phase 1 and 2 of this study, effluent HPC values were significantly higher than the values for the Control PDS. In this case, only microbial water quality was impacted by the change, but not metals as Price and Jefferson observed. Figure 2 shows that copper concentrations were reduced within days of the introduction of inhibitor for PDS 4-6, and this was true in all the Inhibitor PDS. Lead and iron were also stable during the study at levels below the lead action level of 0.015 mg/L, and iron was typically below the secondary standard of 0.3 mg/L (data not shown). Control PDS had Pb and Cu values higher than the Inhibitor PDS, and Fe values equal to, or slightly higher than, the Inhibitor PDS (data not shown). Therefore, all of the inhibitors were able to control metals once introduced to the system, even at the lowest doses used in this study. In contrast, planktonic HPC were increased relative to the Control PDS for the first six months of the study, and did not achieve levels comparable with the Control PDS until six to nine months had passed.

Biofilm HPC densities were greater than the control biofilms in Phase 1 (Table 6); however, after Phase 1 the difference was no longer statistically significant, and by Phase 3 and 4 the median value in the Inhibitor PDS was virtually equal to that in the Control PDS.

The density of the biofilms in the Inhibitor PDS decreased with time and can be seen in Figure 3, which is representative of most of the Inhibitor PDS. Another notable observation was that PVC consistently had a lower HPC density than did the other materials, which was true in both the Inhibitor and Control PDS.

Development of Empirical Models

Bulk Effluent HPC Model for All PDS. The pooled model for Log HPC, with data from all four phases for all 14 PDS, was found to be superior to the segregated models (data not shown). The pooled model is shown in Equation 1. The coefficient matrix is listed in Table 7. This pooled model (Equation 1) permitted estimation of unique coefficients for each inhibitor and for pH control.

Table 7 Coefficient matrix for all PDSs model

Where:

- a = coefficients for different kinds of inhibitors (BOP, OP, ZOP and Silica) and pH control lines
- b = coefficients for bulk water temperature
- *c* = coefficients for bulk water influent total chlorine
- *Temp* = *bulk water temperature* ($^{\circ}C$)

*TCl*_{2-eff} = PDS bulk water effluent total chlorine (mg/L)

The dummy variable coefficients ("a") listed in Table 7 correspond to each of the inhibitors and to the pH control PDS; the values were similar except for the OP PDS. It can be seen that as a result of the greater dummy variable value for the OP PDS, the corresponding coefficients for temperature and residual were lower than for the other PDS. Since the value of the dummy variable and the coefficients both affect the predicted HPC value, the coefficients cannot be used for a direct comparison of the impact of the inhibitors relative to one another. For example, hypotheses tests showed the effluent HPC of the BOP PDS were not significantly different from that of the OP PDS (P value was 0.087); however, the dummy variable coefficient of BOP is approximately one unit less than that of OP. Instead of comparing the coefficients in Table 7, a sensitivity analysis for temperature and residual was conducted using Equation 1. Table 8 shows the sensitivity test for the model. The minimum, average, and maximum values of temperature and effluent residual are the observed values during the yearlong project and are shown for each inhibitor group.

Everything except the BOP PDS and the pH control PDS showed a greater sensitivity to residual than to temperature. The ZOP showed the lowest sensitivity to temperature, while the Si PDS showed the greatest sensitivity to residual. The pH PDS showed the lowest sensitivity to residual of all the PDS.

The main advantage of the pooled PDS model is that it achieved an improvement in the prediction accuracy for all the conditions studied. The fit of the model is shown in Figure 4. The coefficient of determination R² quantifies the strength of the association of the predicted values to the actual values observed. The R² was 0.90, which is an excellent value for microbial enumeration techniques covering a broad range of materials and water quality conditions. The predicted Log effluent HPC range was from approximately 1.90 to 4.30 log while the actual HPC values ranged from 0.49 to 4.49 log. The visual trend of the predicted values versus the actual values was good, and the pooled PDS Model seemed to work acceptably well as a tool for describing the data; however, there was still

	a	b	С
BOP	0.65	0.56	-0.19
OP	1.63	0.26	-0.21
ZOP	0.74	0.50	-0.21
Si	0.90	0.48	-0.35
pH	0.66	0.47	-0.071

Table 8 Sensitivity test for all PDS log effluent HPC (cfu/mL) model

ВОР							
Parameter	Projec	ct Value	s	Model Pre	Range		
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.1	29.1	10.5	3.66	3.22	2.07	1.59
TCl _{eff} (mg/L)	2.32	4.96	0.60	2.78	3.22	4.16	-1.37
OP							
Parameter	Projec	et Value	s	Model Pre	edicted Valu	ues	Range
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.0	29.0	10.4	3.27	3.08	2.50	0.76
TCl _{eff} (mg/L)	2.36	4.44	0.97	2.69	3.08	3.71	-1.01
ZOP							
Parameter	Projec	t Value	s	Model Predicted Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	21.3	24.3	16.4	3.13	2.93	2.57	0.56
TCl _{eff} (mg/L)	2.08	5.55	0.57	2.38	2.93	3.84	-1.46
Si							-
Parameter	Projec	et Value	S	Model Predicted Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.1	29.6	12.3	3.31	2.94	2.17	1.14
TCl _{eff} (mg/L)	2.53	5.35	0.91	2.26	2.94	4.21	-1.95
pН							
Parameter	Projec	et Value	s	Model Predicted Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.4	29.7	11.3	3.07	2.75	1.95	1.12
TCl _{eff} (mg/L)	2.20	6.32	0.62	2.55	2.75	3.00	-0.46

an over-prediction of the lowest values and an under-prediction of the highest values, but the fraction of data affected by this discrepancy was lower than for the segregated models.

Biofilm HPC Model for All PDS. The pooled model for Log BF HPC with data from all the PDS for all study phases was found to be superior to the segregated models and is shown in Equation 2. The coefficient matrix is listed in Table 9. The coefficient for PVC was lower than those of the other materials and hypothesis tests also showed PVC biofilm density was significantly less than the other materials. The Normality Test failed in all the comparisons and the Kruskal-Wallis One-Way Analysis of Variance on Ranks was used. The PVC biofilm density was significantly lower (p-value was less than 0.001) than those of the other materials. The much lower PVC dummy variable coefficient (1.2 to 2.6 log lower than the other coefficients) corresponded to the importance of material in determining biofilm density. From both hypotheses tests on actual observations and the dummy variables coefficients, unlined metals resulted in higher biofilm density, while PVC resulted in the lowest biofilm density (G >UCI >LCI >PVC). The BF HPC increased as temperature increased and decreased as residual increased. For the galvanized steel material, the residual coefficient was a positive number, but it was very low (0.0004) which is for all practical purposes, equal to zero. It indicated the residual had less effect on the biofilm density Continued on page 22





Table Q. Coefficient matrix for All PDS log BE HPC model

Tuble / Coefficient mains for All 1 Do log bit The model						
	a	b	с			
PVC	0.30	1.31	-0.94			
UCI	2.68	0.25	-0.056			
LCI	1.46	0.71	-0.62			
G	2.95	0.20	0.0004			

PVC							
Parameter	Project V	Values		Model Values	3	Range	
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.12	29.7	10.4	5.60	4.03	1.42	4.18
TCl _{inf} (mg/L)	4.93	7.00	1.65	2.90	4.03	11.33	-8.43
UCI	•						
Parameter	Project Values		Model Values	5	Range		
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.12	29.7	10.4	5.73	5.38	4.41	1.32
TCl _{inf} (mg/L)	4.93	7.00	1.65	5.28	5.38	5.72	-0.44
LCI							
Parameter	Project V	Values		Model Predicted Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.12	29.7	10.4	6.06	5.07	2.87	3.19
TCl _{inf} (mg/L)	4.93	7.00	1.65	4.08	5.07	10.01	-5.93
G							
Parameter	Project Values		Model Values	8	Predicted	Range	
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.12	29.7	10.4	5.72	5.45	4.66	1.06
TCl _{inf} (mg/L)	4.93	7.00	1.65	5.45	5.45	5.45	< 0.01

Table 10 Sensitivity test for all PDS for log BF HPC density (cfu/cm²)

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for galvanized steel, while the material impact on density was dominant. The same thing can be said for the other unlined metal, UCI.

$$LogBFHPC = a \ x \ Temp^b \ x \ TCL^{c_{2-inf}}$$
(2)

Where:

- *a* = coefficients for different inhibitors (BOP, OP, ZOP Silica, and pH control)
- *b* = coefficients for average bulk water temperature during incubation
- *c* = *coefficients* for average bulk water influent total chlorine during incubation
- Temp = average bulk water temperature during *incubation* (°*C*)

 $TCL^{c_{2-inf}} = average bulk water influent total$ chlorine during incubation (mg/L)

A sensitivity analysis on Equation 2 was conducted. The PVC and LCI showed sensitivity to temperature (ranges of 4.18 and 3.19 log) and residual (ranges of 8.43 and 5.93 log; Table 10). In contrast, the unlined metals had just over one log of variation due to temperature and no more than 0.44 logs of variation due to residual. This was because most of the predicted BF HPC value came from the much larger coefficient "a" (see Table 9) that the unlined metals had relative to PVC and LCI. This data implies that if unlined metal is used, a dense biofilm will develop regardless of temperature and residual levels. The less dense biofilms of PVC and LCI were sensitive to temperature and residual, however. It may be misleading to assign too much physical significance to the coefficient values from the regression models as they are influenced by the mathematical form of the equations; however, the sensitivity analyses of Equation 2 reinforces the significance of material for the unlined metals.

The main advantage of the pooled PDS model (Equation 2) is that it gives a good predicted versus actual Log BF HPC for all the conditions studied as shown in Figure 5. The coefficient of determination R² quantifies the strength of the association of the predicted values to the actual values observed. The R² was 0.53, which is lower than ideal, but within a reasonable range for microbial enumeration techniques involving detachment, homogenization, and covering a broad range of materials and water quality conditions. Visually, the correlation in Figure 5 looks good. The predicted Log BF HPC range was from approximately 2.86 to 6.85 log, while the actual BF HPC values ranged from 1.5 to 7.5 log. The pooled PDS Model seemed to work acceptably well as a tool for describing the data. As with the pooled PDS model for bulk effluent HPC, the low values were overpredicted and some of the high values were underpredicted.

Comparison of Bulk HPC and Biofilm HPC

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Models. In this study, the average density of suspended cells in the PDS bulk water in all distribution systems was about 2.3 orders of magnitude less (comparing 1 mL to 1 cm²) than the average density of biofilm cells. The average log biofilm density was 5.67 cfu/cm² and the average log effluent bulk HPC was 3.44 cfu/ml; however, the consumer will only see bulk HPC, and the study results showed no correlation between higher biofilm densities and higher bulk HPC values. Instead, bulk HPC values varied with residual and temperature levels.

The biofilm showed lower sensitivity to residual; this was probably because many of the bacteria in the biofilms will be protected from residual due to diffusion limitations as the biofilm gets denser and/or deeper. Looking more closely at the data, there are significant differences in sensitivity to residual between the unlined metals and the lined or PVC pipes. Temperature rather than residual impacted biofilm densities for both unlined materials (UCI and G; Table 10). In contrast, for PVC and LCI, temperature and residual had approximately the same impact, with residual being slightly more important than temperature. The average of biofilm densities in UCI and G were 5.71 and 6.02 cfu/cm², while LCI and PVC were 5.45 and 4.79 cfu/cm². It should also be mentioned that LCI biofilms were the most variable, probably due



Figure 5 Predicted log BF HPC vs. actual project log BF HPC for all PDS model

to differences in the lining with age (i.e., some LCI coupons, presumably the newer ones, had densities approaching those of PVC). The denser biofilm on the unlined metals may have contributed to lower sensitivity to residual since the disinfectant may not have been able to diffuse into the denser biofilms as well, and in addition the residual may have reacted with the unlined metal in areas where the biofilm is "splotchy", which is often the case. Regardless of why, the residual had a greater impact than *Continued on page 24*

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temperature for bulk HPC in all of the Inhibitor PDS, and for BF HPC, on all the pipe materials except the unlined metals. For unlined metals the material effect was the dominant effect, with temperature also having a significant impact. This is not to say that residual levels could be ignored for unlined metals. In this study, a high but stable residual level was maintained so that more extreme conditions (e.g., low residual or residual depletion) were never encountered. In addition, temperatures varied from cool to hot (Table 3), but truly cold temperatures were rarely observed since the PDS were located in Florida.

Conclusions

Both planktonic and biofilm heterotrophic bacteria were significantly higher in the corrosion Inhibitor PDS versus the control PDS during Phase 1. This was true for both phosphorus and silica based inhibitors, and was independent of inhibitor dose; however, between three (biofilms) and nine (planktonic) months, the Inhibitor PDS HPC converged to values comparable to the Control PDS HPC levels. It is unknown if this observed increase would occur in a significant number of full scale systems or not. In spite of the prolonged equilibration period required for planktonic and biofilm bacteria to stabilize in the presence of inhibitors, the beneficial effects of the inhibitors with respect to controlling lead, copper, and to a lesser extent iron, were realized almost immediately.

Three factors beyond addition of inhibitors were identified as being significant for determining bulk and biofilm HPC levels: 1) temperature, 2) residual levels, and, for biofilms only, 3) pipe material. Both bulk HPC and biofilm HPC were impacted significantly by temperature. Bulk HPC were more vulnerable to disinfectant residual than were biofilm HPC. Biofilm HPC were instead most affected by the pipe material that they colonized. Unlined metals resulted in high biofilm density under all the conditions of this study, and PVC always resulted in the lowest biofilm densities. The LCI was variable within the range of observations for PVC and unlined metals. There was no observed correlation between bulk and biofilm HPC, but this may be an artifact of the experimental design since material was not a variable for the bulk HPC data (i.e., all PDS had the same amount of each of the four pipe materials).

Since temperature and pipe material are typically outside the control of utility personnel, residual levels were identified as the most important management tool for maintaining biostability. Residual levels had significant impacts on bulk HPC and the less dense biofilms of the LCI and PVC pipe material. Even with the denser biofilms of the unlined metals, higher residual levels would make it more probable that any sloughed biomass from the biofilm would be inactivated. The reason for the transient increase in bulk HPC with inhibitor addition was never identified, but the data suggests that if unacceptable increases were observed, raising the disinfectant residual might be able to lower HPC levels to their pre-inhibitor levels.

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References

- Abernathy, C.G., Camper, A.K. (1998) The effect of phosphorus based corrosion inhibitors and low disinfectant residuals on distribution biofilms. In : Proceedings of the Water Quality Technology Conferences, San Diego, CA, 1-4 November, American Water Works Association, 1998.
- Appenzeller, B.M.R., Batte, M., Mathieu, L., Block, J.C., Lahoussine, V., Cavard, J., Gatel, D. (2001) Effect of adding phosphate to drinking water on bacterial growth in slightly and highly corroded pipes. Wat. Res., 35(4) :1100-1105.
- Appenzeller, B.M.R., Duval, Y.B., Thomas, F., Block, J.C. (2002) Influence of phosphate on bacterial adhesion onto iron oxyhydroxide in drinking water. Environ. Sci. Technol., 36(4): 646-652.
- Batté, M, Boniface, K., Patrick, L., Mathieu, L., Coallier, J. Prevost, M. (2003) Biofilm responses to ageing and to a high phosphate load in a bench-scale drinking water system. Wat. Res., 37: 1351-1361.
- Butterfield, P.W., Camper, A.K., Biederman, J.A., Bargmeyer, A.M. (2002) Minimizing biofilm in the presence of iron oxides and humic substances. Water Research, 36: 3898-3910.
- Hozalski, R.M., Esbri-Amador, E., Chen, C.F. (2005) Comparison of stannous chloride and phosphate for lead corrosion control. Jour. AWWA, 97(3): 89-103.
- Lyons, J.L., Pontes, J., Karalekas, P. (1995) Optimizing corrosion control for lead and copper using phosphoric acid and sodium hydroxide. In : Proceedings of the Water Quality Technology Conference, New Orleans, 12-16 November, American Water Works Association, 1995.
- Miettinen, I.T., Vartiainen, T., Martikainen, P.J. (1997) Phosphorus and bacterial growth in drinking water. Appl. Environ. Microbiol., 63(8): 3242-3245.
- Price, S., Jefferson, F.T. (1997) Corrosion control strategies for changing water supplies in Tucson, Arizona, Journal New England Water Works Association, September 1997, 285-293.
- Rompre, A., Prevost, M., Coallier, J., Brisebois, P., Lavoie, J., Lafrance, P. (1999) Impacts of implementing a corrosion control strategy on biofilm growth. In : Proceedings of the Conference on Biofilm Systems, New York, 17-20 October IAWQ/IWA, Conference 48, 1999.
- Rompre, A., Prevost, M., Coallier, J., Brisebois, P., Lavole, J. (2000) Impacts of implementing a corrosion control strategy on biofilm growth. Water Sci Technol, 41(4-5) : 287-294.
- Sathasivan, A., Ohgaki, S., Yamamoto, K., Kamiko, N. (1997) Role of inorganic phosphorus in controlling regrowth in water distribution system. Water Sci Technol, 35(8): 37-44.