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Biostability In Drinking Water Distribution Systems In A Changing Water Quality Environment Using Corrosion Inhibitors

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BIOSTABILITY IN DRINKING WATER DISTRIBUTION SYSTEMS IN A CHANGING WATER QUALITY ENVIRONMENT USING CORROSION INHIBITORS

by

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ABSTRACT

In this study, the bacterial growth dynamics of 14 pilot drinking water distribution systems were studied in order to observe water quality changes due to corrosion inhibitor addition. Empirical models were developed to quantity the effect of inhibitor type and dose on bacterial growth (biofilm and bulk water). Water and pipe coupon samples were taken and examined during the experiments. The coupons were exposed to drinking water at approximately 20 °C for at least 5 weeks to allow the formation of a measurable quasi- steady-state biofilm. Bulk water samples were taken every week. In this study, two simple but practical empirical models were created. Sensitivity analysis for the bulk heterotrophic bacteria count (HPC) model (for all 14 of the PDSs) showed that maintaining a chloramine residual at 2.6 mg/L instead of 1.1 mg/L would decrease bulk HPC by anywhere from 0.5 to 0.9 log, which was greater than the increase in bulk HPC from inhibitor addition at 0.31 to 0.42 log for Si and P based inhibitors respectively. This means that maintaining higher residual levels can counteract the relatively modest increases due to inhibitors. Biofilm HPC (BF HPC) was affected by pipe material, effluent residual and temperature in addition to a small increase due to inhibitor addition. Biofilm density was most affected by material type, with polyvinyl chloride (PVC) biofilm density consistently much lower than other materials (0.66, 0.92, and 1.22 log lower than lined cast iron (LCI), unlined cast iron (UCI), and galvanized steel (G), respectively). Temperature had a significant effect on both biofilm and bulk HPC levels but it is not practical to alter temperature for public drinking water distribution systems so temperature is not a management tool like residual.

This study evaluated the effects of four different corrosion inhibitors (i.e. based on either phosphate or silica) on drinking water distribution system biofilms and bulk water HPC levels. Four different pipe materials were used in the pilot scale experiments, polyvinyl chloride (PVC), lined cast iron (LCI), unlined cast iron (UCI), and galvanized steel (G). Three kinds of phosphate based and one silica based corrosion inhibitors were added at concentrations typically applied in a drinking water distribution system for corrosion control. The data showed that there was a statistically significant increase of 0.34 log in biofilm bacterial densities (measured as HPC) with the addition of any of the phosphate based inhibitors (ortho-phosphorus, blended ortho-poly-phosphate, and zinc ortho-phosphate). A silica based inhibitor resulted in an increase of 0.36 log. The biological data also showed that there was a statistically significant increase in bulk water bacterial densities (measured as heterotrophic plates count, HPC) with the addition of any of the four inhibitors. For bulk HPC this increase was relatively small, being 15.4% (0.42 log) when using phosphate based inhibitors, and 11.0% (0.31 log) for the silica based inhibitor. Experiments with PDS influent spiked with phosphate salts, phosphate based inhibitors, and the silicate inhibitor showed that the growth response of P17 and NOx in the assimilable organic carbon (AOC) test was increased by addition of these inorganic compounds. For this source water and the PDSs there was more than one limiting nutrient. In addition to organic compounds phosphorus was identified as a nutrient stimulating growth, and there was also an unidentified nutrient in the silica based inhibitor. However since the percentage increases due to inhibitors were no greater than 15% it is unlikely that this change would be significant for the bulk water microbial quality. In addition it was shown that increasing the chloramines residual could offset any additional growth and that the inhibitors could help compliance with the lead and copper rule. However corrosion inhibitors might result in an increase in monitoring and maintenance

requirements, particularly in dead ends, reaches with long HRTs, and possibly storage facilities. In addition it is unknown what the effect of corrosion inhibitors are on the growth of coliform bacteria and opportunistic pathogens relative to ordinary heterotrophs.

A method was developed to monitor precision for heterotrophic plate count (HPC) using both blind duplicates and lab replicates as part of a project looking at pilot drinking water distribution systems. Precision control charts were used to monitor for changes in assay variability with time just as they are used for chemical assays. In adapting these control charts for the HPC assay, it was determined that only plate counts ≥ 30 cfu per plate could be used for Quality Assurance (QA) purposes. In addition, four dilutions were used for all known Quality Control (QC) samples to ensure counts usable for QC purposes would be obtained. As a result there was a 50% increase in the required labor for a given number of samples when blind duplicates and lab replicates were run in parallel with the samples. For bulk water HPCs the distributions of the duplicate and replicate data were found to be significantly different and separate control charts were used. A probability based analysis for setting up the warning limit (WL) and control limit (CL) was compared with the method following National Institute of Standard and Technology (NIST) guidelines.

To my Mom, my sister and my family for the love, support and encouragement

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LIST OF ABBREVIATIONS

ABBREVIATION	MEANING
AOC	Assimilable Organic Carbon
AWWARF	American Water Works Association Research Foundation
BAC	Biologically Activated Carbon
BAT	Best Available Technology
BDOC	Biochemical Dissolved Organic Carbon
BF HPC	Biofilm Heterotrophic Plate Count
BOM	Biodegradable Organic Matter
CCW	Cypress Creek Wellfield
Cfu	Colony forming unit
CPU	Chloro Patinate Unit
D/DBP	Disinfectants/Disinfection By-Products
DBP	Disinfection By-Products
Diam.	Diameter
DO	Dissolved Oxygen
FC	Fecal Coliform
G	Galvanized Steel
GPD	Gallon Per Day
GW	Ground Water
GWR	Ground Water Rule
HPC	Heterotrophic Plate Count
HRT	Hydraulic Residence Time

Lab	Laboratory
LCI	Lined Cast Iron
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goals
MGD	Million Gallons per Day
NOM	Natural Organic Matter
NPDOC	Non-Particulate Dissolved Organic Carbon
NTU	Nephelometric Titration Unit
PBS	Phosphate Buffer Solution
PDS	Pilot Distribution System
PVC	Polyvinyl Chloride
PWTS	Pilot Water Treatment Systems
QA	Quality Assurance
QC	Quality Control
RO	Reverse Osmosis
RPD	Relative Percentage Difference
SM	Standard Methods
SW	Surface Water
SWTR	Surface Water Treatment Rule
TBW	Tampa Bay Water
TC	Total Coliform
TCP	Tailored Collaborative Project
TCR	Total Coliform Rule
TDS	Total Dissolved Solid

THM	TriHaloMethane
TOC	Total Organic Carbon
TTHM	Total TriHaloMethanes
UCF	University of Central Florida
UCI	Unlined Cast Iron
US	United States
USEPA	United States Environmental Protection Agency
UV	Ultra Violet
WTF	Water Treatment Facility

CHAPTER ONE: INTRODUCTION

This dissertation entitled "Biostability in Drinking Water Distribution Systems in a Changing Water Quality Environment Using Corrosion Inhibitors" is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Civil and Environmental Engineering in the College of Engineering and Computer Science at the University of Central Florida (UCF), Orlando, Florida. The research work published in this document is deemed original work and consists in part of:

One year of research work in the Department of Civil and Environmental Engineering of the University of Central Florida (UCF) on a tailored collaborative project between American Water Works Association Research Foundation (AWWARF), Tampa Bay Water (TBW) and UCF to evaluate the best available inhibitors for maintaining acceptable water quality in the TBW and member government (MG) distribution systems. During this work the following was accomplished:

- Development of a method to monitor the precision of bulk liquid heterotrophic plate count (HPC) measurements. The methodology developed for HPC was also adapted for other biological assays (for example, assimilable organic carbon - AOC).
- Determination of the effects of pipe material, inhibitor type and concentration, temperature, chloramines residual, and other water quality parameters on Biofilm HPC (BF HPC), and bulk HPC, levels in pilot drinking water distribution systems.
- Evaluation of the relationship (if any) between biofilm and bulk/planktonic HPC and subsequent development of an empirical model that describes the relationship between biofilm and bulk HPC and water quality parameters.

This dissertation is divided into several chapters:

- Chapter One provides a general introduction, overview of Tampa Bay Water and the challenges faced by their member governments, and a problem statement and research objectives for both the project and the dissertation.
- Chapter Two provides an extensive literature review on biostability, bacterial regrowth in distribution systems and related issues.
- Chapter Three describes in detail the project experimental plan and the experiment methods.
- Chapter Four to Chapter Seven consist of formatted journal articles intended for publication.
- Chapter Four develops a new method to monitor the precision of bulk liquid and biofilm heterotrophic plate count (HPC) measurements. The traditional National Institute of Standard and Technology (NIST) warning and control limits were used as initial benchmarks for assessment of relative precision, with actual probability bounds being determined once enough data was accumulated.
- Chapter Five investigates the impact of corrosion inhibitors on HPC and BF HPC levels.
- Chapter Six presents non-linear empirical models for bacterial regrowth in distribution systems and discusses their implications.
- Chapter Seven describes the biostability curve to study the two competing effects that determine the regrowth in distribution systems: inactivation due to a disinfectant and growth due to a substrate.
- Chapter Eight provides conclusions and recommendations based on the results discussed in chapters Four to Seven.

Problem Description and Research Objectives

The need for additional water supply and advanced water treatment in response to the increasingly stringent water quality regulations and increased demand due to population increase requires an understanding of how blended water behaves and also the impact of corrosion inhibitors. The most easily treated water supplies are not limitless, and it is crucial to design master water plans both to preserve existing resources and to guarantee sufficient provision of biologically safe drinking water for the future. This concern has been raised in the Tampa Bay area by Tampa Bay Water (TBW) due to the need to utilize new water sources to meet demands for the near future. The following section provides an overview of TBW and the problems they are facing.

Tampa Bay Water and TBW I Project

As with many metropolitan areas, the Tampa Bay area is facing increasing consumer demand and more stringent environmental and drinking water regulations. TBW is responsible for management of drinking water resources in the Tampa Bay area. Ground water, surface water, and brackish water resources are regionally available. Groundwater from the Floridian Aquifer has been used almost exclusively for drinking water supply by all member governments except the City of Tampa which predominately uses surface water. Groundwater use has been cited for adverse environmental impacts and as a consequence, TBW has been directed to reduce groundwater use by the Southwest Water Management District. In order to meet drinking water demands, Tampa Bay Water has developed regional surface water and desalinated supplies which will offset the reductions in groundwater use and provide for growth in the region.

UCF has conducted research regarding the effect of variable finished water quality on distribution system water quality since 2000. From the previous tailored collaboration project

(TCP) entitled "Effects of Blending on Distribution System Water Quality" conducted by Taylor et al. (2005), several observations have been noted on distribution system scale disruptions associated with water quality changes as blends are introduced to the system and these are summarized in the following bulleted text:

- Apparent color, particulate iron, and turbidity were released from cast iron and galvanized pipe when the distributions systems were exposed to water which, in comparison to historical water quality conditions, had lower levels of alkalinity and higher levels of sulfates and chlorides.
- Control of copper corrosion in the available groundwater requires an active corrosion control strategy to meet regulatory requirements for copper because of the high alkalinity of the groundwater.
- Nitrification occurred when the chloramines residual was lost, and probably occurred primarily if not totally in the biofilm on the surface of the distribution system pipes.
- Biofilm growth increased directly with surface roughness of pipe and was greater for galvanized steel and unlined cast iron pipes.

The work demonstrated that control of the scale or film in the existing distribution systems is a primary factor for maintaining acceptable distribution system water quality. The finished water quality received by each member government (MG) will vary because of the use of ground water, surface water, and sea water sources and the associated treatment processes. Not all finished water quality parameters have the same effects on distribution system water quality. The most significant controlling parameters found to date within the variation of water quality expected in the MG distribution systems are alkalinity, sulfates, and chlorides. Changing distribution system water quality can not be eliminated and the study had shown that some aspects of change in water

quality can adversely impact the distribution system scale/film and the associated distribution system water quality.

Research Objectives

One potential method of significantly reducing or eliminating adverse water quality impacts from the disruption of distribution system scale or film is to replace the controlling scale or film with a film that will not be disrupted when exposed to the expected changing water quality. This is possible with surface active agents such as corrosion inhibitors. Corrosion inhibitors offer an opportunity for scale control because they bond directly with the elemental metal or scale on the pipe surface, forming a barrier between the interior pipe surface and the bulk water. This action potentially could reduce surface area for biofilm growth; reduce residual demand; control the release of iron particles, apparent color, and turbidity from the pipe wall; and thus improve distribution system water quality in general. Additionally, the new film will not be dependent on alkalinity, sulfates, chlorides or any other of the water quality parameters that will vary seasonally in the regional finished water. The film will only be dependent on the surface active agent that is added to finished water. The advantage of using a surface active agent such as a corrosion inhibitor is that the concentration of the inhibitor is controlled by application and ideally is not affected by the changing water quality.

However there is little information on the effect of variable water quality on the capability of corrosion inhibitors to function in a distribution system. While it is unreasonable to assume a surface active film can offset all changes in distribution system water quality, it is entirely reasonable to expect an inhibitor generated surface active film to greatly reduce the adverse effects on distribution system water quality. It is probable that maintaining acceptable water quality may

be achieved through the use of corrosion inhibitors. But proper selection of corrosion inhibitors could be critical in maintaining the acceptable water quality in the distribution systems. Therefore, this project is a continuation of the previous TCP and it investigates the best available inhibitors for maintaining acceptable water quality in the TBW and MG distribution systems.

Tampa Bay Water II Project Plan

As described in Chapter Three, eighteen different pilot distribution systems (PDSs) and a number of pilot treatment systems were used in the implementation of the previous project, "Effects of Blending on Distribution System Water Quality" (Taylor et al. 2005). During this project two finished waters were produced at the project site: a conventionally treated groundwater (GW) and a desalinated groundwater (RO), and a treated surface water was also brought to the site to give the three components of the blended water fed to the fourteen hybrid PDSs. The hybrid PDSs consisted of PVC, lined cast iron (LCI), unlined cast iron (UCI), and galvanized steel (G) pipe. The pipes were aged as they were taken from the existing MG distribution systems. Four other PDSs consisted of a single pipe material such as PVC, or all LCI, etc.. The fourteen hybrid PDS were used for the inhibitor study while the other four, the single material lines, were only used to study the effect of Reynolds' number on chloramines decay. Twelve thousand gallons of water a week were used during the project, seven thousand gallons were produced from the two pilot processes and five thousand gallons of surface water (SW) was hauled from the TBW regional surface water treatment plant. The three types of water were blended at predetermined ratios in an individual tank that served as the feed to thirteen (13) of PDS. The other PDS received the same blend; however, its pH was adjusted to the pHs and pHs+0.3. Three UCF

faculty, ten UCF graduate students and two MG field personnel were involved in the operation and maintenance of the field facility.

To conduct the inhibitor study, four different corrosion inhibitors were selected and added to the PDS system: blended ortho-phosphate (BOP), ortho-phosphate (OP), zinc ortho-phosphate (ZOP), and silicate. Each inhibitor was studied at three doses: 0.5, 1, and 2 mg/L as P for all the phosphate type inhibitors. Silicate inhibitor was applied at 3, 6, and 12 mg/L as SiO_2. A delivery system was constructed that included a common blend tank for each inhibitor and separate pumps for each PDS. Physical, chemical, and biological changes resulting from the blended water quality and the addition of inhibitors were monitored through the PDSs.

Dissertation Scope and Objectives

In order to understand all the impacts, both positive and negative, of corrosion inhibitors on drinking water quality, a broad array of water quality parameters were monitored for the PDSs receiving corrosion inhibitors as well as the two parallel control PDSs which did not receive inhibitors.

The impact of corrosion inhibitors on biostability in distribution systems, despite its importance, has not been extensively investigated. Simultaneous study of attached and suspended growth in distribution system under changing water quality scenarios and environmental conditions using corrosion inhibitors has not been reported. For these reasons, the main objectives of the research discussed in this dissertation were:

- To conduct an extensive literature review on biostability in distribution systems, accounting for significant parameters influencing bacterial proliferation and extended to related issues.

- To investigate attached and suspended growth simultaneously in parallel systems allowing the isolation of corrosion inhibitor as an experimental variable.
- To generate empirical models for bulk HPC and biofilm HPC as a function of water quality parameters including evaluation of corrosion inhibitors. Investigation of any link between HPC biofilm and bulk water HPC. Development of a method for monitoring HPC enumeration precision.
- To evaluate if organic carbon was the only limiting nutrient or if instead there were other nutrients that could also stimulate growth in the PDSs.

CHAPTER TWO: LITERATURE REVIEW

Drinking Water Regulations

The purpose of this section is to review how the U.S. Environmental Protection Agency (EPA) has developed federal drinking water regulation that control for pathogens and disinfection by products (DBPs). Modern disinfection practices are largely driven by the dual requirements of disinfection accompanied by avoidance of Disinfection By-Product (DBP) production. Pathogens and DPSs are considered together because control for either one of these groups of contaminates has a direct influence on the other. Federal legislation to control infectious disease in the United States began with the National Quarantine Act of 1878 (Pontius, 2003). The 1912 regulations led to the 1914 Treasury Department standards, which prescribed mandatory limits for bacteria in interstate carrier supplies. Under these standards, the level of Bacteria coli (i.e., coliform bacteria) were limited to less than 2.2 coliforms per 100 mL, and the total bacterial count was not to exceed 100 cfu. Later revisions of the drinking water standards by the U.S. Public Health Service (USPHS) in 1925, 1942, 1946, and 1962 modified the coliform standard and added standards for several inorganic chemicals. In practice, most coliforms are Escherichia coli or species of Klebsiella, Enterobacter, and Citrobacter. Of the many coliform strains, only a tiny minority are pathogenic. The use of the coliform group as a fecal indicator was controversial from the beginning. The difficulty is that most coliforms are also widespread in natural water and soil. Thus a coliform-positive sample does not necessarily indicate fecal contamination. One coliform, Escherichia coli, seldom survives outside the gut for long and thus was considered a satisfactory fecal indicator, but the absence at that time of a suitable culture medium that could distinguish E. coli in a mixture of many other waterborne bacteria precluded its use. Other

organisms were proposed as fecal indicators during this period, but only the enterococci and fecal coliforms were given serious consideration (Geldreich 1966, Kabler and Clark 1960, Committee on Public Health Activities 1961). To clarify terminology, the terms “coliforms”, “coliform group”, “coliform bacteria”, and “total coliforms” are interchangeable. Among the other USEPA standards set in 1975 was one for turbidity. The agency’s primary reason in 1975 for regulating turbidity was to minimize its interference with chlorine disinfection. The Agency also recommended (but did not require) that systems monitor bacterial plate count (also called standard plate count, heterotrophic plate count (HPC), total bacterial counts, etc.). The bacterial plate count is the total number of bacterial colonies per sample volume growing on a specified culture medium under defined incubation conditions; it is used as a rough index of microbial drinking water quality. The premise is that a high HPC, or a sudden increase in HPC, reflects inadequate water treatment. In 1977, the use of a coliform standard and HPC was supported by the National Research Council (NRC 1997). In the 1986 Safe Drinking Water Act (SDWA) reauthorization congress required USEPA to regulate 83 specified contaminants by 1989, including total coliform, turbidity, viruses, Giardia lamblia, Legionella, and heterotrophic plate count (HPC) (Pontius and Clark, 1999). USEPA embarked upon a revision of the 1975 Total Coliform Rule (TCR) to address perceived shortcomings of the rule. The revised TCR, published in 1989, based the maximum contaminant levels (MCLs) on the presence or absence of total coliforms in a 100-mL sample, rather than on coliform density. USEPA defined a reasonably safe drinking water as one that contained coliforms in no more than 10 % of its volume. This led USEPA to set a MCL of 5.0 %; thus, no more than 5.0 % of the total coliform samples collected during a month could be positive.

To respond to the SDWA mandate to control the pathogens mentioned in the previous section (Giardia, Viruses, and Legionella) in surface water and to meet the 1986 SDWA mandate regarding the filtration of systems using surface water sources, USEPA published the surface water treatment rule (SWTR) in 1989. The SWTR also met the SDWA mandates to control HPC and turbidity and, at least for systems using surface water, to require these systems to disinfect. A central issue in developing the SWTR was what minimum level of treatment should be set. Two perspectives were considered in addressing this issue. The perspective was to define the level of treatment that could be attained by a well operated system using conventional treatment (coagulation, flocculation, sedimentation, rapid granular filtration, chlorine disinfection). Such systems rarely had been implicated in a waterborne disease outbreak. Research on filtration and disinfection indicated that such systems could achieve at least a 3log (99.9%) and 4log (99.99%) reduction through the removal and/or inactivation of Giardia and viruses, respectively. In the late 1980s, USEPA had identified a number of disinfection by productions (DBPs). Several animal studies and epidemiology studies suggested that some disinfectants and DBPs might pose a public health risk. These included DBPs from chlorine (e.g., haloacetic acids, haloacetonitriles), from ozone (bromate, aldehydes), from chlorine dioxide (chlorite, chlorate), and from chloramines (cyanogen chloride). In 1998, USEPA published the Stage I Disinfection Byproducts Rule (DBPR). This rule set a treatment technique to reduce the formation of unregulated DBPs in systems that were most likely to have higher levels of these DBPs. This treatment technique required conventionally treated surface water systems to remove specified amounts of organic materials [measured as total organic carbon (TOC)], using enhanced coagulation or enhanced softening. Table 1 contains the basic TOC removal requirements, with compliance based on a running annual average.

Table 1 Required TOC removal by enhanced coagulation/enhanced softening for surface water system using conventional treatment

Source Water TOC (mg/L)	Source Water Alkalinity, mg/L as CaCO3		
	0-60	>60-120	>120
>2.0-4.0	35.0	25.0	15.0
>4.0-8.0	45.0	35.0	25.0
>8.0	50.0	40.0	30.0

In 2000 (USEPA 2000a) USEPA proposed a regulation known as the Ground Water Rule (GWR) that included the following provisions: (1) a periodic state-conducted sanitary survey for every system and a requirement to correct each significant deficiency identified, (2) an initial phase of source water monitoring by undisinfected systems using a fecal indicator (such as E. coli, enterococci, or coliphage), and (3) for each undisinfected system, an assessment of the hydrogeological characteristics to gauge whether the source water might be vulnerable to fecal contamination.

In the United States current disinfection practices are driven largely by TCR, SWTR, and MCL for total trihalomethanes (TTHMs). The approach developed in the United States, instead of relying on removal of, or low, NOM concentrations, relies on alternate primary disinfectant practices when source water NOM is high and increasingly less use of free chlorine for secondary residuals as well. The TCR pertains to both ground waters and surface waters alike, and sets an MCL of zero for Total Coliforms, Fecal Coliforms, and *E. coli* (Pontius, 2000). The Best Available Technology (BAT) for meeting these MCLs is defined as disinfection. There are also many additional details for actually meeting the MCL with respect to the regulatory requirements.

The SWTR establishes MCLs for *Giardia lamblia*, *Legionella* (bacteria), and viruses (Pontius, 2000; U.S.E.PA., 2000). In addition treatment technique requirements are established for meeting HPC and turbidity criteria. Under the SWTR both parameters with and without MCLs have filtration and disinfection defined as the BAT required. There are specific criteria making it possible not to use filtration in some cases however. The Enhanced Surface Water Treatment Rule (ESWTR) contains a proposed MCL of zero for *Cryptosporidium* (protozoal cysts) which will add this parameter to the list of regulated microbiological contaminants (Pontius, 2000).

Current US regulations for disinfection pertain to surface waters and groundwaters designated as under the direct influence of surface water (SWTR). These regulations are largely based on the concept of CT, the product of disinfectant concentration (mg/L) and contact time (minutes). Minimum CT products have been defined from prior studies for inactivation of the microbiological contaminants regulated in the US. Treatment technique requirements pertaining mainly to a) meeting specified CT times, and b) filtration, can sometimes be used in lieu of actual measurements of the maximum contaminant levels (MCLs) for *Giardia lamblia*, *Legionella*, viruses, and for meeting turbidity and heterotrophic plate count requirements also (USEPA, 2000). However specific monitoring requirements for turbidity and other measurements are also outlined, depending on how the plant is categorized according to the regulations. Unless certain criteria are met, filtration is required for treatment of water systems supplied by surface water or surface water influenced groundwater. Under the SWTR effective filtration is assumed to achieve (credited with) 2.5 log removal of *Giardia* and 2 log removal of viruses. Disinfection is required for the remainder of the removal-inactivation (Haas, 2000).

Modern disinfection practice has also recognized that chlorine resistant pathogens, such as protozoans, exist. This has led to a multiple barrier approach that does not rely solely on chlorine

or other oxidants to protect public health for source waters where protozoans or other disinfectant resistant pathogens may be present (Haas, 1999). Other treatment processes such as filtration become important aspects of the overall production of biologically safe drinking water. In addition design and operation of treatment processes prioritizing for the concept of robustness rather than peak performance is an important aspect of insuring the safety of the consumer. Practices such as covering reservoirs and other source protection techniques, maintaining positive pressure and corrosion control in distribution systems, use of flushing, pigging, are all significant aspects of eliminating pathogens and maintaining a biologically stable system (Trussel, 1980).

Secondary disinfectant residual is also an important aspect of practice in the U.S.A. The use of a residual is based on three arguments: 1) suppression of bacterial regrowth, 2) disinfection of exogenous intrusions, 3) as a sentinel to detect intrusions or breaches (Haas, 1999). In Europe elimination of the presumed limiting nutrient, carbon, is often seen as a superior way of suppressing bacterial regrowth (Van der Kooij, 1999; White, 1999), but this point of view is questioned by Haas (1999) who cites several studies in which phosphorus, not carbon, was the limiting nutrient, and he suggests other inorganic nutrients may be limiting in some systems as well. Other practitioners have reported full scale systems where phosphorus rather than carbon was limiting (e.g. Haas *et al.*, 1988).

In the study by Volk and LeChevallier (2000), it was found that coliform occurrences in distribution systems were affected by temperature, disinfectant residual, and Biodegradable Organic Matter (BOM) levels. The temperature threshold values were 15° C, Assimilable Organic Carbon (AOC) > 100 μg/L, and dead-end disinfectant levels < 0.5 mg/L for free chlorine or 1.0 mg/L for chloramines.

Bacterial Growth In Drinking Water Distribution Systems

Biostability is a concept that addresses the overall tendency of the water to promote or suppress microbial proliferation, and can be viewed as an assessment of overall distribution system quality with respect to microorganisms. It pertains to the proliferation of microorganisms in the water distribution system and does not address the ecology of opportunistic or other pathogens or coliforms. Biostability describes aggregate proliferation and does not address the fate of specific subpopulations. As a result biological instability may or may not favor the proliferation of coliforms or a specific pathogen(s) depending on a host of additional factors.

Brazos and O'Connor (1985) proposed specific definitions for two terms that have been synonymously used to describe the unexplained occurrence of blooms or high bacterial population in potable water distribution systems: "regrowth" and "aftergrowth".

Regrowth is the recovery of disinfectant-injured cells which have entered the distribution system from the water source or treatment plant, while aftergrowth is growth of microorganisms native to a water distribution system. These definitions do not clearly discriminate between the two primary mechanisms by which the microorganisms appear in the distribution system, i.e. breakthrough in the treatment plant and growth within the distribution system (van der Wende and Characklis, 1990).

Breakthrough is the increase in bacterial numbers in the distribution system resulting from viable or injured bacteria passing through the disinfection process, which is only meant to suppress pathogenic organisms. Injured cells have the ability to recover and as well as viable cells can inoculate the biofi lms and/or reproduce in the bulk water. Growth is the increase in viable

bacterial numbers in the distribution system (either in biofilms or in the bulk water) resulting from bacterial growth downstream of the disinfection process (van der Wende and Characklis, 1990).

Bacterial Nutrients In Drinking Water Distribution Systems

The bacterial growth in water distribution systems is due to the natural presence in soils and the waters of bacteria. Most of them are participating in one of the following elementary cycles: 1) the carbon cycle, 2) the nitrogen cycle or 3) the sulfur cycle. Iron oxidizing and iron respiring bacteria can also be significant. The bacteria involved in the carbon cycle are heterotrophic organisms, which are very diverse in terms of their metabolism. They can use several electron acceptors (i.e. oxygen, nitrates, nitrites, sulfates, ferric ions). In distribution system, not only heterotrophic organisms exist, also autotrophs. The autotrophs (such as nitrifiers) can initiate the colonization of the inner surface of the distribution systems. The autotrophs convert inorganic carbon to organic carbon and produce a nutrient source for the heterotrophs. So, the autotrophs become the primary initiators of a more complex food chain and make the colonization by heterotrophs possible even under low carbon level conditions. The limitation of carbon source may not be the only nutrient that limits bacterial growth in a distribution system if other nutrients such as ammonia nitrogen and bioavailable phosphorus are present in very low amounts, or alternately if biodegradable organic matter (BOM) is present in large amounts (it is really the C:N:P ratio that determines nutrient limitation). Further it is possible to have multiple limiting nutrients simultaneously since microbial growth rates can be impacted by concentrations of carbon, nitrogen, and phosphorus containing compounds. In addition electron acceptor concentrations can impact growth rates. However carbon (i.e. BOM) is widely accepted as the key limiting nutrient in distribution systems. However there are examples in the literature where phosphorus

was a significant or even the most important limiting nutrient. It was also found that increasing bioavailable phosphorus increased the growth response during this study in some supplemental experiments which probably implies that both carbon and phosphorus were limiting nutrients (multiple nutrient limitations), an observation that has been observed in the literature as well (Haas 1999; Charnock and Kjonno, 2000; Le Puil, 2004).

There are several methods to quantify the potential of bacterial regrowth in water samples. Those methods are divided into two different types: 1) those which directly quantify a limiting nutrient (usually biodegradable dissolved organic carbon, BDOC), and 2) those which directly quantify the bacterial growth in the sample (e.g. AOC) (Le Puil, 2004).

The methods which quantify a limiting nutrient often assume carbon as the limiting nutrient for growth (i.e. BDOC). In addition there are methods that directly measure heterotrophic bacterial growth in a way implying that carbon is the limiting nutrient (i.e. AOC). However it can be found in the literature examples of distribution systems for which other nutrients, such as phosphorus, were limiting. The use of corrosion inhibitors may also provide other nutrients that could impact growth such as phosphate, or maybe even trace AOC or other compounds that could impact growth as well as surface colonization (e.g. silica?). Haas (1999) observed a greater growth with phosphorus addition than with carbon addition, which could be significant since several corrosion inhibitors are based on ortho- and poly-phosphates. In addition, it has been suggested in the literature that the global effect of those inhibitors to prevent the biofilm development is associated with the saturation of adsorption sites at the inner surface of the pipes. This blocks simultaneously the adsorption of natural organic matter (NOM) – which could be used as substrate for fixed biomass – and the adsorption of microorganisms at the surface (Haas, 2000). Certain methods have been specifically developed to determine the effect of phosphorus as major limiting

nutrient (Lehtola *et al.*, 1999). Moreover, certain methods such as AOC sometimes give relatively high results when the samples receive inorganic nutrients (Charnock and Kjonno, 2000) indicating that carbon is not the only limiting nutrient of interest for bacterial regrowth. Recent studies in Japan and Finland have argued that in finished drinking water or in certain samples collected from the distribution system, phosphorus is an important limiting factor for bacterial regrowth (Sathasivan *et al*., 1997; Sathasivan and Ohgaki, 1999; Lehtola and Miettinen, 2001; Lehtola *et al*., 2002a, b, 2004; Miettinen *et al*., 1997; Keinanen *et al*., 2002). Each mg P added to drinking water is reported to support the growth of 1.2×10^8 cells (Sathasivan *et al*., 1997; Sathasivan and Ohgaki, 1999). This is in marked contrast to results from the USA and elsewhere, where studies suggested that orthophosphate dosing to distributed water did not increase biofilm growth on iron in actual or simulated distribution systems (Camper *et al*., 2003; LeChevallier *et al.*, 1993). Instead, in the USA and elsewhere, it is believed that organic matter limits regrowth (van der Kooij, 1992; Camper *et al*., 2003). A key assumption by proponents of the phosphate limitation theory is that "phosphorus can not be introduced into the system unless it is introduced with the water entering the system" (Sathasivan *et al.*, 1997; Sathasivan and Ohgaki, 1999). That is, the water itself is the only possible source of phosphorus for bacteria. If phosphate is limiting and disinfectant concentrations are low, dosing of a phosphate corrosion inhibitor could create problems with regrowth (Miettinen *et al.*, 1997; Morton *et al.*, 2005).

Biofilms in Drinking Water Distribution Systems

Biofilms

A biofilm is a layer of microorganisms in an aquatic environment held together in a polymeric matrix attached to a substratum (Figure 1). The Matrix consists of organic polymers that are

produced and excreted by the biofilm microorganisms and are referred to as extracellular polymeric substances (EPS). The chemical structure of the EPS varies among different types of organisms and is also dependent on environmental conditions (van der and Characklis, 1990).

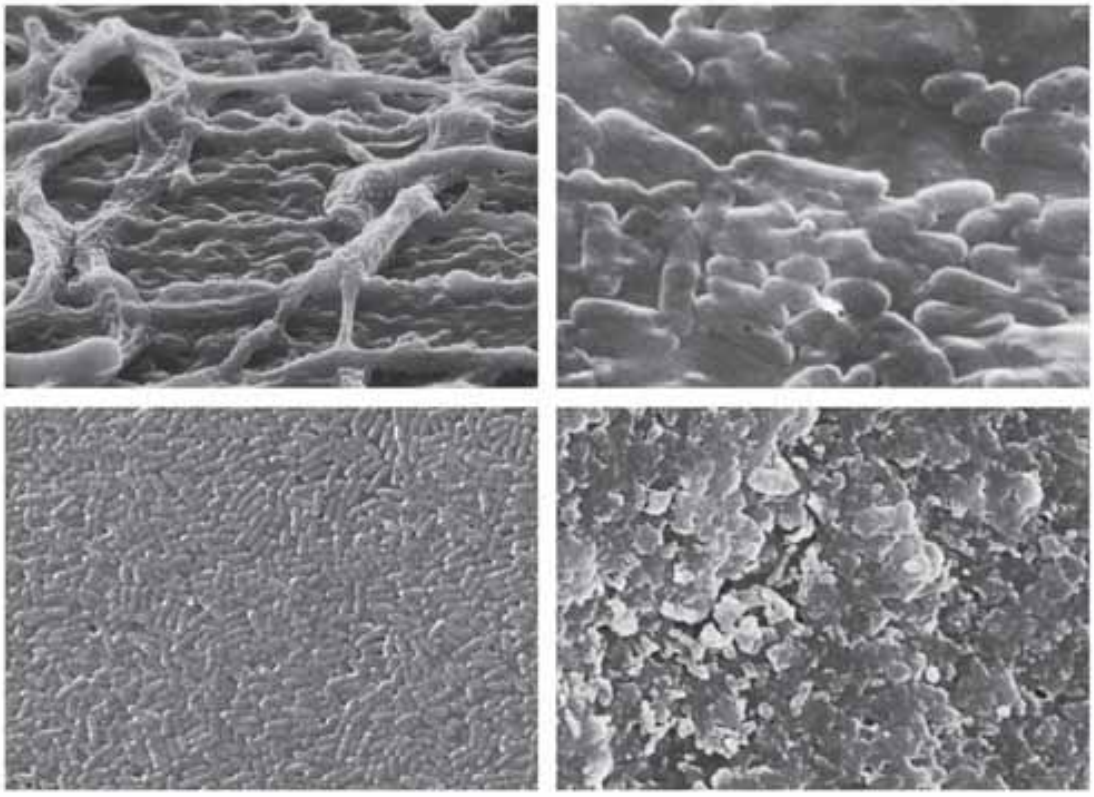

Figure 1 Tansmission Electron Microscopy (TEM) picture of biofilms

A thorough study of biofilm accumulation and biofilm composition throughout drinking water distribution systems is not reported due to the great difficulty to explore the fixed biomass without disturbing it. The biofilm structure is not well described because debris, corrosion products, mineral deposits as well as the formation of corrosion clumps (offering new sites or surfaces to be colonized by biomass) complicate this structure (Allen et al., 1980; Sly et al., 1988;

Flemming and Geesey, 1990; LeChevallier et al., 1987; Ridgway and Olson, 1981 ; Stolzenbach, 1989 ; Tuovinen et al., 1980).

Numerous studies about biofilms' thickness, porosity, density, and fractal dimensions have been generally done with thicker biofilms, generated in laboratory and therefore are not totally adapted to drinking water distribution systems. Biofilms in water distribution systems are thin, reaching maximum thicknesses of perhaps a few hundred micrometers (van der and Characklis, 1990) as shown in Figure 2. When the biofilm (or the micro colonies) are thin (< 40μm), the oxygen-and-nutrient transfer would not be limited and the parameters describing the activity of the global biofilm would be the same as the one used for bacteria in suspension (Bake *et al.*, 1984). When the biofilm (or the colony) is thick (>80μm) the respiratory activity in the deepest layers is reduced (de Beer *et al.*, 1994b). This explains that a fraction of the fixed biomass is less active (Kalmbach *et al.*, 2000; Zhang and Bishop, 1994). The biofilm is composed of a mixture of microorganisms with variable activities, as a function of their position in the aggregate (Rittmann and Manem, 1992). Even microcolonies (50 cells) may represent an association of several genuses (Manz *et al.*, 1993). In Ridgway and Olsons' (1981) study, scanning electron microscopy (SEM) was used to observe the bacterial colonization of a distribution main consisting of galvanized iron pipe with a 0.5 cm thick hard, black lining, presumably cement or porcelain. The surface was examined for microorganisms at high magnification. Many of the observed cells had a coccid shape and the cells were sometimes linked together in chains like streptococci. Filamentous bacteria that bore a clear morphological similarity to the streptomacetes were the most frequently observed microorganisms.

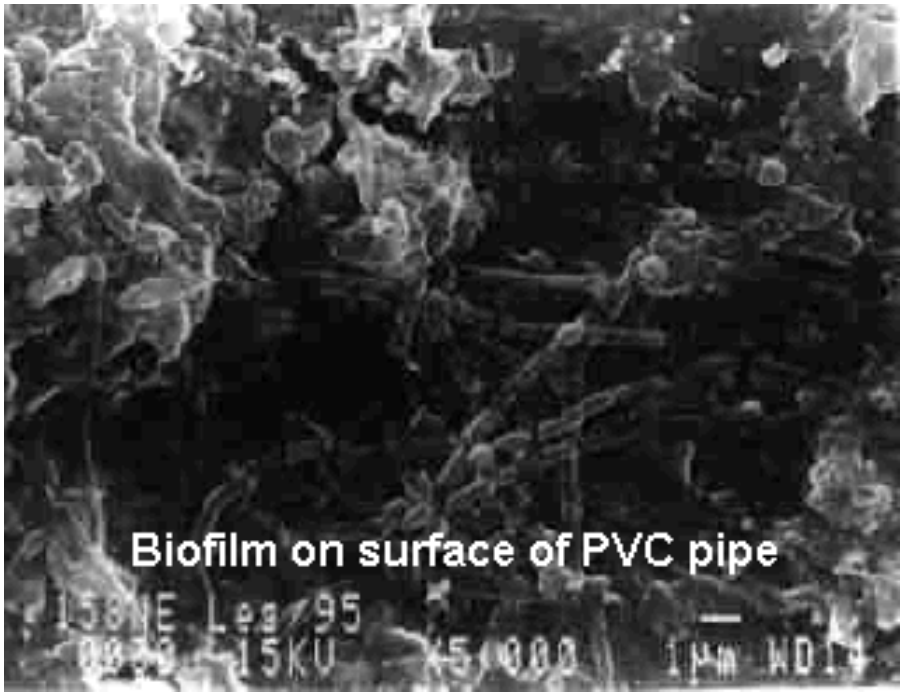

Figure 2 Biofilm on surface of PVC pipe in drinking water distribution system

The biofilm has a non-uniform dispersion structure at the surface of the materials in contact with water because of the discontinuity and the heterogeneity (van der Kooij and Veenendaal, 1992). The aggregates that are distinct from each other in the biofilm are surrounded by canals that occupy up to 50% of the volume of the film and in which there is circulation of water, particles, protozoa, etc (de Beer *et al.*, 1994a et b; Devender, 1995 ; Gjaltema *et al.*, 1994 ; Massol-Deyá *et al.*, 1995 ; Stewart *et al.*, 1993, 1995 ; Stoodley *et al.*, 1994). Diffusion velocity limited the transfer of molecules (oxygen, disinfectant, nutrients). The total microbial count on those surfaces in contact with drinking water is generally high (ranging from 10^6 to 10^8 cfu/ cm^2) (Donlan and Pipes, 1988; Lévi *et al*., 1992; Mathieu *et al*., 1992; Pedersen, 1990). The biofilm stationary phase is never reached in a real distribution system, due to frequent discontinuities: variation in the hydraulic regime, changes in the nature and concentration of nutrients and disinfectants,

introduction of new microorganisms. Zacheus *et al.* (2000) suggested that there is a slow but continuous accumulation of biomass after 5 months of exposure to potable water. Percival *et al.* (1998) observed a switch in the dominant bacterial populations from the "pioneers" which colonize a steel pipe in less than one month to the species existing after 5 months of immersion in the water. Allison *et al*. (2000) also observed by analogy with the actual concepts of differentiation of biofilms during their ageing, there is reorganization of physical biofilm system as it develops there (formation of micro-aggregates, canals, etc.).

Biofilm Measurement

At full-scale, biofilm investigations have examined the treatment conditions that affect the accumulations of biofilm cells (LeChevallier *et al*., 1990; Donlan and Pipes, 1988) and identified cells that attached to the pipe surface (LeChevallier *et al*., 1987). Even if the density of fixed microorganisms is up to $10^6 – 10^8$ cells per cm^2 in most distribution systems, the species diversity and their activity are highly variable and not well known due to obvious technical limitations. One limitation is the difficulty of cultivating these microorganisms (Byrd et Colwell, 1991; Colwell et Grimes, 2000; Rozack et Colwell, 1987). The ability to control environmental (e.g. temperature, organic matter) and operating parameters (e.g. flow rate, chlorine residual) is limited at full-scale. Therefore, many methods and reactors have been developed for assessing and characterizing attached microorganisms from various environmental applications (Cassidy *et al*., 1996; Gjaltema and Griebe, 1995). The Heterotrophic Plate Count method (HPC-R2A USEPA Standard Methods) still remains a reference technique, widely used (Reasoner, 1990), which can be correlated to enumeration techniques based on respiratory activity (INT or CTC marking) (Coallier *et al.*, 1994; Rodriguez *et al*., 1992; Yu et McFeters, 1994), and total direct count (Saby *et al*., 1997).

The methods used to study and describe biofilms are dependent on the information sought. In many cases, regulatory concerns dictate that simple, reliable methods like plate counts be used. Traditionally, environmental bacteria and biofilm have been studied by culture-dependent methods or direct microscopy. Both approaches have limitations. It is now well established that cultural methods underestimate the numbers and diversity of environmental bacteria. Amann *et al*. (1995) suggested that cultural techniques fail to enumerate two classes of organisms: a) known and previously cultured species that have entered a state in which they can no longer be cultured, or not by the method being used; and b) novel species for which no suitable culturing method has been developed. However, they noted that nature is capable of culturing all species. While direct microscopy detects greater numbers of cells than culturing, species generally can not be identified under the microscope based on morphology alone (Trebesius *et al.* 1994).

Factors Controlling Drinking Water Biofilm Formation

An indication of the substantial evidence for interactions is given in the literature about regrowth in distribution systems. Key conditions believed to be conducive to the proliferation of both coliforms and heterotrophs in distribution biofilms have been: 1) temperature effects, especially warm water conditions; 2) the amount of usable carbon for substrate; 3) inefficiencies in the removal/disinfection of organisms in treatment; 4) the presence of corrosion products in distribution systems; 5) disinfectant dose/type; and 6) distribution system hydrodynamics (Figure 3) (Dennus, Godfree and Stewart-Tull, 1999).

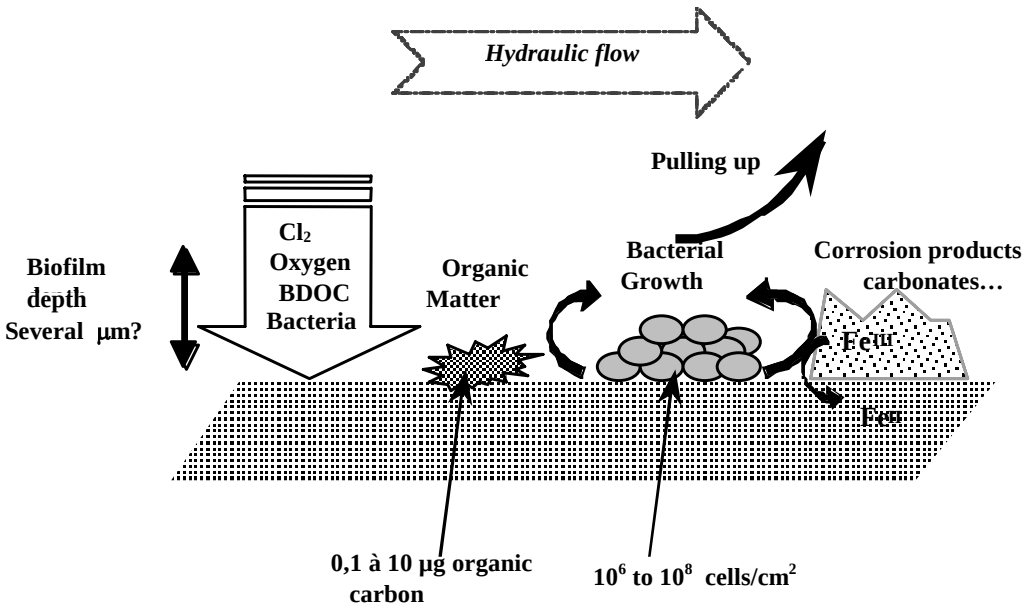

Figure 3 Scheme of several mechanisms taking place in the accumulation of biofilms on a surface in contact with potable water.

The biofilm appears to be a meta stable system, which is fed by the entrance of cells from the bulk (the velocity of deposit/fixation of cells on the surface of materials is correlated to the cell density of moving waters) and by the multiplication of bacteria, and also stabilized by the constant pulling up and release of cells from the biofilm to the bulk (Le Puil, 2004).

Under these conditions, the organization of the biofilm and the structure of the settlements mainly depend on several other factors:

- Various hydraulic regime of the system (Bryers, 1987; Characklis et Marshall, 1990; Characklis et Wilderer, 1989).
- Bacterial species introduced in the system from the treated or non-treated source. An astonishing example is the case of acidic boreal waters, characterized by a high content of humic substances and acid-resistant bacteria. Once treated, these sources allow the

growth of biofilm with classical cell density (about 10^7 cells /cm^2) but with 10^3 Mycobacterium/cm^2 (Iivanainen *et al*., 1999).

- Competition and advantage given to certain species due to their nutritional requirements. As an example, the presence of ammonium in the system supports the development of a strong autotrophic nitrifying population (Lipponen *et al*., 1998).
- Nature of the materials used for drinking water distribution systems. All of them are largely colonized by microorganisms (Niquette *et al.*, 2000; Zacheus *et al*., 2000) but the supporting materials play a significant role in the selection of biomass and its organization (Kerr *et al.*, 1999; Kielemoes *et al*., 2000; Pedersen, 1990; Rogers *et al*., 1994 ; Van der Kooij *et al.*, 1995). Indeed material determines the adsorption efficiency of the "pioneers" and can be source of nutrients or growth factors. Analysis of biofilms adsorbed on glass or polyethylene showed a different proportion of bacteria detected on each material (4 and 26% respectively) by oligonucleotidic probe ALF 1b (Kalmbach *et al.*, 2000). In another situation, polyethylene, PVC, steel and copper display similar cell density but on copper the bacterial activity was a lot less due to the toxicity of Cu ions (Schwartz *et al.*, 2000). Finally, in the case of materials susceptible to corrosion, it has been clearly demonstrated that the presence of iron corrosion products enhances the activity and the production of heterotrophic biomass (Appenzeller *et al*., 2001).
- Resistance of fixed biomass to oxidants (Morin *et al.*, 1999) can be partially explained by the reducing ability of the biofilm, of the fixed organic matter and sometimes of the supporting material, as well as poor diffusion of the oxidant (de Beer *et al.*, 1994a; Stewart and Raquepas, 1995). The exposure of the biofilm bacteria to sub lethal oxidative stresses (Storz *et al*., 2000) leads to a remarkable cellular defense

(surproduction of intracellular glutathione) and an increased resistance of the bacteria to oxidants.

- Disinfections type and reaction with biofilm and pipe material. Free chlorine is the most commonly employed disinfectant because of its remained in water. Nevertheless chlorine decays in large distribution systems, and in areas where the chlorine concentration drops below a minimum desired level, booster chlorination has to be installed in order to maintain a sufficient chlorine level in the water (Lu, Kiene and Levi, 1998). Maul *et al.* (1985a) concluded that the occurrences of the highest bacterial concentrations are attributed to lower levels of chlorine residuals and prolonged retention time of the water in the network. This temporal and spatial consumption of chlorine is caused by chemical reactions of the chlorine with water constituents and with both the biofilm and tubercles formed on the pipe wall, as well as reaction with the pipe wall material itself (Wable *et al*., 1991; Zhang *et al*., 1992; Kiéné *et al.*, 1998; Al-Jasser, 2007). Research has established that higher iron corrosion rates could increase the number of biofilm bacteria, possibly due to protection of bacteria by consumption of disinfectant at the pipe surface via corrosion reactions (LeChevallier *et al*., 1990, 1993). Near the pipe wall where biofilms are attached, chlorine disinfectant concentrations are too low to control bacteria since it is consumed by corrosion and reactions with other constituents such as dissolved natural organic matter. From this perspective, even though monochloramine is a less effective disinfectant than free chlorine, it can be more effective in controlling bacteria on iron since free chlorine is rapidly destroyed by the corroding pipe wall (LeChevallier *et al*., 1990, 1993).

Control of the microbiological quality of waters during their distribution

Considering that 10% of the adults that have episodes of illness lose 1 work day, the economical consequences are not negligible (Paynent, 1997; Garthright *et al.*, 1988). Moreover the image of the product "potable water" and public confidence in it suffers from documented cases of contamination. As a consequence, limiting biological instability in water distribution systems and the growth of organisms in the biofilm is critical for both consumers and producers (Le Puil, 2004).

Since organic carbon has been widely considered to be a limiting nutrient for bacterial growth in distribution systems, assessment of biostability in such systems often relied on biodegradable organic matter (BOM) levels in finished waters. Biodegradable Organic Carbon (BDOC) and assimilable organic carbon (AOC) (either individually or in conjunction) have been used to characterize biostability of drinking water in previous studies. Potential threshold concentrations of assimilable organic carbon have been set at 10 mg C/L for heterotrophs (van der Kooij 1992) and 50 mg C/L for coliforms (LeChevallier *et al.* 1991). Servais *et al.* (1991) have associated biological stability of water with a BDOC level of 0.2 mg/L. Hydraulic residence time is related to carbon, as concentrations of organic carbon capable of assimilation have been shown to be higher at the plant than at increased travel times in the distribution system (LeChevallier *et al.* 1987, 1991; van der Kooij 1992). Decreases in AOC with water age has been noted (van der Wende *et al.* 1988); high AOC levels were associated with heterotrophic regrowth. However, there are instances where there has not been a clear cut correlation between AOC/BDOC and biofilm development. Pilot experiments at Montana State showed a weak correlation between biofilm and influent AOC concentrations, but no correlation with the concentration of AOC in the reactors (Camper 1996). Conflicting information was also obtained in field studies; some systems with

AOC levels less than 100 mg/L experienced regrowth while others with average values less than 100 mg/L also had regrowth events (LeChevallier *et al.* 1996a).

One reason why AOC levels may not be directly correlated with regrowth in distribution systems is that bacterial growth is believed to be balanced with the decay of disinfectant. Since disinfectant concentrations and AOC are often highest at the plant, the growth of organisms may be limited until the disinfectant has decayed sufficiently. Intuitively, elevated levels of chlorine should control regrowth, but this is often not the case (Centers for Disease Control 1985; LeChevallier *et al.* 1987). Historically, utilities have relied on increased disinfectant doses to control regrowth events, with mixed success. It is known that biofilm organisms are less susceptible to disinfectants than suspended cells, especially if they are present on reactive iron surfaces (LeChevallier *et al.* 1990; Chen *et al.* 1993). LeChevallier *et al.* (1993) described results where increased corrosion rates decreased the efficacy of free chlorine on biofilm bacteria.

Another issue related to regrowth and loss of disinfectant is the presence of unlined iron-containing pipe materials in the distribution system. Many older distribution systems contain unlined cast or ductile iron pipes frequently characterized by accumulations of corrosion products or tubercles that can nearly occlude the pipe diameter. Iron surfaces are particularly reactive and contribute the deterioration of water quality through a variety of processes. It has been noted that iron surfaces are prone to substantial microbial colonization and have been implicated as a key component in microbial regrowth in distribution systems (Camper 1996; Camper *et al.* 1996; LeChevallier *et al.* 1993, 1996b). Utilities with a large proportion of unlined ferrous metal pipes that have had coliform regrowth problems are Vancouver British Columbia, Boston, and Washington, DC; and a utility survey has shown a positive relationship between the number of miles of unlined metal pipes and coliform occurrences (LeChevallier *et al.* 1996b). In pilot

distribution systems with varied materials, organisms growing on ferrous metal surfaces were less susceptible to free chlorine than when present on other materials (LeChevallier *et al.* 1987, 1990), presumably because the metal exerts a chlorine demand. The ability for reduced iron to react with disinfectants has been documented (Knocke 1988; Knocke *et al.* 1994; Vasconcelos *et al.* 1996). The importance of surface material on organism numbers, including coliforms, was substantiated in our research. Even in the absence of a disinfectant, mild steel surfaces were consistently colonized by nearly 10-fold more heterotrophs and two to 10-fold more coliforms than polycarbonate when the reactors were operated under the same conditions. The impact extended to the effluent bacterial concentrations as well; elevated counts were found in reactors with mild steel even though only 10% of the reactor surface area is encompassed by the slides. Further, the presence of mild steel affects population densities on polycarbonate surfaces in the same reactor. These surfaces supported the same numbers of bacteria as seen on the steel itself. It therefore appears that the mild steel surface is capable of enhancing biofilm growth, rather than only protecting it from the action of a disinfectant (Camper *et al.* 1996).

It is also known that reactions on ferrous metal surfaces are affected by corrosion control methods (pH adjustment and phosphate addition). Laboratory and pilot work at Montana State University has shown that the number of biofilm bacteria is directly related to the mass of corrosion products present; reduction in biofilm and corrosion product accumulation can be achieved by corrosion control schemes or altering the disinfectant to produce less corrosion. There are reports where implementation of corrosion control in full scale distribution systems apparently has lead to mitigation of coliform regrowth (Martin *et al.* 1982; Hudson *et al.* 1983; Schreppel *et al.* 1997). The mechanism for the ability for corrosion control measures to influence biofilm numbers is not clear cut. LeChevallier *et al.* (1993) showed corrosion control reduced biofilm

numbers but attributed the response to increased chlorine efficacy due to decreased corrosion rates. However, we have noted that at near neutral pH, the presence of low levels of disinfectant actually increases biofilm density on ductile or steel surfaces, presumably because corrosion was enhanced and the disinfectant consumed at the surface (Camper 1996, unpublished data). Martin *et al.* (1982) noted an interaction between pH and chlorine by showing that an elevated pH of 9 reduced bacterial counts substantially. Since chlorine is less effective at high pH, it may be inferred that in this case the reduction in organism numbers was the result of corrosion control and not increased disinfection efficacy.

Another reactive characteristic of iron corrosion products (iron oxides) is that they have a large potential for the adsorption of natural organic matter (NOM; Parfitt *et al.* 1977; McCarthy *et al.* 1993; Zhou *et al.* 1994). Distribution system deposits have been shown to include a variety of iron oxides as well as manganese, carbonate species, and silica (Robinson 1981; Carlson and Schwertman 1987). Under abiotic conditions, humic material is irreversibly held on the surface of iron oxides (Gu *et al.* 1994, 1996). In fact, this property has been used to develop a technique for the removal of NOM from water by coating sand particles used in slow sand filter beds with iron oxides (McMeen and Benjamin 1997). Circumstantial evidence indicates that the bound organic matter is potentially available for biofilm bacteria when these same investigators mentioned that the iron oxide-coated olivine used in their filtration studies continued to remove NOM for a 16-month time period; they suggested that the adsorption sites were being 'bioregenerated'. There is evidence that corrosion products removed from distribution systems are capable of supporting bacterial growth with no other added carbon (Martin *et al.* 1982). Experiments to examine the potential for corrosion products to support microbial growth were done where corrosion products were removed from ductile iron reactors, packed into columns, and fed a sterile humic acid

solution. The corrosion products were then placed in sterile flasks and a population of suspended bacteria in biologically active carbon column effluent added. There was a two log increase in bacterial numbers over a three day time period, again providing circumstantial evidence that the adsorbed humic material was available for microbial metabolism.

Humic substances are the general term for both humic and fulvic acids. The NOM from surface waters has been classed into the general constituents (Malcolm 1991) which indicate that humic substances can make up approximately 50–75% of the NOM in surface waters. These concentrations are less in groundwater. NOM is responsible for chlorine demand in the bulk fluid and is a precursor for disinfection by-products. Humic substances are generally considered to be poorly biodegradable, because of their large molecular size. However, Namkung and Rittmann (1987) have shown that humic substances are in fact biodegradable. Volk *et al.* (1997) have shown that biofilm bacteria in their BDOC columns are capable of using humic substances; these substances are then considered a component of the BDOC. To address this concept, a series of experiments was completed where humic materials were the sole carbon and energy source for biofilm bacteria. The dilution water was biologically treated tap water. Compared to control reactors fed only biologically treated water, there was a two log increase in cell numbers. This is again evidence for biological use of humic substances.

The confusing aspect of NOM utilization by organisms is the relative recalcitrance of the material in the bulk phase. Therefore, it is probable that there must be another mechanism that increases the bioavailabilty of the humic substances. We believe that the humic substances become utilizable when they are adsorbed to surfaces. These molecules can then undergo a conformational change and expose the utilizable attached functional groups. The immobilization on the surface is also likely to permit the cells to use exoenzymes to attack the bonds between the bound amino

acids, sugars, etc. and the backbone of the humic molecule. There is strong evidence to suggest that the adsorption of humic substances allows them to become available for biofilm use. When an assessment of the growth rates of biofilm bacteria grown on humic materials was made, it was found that the growth rate was independent of the added humic carbon concentration (zero order kinetics). This is because of the large amount of humic materials bound in the biofilm; supplementation of additional humic material did not influence the growth rate. There was also visual evidence that the humic material was adsorbed, as these biofilms were a characteristic brown color. This mechanism has profound implications for the water industry. If adsorbed humic materials are utilizable, the prediction of bacterial proliferation using the assimilable organic carbon and biodegradable organic carbon analyses may need to be readdressed, since these methods are believed to measure the quantities of readily available organic carbon in solution. In many conversations with researchers and utility personnel, there has been the feeling that levels of AOC and BDOC in the distribution system may not be associated with bacterial growth, particularly in the presence of ferrous materials. Since iron oxides have such a propensity for adsorption of NOM, the interaction of organics, iron oxides, and bacteria may help explain many of the observations on regrowth in distribution systems. In subsequent experiments using humic material as the sole carbon and energy source, there was no correlation between increased concentrations of added humic material and bacterial production. In these same experiments, it was noted that the humic materials were adsorbed to the biofilm as well as to the surface (Dennus, Godfree and Stewart-Tull, 1999).

CHAPTER THREE: METHODS AND PROCEDURES

Pilot Distribution System Design

The pilot distribution system (PDS) and two pilot treatment systems used in the implementation of this project were designed and built by UCF and TBW-MG personnel in the previous project, "Effects on Blending on Distribution System Water Quality" (Taylor *et al*. 2005). This previous project is sometimes referred to as Tampa Bay Water 1 (TBW1). The PDS was designed to allow flexibility to study water quality changes resulting from blends of significantly different source waters in old distribution pipe systems. The feed water blends represented the typical water chemistry likely to be experienced by TBW member governments. The pipes used in the PDS traditionally received conventionally treated groundwater. Both the physical systems and pipe geometries selected represent typical scenarios experienced in a real distribution system.

The PDSs are composed of 18 different distribution lines. Lines 1 to 14 are hybrid lines that contain segments of four different materials: PVC, unlined cast iron, lined cast iron, and galvanized iron pipes. Lines 15 to 18 contain multiple segments of each of the single material. The PDS was constructed of aged pipes that were obtained from existing utility distribution systems to represent the pipe materials used in the TBW Member Government's distribution systems. The PDS has been maintained with a feed of 100% GW since completion of field activity for TBW1 in the summer of 2005. Introduction of a blend of GW, SW, and RO was initiated in December 2005. Prior to introduction of the blend, field monitoring was resumed in the fall of 2005 to verify uniformity between the PDS effluent for all PDS lines. As learned during the equilibration phase from TBW1, apparent color is a useful parameter for definition of the

attainment of an equilibrated condition in the PDS and was used for this purpose. The project was divided into 4 phases; each of three months duration. The blend used during each phase was different to evaluate the effect of water quality. Similar blends were used during phases I and III to evaluate the effect of seasonal conditions on the PDS.

Pilot Distribution System Components

Relevant characteristics of the individual lines include pipe identification, material of construction, pipe length and diameter, and feed rates. The pilot distribution systems were identified sequentially (PDS01 to PDS18). The pilot distribution lines were operated to maintain a two-day hydraulic residence time (HRT). Standpipes were located at the beginning and end of each PDS. The standpipes were made from translucent plastic pipe that were 48 inches long and had a 4 inches diameter. The retention time in the PDS feed standpipe was 3.1 hours because of the low velocities associated with the two-day HRTs. To avoid bacterial growth, the standpipes were wrapped in a non-transparent material to eliminate direct light exposure and cleaned regularly with a plastic brush and a 0.1% solution of sodium hypochlorite. The sodium hypochlorite solution was allowed a 4-hour contact time in the standpipes. All pilot distribution systems were constructed with a sampling port after each pipe segment to allow an assessment of water quality changes associated with each pipe material.

PDS 1 to 14 were composed of four materials, laid out sequentially as:

- Approximately 20 feet (6.1 m)of 6-inch (0.15 m) diameter polyvinylchloride (PVC) pipe,
- Approximately 20 feet (6.1 m) of 6-inch (0.15 m) diameter lined cast iron (LCI) pipe,
- Approximately 12 feet (3.7 m) of 6-inch (0.15 m) diameter unlined cast iron (UCI) pipe,
- Approximately 40 feet (12.2 m) of 2-inch (0.05 m) diameter galvanized iron (G) pipe

PDS 15 to 18 were composed of a single material each as follows:

- PDS15: Eight reaches of approximately 12 feet (6.1 m) of 6-inch (0.15 m) diameter cast iron each,
- PDS16: Four pipe reaches of approximately 20 feet (6.1 m) of 6-inch (0.15 m) diameter lined cast iron plus 10 feet of 6-inch lined cast iron,
- PDS17: Five pipe reaches of approximately 20 feet (6.1 m) of 6-inch (0.15 m) diameter PVC each,
- PDS18: Eight pipes reaches totaling 135 feet (41.1 m) of 2-inch (0.05 m) diameter galvanized iron pipe.

Pictures of the structures built for the field study are shown in Figure 4 to Figure 15. As shown in Figure 4, a truck and a stainless steel food grade trailer was used to haul surface water to the pilot site. The surface water was obtained from the TBW regional surface water treatment plant. The surface water was stored in two 7000 gallon storage tanks as shown in Figure 5 before being transferred to the finished water tank. The large process area used to prepare the finished waters is shown in Figure 6 and was covered by a 4400 ft2 of 6" cement pad and hurricane rated roof. The trailers shown in Figure 7 contained a reverse osmosis pilot plant, an electro-noise monitoring facility, a storage facility and a field laboratory.

Figure 8 shows the influent standpipes which allowed direct input from the finished storage and inhibitor tanks. Peristaltic pumps were used to control the flow of water and inhibitors to the PDS as well as the flow to the cradles. The inhibitors tanks and the pumps used to feed the PDS and the cradles are shown in Figure 9. Figure 10 shows the pilot distribution system which was followed by coupon cradles shown in Figure 11. The cradles were four inch PVC pipes that housed six-inch PVC pipes, which had been cut in half and supported pipe coupons for surface

characterization and microbiological studies (Figure 12). Finally, the majority of the PDS effluent was directed to a corrosion shed, shown in Figure 13, which contained eighteen loops of copper pipes and lead coupons, as shown in Figure 14 for the copper and lead corrosion study. The rest of the PDS effluent was sent to the electrochemical noise trailer to feed the Nadles (noise cradles), shown in Figure 15, which contained iron, copper, and lead electrodes and coupons.

Figure 4 Truck and stainless steel trailer used to haul raw surface water

Figure 5 Raw surface water storage

Figure 6 Covered tanks for process treatment

Figure 7 Field trailers

Figure 8 Influent standpipes

Figure 9 Inhibitor tanks and feed pumps

Figure 10 Pilot distribution systems

Figure 11 Cradles for housing coupons

Figure 12 Mounted coupons

Figure 13 Corrosion shed

Figure 14 Copper lines with lead coupons

Figure 15 Electrochemical Noise Trailer

Pilot Plant Operation

Finished Source Waters

Three different waters were used as source waters to be blended and fed to the PDS: conventionally treated groundwater (GW), enhanced CSF treated surface water (SW) and desalinated water by reverse osmosis (RO). The surface water was actual finished surface water from the Regional Surface Water Treatment Facility that was transported to the project site and the groundwater and desalinated water were obtained from the pilot water treatment systems at the project site. The description of the three finished source waters and the finished water treatment goals for the project are presented in Table 2 and Table 3.

Groundwater Pilot Plant

The groundwater (GW) pilot unit was designed to simulate the finished water of member governments that utilize conventional treatment of ground water. The GW unit used the Cypress Creek well field as a raw water source. The main unit processes were aeration, disinfection, and

pH stabilization. Before disinfection, GW was aerated to reduce hydrogen sulfide, produce an aerobic water supply, and stabilize the water with respect to calcium carbonate. Aeration was achieved in GW by pumping the raw water to the top of the finished water tank through a nozzle that sprayed the water inside the tank.

Sodium hypochlorite was used as the source of free chlorine for primary disinfection and was dosed to provide a 5 mg/L residual after a 5 minute contact time. Afterwards, ammonium chloride was added to produce a 5 mg/L monochloramine residual. Ammonia was added in the form of NH4Cl at a 5:1 ratio. The NH3:Cl2 ratio was initially 4:1 to protect against DBP formation. This ratio was increased to 5:1 in Phase III to reduce free ammonia. Five thousand gallons of GW were produced every week.

Table 2 Finished source water descriptions

Source Water	Source	System Description
GW	Groundwater	Ground water source. Treatment by aeration, disinfection by free chlorine with a residual of 5 mg/L after a 5 minute contact time. 5.0 mg/L Chloramines residual.
SW	Surface water	TBW treatment plant: Treatment by ferric sulfate coagulation, flocculation, setting, filtration, disinfection by ozonation and chloramination. Project site: adjustment of chloramine residual to 5.0 mg/L chloramines residual.
RO	Groundwater	Treatment by membrane reverse osmosis, aeration, disinfection by free chlorine with a residual of 5 mg/L after a 5 minutes contact time. 5 mg/L chloramines residual.

Table 3 Pilot plant finished water treatment goals

Parameter	Standard	Target
pH units	7.4 min	0.2 above pHs
Alkalinity (mg/L as $CaCO_3$)	40 min	50 min
Calcium (mg/L as $CaCO_3$)	50 min, 250 max	60 mg/L
Total Chlorine (mg/L Cl_2)		5 mg/L
Na (mg/L)	80 max	< 80
Cl (mg/L)	100 max	< 100
Sulfate & Chloride Sum.	3.8 meq/L max	< 3.8 meq/L
TDS (mg/L)	500 max	< 500
Fe (mg/L)	0.15 max	< 0.15
Color (CPU)	15 max	< 15
TOC (mg/L)	3.6 max avg./6.5 max	< 3.6
Ammonia (mg/L as N)	1 max	< 0.5
Turbidity (NTU)	At filter 0.3 max/ 0.1 (95%), 0.25(100%)	< 0.2
	Finished 1 max avg.	< 0.3

Surface Water Pilot Plant

Surface water (SW) was treated at the TBW Regional Surface Water Treatment Facility by enhanced coagulation, ozonation, biologically activated carbon (BAC) filtration, aeration, and chloramination. The SW was hauled weekly to the field facility for use and temporarily stored in two 7000 gallon storage tanks, shown in Figure 5, before being transferred to the SW finished water tank. In the SW finished tank, the chloramines residual was adjusted to 5 mg/L as Cl2.

Reverse Osmosis Pilot Plant

Reverse osmosis (desalination) treatment pilot system produced a finished water that was described as RO. The RO pilot plant was housed in the second trailer from the right as shown in Figure 7 and utilized raw groundwater for the feed stream. The RO treatment pilot system required the addition of TDS, calcium, and alkalinities to the RO permeate to represent the finished water produced by the TBW Regional Desalination Facility. RO pretreatment consisted of 2.7 mg/L antiscalant addition (Hypersperse MDC700TM) followed by 5-micron cartridge filtration.

The RO (high rejection) membrane filtration unit was operated at 72-73% recovery, producing 9.3 gpm permeate flow, which was aerated by a 10" diameter aeration tower filled with tripack plastic packing as previously shown in Figure 6. After aeration, 50 mg/L of sea salt was added to the aerated permeate stream to simulate the TBW desalination process. Calcium chloride and sodium bicarbonate were also added to meet the calcium and alkalinity specifications. The finished was stabilized with sodium hydroxide to 0.1 to 0.3 pH units above pHs.

Blend Finished Water

Three different blends of GW, SW, and RO were investigated during the project. All PDSs received the same blend composition for a three month period or phase. At the end of each three month period the blend composition was changed as shown in Table 4. The blends had different water quality due to the variation of the ratios of the main water sources used in the blends. But the blend ratios during phases I and III were identical. The reason for repeating the blend was to evaluate seasonal effects. The timing of the phases with the repeated blend was designed to capture the largest temperature variation during the year.

Table 4 Blend ratios of GW, SW and RO waters used during projet

Phase	Time Period	% GW	% SW	% RO
I	Feb-May, 2006	62	27	11
II	May-Aug, 2006	27	62	11
III	Aug-Nov, 2006	62	27	11
IV	Nov, 2006-Feb, 2007	40	40	20

The flow diagram for the finished waters is presented in Figure 16. For all the finished waters, a new batch was prepared each week. The water quality analysis schedule required in the field for monitoring finished water storage and process operation is presented in Table 5. Each of the three finished source water storage tanks and the blend storage tank were measured for water stability parameters, disinfection residual, color and UV-254 using field methods. Additionally, each of the storage tanks was measured daily for disinfection residual maintenance. The feed, permeate and concentrate from the membrane process were measured prior to post treatment to evaluate membrane performance and productivity. Additional water quality monitoring was conducted to evaluate specific unit processes within a treatment system. The average water quality for the different source waters are enumerated in Table 6.

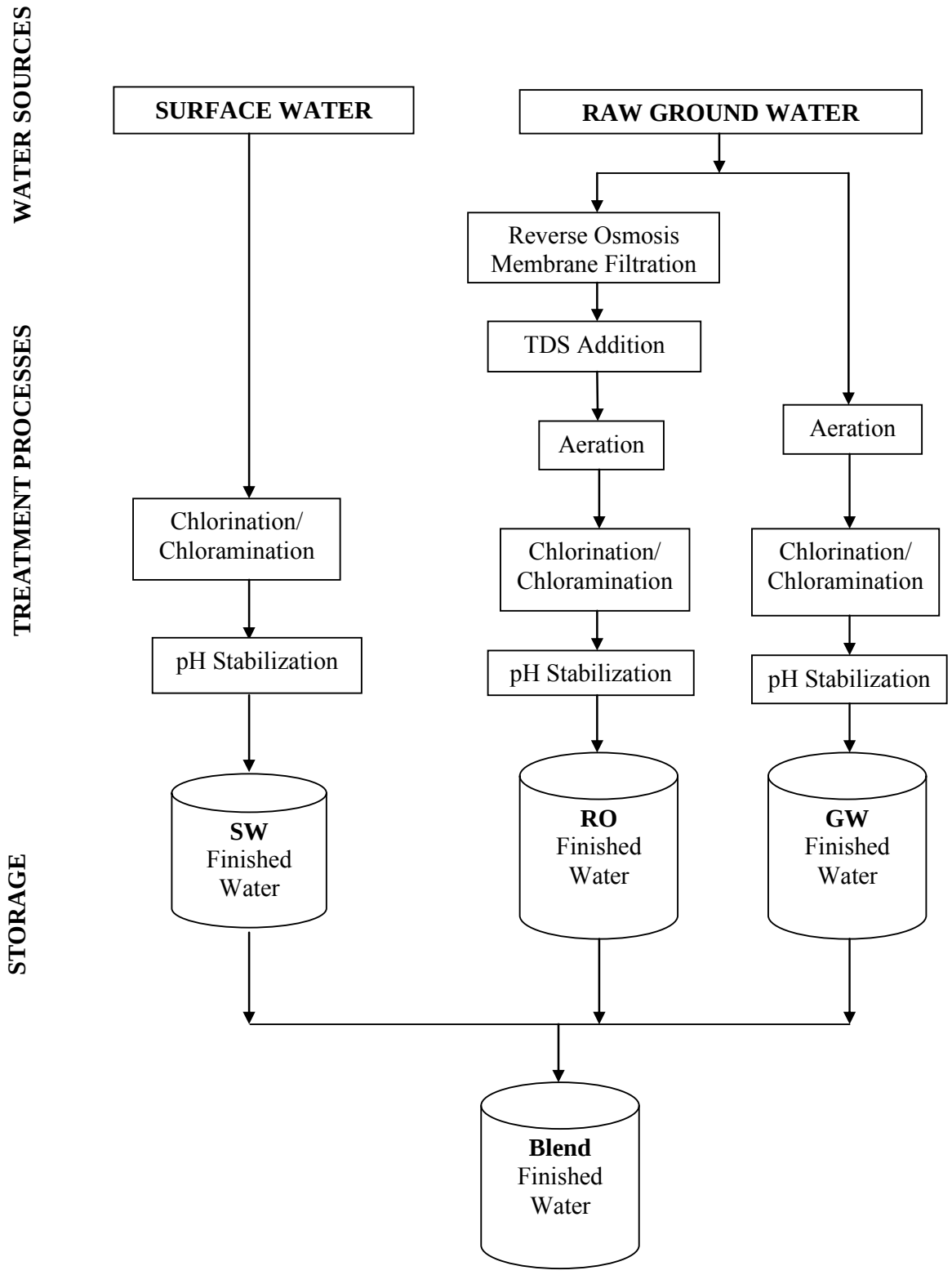

Figure 16 Process train layout

Table 5 Field analysis schedule for water treatment processes

	GW Raw	GW Fin	SW Fin	RO Fin	Blend
Free Cl_2	N/A	D	D	D	D
Total Cl_2	N/A	D	D	D	D
Ammonia	N/A	D	D	D	D
Temperature	B	D	D	D	D
pH	B	B	B	B	B
Alkalinity	B	B	B	B	B
Calcium Hardness (CaH)	B	B	B	B	B
Total Hardness (MgH)	B	B	B	B	B
UV-254	B	B	B	B	B
Color	B	B	B	B	B
Turbidity	B	B	B	B	B
Dissolved Oxygen	B	B	B	B	B
Conductivity	B	B	B	B	B
TDS	B	B	B	B	B
Chlorides	B	B	B	B	B
Sulfates	B	B	B	B	B
Iron	B	B	B	B	B
HPC	n/a	B	B	B	B

Note: D = Daily, B = Batch (after each batch is finished). UV-256 is filtered. MgH is determined by difference between CaH and TH. pH is determined after all chemicals are added to adjust water quality to targeted goals. TDS was measured by conductivity probe.

Process Finished Water Quality

Water quality was monitored after each batch was produced to ensure they met target water quality objectives designed to mimic the actual and expected TBW blended water quality shown in Table 6. Some variations in average water quality were observed for the surface water. The variations somehow follow the dry season (December-April) and wet season (May-November) that are characteristic of Florida. During the dry season (February-March), the average alkalinity and calcium were 89 and 250 mg/L as $CaCO_3$, respectively. During part of the raining season (April to August), the alkalinity and calcium dropped to 76 and 195 mg/L as $CaCO_3$. At the end of the rainy season (September-November), the alkalinity stayed at 78 mg/L as $CaCO_3$ while calcium returned to almost dry season levels of 234 mg/L as $CaCO_3$. The water quality of the surface water during the following dry season was different from the previous with lower alkalinity (73 mg/L as $CaCO_3$) and lower calcium (160 mg/L as $CaCO_3$). Sulfates followed a similar pattern, with averages of 208 mg/L, 166 mg/L, 195 mg/L, and 175 mg/L for the mentioned time periods. Selected water quality parameters for the surface water are presented in Table 7.

Table 6 Water quality objectives for finished process waters

Parameters	Finished water type			
	GW	SW	RO	Blend
pH	pHs + 0.3	> pHs	> pHs	> pHs
Total Hardness (mg/L as $CaCO_3$)	240	60 min	60 min	60 min
Total Cl_2 (mg/L as Cl_2)	4.0	4.0	4.0	4.0
Na (mg/L)	80 max	80 max	80 max	80 max
SO_4 & Cl^- (meq/L)	3.8 max	3.8 max	3.8 max	3.8 max

TDS (mg/L)	500 max	500 max	500 max	500 max
Color (CPU)	15 max	15 max	15 max	15 max
TOC (mg/L)	3.6 max	3.6 max	3.6 max	3.6 max

Table 7 Seasonal variations in water quality for finished surface water

	Water quality parameters									
Time Period	pH	Alk	Ca	Color	UV254	Turb	DO	TDS	SO4	Temp
		mg/L	mg/L	CPU	cm^{-1}	NTU	mg/L	mg/L	mg/L	°C
01/25/06-03/30/06	7.7	89	250	1	0.051	0.3	9.7	444	208	180.3
04/06/06-08/18/06	7.8	76	195	1	0.058	0.2	8.2	396	166	25.0
08/25/06-11/30/06	7.8	78	234	1	0.063	0.2	8.3	463	195	23.1
12/07/06-02/08/07	7.6	73	160	1	0.060	0.4	9.7	416	175	18.5

Note: Alkalinity (Alk) and Ca are mg/L as $CaCO_3$. DO is mg/L as O_2.

The water quality of the raw ground water had little variation during the project as shown in Table 8. The average alkalinity was 215 mg/L as $CaCO_3$ with a standard deviation of 3 mg/L as $CaCO_3$, calcium hardness was 216 mg/L as $CaCO_3$ with a standard deviation of 8 mg/L as $CaCO_3$, color was 8 CPU with a standard deviation of 3 CPU and TDS was 318 mg/L with a standard deviation of 26 mg/L.

Table 8 Water quality averages for the water pilot treatment processes

Process Statistic	Free Cl2 mg/L Cl2	TotalCl2 mg/L Cl2	NH3 mg/L NH3	pH	Alkalinity mg/L CaCO3	Ca H mg/L CaCO3	TH mg/L CaCO3	HPC cfu/ml	UV254 cm-1	Color CPU	Turbidity NTU	D.O. mg/L	Cond. µS/cm	TDS mg/L	Temp oC	Cl- mg/L	SO4 mg/L	Fe mg/L
GW																		
Average	0.07	4.9	0.15	7.7	211	214	242	218	0.073	4	0.2	7.4	540	357	23	36	29	0.06
Std Dev	0.02	1.1	0.07	0.2	7	9	7	510	0.016	1.4	0.1	0.5	32	20	2.2	7.4	4.3	0.04
SW																		
Average	0.07	5.1	0.16	7.8	79	210	242	1016	0.058	1	0.3	8.8	647	428	22	51	184	0.03
Std Dev	0.02	1.2	0.07	0.2	8	4	4	2041	0.014	1.1	0.1	0.9	57	38	3.8	9.5	22.9	0.01
RO																		
Average	0.07	5.0	0.10	7.9	70	63	69	309	0.029	0	0.1	8.3	431	285	23	92	2	0.01
Std Dev	0.02	0.9	0.05	0.2	7	6	7	1207	0.009	0.2	0.1	0.6	34	22	2.8	9.4	2.1	0.01
pHs + 0.3 (Phase 1)																		
Average	0.07	5.0	0.11	7.9	163	202	231	700	0.071	3	0.3	8.7	549	365	21	47	72	0.07
Std Dev	0.02	0.7	0.05	0.1	4	8	8	1514	0.009	1.1	0.1	0.6	20	13	1.8	5.3	5.2	0.05
pHs (Phase 1)																		
Average	0.07	4.9	0.10	7.4	129	192	216	43	0.078	3	0.2	8.1	617	407	24	83	44	0.03
Std Dev	0.01	0.6	0.04	0.0	15	7	3	N/A	0.002	0.7	0.1	0.2	43	29	0.1	20.0	34.7	0.01
pHs + 0.3 (Phase 2)																		
Average	0.08	6.2	0.13	7.9	109	185	213	142	0.069	2	0.2	8.0	588	388	26	59	112	0.04
Std Dev	0.02	0.7	0.05	0.1	4	8	9	78	0.012	0.7	0.0	0.2	24	16	1.36	6.29	6.76	0.01
pHs (Phase 2)																		
Average	0.08	6.1	0.14	7.5	101	185	211	66	0.069	2	0.2	8.1	613	405	26	72	111	0.04
Std Dev	0.02	0.6	0.06	0.1	6	8	6	97	0.012	1.2	0.0	0.4	39	26	1.6	10.4	5.1	0.01
pHs + 0.3 (Phase 3)																		
Average	0.08	6.8	0.10	7.9	154	206	229	19	0.077	2	0.2	8.0	626	413	25	65	74	0.05
Std Dev	0.02	0.4	0.06	0.3	3	9	7	16	0.012	0.8	0.1	0.3	29	19	1.8	8.3	7.6	0.01
pHs (Phase 3)																		
Average	0.08	6.7	0.12	7.5	151	203	226	22	0.074	2	0.2	8.1	674	445	24	83	74	0.05
Std Dev	0.02	0.5	0.07	0.1	9	8	7	21	0.013	0.8	0.1	0.5	43	28	2.0	11.2	6.4	0.01
pHs + 0.3 (Phase 4)																		
Average	0.09	6.9	0.08	7.8	127	168	194	151	0.063	2	0.2	9.1	573	378	20	56	85	0.04
Std Dev	0.04	0.9	0.04	0.1	6	14	12	144	0.012	0.8	0.1	0.3	19	13	1.7	6.3	2.9	0.01
pHs (Phase 4)																		
Average	0.09	6.6	0.08	7.5	124	169	195	745	0.058	2	0.2	9.1	597	394	19	65	85	0.04
Std Dev	0.04	0.8	0.04	0.1	6	11	11	824	0.011	0.8	0.1	0.4	31	20	2.1	9.6	2.5	0.00
Raw - GW																		
Average				7.4	215	216	244		0.077	8	0.1	0.3	476	318	23	14	30	0.06
Std Dev				0.1	3	8	6		0.016	2.6	0.3	0.2	14	26	3.4	2.2	4.8	0.02

The variation in water quality for the finished source waters was minimal as shown by the standard deviation value presented in Table 8. The average alkalinity of GW, SW, and RO was 211, 79, and 70 mg/L as CaCO3 with standard deviations of 7, 8, and 7 mg/L as CaCO3, respectively. The average total hardness was 242 mg/L as CaCO3 for GW and SW, while RO had an average of 63 mg/L as CaCO3. Turbidity and TDS averaged 0.2 NTU and 357 mg/L for GW, 0.3 NTU and 428 mg/L for SW and 0.1 NTU and 285 mg/L for RO, respectively. The chloramine residual averaged 5 mg/L as Cl2 for GW, SW, and RO. Table 9 shows results of one time samples analyzed for chlorides, sulfates, and NPDOC at the UCF laboratory on January 6, 2006 as well as silica results obtained from the finished source waters on May 25, 2006. The HPC values shown on Table 9 are the average for the project.

Table 9 January 6, 2006 water quality

Parameter	units	GW	SW	RO
Chlorides	mg/L	22*	38*	81*
Sulfates	mg/L	27*	242*	6*
NPDOC	mg/L as C	2.2*	1.5*	0.1*
Silica	mg/L as SiO_2	14.1+	1.2+	0.4+
HPC	cfu/mL	218	1016	309

Notes: *Measured once at UCF laboratory. Should not be compared to average values.
+Measured once on project site.

The averages for the control waters are shown by phase because the blending percentages were changed as was presented in Table 4. As can be seen in Table 8, the main difference between the pHs and the pHs+0.3 waters is the pH. The average pH for the pHs water was 7.4 to 7.5 and was always 0.3 to 0.4 pH units below the average pH for the pHs+0.3 water (7.8 to 7.9). The alkalinity and chlorides concentration were also slightly different due to the addition of HCl to

the pHs water to lower the pH. For the pHs and pHs+0.3 waters, the average chloramine residual was 5 mg/L as Cl2 during Phase I but it was increased above 6 mg/L as Cl2 for the remaining of the project to ensure residual maintenance.

Pilot Distribution System Operation

The PDS were maintained with a feed of 100% GW since completion of field activity for TBW1 in the summer of 2003. Introduction of a blend of GW, SW, and RO was initiated in December 2005, this blend was identical to the blend required for Phase I as shown in Table 4. The corrosion inhibitors feed was started on January 19, 2006 and Phase I was started on January 30, 2006. The water quality was changed every three months for all the PDSs. Fourteen hybrid PDSs were used for this investigation. These PDSs are identical and consist of increments of PVC, lined cast iron, unlined cast iron and galvanized steel pipes connected in series. PDS 01-12 contained 3 different doses of the 4 corrosion inhibitors, while PDS 13 and PDS 14 were adjusted for pH control. One set of water quality conditions was repeated in one phase to evaluate seasonal effects.

The selection of inhibitor for optimization of distribution system water quality investigated (a) corrosion inhibitor, (b) dose, (c) change of blend, and (d) season. It was a 2 year project involving 1 year of data collection and PDS operation. Four different corrosion inhibitors were investigated: orthophosphates, blended ortho and polyphosphates, zinc orthophosphate and silicates. These inhibitors provided a wide spectrum of currently available corrosion control inhibitors for various water quality conditions. Each of these inhibitors has been shown to successfully control copper, lead and iron corrosion and corrosion products under specific

environments. Once the inhibitor and dose was established for a given PDS, the inhibitor and dose was held constant for one year of investigation.

The nominal hydraulic residence time (HRT) in the hybrid pipe system was set to two days. Results from the prior study documented difficulties in maintenance of a combined chlorine residual in the PDS during elevated temperature conditions if the HRT exceeded two days (Taylor *et al*. 2005). This configuration is intended to mimic dead zones that would be encountered in a prototype system. The PDS feed and inhibitor pumps were fitted with back pressure devices to ensure consistent flows and associated maintenance of consistent HRTs in the PDS. Input flow measurements and adjustments were completed every two weeks to regulate the HRT at the desired value.

The PDS feed was produced as a blend of treated groundwater, reverse osmosis and treated surface water from the TBW regional facility. The individual sources were chloraminated to produce a residual of approximately 4.5 to 5.0 mg/L. The sources were blended in a storage tank to obtain the required blend to feed the PDS. Regular chlorine additions were made to the blend to maintain the desired combined chlorine residual at a value slightly in excess of 5 mg/L.

The PDS lines were flushed at an interval of two weeks. The flushing event achieved a velocity of 1 ft/sec for several minutes. This duration was selected to pass three pipe volumes of flush water through the hybrid lines. The nominal length of the hybrid lines is only 100 feet, thus the normal system velocities are very small. The objective of the flushing exercise is periodic removal of excessive films by hydraulic scour that may otherwise accumulate on the pipe interior during the nearly stagnant conditions associated with the 2-day HRT. The influent and effluent standpipes were cleaned at the same frequency to remove biological growths. Flushing and standpipe cleaning operations were concluded at the end of the week (Thursday or Friday).

The PDS sampling activity was undertaken at the beginning of the week. PDS influent samples were obtained at the beginning of the week (typically a Monday) corresponding with a new batch of process water. PDS effluent samples were not collected until at least 60 hours had elapsed after a flushing event in order to provide the desired two day HRT prior to sample collection.

Corrosion Inhibitors

The four types of inhibitors selected for project use were: orthophosphates, blended ortho and polyphosphates, zinc orthophosphate and silicates. The doses utilized were selected by combining information of recommended doses from the inhibitor manufacturers specific for the blends of GW, SW and RO used in the project as well as a survey of utilities that use the same types of inhibitors. However, the methods used by the manufacturers and utilities appeared to be based on experience and were qualitative rather than quantitative. This variation of independent variables provided an evaluation of inhibitor, dose and season in a varying water quality environment for control of color, turbidity and total iron release, residual maintenance, biofilm stability and bulk water biostability. The variation of water quality and inhibitors by PDS is shown in Table 11.

The target doses of the inhibitors were defined as: 0.5, 1.0, and 2.0 mg/L as P for the phosphorus-based inhibitors and 10, 20, and 40 mg/L as SiO2 above the background concentration for the silicate-based inhibitor. After 1 month of operation, the doses of the silicate inhibitor had to be changed to 3, 6 and 12 mg/L because the 20 and 40 mg/L doses were causing $CaCO_3$ within the PDS. The stock solutions of the corrosion inhibitors were diluted in a 40 gallon feed tank prepared twice a week, and the feed rates of the diluted inhibitor solution were calibrated to

delivered the low, medium, and high inhibitor target concentration. The water used during the flushes was also dosed with the required type and amount of inhibitor to mimic the operating conditions of the respective PDS. The diluted inhibitor tanks were cleaned every week and maintained with a free chlorine residual enough to minimize bacteriological growth.

The relevant properties of each inhibitor used during the project are shown in Table 11. The selected blended orthophosphate (BOP) product is called Sodium Polyphosphate SK-7641 (Stiles-Kem/Met-Pro Corporation, Waukegan, IL). Manufacturer claimed that when produced the BOP product contained approximately 40% orthophosphate and 60% polyphosphate. However, monitoring of BOP dose administered during operation indicated a 60-80% orthophosphate. Periodic determination of the actual ratio was conducted to administer the correct dose of the product. The ortho-phosphate inhibitor was Inhibit-All WSF-36 (SPER Chemical Corporation, Clearwater, FL). The zinc orthophosphate inhibitor was CP630 (Sweetwater Technologies, Temecula, CA). It is made by dissolving zinc sulfate into phosphoric acid solution in a 1:5 Zinc to PO4 ratio. The silica inhibitor, product name N, was a sodium silicate solution (PQ Corporation, Valley Forge, PA).

Table 10 Inhibitor product properties

Parameter	BOP	OP	ZOP	SiO_2
Percent Active Product	36%	36%	44%	37.5%
Bulk Density (lbs/gal)	11.5	11.25	10.8	11.6
Specific Gravity (at 72°F)	1.3	1.35	1.45	1.39
pH 1% solution (at 72ºF)	6.3-6.6	5.1-5.4	<1	11.3
Recommended Dose (mg/L)	1-2 as P	1-4 as P	2-3 as P	4-12 as SiO_2
Recommended pH Range	6.0-8.5	6.8-7.8	7-8	N/A
Solubility in water (g/g H_2O)	60/100	N/A	N/A	Miscible
Shelf Life (months)	6	None	6	None
Storage Limitation	Indoors	None	None	None

Table 11 Variations of water quality, inhibitor and dose by project phase

Ind. Var.	Water Quality 1													
Phase I	PDS 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10*	P 11*	P 12*	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 –SiO2	6 - SiO2	12 - SiO2	0	0
	Water Quality 2													
Phase II	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 SiO2	6 SiO2	12 SiO2	0	0
	Water Quality 1													
Phase III	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 SiO2	6 SiO2	12 SiO2	0	0
	Water Quality 3													
Phase IV	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 SiO2	6 SiO2	12 SiO2	0	0

*Original dosages of 10, 20, and 40 mg/L were changed to reported dosages after 3 weeks

Data Collection

Samples were collected and analyzed in the field and at the UCF laboratory. Monitoring and analyses of many physical, chemical and biological water quality parameters was carried out on the influent and effluent to each of the fourteen PDS, cradles and corrosion lines. Table 12 shows the different parameters that were monitored during the study. The data collection was done for one year of operation. Data collection for one year is critical to understanding the seasonal variation of the blends, microbial activity and water quality parameters.

Weekly analyses were completed in the field laboratory and samples were also brought back to UCF laboratory for analysis. Quality assurance and quality control of both the laboratory and field determinations of water quality parameters was established by duplicating analyses of at least 10% of the samples. Where appropriate standards were available, 10% of the samples were spiked with known concentrations of the parameter being analyzed and the recovery measured. Blind duplicates and spikes were also used to determine the accuracy of measurements. Dynamic control charts were used to determine whether the results were acceptable.

PDS Chemical and Physical Water Quality Characterization

The fourteen PDSs and stored source waters were monitored for physical and chemical water quality as shown in Table 12. Additional biological and surface characterization analyses are described later in the data collection section. The general water quality varied in sets of four, as there are four phases to the project. The feed water quality was similar for all PDSs except for pH, inhibitor type and concentration and sometimes alkalinity. Parameters such as free and total

chlorine, free ammonia, ortho-phosphorus and silica were monitored constantly and at a minimum 3 times every week.

Table 12 Influent and effluent chemical monitoring for each of 14 PDSs and cradles

			Frequency in phase I		Frequency in phase II, III & IV			
			PDS 1-14		PDS 1-12		PDS 13 and 14	
Parameter	Location	Method	Inf	Eff	Inf	Eff	Inf	Eff
Alkalinity	Field lab	titration	W	W	BW	BW	W	W
Ammonia	Field lab	NH3 probe	W	W	BW	BW	W	W
Chlorine, Free	Field lab	spectrophotometer	W	W	W	W	W	W
Chlorine, Total	Field lab	spectrophotometer	W	W	W	W	W	W
Color, Apparent	Field lab	spectrophotometer	W	W	BW	BW	W	W
Conductivity	Field lab	Conduct probe	W	W	BW	BW	W	W
Nitrite-N	Field lab	spectrophotometer	no	W	no	W	no	W
ORP	Field lab	Redox probe	W	W	BW	BW	W	W
Oxygen, Dissolved	Field lab	DO probe	W	W	W	W	W	W
pH	Field lab	pH probe	W	W	W	W	W	W
Phosphorus, Ortho	Field lab	spectrophotometer	3/W	3/W	3/W	3/W	3/W	3/W
Silica	Field lab	spectrophotometer	3/W	3/W	3/W	3/W	3/W	3/W
TDS	Field lab	TDS probe	W	W	BW	BW	W	W
Temperature	Field lab	probe	W	W	W	W	W	W
Turbidity	Field lab	turbidimeter	W	W	W	W	W	W
UV-254	Field lab	spectrophotometer	W	W	W	W	W	W
Aluminum	UCF lab	ICP	W	W	BW	BW	W	W
Calcium	UCF lab	ICP	W	W	BW	BW	W	W
Chloride	UCF lab	IC	W	W	BW	BW	W	W
Copper, Dissolved	UCF lab	ICP	no	W	no	BW	N/A	W
Copper, Total	UCF lab	ICP	no	W	no	BW	N/A	W
Iron, Dissolved	UCF lab	ICP	W	W	BW	BW	W	W
Iron, Total	UCF lab	ICP	W	W	BW	BW	W	W
Lead, Dissolved	UCF lab	ICP	no	W	no	BW	No	W
Lead, Total	UCF lab	ICP	no	W	no	BW	no	W
Magnesium	UCF lab	ICP	W	W	BW	BW	W	W
NPDOC	UCF lab	TOC Instrument	W	W	BW	BW	W	W
Phosphorus, Total	UCF lab	ICP	W	W	W	W	W	W
Silica	UCF lab	ICP	W	W	W	W	W	W
Sodium	UCF lab	ICP	W	W	BW	BW	W	W
Sulfate	UCF lab	IC	W	W	BW	BW	W	W
TKN	UCF lab	Digestion	Q	Q	Q	Q	Q	Q
Zinc, Dissolved	UCF lab	ICP	W	W	BW	BW	W	W
Zinc, Total	UCF lab	ICP	W	W	BW	BW	W	W
AOC	UCF lab		Q	Q	Q	Q	Q	Q
HPC, bulk	UCF lab		W	W	W	W	W	W

3/W = three times a week
W = once a week
BW = every two weeks
Q = quarterly, four times a year
All the methods of analysis are described later

Pipe Coupon Study

Biofilm and surface characterization investigations were conducted using coupons made from the same MG pipes that were used to construct the PDSs. The coupons were housed in cradles following every PDS as shown in Figure 11 and Figure 12 each receiving one of the

fourteen PDS waters in parallel to its corresponding PDS. Each cradle consisted of 4" PVC pipe housing and sliding pipe coupon holders made of 6-inch diameter PVC pipe cut length-wise (Figure 12). The length of the cradles was approximately 12 feet. The pipe coupon holders were easily removed from the cradles and replaced after each experimental phase for bio-film and chemical deposit analysis. The holders were transported in a large plastic bin with the coupons still in place. The humidity in the bin was elevated by placing a wet sponge inside. Upon arrival at the UCF labs the coupons were harvested for surface or biofilm studies.

The coupons for biofilm studies were circular while the surface characterization coupons were rectangular. The surface characterization coupon holders (containing much larger coupons) were placed downstream of the biological cradles to minimize transport of corrosion products onto the biofilm coupons. Cu and Pb/Sn coupons, were placed in the PVC electrochemical noise cradles or "nadles." These two materials were not originally part of the experimental plan for roughness, but were added in Phases III and IV when it was found that they could be measured even more quickly than other pipe materials. Examination of Cu and Pb/Sn surface roughness may provide insight into changes in the surfaces of Cu and Pb/Sn under exposure to varying source water blends and corrosion inhibitor treatments.

Biological Film Characterization

Bioassays were conducted on the PDSs and cradles using coupons from all pipe materials as shown in Table 13. Pilot distribution system pipe coupons for biological sampling were approximately 3 cm in diameter with a small PVC peg made from PVC welding rod attached to what was the outer surface of the pipe (Figure 12). The material is drilled from aged or pristine pipe and then de-burred to give smooth edges. In each integrated pilot distribution system cradle,

there were duplicate aged coupons of each of the four materials. The sequence of the coupons in these cradles from upstream to downstream was PVC, LCI, UCI and finally G coupons.

The coupons were placed in this order to avoid transport of corrosion materials downstream to contaminate the less easily corroded materials such as PVC and CICL. Thus the two more easily corroded materials (i.e. CI and G) were placed in the downstream locations. The aged pipe coupons were obtained from used pipe segments from actual member government networks. Biofilm were generated on the coupons that were housed in the cradles and were harvested after 5-6 weeks of growth. The biofilms were assessed using biofilm HPC with units of HPCs/cm2 and bulk biological stability was assessed using HPCs and AOC.

Table 13 Biological monitoring

Cradle Coupons	Wkly	Biwkly	6 Weeks	Quarterly
Cast Iron				
BFHPC			X	
BF-TC/E. Coli			X	
Galvanized Steel				
BFHPC			X	
BF-TC/E. Coli			X	
Lined Cast Iron				
BFHPC			X	
BF-TC/E. Coli			X	
PVC				
BFHPC			X	
BF-TC/E. Coli			X	
PDS Bulk				
AOC	All finished waters and four PDS I/O at start up for 15 total AOC			
HPC	X[1]			

[1]HPC will be sampled daily during some special periods

Quality Assurance

This portion is intended to provide a comprehensive overview of quality control procedures, analytical results and supplementary discussion of associated PDS sampling events and process sampling events over the duration of this entire project.

Quality control as discussed in this document refers to the practices of the UCF Civil and Environmental Engineering laboratories, both main and field facilities, for sample bottle preparation, sample storage after receipt from the field, analysis, and data reporting. General laboratory practices include the use of suitable grades of reagents, gases, glassware, and standard materials. All reagents were of at least reagent grade for the inorganic and NPDOC analyses. Gases and standards were UHP (ultra high purity) grade or equivalent.

Primary standard materials were purchased fresh at least every six months (or more often as needed) from an external vendor. Working standard solutions were replaced in accordance with the particular analytical methods. All volumetric glassware was of Class A grade. Periodic checks on performance of the laboratory equipment were performed regularly as a part of the quality control program. Likewise the performance of the analytical balances was monitored on at least an annual basis. They were calibrated annually by weighing a series of Class S weights. If a deviation of more than 0.0005 grams, or other malfunction was observed, an external maintenance firm specializing in the calibration of analytical equipment was to be consulted. No such errors or malfunctions were observed in the use of any of our analytical balances throughout the course of this project.

The total number of samples, spiked samples, duplicates and blind analyses varied by parameter, mainly because different parameters were analyzed at different locations of the experimental system (i.e. PDS ports, corrosion loops, process waters, stock tanks etc.).

The following section addresses analytical QA/QC issues and other laboratory function and information issues pertinent to those samples that were analyzed in the field as well as those that were brought back to the main ESEI lab facility for analysis.

Quality Control Methods

The following describes quality assurance and quality control (QA/QC) measures that were always taken in order to insure that all data were of known and acceptable quality throughout the entire study. The master QA/QC plan for all data analysis and handling has followed all guidelines for chemical sampling and analysis as presented in the 20th Edition of Standard Methods for the Examination of Water and Wastewater Analysis (APHA, 1999), hereafter referred to as "Standard Methods".

Table 14 Methods and reporting limits for inorganic analysis in the main laboratory during the entire project

Parameter	Method	Reporting Limit
Aluminum	SM 3120B pages 3-38 to 3- 43 ICP Method	0.001 mg/L
Bicarbonate	SM 2320B, pages 2-26 to 2-29 Titration Method	5 mg/L
Calcium	SM 3120B pages 3-38 to 3- 43 ICP Method	0.1 mg/L
Chloride	SM 4110 pages 4-2 to 4-8, Ion Chromatography with Chemical Suppression of Eluent Conductivity	0.1 mg/L
Copper	SM 3120B pages 3-38 to 3- 43 ICP Method	0.001 mg/L
Iron	SM 3120B pages 3-38 to 3- 43 ICP Method	0.001 mg/L
Lead	SM 3120B pages 3-38 to 3- 43 ICP Method	0.001 mg/L
Magnesium	SM 3120B pages 3-38 to 3- 43 ICP Method	0.1 mg/L

Nitrogen (NH3,TKN)	SM 4500-Norg pages 4-123 to 4-125 Macro-Kjeldahl Method	0.1 mg/L
pH	SM 4500-H+ B pages 4-86 to 4-91 Electrometric Method	± 0.01 pH units
Phosphorus	SM 3120B pages 3-38 to 3- 43 ICP Method	0.001 mg/L
Silica	SM 3120B pages 3-38 to 3- 43 ICP Method	0.001 mg/L
Sodium	SM 3120B pages 3-38 to 3- 43 ICP Method	0.1 mg/L
Sulfate	SM 4110 pages 4-2 to 4-8, Ion Chromatography with Chemical Suppression of Eluent Conductivity	0.1 mg/L
Zinc	SM 3120B pages 3-38 to 3- 43 ICP Method	0.001 mg/L

*All techniques designated with "SM" referenced in Standard Methods are from the 20th Edition (1999).

Table 15 Methods and reporting limits for aggregate properties and NPDOC measured in the main laboratory during the entire project

Parameter	Method	Reporting Limit
Color	SM 2120A pages 2-1 to 2-3 Or Hach 8025 Cobalt-Platinate Method (with spec)	1 cpu
Conductivity	SM 2510B pages 2-44 to 2-47 Laboratory Method	1 μmho/cm
NPDOC	SM 5310C, pages 5-22 to 5-24	0.1 mg C/L
Solids (TDS)	Estimation of TDS by major ion sum	1 mg/L
Turbidity	SM 2130B pages 2-8 to 2-10 Nephelometric Method	0.01 NTU
UV-254	SM 5910 pages 5-65 to 5-68 UV Absorption at 254 nm	0.0001 cm^{-1}

*All techniques designated with "SM" referenced in Standard Methods are from the 20th Edition (1999).

Chemical Data - Field Laboratory

Maintenance of good analytical practice in the field was carried out according to the same relevant procedural and statistical principles that were used in the main laboratory. While facilities available in a field laboratory are somewhat less sophisticated than those in a full-scale laboratory, the same guidelines were applied to obtain the maximum quality possible from the data gathered in the field. The methodologies used in the field are summarized in Table 16. Many of these techniques were the same as those used in the laboratory (such as the alkalinity determination). Others were modifications of known standard laboratory methods that were commercially available for application to field usage (such as the Hach Company application for chlorine, reactive phosphorus, and reactive silica among others).

In all cases involving water quality analysis during the course of this project, the commercial modifications cited and used here were widely accepted and available applications of existing standard protocols described in *Standard Methods*. They were not novel, obscure, or untested methods; they are broadly accepted and used by utilities all over the U.S.

Table 16 Methods and reporting limits for inorganic analysis in the field laboratory during the project

Parameter	Method	Method Reference	Approx. Range
Alkalinity	Titration	SM 2320 B	5 – 500 ppm
Ammonia-N	Membrane Probe Method	SM 4500-NH3 C	0.1 – 3 ppm
Chloride	Argentometric Titration	SM 4500-Cl^- B	1 – 100 mg/L
Chlorine, free	DPD colorimetric	SM 4500-Cl G or Hach 8021	0.1 – 2 ppm
Chlorine, total	DPD colorimetric	SM 4500-Cl-G or	0.1 – 2 ppm

		Hach 8167	
Color, apparent	Visual Comparison (by spectrometer)	SM 2120 B	1 – 50 cpu
Conductivity	Conductivity Bridge	SM 2510 B	variable
Hardness (total, calcium)	EDTA Titration	SM 2340 C	5 – 500 mg/L
Nitrate	Cadmium reduction	Hach 8192	0.1 – 0.5 mg/L
Nitrite	Diazotization	Hach 8507	0.1 – 0.3 mg/L
Oxygen, Dissolved (DO)	Membrane probe	SM 4500-O G	0.1 – 20 mg/L
pH	Electrometric	SM 4500-H^+ B	2 – 13
Phosphate-P (Reactive)	Ascorbic Acid Method	SM 4500-P E.or Hach 8048	0.1 – 2.5
Silica, SiO_2 (reactive)	Molybdosilicate Method	SM 4500-SiO_2 or Hach 8185	0.1 – 100 mg/L as SiO_2
Temperature	Direct reading		0 - 100 deg C
Turbidity	Nephelometric	SM 2130 B	0.02 - 200 ntu
UV254	UV spectrometry	SM 5910 A	0-0.5200 cm^{-1}

*All techniques designated with "SM" referenced in Standard Methods are from the 20th Edition (1999).

Statistics (accuracy, precision, etc.) were monitored in the same fashion as in the main laboratory, where applicable. Some parameters (UV254, pH, etc.) can not be monitored for accuracy under field conditions (i.e. can not be accurately spiked with a known increment of a primary standard). In such cases as these, either a primary standard does not exist (UV254), is not applicable (turbidity, color), or is not sufficiently stable, particularly under field conditions (DO, sulfide). Precision was monitored for these parameters. For reasons such as these, surrogate or secondary standards were also used for alkalinity, turbidity, and pH to further assist with

assessment. External primary standards were purchased for all relevant parameters, including those measured on site. These were used for instrument calibration as well as for check standards (when two separate vendors/sources were available).

Biological Data

In order to insure quality of the biological data for this study, several measures were taken to minimize and quantify the variability and analytical errors in the bioassays. These measures can be divided into three categories: (i) sampling protocol, (ii) preparation of reagents and microorganisms, and (iii) analysis of the samples. In each category, the fate of both bulk liquid and biofilm samples is further discussed.

For all the bulk liquid assays (i.e. AOC and HPC) samples were taken using sterile gloves and sterile containers (either pre-purchased sterile disposable 100-mL Coliform test sampling containers or 1-L Pyrex bottles that had been muffled at 525^{o}C for 6 hrs). After collection, sample bottles were capped with persulfate washed caps. The caps were water bathed in 20% persulfate solution at 70 °C for 1 hour, then washed with DI water four times, dried at room temperature and then autoclaved for 20 minutes. Bulk liquid samples were then placed in a cooler with ice for transport and brought back to the laboratory where they were stored in a 4^{o}C walk-in cooler until preservation and/or analysis. Storage of bulk liquid samples did not exceed 24 hrs prior to analysis unless proper preservation measures were taken. For biofilm assays (i.e. BFHPC and BF TC/E.Coli), coupons affixed to PVC holders were handled with sterile gloves and transported in air-tight PVC containers, in which the moisture level was maintained with sponges soaked with the same PDS water the coupons had been immersed in prior to sampling. The soaked sponges prevented desiccation of the biofilms during transport. For the preparation of

reagents in contact with bulk liquid samples and/or microorganisms, strict aseptic technique was used. Sterilization of agar and reagents (such as distilled water, dilution buffer and control reagents) was performed by autoclaving at 121^{o}C and 15 psi for 20 minutes. Preservation of AOC samples was carried out by pasteurization in a water bath at 70^{o}C for 1 hr or with the alternate pasteurization technique described by Escobar and Randall (2000) for extended storage. Aliquots of AOC samples were added to 40-mL EPA vials, previously muffled at 525^{o}C for 6 hrs, and capped with persulfate washed caps. To prevent contamination, handling and manipulating of samples and reagents requiring aseptic conditions was carried out under a laminar flow hood, previously wiped with bactericidal detergent (IDEXX Laboratories, Inc. Westbrook, Maine, US) and irradiated with a UV lamp for 5 minutes. For biofilm samples, a sterilized (with 70% ethanol solution) cell scraper and tissue homogenizer were used for cell detachment and homogenization of suspended materials respectively.

Analysis of samples was mainly carried out under a laminar flow hood (kept sterile as described above). In order to check aseptic conditions and proper sterility of the reagents, control measures included the following three steps: (i) laminar flow hood air sterility was checked using triplicate open agar plates during spreading, (ii) dilution buffer sterility was checked by spreading triplicate plates with dilution buffer alone as blanks during the same time as the spreading of the actual samples, and (iii) sterility of the agar was verified by inclusion of triplicate covered, non-inoculated plates in each run. These nine plates (triplicate for each control) were incubated along with the samples. A glass spreader (i.e. bent glass rod) was sterilized with 70% ethanol solution and flamed with a Bunsen burner. Sterile disposable pipette tips, sterilized powder free gloves and sterilized dilution vials were used to prevent contamination during experiments. For AOC, sterile needles and syringes were used to inoculate samples with *Pseudomonas fluorescens*

P17 and *Spirillum* NOX (LeChevallier *et al.* 1993). Time series enumeration data were used to quantify the time to stationary phase, and purity of the inocula was checked by inspecting the uniformity of observed colonies. P17 and NOX spread-plates were made to monitor growth of P17 and NOX working cultures. P17 and NOX pure cultures were previously frozen in a cryogenic freezer (-80°C) in 1-2 mL aliquots mixed with 2 % peptone and 20% glycerol solution. Incubation of samples was performed in a temperature-controlled incubator. The incubation times and temperatures are listed in Table 17. For bioassays involving plate spreading techniques (HPC, BFHPC and AOC), serial dilutions were always performed in duplicate and plate counts in the range of 30-300 cfu were selected whenever possible. However in the case of low (less than 30 cfu at 10^{-1} dilution) or high (above 300 cfu at highest dilution) density samples, plate counts out of this range have been used for interpretation of samples but only counts > 30 cfu were used for QC purposes. The variability of counts between 30 and 300 and above 300 were not statistically different but variability for counts below 30 cfu was significantly greater. Since the sample analysis and assessment required 7 to 10 days to complete, it became impracticable to do a second analysis of the sample if the first analysis run failed to produce an acceptable number. This was due to the excessive storage time that would have resulted.

Table 17 Summary of incubation temperature and time.

	Incubation Temperature	Incubation Time (days)
HPC	26	3-5
BF HPC	26	3
BF TC/E. Coli	35	24 hours
AOC	26	3

Bioassays

HPC Measurement

HPC was measured with spreadplates on R2A agar. Label all plates with sample number and location. Using a 10-100μL pipet, pipet 0.1 mL sample onto surface of predried agar plates. Using a sterile bent glass rob as spreader, distribute inoculums over suface of the medium by rotating the dish on a turntable. Before spread plating, dip the glass spreader into isopropyl alcohol and flame. Cool spreader under the hood on another sterile agar plate, and then place in the middle of the sample and gently move back and forth as you spin the turntable and evenly spread the sample over the entire plate. After completion of the final plate, wait 15 minutes and invert the plates. Incubation is at 25 degrees C for at least 48 hours, with triplicate plates for each dilution of a sample. The colonies are enumerated after incubation. There are two dilutions for each sample, including the blind duplicates. Four dilutions are used for lab replicate QA samples.

Biofilm HPC Measurement

Biofilm HPC is measured the same way as bulk HPC except the samples are detached from the coupons using a sterile spatula. Coupons colonized by biofilm are sampled and rinsed carefully with phosphate buffer solution (PBS) twice. The biofilm is manually detached from the coupon using a sterile spatula (sterilized by 70% ethanol and flame) into 10 mL of sterile PBS. Homogenize the sample by using a tissue blender (Tissue Tearor^{TM}, Biospec products, Inc) at 5000 rpm for 2 minutes. Clean the blender top in 10% bleach solution for 15 seconds and then in DI water for 15 seconds between samples. The sample is then placed on the R2A agar plate after serial dilution as described in the HPC measurement section. Incubation was at 25 degrees C for at least 5 days, with triplicate plates for each dilution of a sample. The colonies are enumerated after

incubation. There are two dilutions for each sample, including the blind duplicates. Four dilutions are used for lab replicate QA samples.

AOC Measurement

AOC refers to the most readily degradable fraction of BDOC/BOM, which is a fraction of the total organic carbon (TOC) and can be utilized by specific strains or defined mixtures of bacteria. Pure bacterial *Pseudomonas flourescens* P17 and *Spirillum* NOx were cultivated in laboratory conditions for use in the AOC bioassay. These inocula were used for both the combined method, with samples inoculated with both P17 and NOx into the same sample vial, and the separate method with P17 and NOx inoculated into different vials. Both methods were compared and found to have similar accuracies (i.e. similar yields with sodium acetate standards). In this study the combined method was used since it required less glassware and labor. The combined method is also the method described in Standard Methods. There were some modifications to the method as described in Standard Methods including a higher inoculation density of P17 and NOx, and a higher incubation temperature at 25 degrees C. Spreadplates with R2A agar were used with plate counts being conducted on days 3, 4, and 5. Instead of using the maximum day count as an estimation of cell density at stationary phase, which in effect picks the outlier value of the data set, the average of the counts obtained on all 3 days was used. Many of the modifications were derived from LeChevallier's rapid AOC method but without adopting the use of luciferase (i.e. ATP quantification) for enumeration rather than plate counts. Standard curves were set up to address the accuracy of AOC measurements. These standard curves were run in parallel with samples on all AOC runs. Standard concentrations were 0, 50, 100, and 200 μg/L of NaAc as acetate.

Biofilm Total Coliform-E.Coli (BF TC/Ecoli)

TC/ *E. coli* was measured by colilert and Quanti-Tray/2000 (IDEXX) which simultaneously detects total coliforms and *E. coli* in the samples. The procedure is as follows:

- Add contents of one pack colilert to a 100 ml water samples in a EPA standard 100 ml vessel.
- Cap vessel and shake until dissolved.
- Pour sample/reagent mixture into a Quanti-Tray/2000 (IDEXX) and seal in an IDEXX Quanti-Tray Sealer.
- Place the sealed tray in a 35°±0.5°C incubator for 24 hours.
- Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number per 100 mL.
- Adjust for dilution when appropriate.

Table 18 Result Interpretation

Appearance	Result
No Yellow wells (less yellow than positive control)	negative for total coliforms and *E.coli*
Yellow equal to or greater than the positive control	positive for total coliforms
Blue fluorescence equal to or greater than the positive control	positive for *E.coli*

Quality Assessment

The discussion and respective tables on the following pages provide a summary of the accuracy and precision of laboratory analysis during the entire project, in the main UCF laboratory facilities, as well as at the field site laboratory.

Chemical Data – UCF Main Laboratory

Accuracy. The mean recoveries for the inorganic parameters ranged from about 90% to 101%, as shown in Table 19. Metal recoveries ranged from 90 to 101% recovery. Recoveries of the other inorganics fell over a range of 95 to 101%. NPDOC showed about 93% recovery. Spike recoveries were tracked using Shewart control charts in accordance with Standard Methods. The total number of PDS samples taken and measured for most of these parameters throughout the entire project was approximately 1500 analyses of each parameter. The actual number of spikes taken for each relevant parameter as shown in the "N" column exceeds the required 10% (150 spikes in the majority of cases) called for by the Quality Assurance Plan in all cases. Those parameters measured less than 1500 times (such as TKN's) also complied with this policy, and had at least 10% of their samples spiked, and another 10% duplicated. Table 19 also summarizes the warning and control limit information gathered in the accuracy control charts for all applicable parameters analyzed in the main laboratory during the entire project.

Table 19 Accuracy summary for inorganic parameters and NPDOC analysis in the UCF main laboratory during the entire project.

Parameter	Method	Units	N	Avg Pct Rec	Pct. RSD	Max Pct Rec	Min Pct Rec	No. >WL	No. > CL	Pct. <WL	Pct. <CL
PDS											
Aluminum	ICP	mg/L	167	101.4	13.0	133.1	76.4	8	0	95.2	100
Calcium	ICP	mg/L	171	100.1	14.5	138.8	78.2	8	0	95.3	100
Iron, diss	ICP	mg/L	56	92.2	6.2	109.3	81.0	3	0	94.6	100
Iron, total	ICP	mg/L	169	97.9	11.4	131.7	76.6	6	0	96.4	100
Magnesium	ICP	mg/L	168	96.6	11.4	127.5	79.9	8	0	95.2	100
Sodium	ICP	mg/L	161	95.0	12.3	132.4	73.0	7	0	95.7	100
Zinc, total	ICP	mg/L	160	97.8	10.9	139.7	73.0	4	1	97.5	99.4
Corrosion Loops											
Copper, Diss.	ICP	mg/L	100	99.0	8.6	124.7	78.7	8	0	92.0	100
Copper, Total	ICP	mg/L	100	96.9	8.0	121.5	78.7	9	0	91.0	100
Lead, Diss.	ICP	mg/L	100	90.4	8.5	118.6	75.2	4	0	96.0	100
Lead, Total	ICP	mg/L	100	92.5	8.2	117.5	78.0	1	0	99.0	100
Other Inorganics											
Chloride	IC	mg/L	177	95.1	6.5	117.6	80.7	9	0	94.9	100
PO_4-P (tot)	ICP	mg/L	188	96.4	13.7	144.0	72.5	6	0	96.8	100
Silica	ICP	mg/L	208	89.7	7.3	111.4	72.0	7	3	96.6	98.6
Sulfate	IC	mg/L	167	96.5	6.2	112.0	83.4	10	0	94.0	100
TKN (insufficient data)	Kjeldahl	mg/L	17	100.6	12.9	120.1	73.5	N/A	N/A	N/A	N/A
Organics											
NPDOC	UV/oxidn	mg/L	177	92.9	8.2	111.1	74.6	10	0	94.4	100

The most typical source of error with accuracy in both lab and field was analyst error, usually from use of a faulty spiking solution, or some other error due to faulty standard curve or spike preparation. For example, many metal concentrations encountered in this project were in the low ppb range. This requires several dilutions (for standards, spiking solutions, etc.) to prepare. It is expected that occasionally an analyst would make dilution errors, or easily cross-contaminate a sample or standard of low concentration. When any accuracy error was encountered, the first step taken was to prepare an extra spiked sample with a freshly prepared spiking solution. This new spiked sample would be included at the end of a batch. If this new value was within acceptable limits, the data set was accepted and the next batch was analyzed. If this new value was faulty, results were rejected and an investigation into instrument function or maintenance would be carried out. The vast majority of accuracy errors were corrected with the use of a new spiking solution.

Errors not corrected with the new spike preparation were investigated further. A new standard curve (i.e. new set of diluted standards) was prepared and analyzed. If the new curve was acceptably linear ($R > 0.99$), spiked samples were run to verify correction of the problem. If the results of the spiked samples were within control, the batch was re-run. Errors beyond this would result in the purchase of a new external standard, but this need was never encountered.

Errors that revealed instrument malfunction issues were immediately dealt with before any further samples were run. This occurred once during the project with the ICP, which was found to need a new nebulizer. The part was replaced and the unit was returned to functional status within two weeks. Accuracy of analysis was restored to previous levels by the instrument repair.

Precision. Average RPD values for NPDOC as well as all inorganic parameters analyzed in the laboratory during the entire project are shown in Table 20. The precision control chart data for these parameters are also provided in Table 20.

Most of the warning and control violation incidents that were encountered were explainable by one of a few common sources. One of the key error sources for precision involved values that were measured at concentrations near or at the detection limit for a given technique, and that could not be accurately resolved further. For example, PDS influent zinc measurements usually ranged between 0 and 0.005 mg/L in magnitude. Duplicate results such as these inevitably gave rise to a high RPD between analyses.

Another source of positive error typically arose around the time of a phase change. The magnitude of some parameters would change some 5-10 (or more) times over what they were in the previous phase. This would send the limits of the precision control charts on a sharp upward or downward trend, resulting in the analyst exceeding the control limit, even though this was most likely not a true violation. Up to six weeks were shown to be necessary for the limits to stabilize for the new ambient matrix concentrations of these parameters. A good example of this is provided by sodium, during the change from Phase I to Phase II (around May 9, 2006). At this time, over the entire PDS system, sodium increased from about 8-9 mg/L to about 53-55 mg/L, a six-fold change. The control chart showed some seven precision violations during this change. Two months (another 20-22 duplicate sets) were required for re-stabilization of the warning and control limits to values more representative of the data set. Even so, from Phase II onward (where this expansion occurred), we still obtained an average duplicate range of 0.7 mg/L. The maximum range value was 5.5 mg/L for a sample containing about 30 mg/L sodium. Over 75% (126/165) of the known sodium duplicates had a range of 1.0 mg/L or below. The vast majority

(163/165) had %RPD values below 10%. All of these values indicate that sodium precision was most likely in control (i.e. acceptable) during this time. The precision control chart however continued to experience the mathematical effects of the large range change (on May 9) until that value is no longer averaged in to obtain the mean range. This becomes an unavoidable artifact of the water quality change at the phase change, and did not originate from a problem with quality control.

Table 20 Precision summary for inorganic parameters and NPDOC analysis in the UCF main laboratory during the entire project

Parameter	Method	Units	N	Mean Range (mg/L)	Range RSD (mg/L)	Max (mg/L)	Min (mg/L)	Tot > WL	Tot > CL	Pct. < WL	Pct. < CL
Metals											
PDS											
Aluminum	ICP	mg/L	170	0.001	0.001	0.007	<0.001	8	3	95.3	98.3
Calcium	ICP	mg/L	170	1.4	1.2	5.5	<0.1	13	0	92.3	100
Iron, diss	ICP	mg/L	56	0.001	0.001	0.005	<0.001	4	3[b]	92.9	94.6
Iron, total	ICP	mg/L	172	0.006	0.009	0.449	<0.001	18	10[a]	89.7	94.3
Magnesium	ICP	mg/L	170	0.1	0.1	0.8	<0.1	9	1	94.8	99.4
Sodium	ICP	mg/L	165	0.7	1.0	5.5	<0.1	11	10[a]	93.3	93.9
Zinc, total	ICP	mg/L	163	0.002	0.004	0.029	<0.001	9	5	94.5	96.9
Corrosion Loops											
Copper, Diss.	ICP	mg/L	101	0.012	0.012	0.065	<0.001	10	1	90.1	99.0
Copper, Total	ICP	mg/L	101	0.013	0.016	0.088	<0.001	5	2	95.0	98.0

Lead, Diss.	ICP	mg/L	101	0.000	0.000	0.000	0.001	10	9 [b]	90.1	91.1
Lead, Total	ICP	mg/L	101	0.000	0.001	0.005	<0.001	4	1	96.0	99.0
Other Inorganics											
Chloride	Titrn	mg/L	177	0.5	0.5	2.6	<0.1	11	1	93.8	99.0
PO_4-P(tot)*	ICP	mg/L	195	0.03	0.03	0.19	<0.01	13	2	93.3	99.0
Silica	ICP	mg/L	193	0.08	0.08	0.44	<0.1	10	4	94.8	97.9
Sulfate	IC	mg/L	168	1.3	0.5	3.1	<0.1	9	3	94.6	98.2
TKN*	Kjeld	mg/L	17	0.1	0.1	0.5	<0.1	N/A	N/A	N/A	N/A
Organics											
NPDOC	UV/ox	mg/L	177	0.12	0.11	0.57	<0.1	9	2	94.9	98.9

*Insufficient data to construct control chart for this parameter.
[a] Drastic concentration change (due to phase change) resulted in majority of control violations.
[b] Average range extremely narrow (usually zero); violations physically insignificant (< 0.001 mg/L)

Similar trends during the same date/event were clearly visible in several other parameters as well, including total iron and calcium. This information was consistent with good QA/QC for sodium in spite of the range changing issue, and further suggests that most of the violations were the results of the drastic limit change.

As an added measure of precision, blind duplicates were collected on site with every sampling event. With each batch of samples, usually two to four blind duplicates were also collected. These samples were submitted as routine samples, along with their respective sample batch, in the same work order, and analyzed accordingly. This practice assured that blind duplicates were analyzed using the same method, handling, and treatment over the same time period as the corresponding known samples. After the results for each parameter were submitted to the lab manager for the entire work order, the blind duplicate results were assessed. A

summary of the project-wide assessment of blind duplicate's precision for all laboratory parameters is shown in Table 21.

Blind duplicates served a number of useful purposes. First, instead of merely demonstrating repeatability of a measurement on replicate aliquots from the same bottle (i.e. an analytical duplicate), they also helped monitor the consistency of the sample custody and treatment process as a whole. A second purpose they served was to demonstrate ability to deal with the aggregate nature of some of the sample parameters. For example, the values of some of these parameters will differ from one collected aliquot to the next, because they are aggregate in nature. Total metals (copper, iron, lead, zinc) provide clear examples of this phenomenon, since by virtue of the term "total", we understand that some of the metal in question may be in solution (dissolved) and some may be present in particulate form. When a measured species can be present (even partially) in particulate form, it is then by nature, not homogenous throughout the bulk of the sample. This is where its aggregate nature arises from. So, during an iron release event, if several fragments of oxidized iron particulate dislodge from a pipe wall, they create heterogeneity in the bulk sample. One bottle may catch some of this particulate matter, another in succession may not. Furthermore, one bottle may capture a different number of iron particles that happen to be traveling through the pipe at a given time, while its blind duplicate might capture a different number of particles. These particles do not have to be the same size or shape. When each blind duplicate is digested for total metals, this will inevitably introduce error. Blind duplicates help with assessing repeatability in the face of this phenomenon, in the same sense that an experimental duplicate does, with the additional advantage of not introducing bias on behalf of the analyst (i.e. foreknowledge of what the "correct" concentration might be).

Table 21 Blind dupes precision summary for all parameters for UCF main laboratory during the entire project

Parameter	Method	Units	N	Mean Range (mg/L)	Range RSD (mg/L)	Max (mg/L)	Min (mg/L)	No. > WL	No > CL	Pct. < WL	Pct. < CL
PDS											
Aluminum	ICP	mg/L	170	0.003	0.004	0.013	<0.001	8	4	95.3	97.8
Calcium	ICP	mg/L	170	1.8	2.0	17.3	< 0.1	11	7	93.5	95.9
Iron, diss	ICP	mg/L	53	0.002	0.004	0.020	<0.001	2	1	96.2	98.1
Iron, total	ICP	mg/L	170	0.016	0.038	0.319	<0.001	11	9	93.5	94.7
Magnesium	ICP	mg/L	170	0.2	0.2	0.9	< 0.1	10	7	94.1	95.9
Sodium	ICP	mg/L	170	1.0	1.5	9.8	< 0.1	10	7	94.1	95.9
Zinc, total	ICP	mg/L	170	0.008	0.023	0.159	<0.001	9	7	94.7	95.9
Corrosion Loops											
Copper, Diss	ICP	mg/L	98	0.030	0.059	0.359	< 0.001	4	4	95.9	95.9
Copper, Total	ICP	mg/L	102	0.042	0.082	0.474	< 0.001	7	4	93.1	96.1
Lead, Diss	ICP	mg/L	101	< 0.001	0.001	0.006	< 0.001	4	3	96.0	97.0
Lead, Total	ICP	mg/L		< 0.001	0.001	0.006	< 0.001	4	3	96.0	97.0
Other Inorganics											
Chloride	IC	mg/L	228	1.0	1.0	6.7	< 1.0	15	7	93.4	96.9
PO_4-P, total	ICP	mg/L	170	0.02	0.03	0.22	< 0.01	12	3	92.9	98.2
SiO_2, reactive	ICP	mg/L	170	0.17	0.29	2.61	< 0.01	8	4	95.3	97.6
Sulfate	IC	mg/L	204	1.3	1.2	7.6	< 1.0	11	6	94.6	97.1
TKN	Kjehl	mg/L	15	0.2	0.1	0.4	< 0.1	N/A	N/A	N/A	N/A
Organics											
NPDOC	UV/ox	mg/L	205	0.30	0.46	3.57	< 0.1	9	3	95.6	98.5

Chemical Data - Field Laboratory

Table 22 provides a summary of the accuracy (when applicable) of field analysis during the entire project. Table 23 provides a similar summary of precision in the field. The information provided here is analogous to that provided for the data analyzed in the main UCF laboratory. The only prevailing difference is that data summarized here were gathered directly at the field site. Error sources were similar, and were responded to in similar fashions.

Table 22 Accuracy summary for field parameters during the entire project

Parameter	Method	Units	N	Avg % Rec	RPD	Max	Min	Tot >WL	Tot > CL	Pct. <WL	Pct. <CL
Alkalinity	Titr'n	$mgCaCO_3/L$	182	100.0	6.7	173.0	88.2	14	5	92.3	97.3
Ammonia	Probe	mg N/L	251	101.7	16.7	148.4	43.2	12	1	95.2	99.6
Chloride	Titr'n	mg/L	53	100.7	3.5	120.5	95.8	3	1	94.3	98.1
Cl_2-Free [a]	Spec	mg/L	357	99.6	2.0	105.6	94.4	37	21	89.6	94.1
Cl_2-Total [a]	Spec	mg/L	468	99.7	2.1	105.6	88.9	40	11	91.5	97.6
Color, App [a]	Spec	cpu	184	99.6	4.2	113.6	80.0	25	14	86.4	92.4
Conductivity [a]	Probe	µmho/cm	462	98.8	1.8	104.1	94.5	29	5	93.7	98.9
Hard, Ca	Titr'n	$mgCaCO_3/L$	60	99.5	3.1	108.3	91.7	5	0	91.7	100
Hard, Total	Titr'n	$mgCaCO_3/L$	61	99.9	9.4	160.0	77.0	6	4	90.2	93.4
Iron, Diss.	Spec	mg/L	62	96.9	8.9	110.6	80.4	4	0	93.5	100
Nitrite-N	Spec	mg N/L	129	95.9	6.8	112.1	78.4	12	2	90.7	98.4
ORP [a]	Probe	mV	163	97.3	4.8	117.5	83.0	13	5	92..0	96.9
Oxygen, Diss	Probe	mg/L	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
pH [a]	Probe	(unitless)	536	96.4	5.1	117.5	81.3	28	5	94.8	99.1
Phosphate, o-	Spec	mg P/L	275	101.0	9.2	166.3	39.5	19	4	93.1	98.5
Silica	Spec	mg SiO_2/L	126	100.1	15.8	129.4	80.6	5	2	96.0	98.4
Sulfate	Spec	mg/L	60	104.5	5.2	114.4	84.6	5	1	91.7	98.3

Turbidity	Turb	ntu	542	98.0	11.6	153.3	59.0	67	33	87.6	93.9
UV254	Spec	cm^{-1}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

*Insufficient data to construct control chart for this parameter.`
[a] Accuracy by matrix spike not valid for this parameter; assessed by use of check standards/"DI spikes".

Table 23 Precision summary for field parameters during the entire project

Parameter	Method	Units	N	Avg Range	RPD	Max	Min	Tot > WL	Tot > CL	Pct. < WL	Pct. < CL
Alkalinity	Titr'n	$mgCaCO_3/L$	235	2	3	26	< 1	14	6	94.0	97.4
Ammonia	Probe	mg N/L	301	0.02	0.03	0.52	<0.01	29	14	90.4	92.0
Chloride	Titr'n	mg/L	53	0.7	0.5	2.0	< 0.1	2	0	96.2	100
Cl_2-Free	Spec	mg/L	372	0.02	0.09	1.65	<0.01	47	31	87.4	91.7
Cl_2-Total	Spec	mg/L	447	0.04	0.08	0.96	<0.01	41	25	90.8	94.4
Color, App	Spec	cpu	242	0.29	0.48	2.00	<0.01	15	3	93.8	98.8
Conductivity	Probe	µmho/cm	252	2	2	12	< 1	10	3	96.0	98.8
Hard, Ca	Titr'n	$mgCaCO_3/L$	57	1	1	4	< 1	2	0	96.5	100
Hard, Total	Titr'n	$mgCaCO_3/L$	60	1	1	4	< 1	3	0	95.0	100
Iron, Diss.	Spec	mg/L	69	0.003	0.002	0.009	<0.001	1	0	98.6	100
Nitrite-N	Spec	mg N/L	155	0.001	0.001	0.007	<0.001	10	2	96.7	98.7
ORP	Probe	mV	187	4.2	6.0	50.2	0.1	24	17	87.2	90.9
O_2, Diss	Probe	mg/L	222	0.03	0.02	0.13	<0.01	13	4	94.1	98.2
pH	Probe	(unitless)	338	0.03	0.11	1.22	<0.01	22	7	93.5	97.9
Phosphate, o-	Spec	mg P/L	379	0.013	0.034	0.408	<0.001	39	27	89.7	92.9
Silica	Spec	mg SiO_2/L	135	0.277	0.246	1.800	<0.001	4	2	97.0	98.5
Sulfate	Spec	mg/L	59	0.65	0.99	7.20	< 0.01	2	1	96.6	98.3
Turbidity	Turb	ntu	262	0.012	0.022	0.275	<0.001	15	9	94.3	96.6
UV254	Spec	cm^{-1}	240	0.0008	0.0013	0.0120	<0.0001	14	7	94.2	97.1

*Insufficient data to construct control chart for this parameter.

Biological Data

For plate counting techniques (HPC, BF HPC, and AOC), all samples were quantified using triplicate plates for each dilution on each day of spread plating. For each individual plate within each sample, the counts were assessed and then the triplicate plate counts were averaged. An additional 10% of the samples were run in replicate (two sets of triplicate plates from two identical aliquots coming from the same sample container).

It should be noted that in the USEPA guidelines for biological assays such as microbial enumeration, no quantitative assessment of precision is required or cited, and no QC control charts have been established in the literature for bioassays. This aspect of the project was developed as an original contribution by the project team. A summary of biological quality control completed during the project is presented in Table 24, which shows that 599 samples were analyzed for quality control of HPCs, BFHPCs, TC/E Coli and AOC. 56 QC samples, or 9.3% of the total, were in violation based on the control and warning limits defined using the same method developed for non-biological assays. Biological analyses have sources of variability that non-biological assays do not, and 9.3 % violation was considered acceptable given the nature of these analyses. These violations are discussed in the following sub-sections.

Both blind duplicates from the field (sample identity unknown to the lab analyst) and lab replicates selected by the analyst were used to quantify precision. The values of raw plate counts usable for QC purposes were the same for HPCs, AOC, and BFHPC, i.e. $\geq$ 30 cfu per plate. Dilutions were selected to increase the likelihood of counts in the optimal range of 30 to 300 cfu. To insure that this occurred from 2 to 4 dilutions (i.e. 10^{-1} to 10^{-4} for HPC, 10^{-2} to 10^{-4} for BF HPC, 3×10^{-3} and 10^{-4} for AOC) were used for all duplicates and lab replicates. Counts over 300 were

accepted for QC purposes. Analysis early in the study showed that high counts had similar variability to counts between 30 and 300, while low counts had a significantly higher variability.

Table 24 Summary of biological quality control sampling.

Parameter	Type	Total QA Samples	Violations
HPC	Lab Replicate	183	31
	Blind Duplicate	190	21
BFHPC	Lab Replicate	45	2
	Duplicate Coupon	36	1
TC/E Coli	Lab Control	66	0
	Lab Replicate	36	0
AOC	Lab Replicate	32	0
	Blind Duplicate	11	1
Total		599	56

HPC

Duplicate and replicate QC samples were analyzed in separate control charts since it was shown early in the study that there was a significant difference in the range values and variability of these two types of QC samples. The analysis of the total project data confirmed this observed difference. The mean of the blind duplicate data range was 0.17 log higher than the replicate data range which was 0.16 log. Since the normality test failed (p value was less than 0.05), the Mann-Whitney Rank Sum Test was used to compare the difference between these two sets of data. The results are shown in Table 25. The results showed that the difference in the median values between the two groups is greater than would be expected by chance with a P value of 0.001. As a

result it was concluded that the duplicate data had higher variability than the replicate data which was logical since duplicates have additional sources of variability (e.g. different sample containers, etc…). Pooled analysis of the duplicate and replicate data was not appropriate and separate control charts for blind duplicates and lab replicates were used to monitor method precision (Figure 17 and Figure 18).

Table 25 Mann-Whitney rank sum test for HPC duplicates and replicates

Group	N	Missing	Median	25%	75%
Duplicate	168	0	0.183	0.0859	0.352
Replicate	165	0	0.100	0.0462	0.194

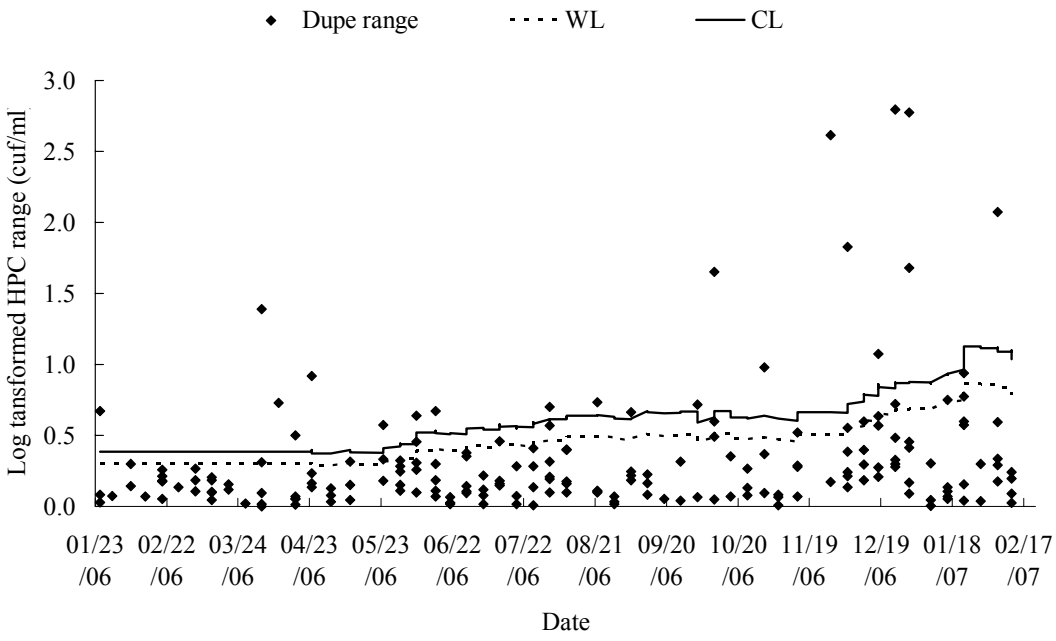

Figure 17 HPC precision duplicate data control chart

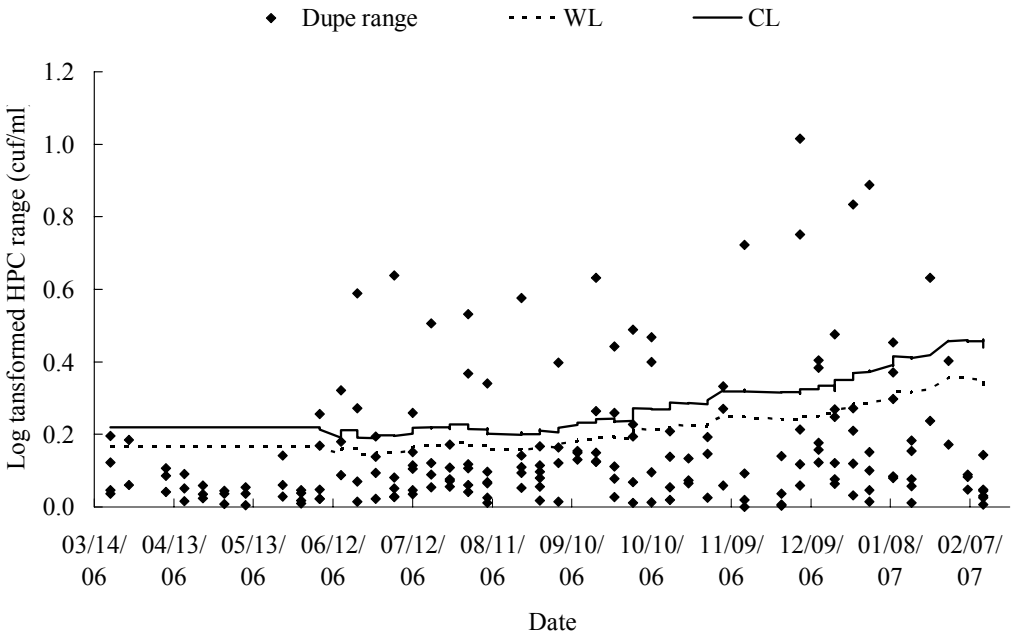

Figure 18 HPC precision replicate data control chart

In total, twenty-one duplicates were found to be control violations, as shown in Figure 17, and 31 replicates were also found to be control violations (Figure 18).

The QC data for HPC showed increased variability during the last 2 to 3 months of the project. When this trend was identified from the QC control charts a cause was sought. It turned out that some of the samples were stored longer than 48 hours. Since this problem was only discovered near the end of the project, no effective corrective action could be implemented. The precision was decreased by roughly 20% during this final period but statistical analysis showed that the increased variability was not enough to give the data significantly different characteristics for purposes of data analysis and interpretation. In this analysis, data arising from samples with storage times of 48 hours or less were separated from data from samples whose storage exceeded

48 hours. (Standard Methods specified a storage time of 24 hours; while other published literature indicated that storage times up to 48 hours will not typically affect enumerations). These two sets of data were cross-compared, i.e. data with normal storage time were modeled and applied to predict the suspect data, and vice versa. The results from the comparison between predicted data and actual data indicated no statistical difference between the two data sets. Moreover, several additional tests between suspect data and the whole data set as well as subsequent sensitivity analysis all verified the fact that we could analyze the data together in spite of the unintended excess storage time.

The distribution of the entire QC data was tested for normality and it was found that it was not normally distributed. As a result the control and warning limits can not be assigned a probability based on a normal distribution (which is typically done for non-biological assays). In addition it can be observed that control limit violations were more probable than warning limit violations which would not be expected for normally distributed data either. However the control chart did serve a useful function for monitoring purposes, i.e. the control chart made it possible to identify changes in measurement system variability over time (e.g. Is the system as precise now as it was 6 months ago?). This allowed us on at least two occasions to identify a new source of variability, in the first case that a graduate student was not spreading aliquots immediately after pipetting them onto the agar and in the second case a graduate student stored samples an excessive amount of time. In the first case corrective action was taken and the source of variability removed. In the second case identification of the variability source came right after the last sampling event so corrective action could not be implemented.

The distribution of the QC data as shown in Figure 17 and **Error! Reference source not found.** implies that there is a periodic source of variability that causes between 11 and 17% of the replicate pairs to have a significantly higher range value (i.e. a violation of the UCL). In contrast warning limit violations were only about half as frequent. In many cases the UCL violations showed log range values double that of the corresponding observations that were not control violations. It is probable that this type of behavior was caused by non-homogeneity of dispersions of microbial cells. With a dissolved constituent we would expect the assumption of homogeneity to hold very well and thus this would not be a source of variability for replicate or duplicate analysis. However in this case we have a colloidal dispersion, and further the cells per particle would be expected to vary significantly as cells aggregate due to interparticle bridging resulting from extracellular polymers. In addition some bacteria are mobile and there are a host of other phenomena that would not normally affect other colloidal dispersions. Analysis indicated that the distribution of the data was not normal. At the end of this section the distribution of the data from bulk HPC, BFHPC, and AOC is evaluated to determine what distribution fits each data set, what the NIST UWL and UCL calculations mean in terms of probability (95% and 99.7% for a normal distribution but the data was not normal). In addition a modified approach is given for bioassays.

BF HPC

Since both replicates and duplicates were used any potential difference in the variability of the blind duplicates versus lab replicates was investigated. The mean of the blind duplicate data range was 0.27 log, higher than the replicate data at 0.21. Statistical tests by Sigmaplot® confirmed that the QC data were not normally distributed (i.e. normality test failed, p value was

less than 0.050). A Mann-Whitney Rank Sum Test (Table 26) was used to compare the data. It showed that the difference in the median values between the two groups was greater than would be expected by chance; i.e. there was a statistically significant difference ($p = 0.001$) between the replicate and duplicate data. The variability was much lower for replicates than duplicates because of the additional sources of error applicable to duplicates (e.g. variability between duplicates due to sampling, different sample containers, different coupons, etc.).

Separate control charts were developed to monitor method precision (Figure 19 and Figure 20). One violation was observed in the duplicate data (Figure 19) and two for the replicate data (Figure 20). The number of violations is so small in both data sets that there is probably no significance to the fact that the replicate controls had more violations than the duplicate controls. As listed in Table 27, the percentages of the violations out of observations for replicates and duplicates are 4.44% and 3.13%. There is no significant difference between these percentages. The most notable difference between the two data sets is that the warning and control limits (and thus the variability) of the replicates was about 0.3 log lower than the duplicate QC data.

Table 26 Mann-Whitney rank sum test for BF HPCduplicates and replicates

Group	N	Missing	Median	25%	75%
Duplicate	32	0	0.263	0.13	0.387
Replicate	45	0	0.0952	0.0544	0.233

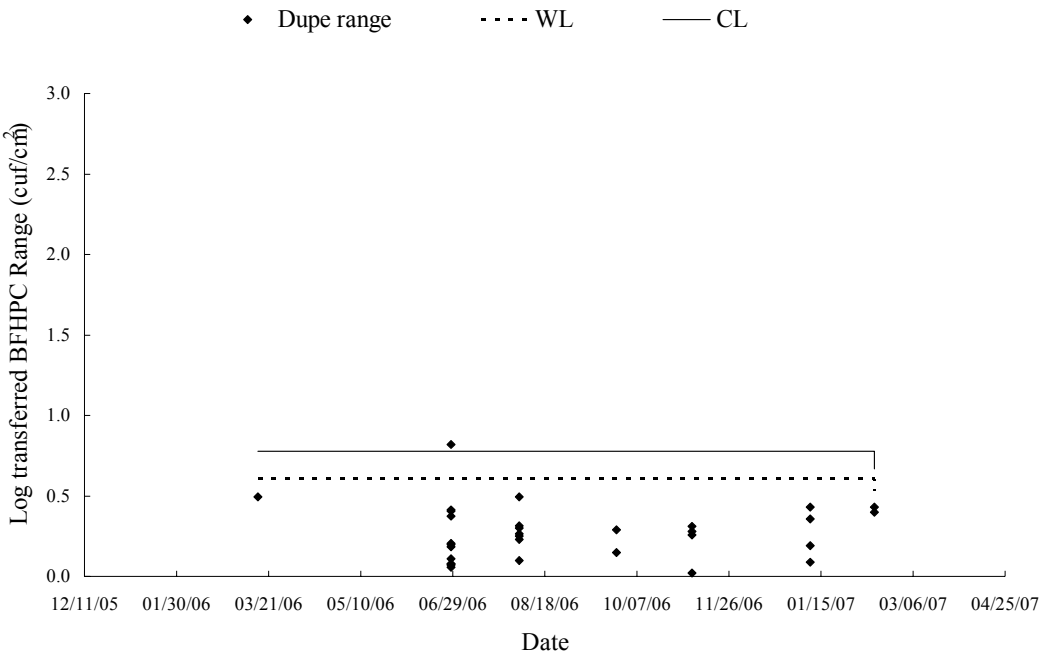

Figure 19 BFHPC precision duplicate data control chart

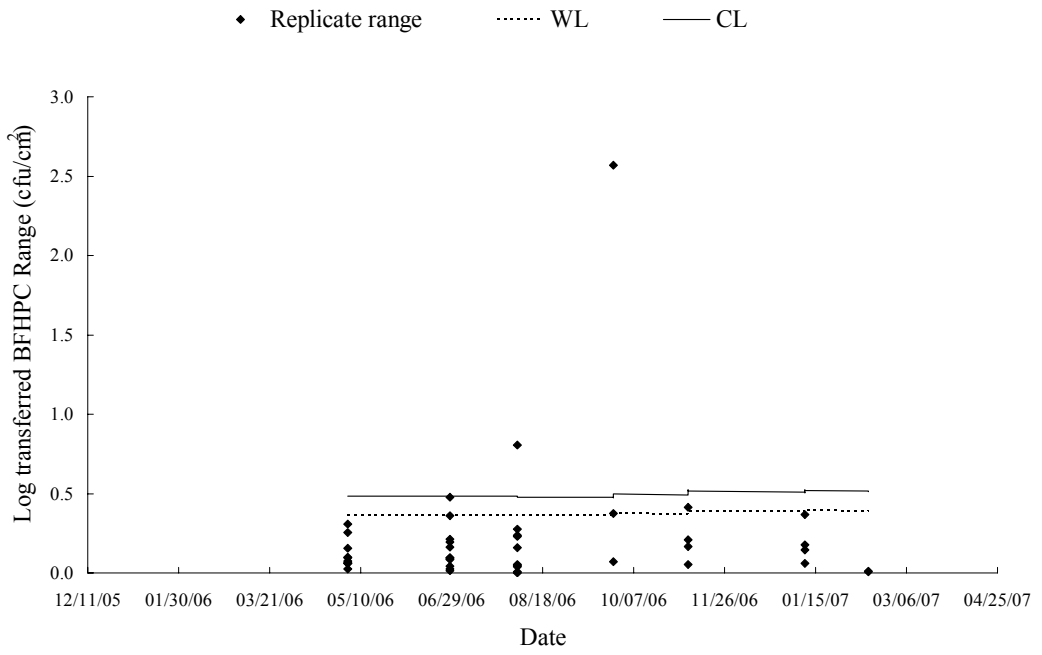

Figure 20 BFHPC precision replicate data control chart

Table 27 Violations for BF HPC duplicates and replicates

Group	Violations	Total Observations	Percentage
Duplicate	1	32	3.13%
Replicate	2	45	4.44%

Biofilm TC/E. Coli

No biofilm samples tested positive for coliforms during the entire study until Phase IV. The first coupon sampling event for Phase IV tested positive for coliforms in the PDS 11 unlined cast iron coupon biofilm and the PDS 14 galvanized coupon. Both were negative for E. Coli. The coliform densities of the coupons were 154 and 1.1 MPN/cm^2 which was an extremely small fraction of the total biofilm biomass (several orders of magnitude less). Table 28 shows that control data for the run behaved perfectly.

Table 28 Biofilm total coliform/*E. Coli* control data from Phase IV sampling event 2 (02/12/07)

		Without UV (Coliforms)			With UV (E. Coli)		
Control	DF		Diluted MPN (MPN/ 100mL)	MPN of Sample (MPN/100mL)		Diluted MPN (MPN/ 100mL)	MPN of Sample (MPN/100mL)
EC	1.00E+02	>	2419.2	2.42E+05	>	2419.2	2.42E+05
(+/+)	1.00E+04	>	2419.2	2.42E+07	>	2419.2	2.42E+07
	1.00E+06	=	35	3.50E+07	=	35	3.50E+07
PA	1.00E+02	<	1	1.00E+02	<	1	1.00E+02
(-/-)	1.00E+04	<	1	1.00E+04	<	1	1.00E+04

	1.00E+06	<	1	1.00E+06	<	11	1.00E+06
KP	1.00E+02	>	2419.2	2.42E+05	<	1	1.00E+02
(+/-)	1.00E+04	>	2419.2	2.42E+07	<	1	1.00E+04
	1.00E+06	=	54.5	5.45E+07	<	1	1.00E+06

AOC

The AOC precision QC chart is shown in Figure 21. Both blind duplicates from the field (sample identity unknown to the lab analyst) and lab replicates selected by the analyst were used to quantify precision. Unlike the other biological parameters the AOC QC replicates and duplicates were analyzed together (i.e. pooled QC data). The statistics test by Sigmaplot® confirmed that the QC data were normally distributed (normality test passed, p value was 0.110 and α value was 0.10). An F-test showed that the two sets of data had equal variances (Table 29). A t-test was used to compare the means of the two data sets and it showed that there was not enough evidence to conclude the duplicate and replicate means were different. Since there were only 24 duplicate and 17 replicate data points it was less likely that any difference between the replicates and duplicates would be statistically significant (Table 30). It is unknown if they would have been different had there been more AOC QC data. The values of raw plate counts (both P17 and NOx) usable for QC purposes were the same as those adopted for HPC, i.e. ≥ 30 cfu per plate. Dilutions were selected to increase the likelihood of counts in the optimal range of 30 to 300 cfu. To insure that this occurred, dilutions of 3×10-3 and 10-4 were used for all lab replicates. There was only one violation in the AOC control chart (Figure 21). The average RPD for AOC QC data was 21%. RPD was adopted as the statistic for AOC rather than log range since it allows comparison with

prior research where the source water AOC can vary from 10 to 250 µg/L, while a log transformation was not appropriate for this parameter since it does not vary by orders of magnitude as HPC and BFHPC do.

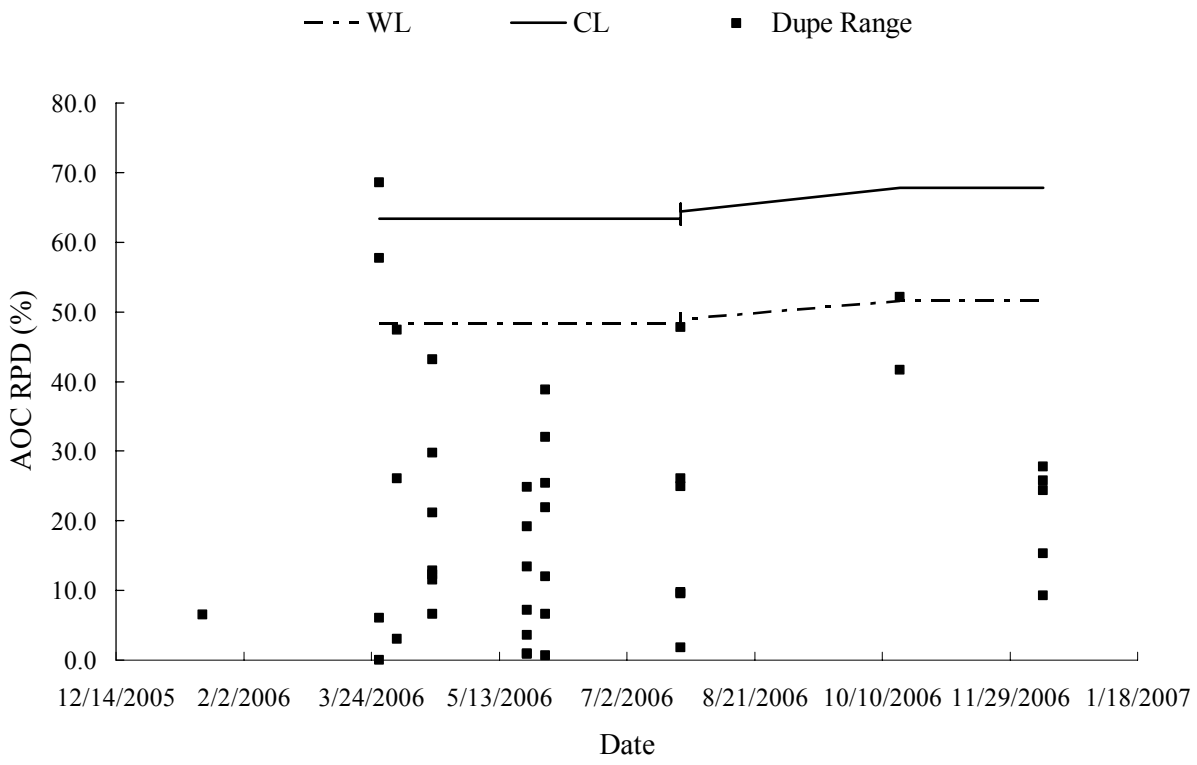

Figure 21 AOC precision pooled data control chart

Table 29 F-Test two-sample test for unequal variances

	Duplicate	Replicate
Mean	22.01	18.96
Variance	395.44	181.10
Observations	24	17
df	23	16
F	2.18	
P(F<=f) one-tail	0.06	
F Critical one-tail	2.24	

Table 30 t-Test comparing means of duplicates and replicates: Two-sample assuming equal variance

	Replicate	Duplicate
Mean	22.01	18.96
Variance	395.44	181.10
Observations	24	17
Pooled Variance	307.51	
Hypothesized Mean Difference	0	
df	39	
t Stat	0.55	
P(T<=t) one-tail	0.29	
t Critical one-tail	1.68	
P(T<=t) two-tail	0.59	
t Critical two-tail	2.02	

CHAPTER FOUR: QUANTIFICATION OF DRINKING WATER BIOSTABILITY: METHOD PRECISION FOR HETEROTROPHIC PLATE COUNT

Introduction

Currently, many of the quantitative quality assurance (QA) tools used for monitoring accuracy and precision of chemical assays are not used for microbiological measurements. There is typically no established analytical standard for bacterial enumeration allowing development of accuracy control charts, in contrast with most chemical parameters. Since variability between duplicates or replicates can be used to quantify precision, it should be possible to adapt precision control charts for monitoring bioassays. National Institute of Standards and Technology (NIST) guidelines for precision (ASTM, 1977) specify warning limits and control limits at 2 and 3 standard deviations above the average range (Range = the absolute value of the difference between two duplicate or replicate measurements). Thus the formulas specified for the limits are:

Warning Limit (WL) = Average Range + 2 x Standard Deviation of Range

Control Limit (CL) = Average Range + 3 x Standard Deviation of Range

The NIST specified limits may be adopted independently of any assumed distribution of the data. If there is no knowledge of the distribution of the data, the limits serve as an indicator of relative change in the variability of a measurement system. If the distribution is known, then it is possible to determine the probability that corresponds to the limits as defined by NIST. For application of these warning and control limits to data with a normal distribution, 5 % of valid observations will exceed the warning limit and 0.3 % of the valid observations will exceed the

control limits. Out of control conditions are judged to be present when a single event exceeds the control limit or when two consecutive events exceed the warning limit.

In this paper, the methodology for precision control charts used for chemical assays has been adapted for monitoring changes in variability for HPC measurements without any assumption concerning data distribution. In this case, the limits serve as monitoring benchmarks of unusual events with the measurement system compared to past performance rather than to known probability boundaries. Analysis of the probability distribution of the complete data set also allowed a WL and CL with probabilities equal to 5 % and 0.3 % respectively to be determined and compared to the NIST formula WL and CL.

Materials and Methods

HPC Measurement

HPC was measured with spread plates on R2A agar. Incubation was at 25 degrees Celsius for 5 days, with triplicate plates for each sample dilution. There were two dilutions for each sample. Four dilutions were used for lab replicate QA samples and for the known blind duplicates (since the samples that had blind duplicates were unknown only two dilutions were used just as other samples).

Biofilm HPC Measurement

Biofilm HPC was measured the same way as bulk HPC except the samples were scraped from the coupons. Coupons colonized by biofilm were sampled and rinsed carefully with phosphate buffer solution (PBS) twice. The biofilm was manually detached from the coupon using a sterile

cell scraper (sterilized with 70% Ethanol and flamed) into 10 mL of sterile PBS. Samples were homogenized by using a tissue blender (Tissue Tearor^{TM}, Biospec products, Inc, Bartlesville, OK, USA) at 5000 rpm for 2 minutes. The tissue homogenizer probe was cleaned in 10% bleach solution for 15 seconds and then in DI water for 15 seconds between two samples. The sample was then diluted and spread on R2A agar plate as described in the preceding HPC measurement section. Incubation was at 25 degrees Celsius for at least 5 days, with triplicate plates for each dilution of a sample. There were two dilutions for each sample, including the samples with corresponding blind duplicates. Four dilutions were used for lab replicate QA samples and the known half of the blind duplicates.

Results and Discussion

HPC Duplicates and Replicates Distribution Tests

After completion of the study it was possible to go back and determine the type of distribution that described the bulk HPC and BFHPC. In addition it was obvious that the NIST calculated warning limit (WL) and control limit (CL) did not correspond to 95% and 99.7% probability (e.g. only a 5% probability that a valid observation would exceed WL) as they would for a normal distribution. This made it desirable to develop a method to select a WL and CL for these non-normal distributions where the probability was the same as the NIST WL and CL for a normal distribution once sufficient data has been gathered (initially the NIST WL and CL can be used as a starting point).

Statistical tests during the study showed that the log range (which was used as the QC statistic) of duplicates was more variable than those of replicates. As a result the QC analysis was conducted separately for duplicates and replicates. Separate precision control charts are provided

for blind duplicates and lab replicates in Figure 22 and Figure 23, respectively. As indicated in these figures, the warning and control limits were initially established from the first 30 ranges not exceeding the CL. Thereafter, the limits were based on the most recent set of 30 observations that did not violate the control limit. When an outlier showed up in the chart, it would not be used in the WL and CL calculation. The separated duplicate and replicate control chart worked well for this study. Since December 5th (as shown in Figure 22 and Figure 23), there was an upward trend in both control charts based on the duplicates and replicates. In January the increase became significant enough to trigger an investigation into possible problems in the analytical system. The reason was found to be a change in storage times for the samples by a new analyst, with the excessive storage times (from 24-48 hours in the past to over a week with the new analyst) being the probable explanation for the higher variability.

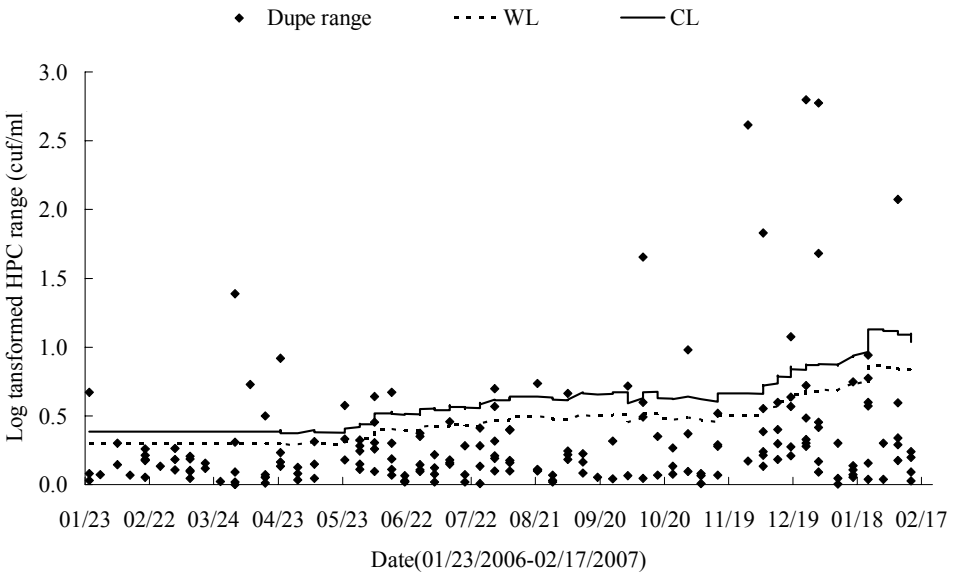

Figure 22 Bulk HPC precision control chart based on duplicates

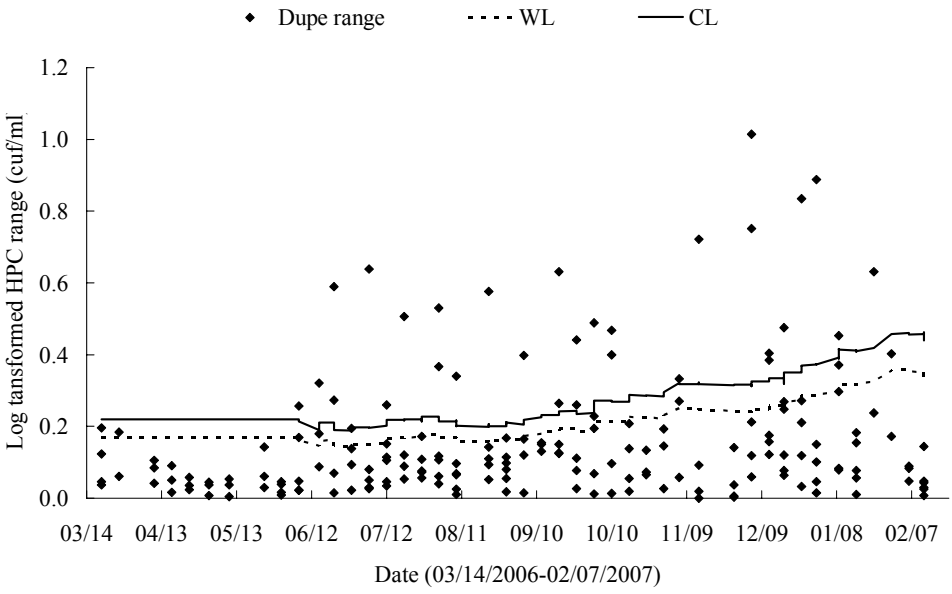

Figure 23 Bulk HPC precision control chart based on replicates

EasyFit®(MathWave Technologies, Dnepropetrovsk Ukraine) was employed to test the HPC QC data's distribution. Duplicate and replicate log HPC ranges were used in the distribution test. Through Kolmogorov Smirnov, Anderson Darling and Chi-Squared distribution fit tests, it is reasonable to use the Log-logistic distribution as models for both duplicate and replicate log HPC ranges as shown in Figure 24 and Figure 25. The log-logistic distribution has certain similarities to the logistic distribution. A random variable is log-logistically distributed if the logarithm of the random variable is logistically distributed. For a continuous function, the probability density function (PDF) is the probability that the variate has the value x. Since for continuous distributions the probability at a single point is zero, this is often expressed in terms of an integral between two points. In these two figures, the empirical probability density function (PDF) was displayed as a histogram consisting of equal-width vertical bars, each representing the number of sample data values falling into the corresponding interval, divided by the total number of data points. The theoretical PDF is displayed as a continuous curve properly scaled depending on the number of intervals. The scaling means multiplying the PDF values by the interval width. Duplicate and replicate data were divided into 20 intervals in Figure 24 and Figure 25.

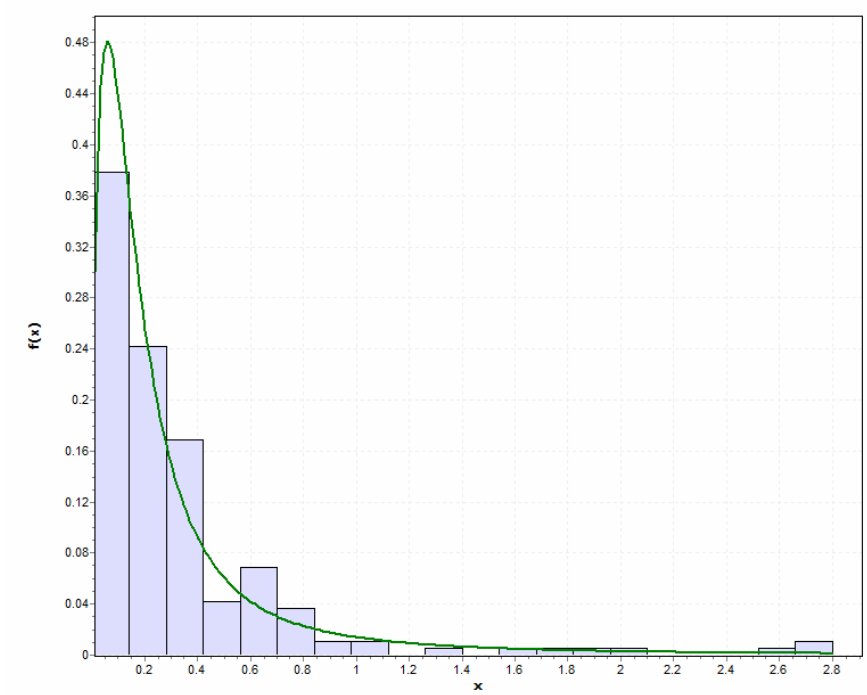

Figure 24 HPC duplicate QC data probability density distribution (log-logistic)

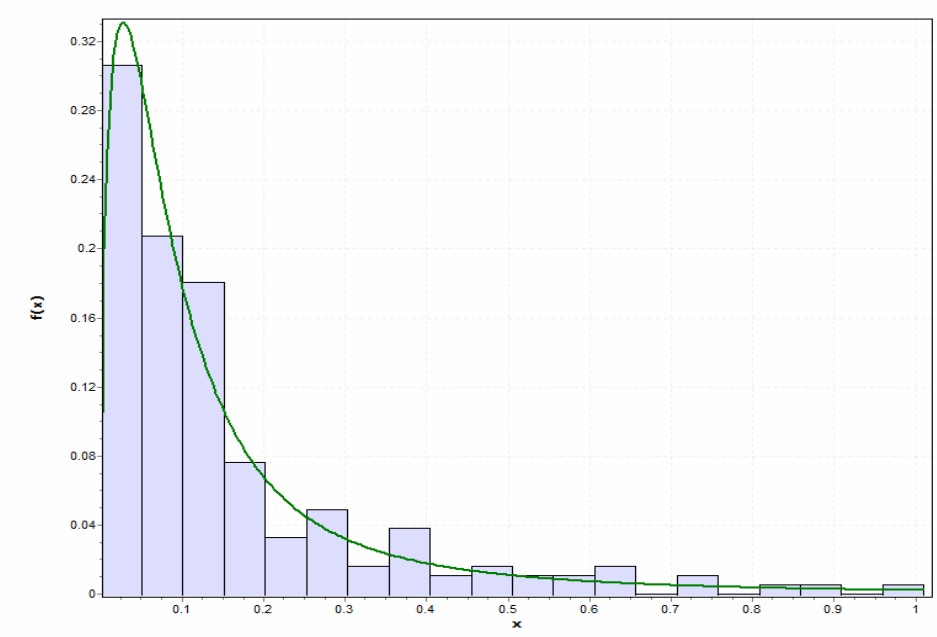

Figure 25 HPC Replicate QC data probability density distribution (log-logistic)

For application of NIST guideline warning and control limit probabilities, it has been assumed that 5 % of valid observations will exceed the warning limit and 0.3% of the valid observations will exceed the control limits. Control violations occur when a single event exceeds the control limit or when two consecutive events exceed the warning limit. For a normal distribution, it is assumed that 5 % of valid observations will exceed the warning limit (average±2×standard deviation) and 0.3 % of the valid observations will exceed the control limit (average±3×standard deviation).

Using the full complement of duplicate and replicate QC analysis for the project according to the NIST standard, the associated control limits and warning limits for duplicate ranges were 1.04 and 0.79 (log transform). The control limits and warning limits for replicate ranges were 0.44 and 0.33 (log transform). In Figure 26 and Figure 27, cumulative distribution plots were used to determine the actual probabilities of the NIST WL and CL. The cumulative distribution function (CDF) was the probability that the variate takes on a value less than or equal to x. From Figure 26, 10.0 % of valid observations exceeded the NIST warning limit from Figure 22 of 0.79 and 6.7 % of the valid observations exceeded the NIST control limit from Figure 22 of 1. 04. With replicates QC samples, the percentage over the NIST warning limit and NIST control limit (Figure 23) was 14.4 % and 9.4 %. To compare these NIST WL and CL values with the actual probability bounds for this log-logistic distribution the 95 % and 99.7 % probability bounds were assigned on Figure 26 and Figure 27 also. The warning limits at 95% probability (i.e. a 5% probability of a valid measurement exceeding this value) for duplicate and replicate HPC ranges were 1.31 log and 0.72 log respectively. For both duplicates and replicates the actual 95% probability boundary was higher than the NIST control warning limit. The control limits corresponding to 99.7% probability

were both off of the chart, and were higher than the highest observed values in Figure 22 and Figure 23. As a consequence the NIST WL and CL were useful for relative comparison of HPC precision but were extremely conservative with respect to actual probabilities (i.e. they would indicate a QC violation when there was nothing wrong). Thus the identification of trends or of higher frequency of NIST control violations over time were rational ways to identify possible control violations, but the normal NIST rules for control violations (2 consecutive observations over the NIST WL, or one observation over the NIST CL) would be overly conservative, for HPC enumeration.

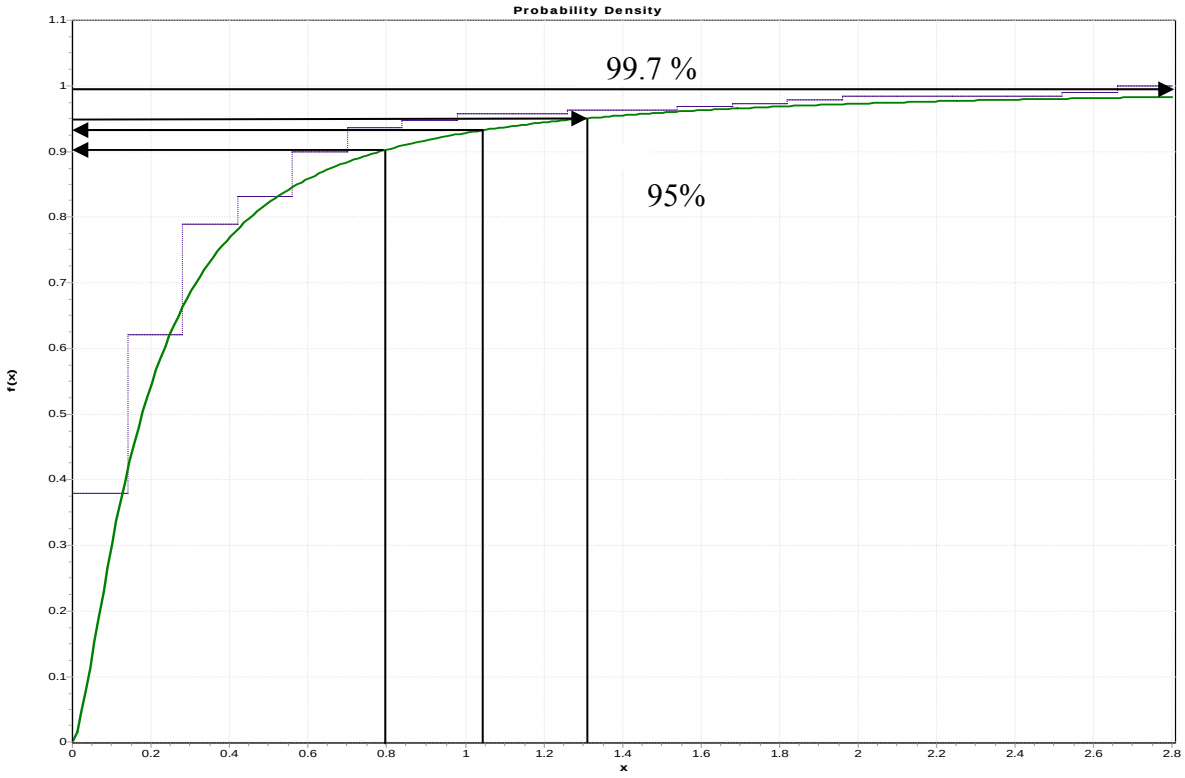

Figure 26 HPC QC data cumulative probability distribution function plot (based on duplicates)

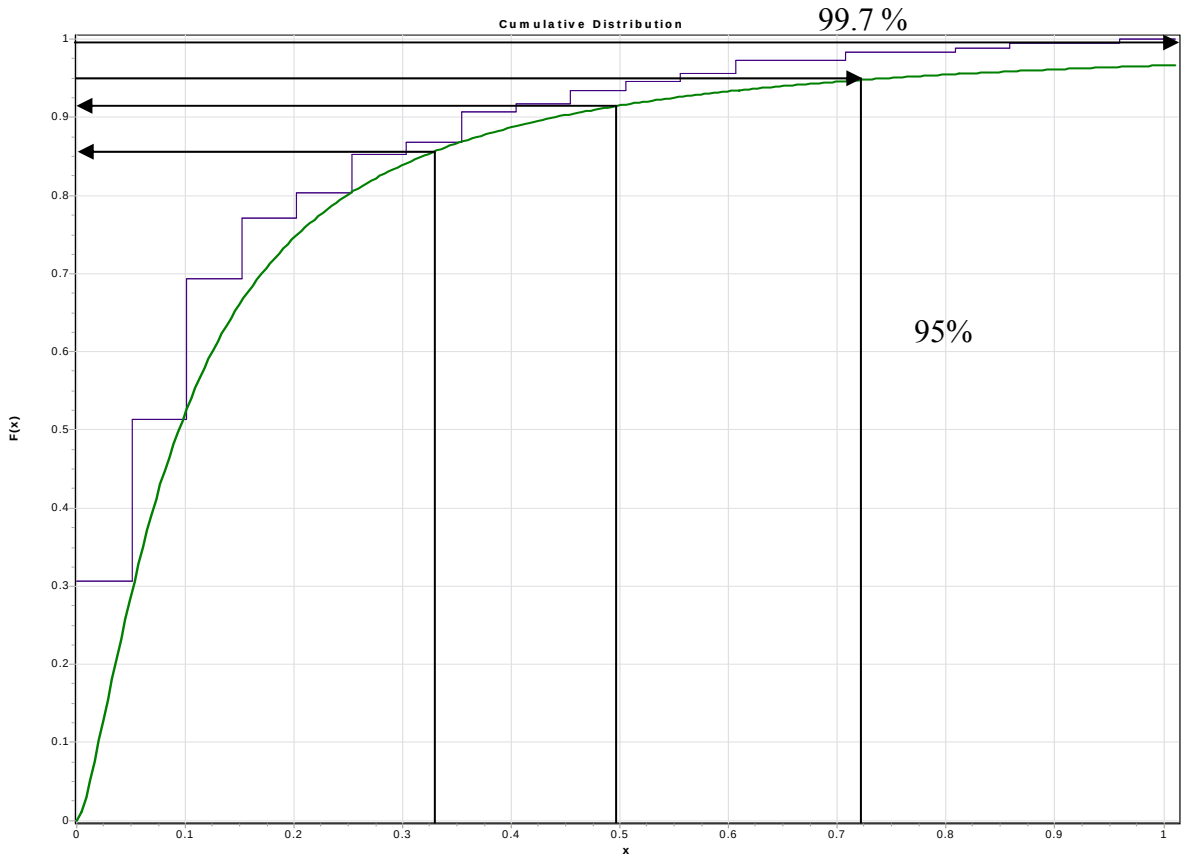

Figure 27 HPC QC data cumulative probability distribution function plot (based on replicates)

The NIST specified limits may be adopted independently of any assumed distribution of the data as an arbitrary baseline. If there is no knowledge of the distribution of the data, the limits serve as an indicator of relative change in the variability of a measurement system. If the distribution is known, then it is possible to determine the actual probability that corresponds to the limits as defined by NIST, or to determine the WL and CL based on probability rather than the NIST calculation. When starting however, a certain amount of data must be obtained before this can be accomplished, and for many studies there may be insufficient observations to determine the

CL since the probability of 0.3% (3 out of a thousand) implies that as a minimum many hundreds of observations would be needed to observe even one true CL violation based on probability.

Table 31 HPC duplicate and replicate NIST WL and NIST CL data summary

	Total Number of Observations	Number > WL		WL<Number<CL		Number>CL	
	No.	No.	%	No.	%	No.	%
HPC duplicates	190	38	20.0 %	17	9.0 %	21	11.1 %
HPC replicates	183	45	24.6 %	14	7.7 %	31	16.9 %

Table 32 Comparison of normal distribution WL and CL with real distribution WL and CL

	WL from chart (+/- 2s)	CL from chart (+/- 3s)	WL from Cumulative Probability Density Function	CL from Cumulative Probability Density Function	Percent Change of the WL vs. the NIST WL	Percent Change of the CL vs. the NIST CL
HPC duplicates	0.79	1.04	1.31	2.80	39.69%	62.86%
HPC replicates	0.33	0.44	0.72	1.01	54.17%	56.44%

Table 31 describes the total number of duplicate and replicate samples and the numbers over the warning limit, over the control limit and between these two limits. It can be seen that much more than 5 % and 0.3 % of the observations exceeded the NIST WL and CL. The warning limit and control limit from the NIST calculations used during the project were compared with those

obtained from the Cumulative PDF (CDPF in Figure 26 and Figure 27) and are shown in Table 32. It suggested if it was assumed 5 % of the observations would exceed the warning limit, the warning limit should be 1.31 for duplicate samples and 0.72 for replicate samples, which is significantly different from the NIST CL and WL used during the study (0.79 and 0.33 respectively). The CPDF warning limits were 39.69 % and 54.17 % greater than the NIST warning limits for duplicates and replicates. The CPDF control limits were 62.86 % and 56.44 % more than the NIST limits.

Biofilm HPC (BFHPC) Pooled QC data Distribution Fit Tests

Although for BFHPC, duplicate and replicate warning limits and control limits were statistically different, the data sets were not sufficient to separate them and still be able to test the distribution. BF HPC pooled duplicate and replicate data distribution was tested and Wakeby distribution is reasonable to be used as a model for the data as shown in Figure 28.

The actual warning limit and control limit according to NIST formulas were 0.47 and 0.61 (log transform). However, from Figure 29, 4 % of the observations would exceed the actual control limit and 7.5 % the WL. Thus they had very different probabilities than a normal distribution (0.3 % and 5 % from the NIST standard).

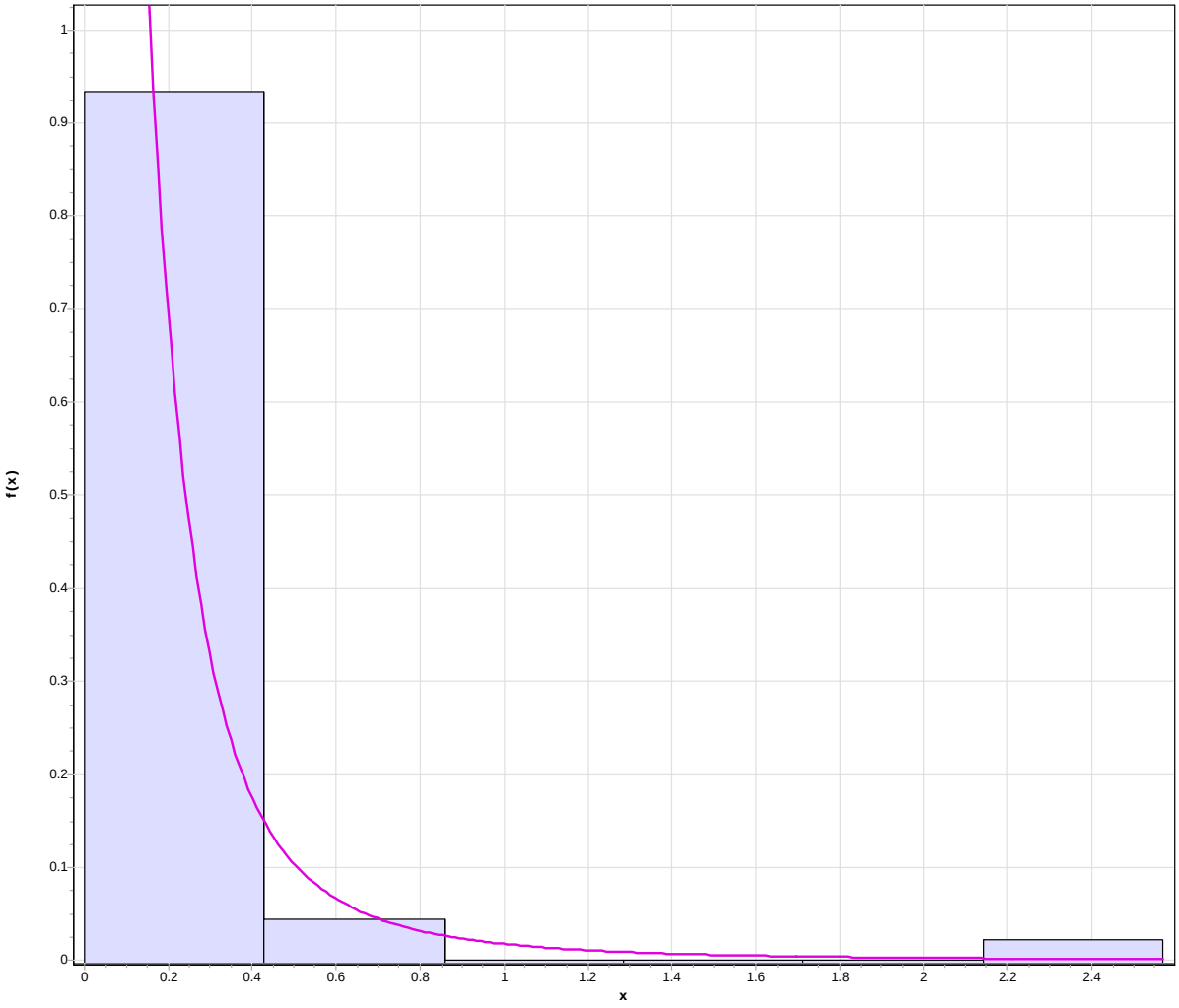

Figure 28 BF HPC pooled QC data probability density distribution (Wakeby distribution)

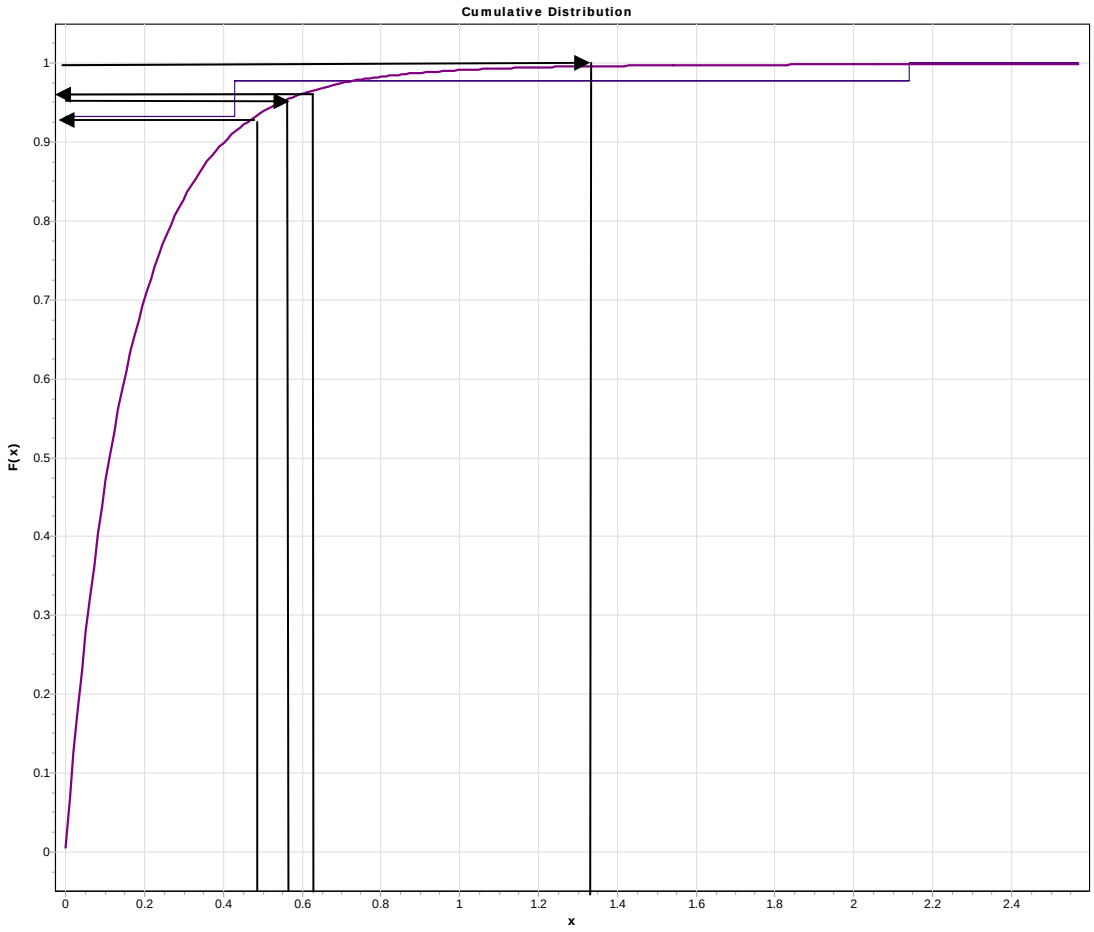

Figure 29 BFHPC pooled QC data cumulative distribution function plot

BFHPC QC data was analyzed using the pooled total number of duplicate and replicate samples. The range values were categorized with respect to the NIST WL and CL used in the study (Table 33). The WL and CL from the cumulative distribution function were 0.54 and 1.32, which were 12.96 % and 53.79 % greater than the NIST limits.

Table 33 BF HPC pooled, duplicate and replicate NIST WL and NIST CL data summary

	Total Number of Observations	Number > WL		WL<Number<CL		Number>CL	
	No.	No.	%	No.	%	No.	%
BF HPC Pooled	77	5	6.49%	2	2.60%	3	3.90%
BF HPC duplicates	32	1	3.13%	0	0.00%	1	3.13%
BF HPC replicates	45	4	8.89%	2	4.44%	2	4.44%

Suggested HPC QC chart set-up

NIST WL and CL are the most practical boundaries to use for monitoring precision for HPC enumeration at the beginning of a study. However, since HPC data was not normally distributed the definitions of control violations based on the NIST WL and CL, which are associated with 5 % and 0.3 % probabilities, are inappropriate. By using a traveling WL and CL based on the last 30 log ranges the relative change in variability can be monitored. Thus a consistent trend of increasing variability would be the observation that will alert the investigators to look for the source of the increase. As the cumulative data increases, it should also be possible to determine a $WL_{5\,\%}$ (i.e. the WL based on 5 % probability) versus the NIST WL (average + 2 ×standard deviation). The $WL_{5\,\%}$ could be used in conjunction with the NIST WL and CL. However determining the $CL_{0.3\,\%}$ will not be practical for many studies due to the large number of observations needed to determine

it. Thus HPC QC monitoring should be based on the following control violations: a) consistent trend of increasing variability (i.e. higher NIST WL and CL) over 7 sampling events (similar to the NIST definition of control violation due to trends) or b) two consecutive observations exceeding the $WL_{5\,\%}$. In addition if it is noted that the periodic NIST CL violations have increased as a fraction of the total QC observations (i.e. a trend of more NIST CL violations than in the past) this would also be considered a control violation that should be investigated.

The first 60 pairs of sample and duplicate data were used here to make an example of setting up the QC chart. The initial NIST WL and CL were obtained at 0.300 and 0.378 using the first 30 pairs of data that were not excluded as CL violations (in this example 4 pairs of data were excluded which exceeded the NIST CL calculated as each new duplicate pair came in).

To compare with the $WL_{5\,\%}$ gained from the CDF plot, the cumulative distribution function plot calculation is listed in Table 34 by using the 60 initial observations from the duplicate HPC QC data. 60 observations were separated into 10 groups and the bins were 0.14.

Table 34 Simple example for HPC duplicate QC chart set-up (cumulative distribution function calculation)

Data Characteristics	
max	1.39
min	0.01
N	60

Cumulative Distribution Function Estimation

Bins	Count	Total
0	0	0
0.14	28	0.47
0.28	17	0.75
0.42	7	0.87
0.56	1	0.88
0.7	4	0.95
0.84	1	0.97
0.98	1	0.98
1.12	0	0.98
1.26	0	0.98
1.4	1	1

The $WL_{5\%}$ can be obtained from the cumulative distribution function plot as shown in Figure 30. The warning limit was 0.700 (log transformed). The observations exceeding this $WL_{5\%}$ were only 3 and exactly 5 % of valid observations are expected to exceed the $WL_{5\%}$. The NIST WL for the first 60 duplicate QC data was 0.409. In those 60 observations, there were 8 observations exceeding the NIST WL, which was 13.3 % of total observations. Thus if sufficient historical data exists the $WL_{5\%}$ can be estimated with well under 100 duplicate pairs and used for QC purposes.

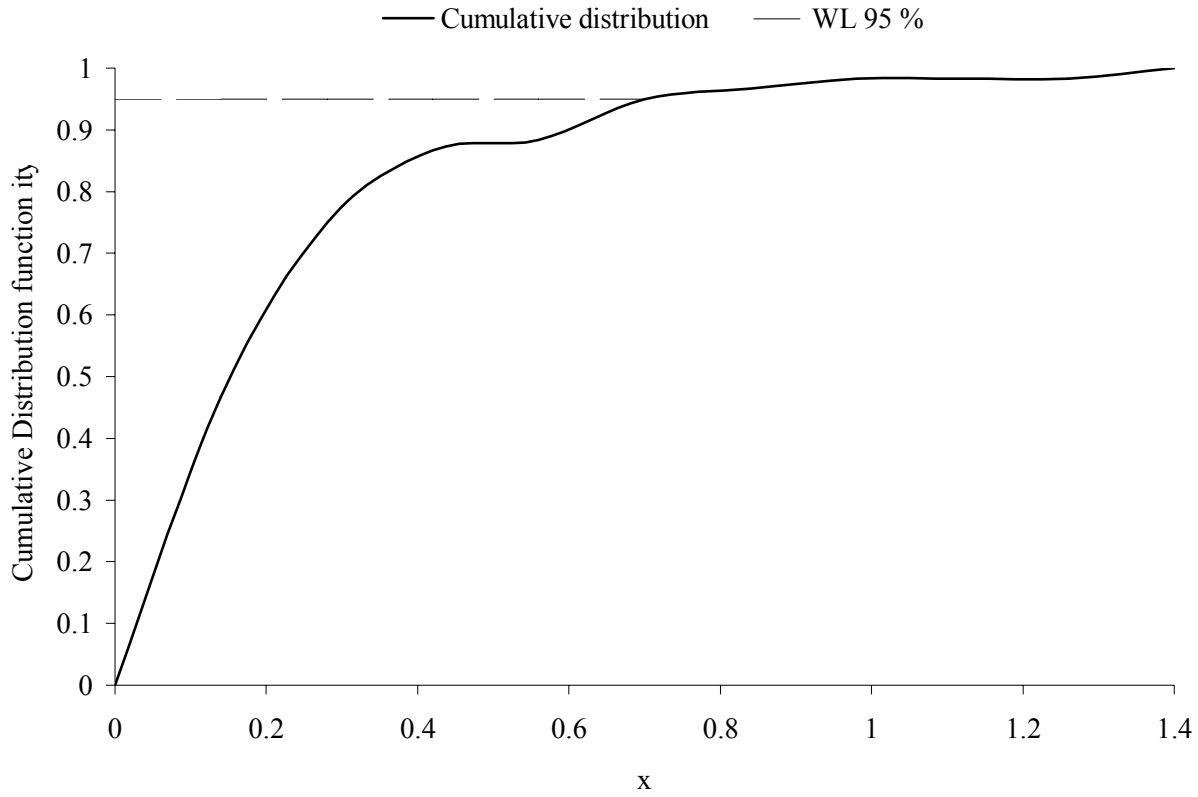

Figure 30 HPC duplicate data setup new QC chart by cumulative distribution function plot

Conclusions

- A quality control method was developed which allows monitoring of HPC for precision. The method involves a modification of traditional methods that use range for assessment

of precision. In this case, a log transform of the observations is used to generate a range statistic that is homogeneous over a large variation in HPC values. NIST WL and CL are used to monitor relative changes in variability rather than using as probability boundaries.

- HPC precision was quantified only for HPC values derived from raw plate counts ≥ 30 cfu. Four dilutions were used on all known QC related samples.
- Laboratory replicate samples yield a lower variability than blind duplicates for HPC, presumably due to elimination of variance associated with collection of separate field samples.
- HPC duplicate and replicate data distributions were investigated. Log-logistic distribution fit the data the best.
- BF HPC pooled duplicate and replicate data distributions were best fit by Wakeby distribution.
- NIST WL and CL are the most practical boundaries to use for monitoring precision for HPC enumeration. However, since HPC data was not normally distributed the definitions of control violations based on the NIST WL and CL, which are associated with 5 % and 0.3 % probabilities are inappropriate.
- HPC QC monitoring should be based on the following control violations: a) consistent trend of increasing variability (i.e. higher NIST WL and CL) over 7 sampling events (similar to NIST definition of control violation due to trends) or b) two consecutive observations exceeding the $WL_{5\ \%}$. However care must be exercised since an accurate estimation of $WL_{5\%}$ is only possible after a very significant amount of data has been

obtained. For example with 60 replicate or duplicate pairs there would be somewhere on the order of an average of 3 pairs exceeding the $WL_{5\%}$ but it would also be possible for this number to vary significantly with only 60 pairs. The estimation of $WL_{5\%}$ would continue to improve as more replicate or duplicate pairs were obtained. A suggested minimum would be 60 pairs to provide an initial estimation of $WL_{5\%}$, and as more pairs come in to continue to improve the $WL_{5\%}$ estimate using all data that do not exceed the current NIST CL, which should be based on the most recent 30 pairs of data.

References

ASTM (1977). Standard Practice for Determination of Precision and Bias of Methods. Committee D-19 on Water, Designation D2777-77, American Society for Testing and Materials, Philadelphia, PA.

CHAPTER FIVE: BIOFILM FORMATION AFFECTED BY A HIGH PHOSPHATE AND SILICA LOAD IN A PILOT-SCALE DRINKING WATER DISTRIBUTION SYSTEM

Introduction

Growth of bacteria in drinking water distribution systems can lead to deterioration of water quality (taste, odor), violation of quality standards and increased operating costs (Rice *et al*, 1991; Charnock and KjØnnØ, 2000). Typically, in biologically unstable water, bacterial populations proliferate as a biofilm becomes attached to the pipe wall. In the biofilm, immobilized cells frequently are embedded in an organic polymer matrix of microbial origin (Charackilis and Marshall 1990). The accumulation and proliferation of fixed bacteria under the form of biofilms is usually controlled by a large number of parameters, more or less well studied at bench scale and more rarely in the field at full scale (e.g. hydraulics, temperature, nature and concentration of nutrients, bacterial density and species introduced into the system, nature of pipe materials, predators). Biofilm heterotrophic plate count (biofilm HPC) is the parameter being used to quantify biofilm density in this study, and the biofilms are being compared on four different materials, PVC, lined cast iron (LCI), unlined cast iron (UCI) and galvanized steel (G). Recent research suggests that using phosphate-based inhibitors for corrosion control contributes to improving the microbial quality of distributed water and enhancing compliance records, which may be the result of better maintenance of the chlorine residual along with the limitation of corrosion deposits (M. Batté *et al*. 2003; LeChevallier *et al*. 1996; Olson 1996). However in some studies phosphorus has been observed to be a limiting nutrient which stimulates bacterial growth (Sathasivan *et al.* 1997 and 1998). The limitation of an organic carbon source may not be the only nutrient that limits bacterial growth in a distribution system if other nutrients such as

ammonia/nitrogen and bioavailable phosphorus are present in very low amounts, or alternately if biodegradable organic matter (BOM) is present in large amounts (it is really the C:N:P ratio that determines nutrient limitation with respect to normal microbial growth). Further it is possible to have multiple limiting nutrients simultaneously.

Materials and Methods

Distribution Networks

The experimental system for the project consists of fourteen pilot distribution system (PDSs) being fed a blend of groundwater, surface water, and RO permeate from desalination which is representative of the blend anticipated in the near future for the utilities involved in the study. Four corrosion inhibitors were evaluated at three different inhibitor doses each (PDS 1 through 12). In addition there were 2 PDSs that received no inhibitor but were pH controlled. The inhibitors tested were blended ortho and polyphosphates, orthophosphates, silicates and zinc orthophosphate. The experimental system is described briefly below and the inhibitor doses used are shown inTable 35

The PDSs were identical and consisted of sections of PVC, lined cast iron, unlined cast iron and galvanized steel pipes connected in series. The pipe materials were used pipes excavated from the full scale distribution systems of the participating utilities.

The fourteen PDSs were divided into three sets of four with the remaining two PDSs used as controls. The maximum dosage of the corrosion inhibitors were added to PDS 2, PDS 5, PDS 8 and PDS 11. PDS 13 and 14 were control systems with no inhibitor but with one operated at saturation (with respect to calcium carbonate) i.e. at pHs, and the other was operated slightly above the pHs.

Each set of four PDSs received one blend for a three month period. At the end of each three month period the blends for each PDS were changed but the corrosion inhibitor and the dose of corrosion inhibitor were held constant.

Seasonal effects were evaluated by repeating the blend scenarios for the first three month phase (Phase I) and the third three month phase (Phase III) 6 months later.

Table 35 Inhibitor Dosage in Fourteen PDSs

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 SiO2
11	Silica	6 mg/L as SiO2
12	Silica	12 mg/L as SiO2
13	pHs	None

14	pHs+0.3	None

Coupons were held in a PVC cradle that fit into a section of PVC pipe receiving PDS influent. PDS pipe coupons for biological sampling were approximately 3 cm in diameter with a small PVC peg made from PVC welding rod attached to what was the outer surface of the pipe. The coupons were drilled from aged pipe and then de-burred to give smooth edges. In each integrated pilot distribution system cradle, there were duplicate aged coupons of each of the four materials. The sequence of the coupons in these cradles from upstream to downstream was PVC, LCI, UCI and finally G coupons.

The coupons were placed in this order to avoid transport of corroded materials downstream to contaminate the less easily corroded materials such as PVC and LCI. Thus the two more easily corroded materials (i.e. UCI and G) were placed in the downstream locations. Biofilms on the coupons that were housed in the cradles were analyzed after 5-6 weeks of growth. The biofilms were assessed using biofilm HPC with units of HPCs/cm^2. Biodegradable organic matter (BOM) was quantified using AOC.

Biofilm and Water Quality Analyses

Biofilm HPC Measurement

Biofilm HPC was measured the same way as bulk water HPC except the samples were scratched from the coupons. Coupons colonized by biofilm were sampled and rinsed carefully with phosphate buffer solution (PBS) twice. The biofilm was manually detached from the coupon using a sterile cell scraper (sterilized by 70% Ethanol and flaming) in 10 mL of sterile PBS. The

detached biofilm was homogenized by using a tissue blender (Tissue Tearor™, Biospec products, Inc) at 5000 rpm for 2 minutes. The blender top was cleaned in 10% bleach solution for 15 seconds and then in DI water for 15 seconds between each two samples. The sample was then placed on R2A agar plates after serial dilution. Incubation was at 25 degrees C for at least 5 days, with triplicate plates for each dilution of a sample. The colonies were enumerated after incubation. There were two dilutions for each sample, including blind duplicates used for quality control purposes. Four dilutions were used for lab replicate quality control samples.

AOC Measurement

AOC refers to the most readily degradable fraction of BOM, which is the fraction of the total organic carbon (TOC) that can be utilized by specific strains or defined mixtures of bacteria. Pure cultures of *Pseudomonas fluorescens* 17 and *Spirillum* NOx were cultivated in laboratory conditions to run AOC using a combined method (P17 and NOx inoculated into the same sample vial) as described in Standard Methods. There were some small modifications of the method, i.e. the inoculation density of P17 and NOx was higher than that used in Standard Methods, and the incubation temperature was 25 degrees C. Spreadplates with R2A agar were used with plate counts being conducted on day 3, 4, and 5. Instead of using the maximum day count as an estimation of the cell density at stationary phase, which in effect picks the outlier value of the data set, the average of the counts obtained on all 3 days was used. Many of the modifications were derived from LeChevallier's rapid AOC method although we did not adopt the use of luciferase (i.e. ATP quantification) for enumeration but instead we stayed with plate counts. Standard curves were set up and the accuracy and precision of AOC measurements were quantified. Standard curves were run in parallel with samples on all AOC runs. Standard concentrations

were 0, 50, 100, and 200 µg/L of Sodium Acetate as acetate. A typical standard curve is shown in Figure 31.

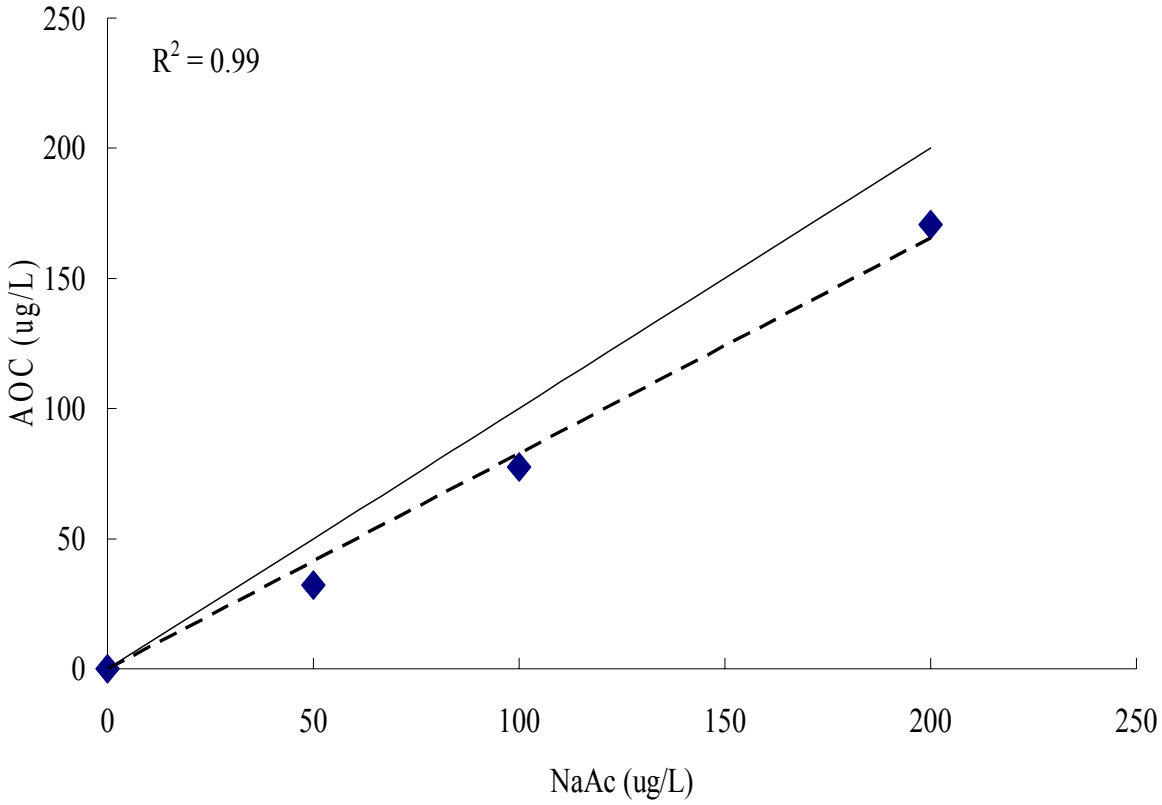

Figure 31 Combined method AOC standard curve relative to blank (Phase IV standard curve)

Results and Discussion

Statistical Comparison of Biofilm Density of Control PDSs and Inhibitor PDSs

The experimental facilities allowed for side-by-side testing of fourteen separate PDS hybrid lines (each with four pipe materials) that received the same finished water blend. The individual hybrid lines received a different corrosion control strategy consisting of an inhibitor at varying

dosages or pH control without addition of an inhibitor. Since PDS 13-14 were the control pipes, PDS 1-9 (phosphate based inhibitors) and 10-12 (Si based inhibitors) were compared relative to the control PDSs. The data was also examined for a possible difference between phosphate based inhibitors PDSs and Si based inhibitor PDSs. The Normality Test failed in PDS 13-14 data and the Kruskal-Wallis Test was used instead of instead of multiple t-test and Mann Whitney tests. The Kruskal-Wallis Ranks Test is a nonparametric test that does not require assuming all the samples were normal distributed with equal variances. The results are shown in Table 36 and Table 37. α values used in both normality test and Kruskal-Wallis Ranks Test were 0.10.

Table 36 Normality test for all the data groups

	P value	Passed Normality Test?
PDS 1-9	0.177	YES
PDS 10-12	>0.200	YES
PDS 13-14	0.065	NO

Table 37 Biofilm HPC Data Statistical Comparison (Kruskal-Wallis Test, p = 0.041)

Comparison	Difference of Ranks	Statistical Critical Value	Significantly Different
PDS 1-9 vs PDS 13-14	29.29	26.86	Yes
PDS 10-12 vs PDS 13-14	34.43	31.50	Yes
PDS 1-9 vs PDS 10-12	5.13	23.09	No

The difference in the median values between PDS 1-9 and PDS 13-14 in Table 38 Comparison of log BF HPC (cfu/cm^2) for PDS was greater than would be expected by chance; there was a statistically significant difference. The PDSs with phosphate inhibitor had 0.34 log greater median values for biofilm HPC than the control PDSs in Table 38. The distribution also

showed that the lowest 25% of the values in PDS 1-9 were 0.44 log higher than the same value in the control PDSs. The lowest 75% of the observed values in PDS 1-9 were 0.38 log higher than the control PDSs. Thus through the entire distribution of the data the phosphate based inhibitors resulted in a statistically significant increase in biofilm density.

The difference in the median values between PDS 10-12 and PDS 13-14 in Table 38 was greater than would be expected by chance; there was a statistically significant difference. PDSs with silica inhibitor had 0.36 log greater median values than the control PDSs. The distribution also showed that the lowest 25% of the values in PDS 1-9 were 0.34 log higher than the control PDSs, and the lowest 75% of the values were 0.55 log higher. Again the addition of inhibitors corresponded to a significantly greater biofilm density, and the increase with Si based inhibitors was at about the same level as was observed with phosphate based inhibitors.

From the table, the difference in the median values between PDS 1-9 and PDS 10-12 was not great enough to exclude the possibility that the difference was due to random sampling variability; there was not a statistically significant difference. The median value of PDS 1-9 was only 0.02 log lower than PDS 10-12. At 25% level, the difference in the median value was 0.10 log and at the 75% level, it was 0.17 log difference. The biofilm density with phosphate-based inhibitors was about the same as Si based inhibitors and there was no statistically significant difference between the biofilm densities observed.

Table 38 Comparison of log BF HPC (cfu/cm^2) for PDSs

Group	N	Missing	Median	25%	75%
PDS 1-9	144	0	5.26	4.65	5.59
PDS 10-12	48	1	5.28	4.55	5.76

PDS 13-14	32	0	4.92	4.21	5.21

Note: Missing denotes the number of missing values for that column or group.

Growth Response to Inhibitors and AOC Growth Control and Yield Control Analysis

To investigate the corrosion inhibitors impact on bacterial proliferation several supplemental experiments were run using the P17 and NOx cultures for AOC (some AOC growth and yield controls had already implied that organic carbon was not the only limiting nutrient). This was done twice in Phase IV. The spiked sample IDs are shown in Table 39. There was not enough data to conduct a statistical comparison (e.g. t-test) and only the range and relative percentage difference (RPD) of each pair of samples was calculated (Table 40). Since blend tank water showed inhibition with respect to the AOC assay in Phase IV, the average AOC (based on duplicate AOC measurements) of the unspiked blend water was very low in both experiments (Table 40). In the first experiment all the spikes resulted in a very significant increase in bacterial growth (the higher plate counts were then expressed as AOC counts using the P17 and NOx yields from Standard Methods). Trace carbon could not be ruled out for the inhibitors added, but the potassium monophosphate spike was almost certainly carbon free and it can be seen that it stimulated growth also. If carbon was the only limiting nutrient then it should have been impossible to elevate the plate counts by adding K_2HPO_4 since this used a reagent grade chemical that should not have had any trace BOM.

The average range between duplicates for AOC during this study was 46 µg/L, so the increases in AOC in the first experiment in Error! Reference source not found. were clearly significant. This seemed to imply that inorganic nutrients either neutralized the inhibitory compounds/effect in Phase IV samples, or that some of the inorganic nutrients present stimulated

growth. Taking into account the higher bulk and biofilm HPCs observed in the inhibitor PDSs throughout the rest of the study (Phase 1 and 2, and most of 3, did not have inhibitory characteristics for the most part) it seems likely that the nutrients stimulated growth rather than neutralized something inhibitory. Thus at the very least phosphorus was also a significant limiting nutrient in addition to BOM. The fact that the silica inhibitor also stimulated growth implies that other inorganic also may have functioned as limiting nutrients, or that the silica inhibitor had significant trace carbon. The second experiment gave more ambiguous results where all the spiked AOC values seemed to still be inhibited except for the BOP sample, which showed about the same change in AOC/growth that it showed in experiment one. The increase in AOC for the silica inhibitor in experiment two was smaller than the average range between duplicate AOC samples, so it could not be concluded that it stimulated growth in the second experiment. The results reinforced the evidence that there was something inhibitory in the influent in Phase IV, and also implied that inorganic nutrients, including but not limited to phosphorus, stimulated growth for the Phase IV water blend. All the inhibitors studied delivered nutrients stimulating growth, and this reinforces the conclusions from the PDS data. This implies than biodegradable carbon, and phosphorus, were probably the nutrients stimulating biofilm growth in the inhibitor PDSs. It is also possible that other inorganic nutrients stimulated growth as well. The data from this study suggests strongly that future analysis of drinking water systems for biostability should no longer view systems as having a single limiting nutrient. Fresh water ecosystems, estuaries, and saline waters are commonly though of as having multiple limiting nutrients (e.g. both N and P, iron and carbon limited, etc.) and to view biodegradable carbon as the only limiting nutrient is probably an oversimplification for a significant fraction of distribution systems. It is common for

Environmental Engineers to use multiple limiting nutrients in kinetic equations describing microbial growth (e.g. multiple monod-like functions for electron acceptors, electron donors, phosphorus, nitrogen, etc.) in design equations and mechanistic modeling. The concept of multiple limiting nutrients, any one of which can stimulate microbial growth, is an important concept for distribution systems as well, and AOC or BDOC data also needs to include controls for any suspected inorganic nutrients or inhibitory compounds. Likewise the effect of even inorganic chemical streams with no trace carbon impurities to treated water needs to be evaluated for its' potential impact on biostability in the distribution system.

Table 39 Corrosion inhibitor spiked blend water IDs

BOP	Blend Tank water spiked with BOP inhibitor tank water to make 2mg/L-P in the sample
OP	Blend Tank water spiked with OP inhibitor tank water to make 2mg/L-P in the sample
ZOP	Blend Tank water spiked with ZOP inhibitor tank water to make 2mg/L-P in the sample
Silica	Blend Tank water spiked with Si inhibitor tank water to make 12mg/L-SiO_2 in the sample
K_2HPO_4	Blend Tank water spiked with K_2HPO_4 to make 2mg/L-P in the sample

Table 40 AOC of spiked blend tank samples

	Spike ID	Sample	Unspiked (µg/L)	Sample	AOC	Spiked AOC (µg/L)	Sample	Increase in AOC (µg/L)
First Run	BOP		12			116		104

	OP	12	215	203
	ZOP	12	145	133
	Silica	12	329	317
	K_2HPO_4	12	354	342
	BOP	6	138	132
	OP	6	9	3
Second Run	ZOP	6	0	-6
	Silica	6	25	19
	K_2HPO_4	6	0	-6

Conclusion

Increases in biofilm density in the PDS lines receiving inhibitors were about the same level, with an increase of 0.34 log for phosphate based inhibitors, and 0.36 log for silica based inhibitors. There were also similar increases in bulk HPC in the inhibitor PDSs. AOC was stable as it passed through the PDSs during the study. However the AOC quality control data specified by Standard Methods (e.g. growth controls, yield controls) showed that in some cases carbon was not the only limiting nutrient. The AOC cultures P17 and NOx were then used to conduct supplemental experiments to determine if inorganics could stimulate growth and provide an explanation for the higher biofilm and bulk HPC counts in the inhibitor PDSs. The results showed that while adding carbon (as acetate) did stimulate growth, so did the addition of phosphate salts, phosphate based inhibitors, and silica based inhibitors. Using the data from this study one must conclude that there was, for this blend/system combination, often more than one limiting nutrient. Phosphorus was

identified as one of those nutrients and there was also an unidentified nutrient in the Si based inhibitor.

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CHAPTER SIX: MODLEING OF BULK AND BIOFILM HPC IN PILOT DISTRIBUTION SYSTEMS USING CORROSION INHIBITORS

Introduction

Maintaining microbiological quality in drinking water distribution systems as a complex ecosystem is of considerable interest to water utilities world-wide. In most distribution systems, the density of suspended cultivable bacteria increases between the plant and the consumer's tap as a function of disinfectant decay, substrate uptake and the presence of corrosion deposits (Kerneïs *et al.,* 1995; Prévost *et al.*, 1998; Power and Nagy, 1999). The inter-relationship between corrosion and bacterial regrowth has been known for a long time (Larson, 1939) and it was suggested that the distribution systems can contribute to water borne disease because of biofilm detachment (Payment *et al.*, 1997). Bacterial growth may affect the turbidity, taste, odor and color of the distributed water (Servais *et al.,* 1995). Coliform bacteria have been associated with a high abundance of heterotrophic bacteria and biofilms, producing a possible health risk (Goshko *et al.*, 1983). There is widespread controversy as to how microbial regrowth of heterotrophic bacteria is best controlled (Morton, Zhang and Edwards, 2005). The previous research has suggested that corrosion rate could increase the number of biofilm bacteria, possibly due to protection of bacteria by consumption of disinfectant at the pipe surface via corrosion reactions (LeChevallier *et al.*, 1993). Chlorine disinfectant concentrations are too low to control bacteria near the pipe wall with biofilms attached. This is because the chlorine disinfectant is consumed by corrosion and reactions with other constituents such as dissolved natural organic matter (LeChevallier *et al.*, 1990). The biofilms which can develop in distribution systems are composed of bacteria held in a polymeric matrix and can exert a chlorine demand, reducing the protection afforded by residual disinfectant

(Lu *et al.*, 1999). One potential and feasible method of significantly reducing or eliminating adverse water quality impacts from the disruption of distribution system scale or film is to replace the controlling scale or film with a film that will not be disrupted when exposed to the expected changing water quality. This is possible with surface active agents such as corrosion inhibitors. Blended phosphates are corrosion inhibitors which are frequently used to control corrosion in drinking water distribution systems. Orthophosphates, and possibly polyphosphates, are bacterial nutrients and thus may stimulate the growth of fixed and suspended bacteria. Recent research suggests that using phosphate-based (P-based) inhibitors for corrosion control contributes to improving the microbial quality of distributed water and enhancing compliance records, which may be the result of better maintenance of the chlorine residual along with the limitation of corrosion deposits (M. Batté *et al.* 2003; LeChevallier *et al*. 1996.; Olson, 1996).

The influence of pipe material on biofilm density has been observed by a number of authors. Ridgeway and Olson (1981) conducted an extensive survey of biofilm formed on a section of cement lined, galvanized iron pipe removed from a distribution system. The internal surface of the cement lining was almost completely concealed beneath a mineral encrustation 10-100 μm in thickness. Colonies of microorganisms were randomly and sparsely distributed along the surface of the pipe and were frequently associated with crevices in the mineral layer. Another study by Van der Kooij (1992) removed sections of 100 mm diameter PVC pipe from a number of distribution systems and the biofilm density was determined to be in the range from 40 to 200 pg ATP/ cm^2. Also the biofilm accumulated on a PVC pipe in unchlorinated water was examined by Characklis *et al.* (1998). A readily visible biofilm had covered the entire surface producing cell densities of 5×10^{10} cells/cm^2.

This study determined the effects of pipe material, inhibitor type and concentration, temperature, chloramines residual, and other water quality parameters on biofilm HPC (BF HPC), and bulk HPC, levels in pilot drinking water distribution systems and evaluation of the relationship (if any) between biofilm and bulk/planktonic HPC. Empirical models that describes the relationship between water quality parameters and biofilm HPC and bulk HPC, were developed.

Materials and methods

Pilot Distribution Systems

The fourteen pilot distribution systems (PDSs) and two pilot treatment systems used in the implementation of this project were designed and built by the University of Central Florida and Tampa Bay Water-Member Government (TBW-MG) personnel in the previous AWWARF –TBW project, "Effects of Blending on Distribution System Water Quality" (Taylor *et al*. 2005). The PDS was designed to simulate water quality changes resulting from single sources and blends of significantly different source waters in distribution systems historically receiving mostly groundwater.

The experimental system for the project consisted of fourteen pilot distribution system (PDSs) being fed a blend of groundwater, surface water, and RO permeate from desalination. Figure 32 shows the pilot systems consisting of four pipe segments of PVC, LCI, UCI and G connected in series. Four corrosion inhibitors were evaluated at three different inhibitor doses each (PDS 1 through 12). The inhibitors were blended ortho and polyphosphates, orthophosphates, zinc orthophosphate and silicates. Two PDSs (PDS 13 and 14) did not receive inhibitors but were controlled at pH_s and pH_s+0.3. All pilot distribution lines were operated to maintain a two-day hydraulic residence time (HRT). The retention time in the PDS feed standpipe was 3.1 hours

because of the low velocities associated with the two-day HRTs. To avoid bacterial growth, the standpipes were wrapped in a non-transparent material to eliminate direct light exposure and cleaned regularly with a plastic brush and a 0.1% solution of sodium hypochlorite. All pilot distribution systems were constructed with a sampling port after each pipe segment to allow an assessment of water quality changes associated with each pipe material. Three different blends were used during the study based on the expected blend of actual finished waters from (a) the Regional Surface Water Treatment Facility, (b) the TBW Desalination Facility and (c) the Cypress Creek Groundwater Treatment Facility. The variation in water quality is listed in Table 41. Each set of four PDSs received one water quality blend for a three month period. At the end of each three month period the blends for the PDSs was changed but the corrosion inhibitor and the dose of corrosion inhibitor were held constant (Table 42). The effects of seasonal temperatures were evaluated by repeating the blend scenarios for the first three months (phase I) for the six months later (phase III).

Figure 32 Pilot distribution systems

Table 41 Blend ratios of GW, SW and RO waters used during project

Phase	Time Period	% GW	%SW	%RO
1	Feb-May 2006	62	27	11
2	May-Aug 2006	27	62	11
3	Aug-Nov 2006	62	27	11
4	Nov 2006-Feb 2007	40	40	20

Table 42 Variations of Water Quality, Inhibitor and Dose by Project Phase

Ind. Var.	Water Quality 1													
Phase I	PDS 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10*	P 11*	P 12*	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 –SiO2	6 - SiO2	12 - SiO2	0	0
	Water Quality 2													
Phase II	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 SiO2	6 SiO2	12 SiO2	0	0
	Water Quality 1													
Phase III	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 SiO2	6 SiO2	12 SiO2	0	0
	Water Quality 3													
Phase IV	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14
Inhibitor	BOP	BOP	BOP	OP	OP	OP	ZOP	ZOP	ZOP	Si	Si	Si	pHs	pHs+ 0.3
Dose (mg/L)	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	0.5 - P	1.0 - P	2.0 - P	3 SiO2	6 SiO2	12 SiO2	0	0

*Original dosages of 10, 20, and 40 mg/L were changed to reported dosages after 3 weeks

Bioassays were conducted on the PDS influent and effluent and for biofilm cradles using coupons from all pipe materials as shown in Figure 33 and Figure 34. Pilot distribution system pipe coupons for biological sampling were approximately 3 cm in diameter with a small PVC peg made from PVC welding rod attached to what was the outer surface of the pipe. The coupons were drilled from aged pipe and then de-burred to give smooth edges. In each integrated pilot distribution system cradle, there were duplicate aged coupons of each of the four materials. The sequence of the coupons in these cradles from upstream to downstream was PVC, LCI, UCI and finally G coupons. The coupons were placed in this order to avoid transport of corrosion materials downstream which might contaminate the less easily corroded materials such as PVC and LCI. Thus the two more easily corroded materials (i.e. UCI and G) were placed in the downstream locations. The aged pipe coupons were obtained from used pipe segments from actual member government distribution systems. Biofilms were generated on the coupons that were housed in the cradles and were harvested after 5-6 weeks of growth. The biofilms were assessed using HPC after detaching and homogenizing the biofilms. Biofilm density was expressed in units of cfu/cm^2 and bulk biological stability was assessed using HPCs expressed in units of cfu/mL. As one of the primary monitoring parameters of microbial water quality, bulk fluid HPCs were analyzed weekly for both influent and effluent of the 14 PDSs, 4 inhibitor tanks, three storage tanks and the blend tank (Figure 35 and Figure 36). During each sampling event, six blind duplicate samples were tested with one from the inhibitor tanks, one from the storage tanks, two from the PDS influents and two from the PDS effluents. At the same time, replicates of four randomly picked PDS samples were examined in the laboratory.

Figure 33 Mounted coupons

Figure 34 Cradles for housing coupons

Figure 35 Raw surface water storage

Figure 36 Inhibitor tanks and feed pumps

Water Analyses

HPC Measurement

HPC was measured with spreadplates on R2A agar. The plates were labeled with sample number and location. Using a 10-100µL pipet, 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 spreaded 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 plates were measured following incubation. There were two dilutions for each sample, including the blind duplicates. Four dilutions were used for lab replicate quality assurance (QA) samples.

Biofilm HPC Measurement

Biofilm HPC was measured the same way as bulk HPC except the samples were scraped from the coupons. Coupons colonized by biofilm were sampled and rinsed carefully with phosphate buffer solution (PBS) twice. The biofilm was manually detached from the coupon using a sterile spatula as a cell scraper (sterilized with 70% Ethanol and flamed) into 10 mL of sterile phosphate buffer solution (PBS). Samples were homogenized by using a tissue blender (Tissue Tearor™, Biospec products, Inc, Bartlesville, OK, USA) at 5000 rpm for 2 minutes. The tissue homogenizer probe was cleaned in 10% bleach solution for 15 seconds and then in DI water for 15 seconds between two samples. The sample was then diluted and spread on R2A agar plate as described in the preceding HPC measurement section. Incubation was at 25 degrees Celsius for at least 48 hours, with triplicate plates for each dilution of a sample. There were two dilutions for each sample, including the samples with corresponding blind duplicates. Four dilutions were used for lab replicate QA samples and the known half of the blind duplicates.

Model Development

Bulk HPC Model Development

An empirical model was developed using the entire dataset (all Phases and all PDSs). 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. A power form of the model was used. The water quality parameters monitored in the PDSs were evaluated using ANOVA procedures to identify statistically significant parameters. Non-linear least squares regression techniques were used to estimate parameters in the empirical models. Those independent variables not shown to be significant at a 95 % confidence level were eliminated from the model. Initial model development segregated the data based on inhibitor type. 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 the PDSs 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.

An empirical model was developed using the entire dataset (all Phases and all PDSs). The objective of the model was to quantify the impact of water quality on the biofilm density in the distribution systems. Dummy variables (PVC, LCI, UCI and G) for each pipe material were incorporated into the model. Water quality parameters including influent dissolved zinc, total phosphate and silica were used to evaluate the effect of inhibitors on BF HPC. The use of dummy variables allowed estimation of a single parameter that is associated with each of the four different materials.

The biofilm HPC (BF HPC) model was set up in the same way as the bulk HPC model. However, four different kinds of materials (PVC, UCI, LCI and G) were used as the dummy variables instead of dummy variables for the inhibitors and pH control lines (BOP, OP, ZOP, Si and pH control) as was done with the bulk HPC model. The empirical models presented in this paper are intended to provide information regarding the benefit of inhibitor addition for control of 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. All remaining independent variables, i.e. material, temperature, residual and water quality parameters were statistically significant at a 95 % level of confidence in the reported models.

Results and discussion

Bulk HPC Modeling

Effluent HPC as a Function of Different Doses of Inhibitors

The averages of effluent HPC in four phases are shown in Figure 37 (BOP inhibitor), Figure 38 (OP inhibitor), Figure 39 (ZOP inhibitor), and Figure 40 (Si inhibitor). The graphs do not show any consistent upward or downward trends corresponding to dosage for any of the inhibitors.

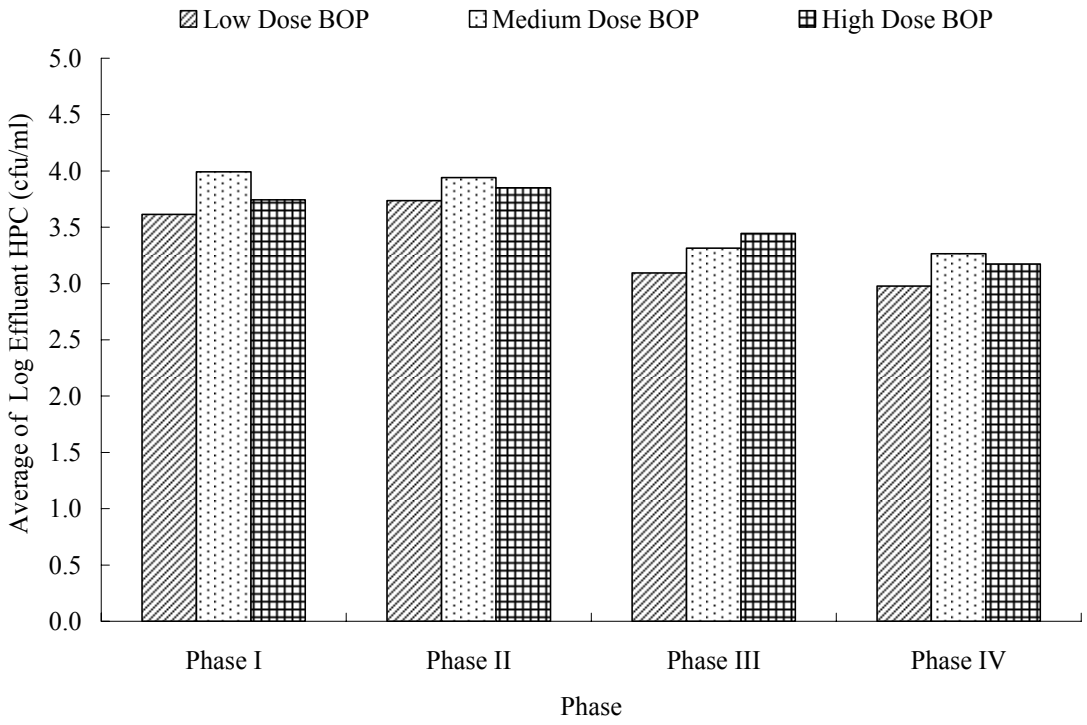

Figure 37 Average log effluent HPC for four phases using different doses of BOP inhibitor

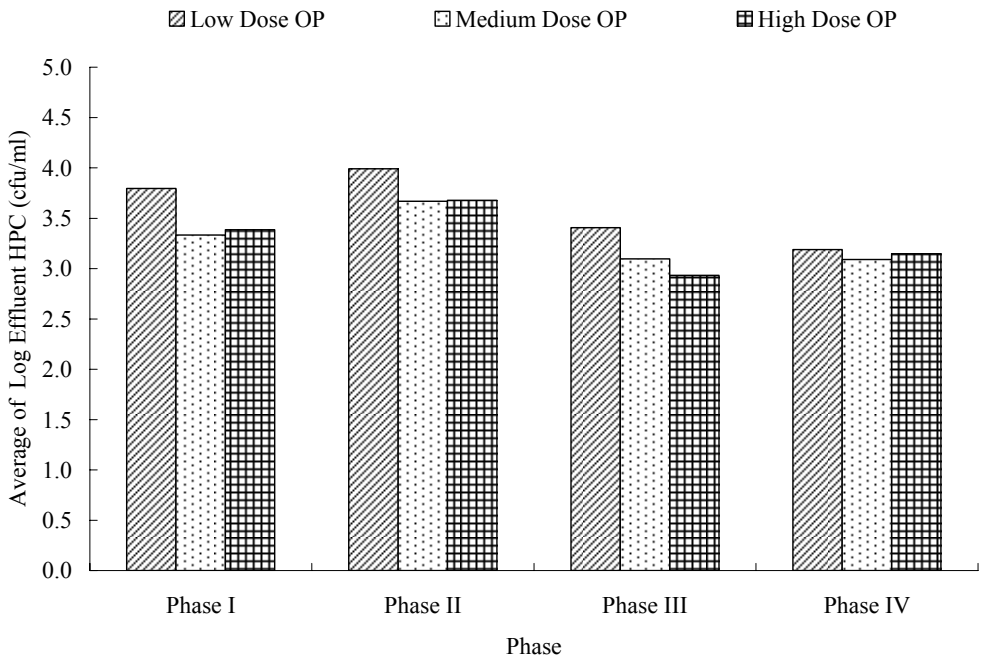

Figure 38 Average log effluent HPC for four phases using different doses of OP inhibitor

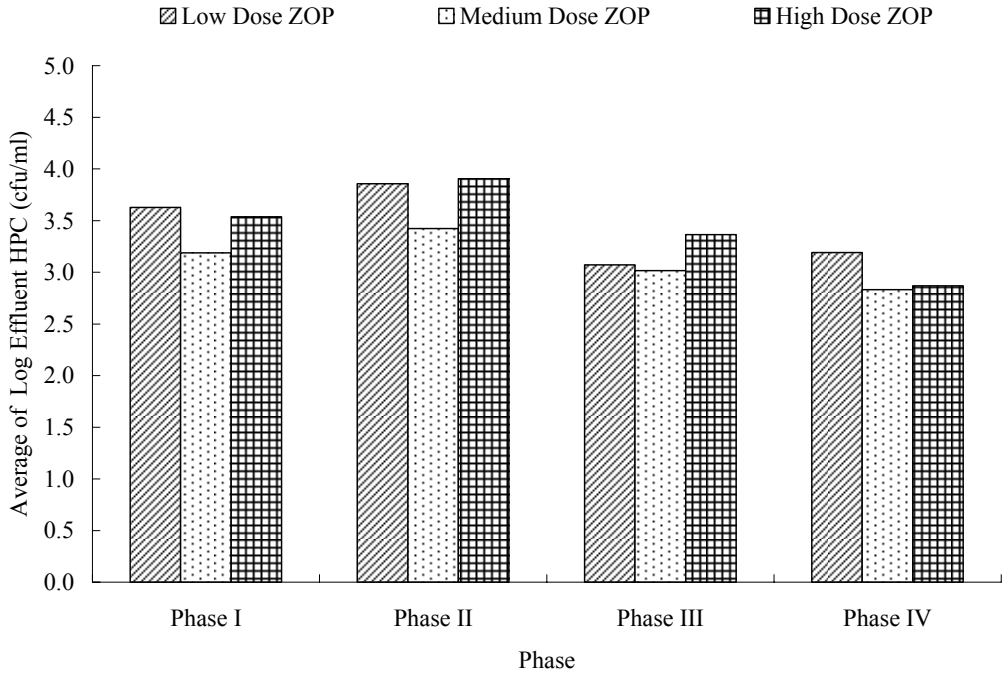

Figure 39 Average log effluent HPC for four phases using different doses of ZOP inhibitor

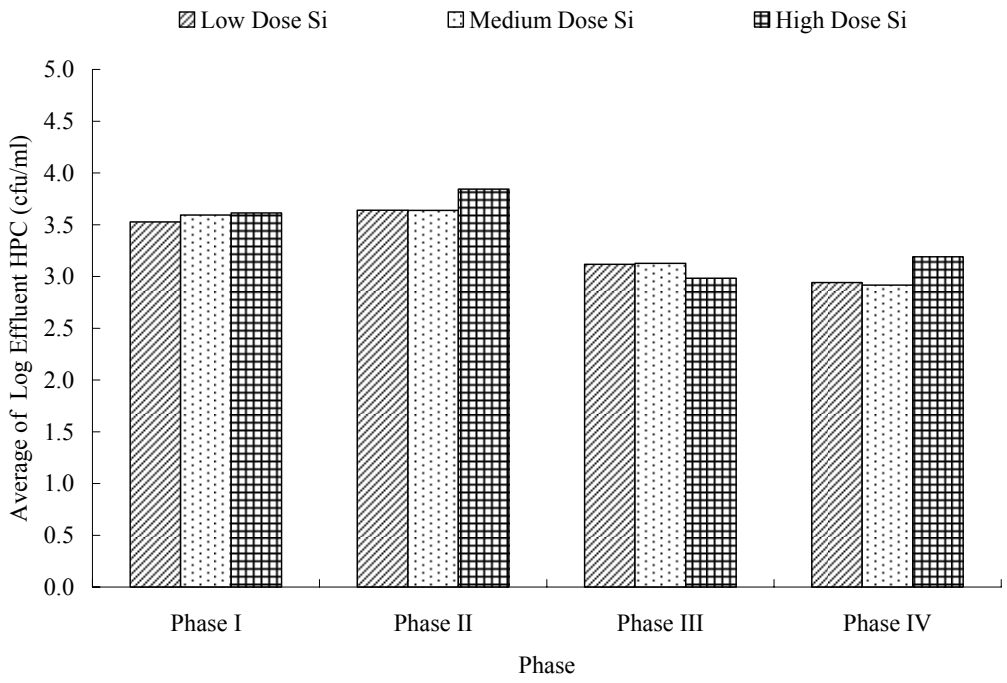

Figure 40 Average log effluent HPC for four phases using different doses of Si inhibitor

Statistical Comparison of Effluent HPC between different PDSs

Since PDS 13-14 were the control pipes, PDS 1-9 (phosphate based inhibitors) and 10-12 (Si based inhibitors) were compared relative to the control PDSs. The data was also examined for a possible difference between phosphate based inhibitors PDSs and Si based inhibitor PDSs. The Normality Test failed in PDS 13-14 data and the Kruskal-Wallis Test was used instead of instead of multiple t-test and Mann Whitney tests. Phosphate based inhibitor lines and Si based inhibitor lines were significantly different from the control lines but they are not different from each other. PDSs with phosphate inhibitor had 0.32 log greater median values for effluent HPC than the control PDSs. PDSs with silica inhibitor had 0.30 log greater median values than the control PDSs.

Bulk Effluent HPC Model for All PDSs

The model for Log HPC with data from all four phases for all 14 PDSs 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 43. This pooled model (Equation 1) permitted estimation of unique coefficients for each inhibitor and for pH control.

$$LogHPC_{eff} = a \times Temp^{b} \times TCl_{2-eff}^{c} \quad \text{Equation 1}$$

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 (°C)
TCl_{2-eff} = bulk water influent total chlorine (mg/L)

Table 43 Coefficient matrix for log effluent HPC (cfu/mL) model

	a	b	c
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	-0.07

The dummy variable coefficients ("a") listed in Table 43 correspond to each of the inhibitors and to the pH control PDSs. The values were similar except for the OP PDSs. It can be seen that as a result of the greater dummy variable value for the OP PDSs, the corresponding coefficient for temperature was lower than for the other PDSs. 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 PDSs were not significantly different from that of the OP PDSs (p value was 0.087). However the dummy variable coefficient of BOP is approximately 1 unit less than that of OP. Instead of comparing the coefficients in Table 43, a sensitivity analysis for temperature and residual was conducted using Equation 1. Table 44 shows the sensitivity test for the model. The minimum, average, and maximum values of temperature and effluent residual are the observed values during the year long project and are shown for each inhibitor group.

Everything except the BOP and pH control PDSs showed a greater sensitivity to residual than to temperature. The ZOP PDSs showed the lowest sensitivity to temperature, while the BOP

PDSs showed the greatest sensitivity to temperature. The pH PDSs showed the lowest sensitivity to residual of all the PDSs, while Si PDSs showed the greatest sensitivity to residual.

Table 44 Sensitivity test for all PDSs log effluent HPC (cfu/mL) model

BOP							
Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^{o}C)	23.1	29.1	10.5	3.7	3.2	2.1	1.6
TCl_{eff} (mg/L)	2.3	5.0	0.6	2.8	3.2	4.2	-1.4
OP							
Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^{o}C)	23.0	29.0	10.4	3.3	3.1	2.5	0.8
TCl_{eff} (mg/L)	2.4	4.4	1.0	2.7	3.1	3.7	-1.0
ZOP							
Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^{o}C)	21.3	24.3	16.4	3.1	2.9	2.6	0.56
TCl_{eff} (mg/L)	2.1	5.6	0.6	2.4	2.9	3.8	-1.5
Si							
Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^{o}C)	23.1	29.6	12.3	3.3	2.9	2.2	1.1
TCl_{eff} (mg/L)	2.5	5.4	0.9	2.3	2.9	4.2	-2.0
pH							

Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^{o}C)	23.4	29.7	11.3	3.07	2.8	2.0	1.1
TCl_{eff} (mg/L)	2.2	6.3	0.6	2.6	2.8	3.0	-0.5

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 41. The coefficient of determination R^2 quantifies the strength of the association of the predicted values to the actual values observed. The R^2 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 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.

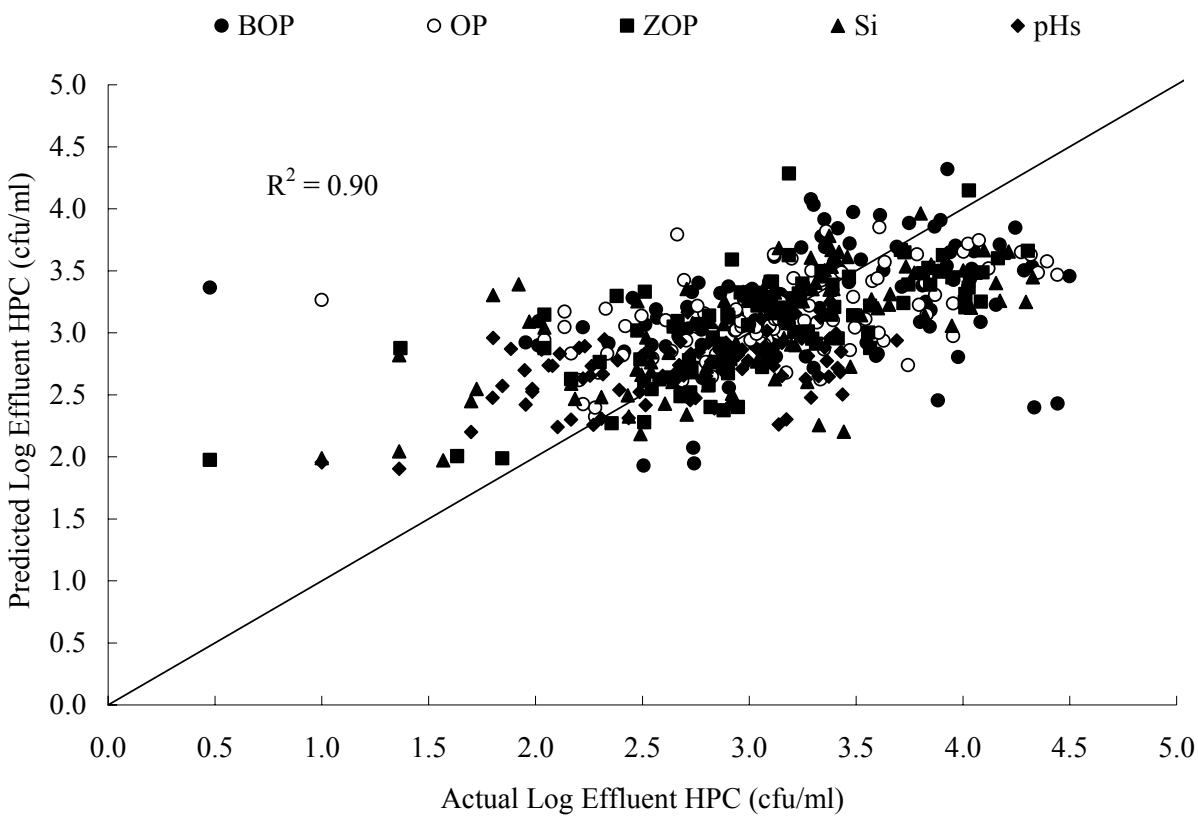

Figure 41 Predicted log effluent HPC vs. actual project log effluent HPC for all PDSs bulk effluent HPC model

Biofilm HPC Modeling

Biofilm Density Growth on Different Doses of Inhibitors

The averages of biofilm density in four phases for different materials are shown in Figure 42 (BOP inhibitor), Figure 43 (OP inhibitor), Figure 44 (ZOP inhibitor), and Figure 45 (Si inhibitor). The graphs do not show any upward or downward trends with dosage for any of the inhibitors except for the silica inhibitor. For the Si inhibitor, biofilm density decreased with the inhibitor dose increases on PVC material.

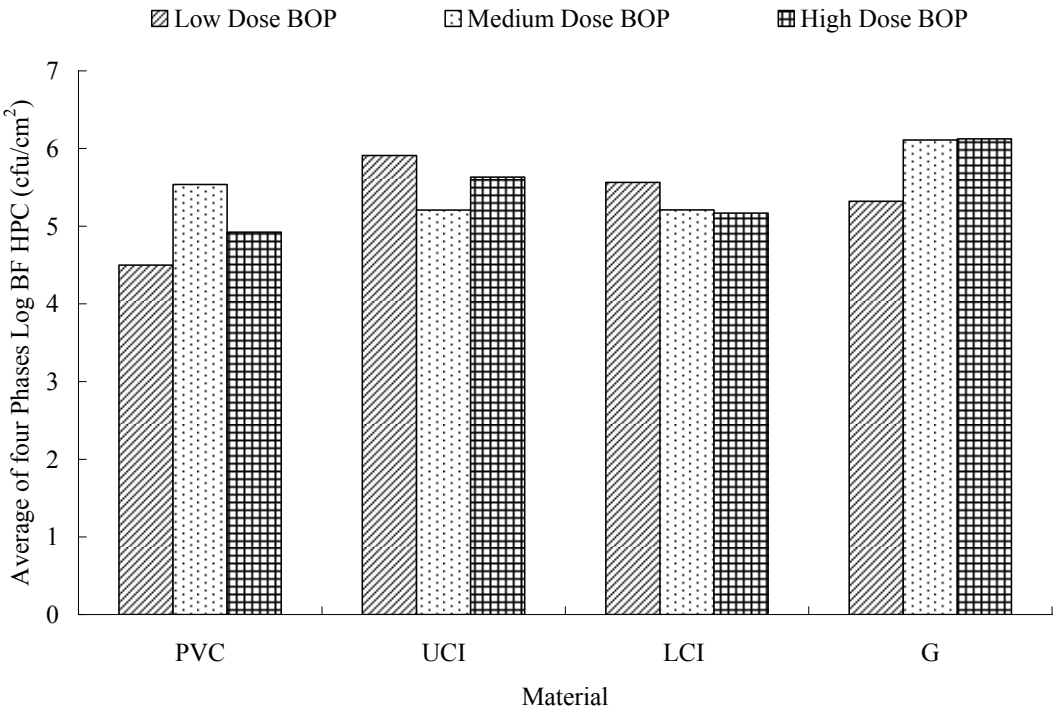

Figure 42 Biofilm density of four kinds of Materials on different doses BOP inhibitor

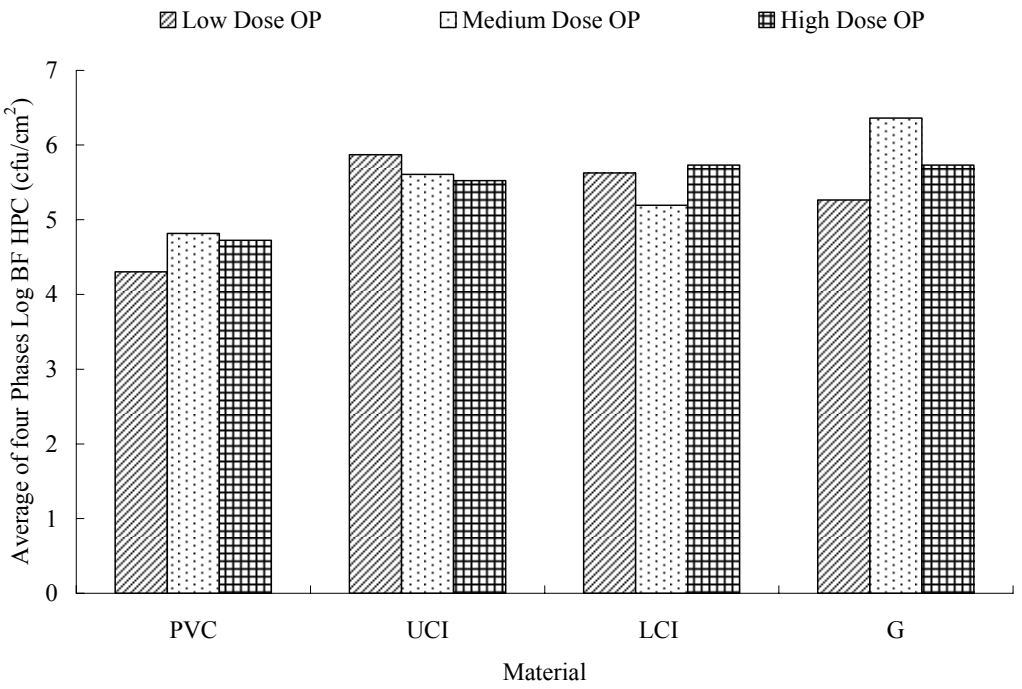

Figure 43 Biofilm density of four kinds of Materials on different doses OP inhibitor

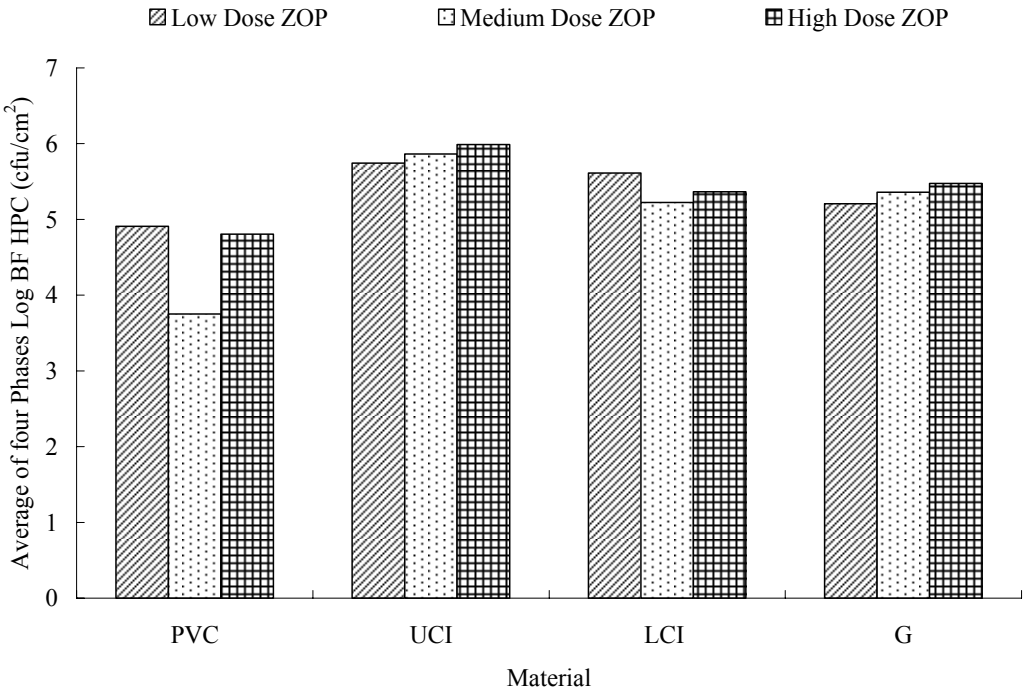

Figure 44 Biofilm density of four kinds of Materials on different doses ZOP inhibitor

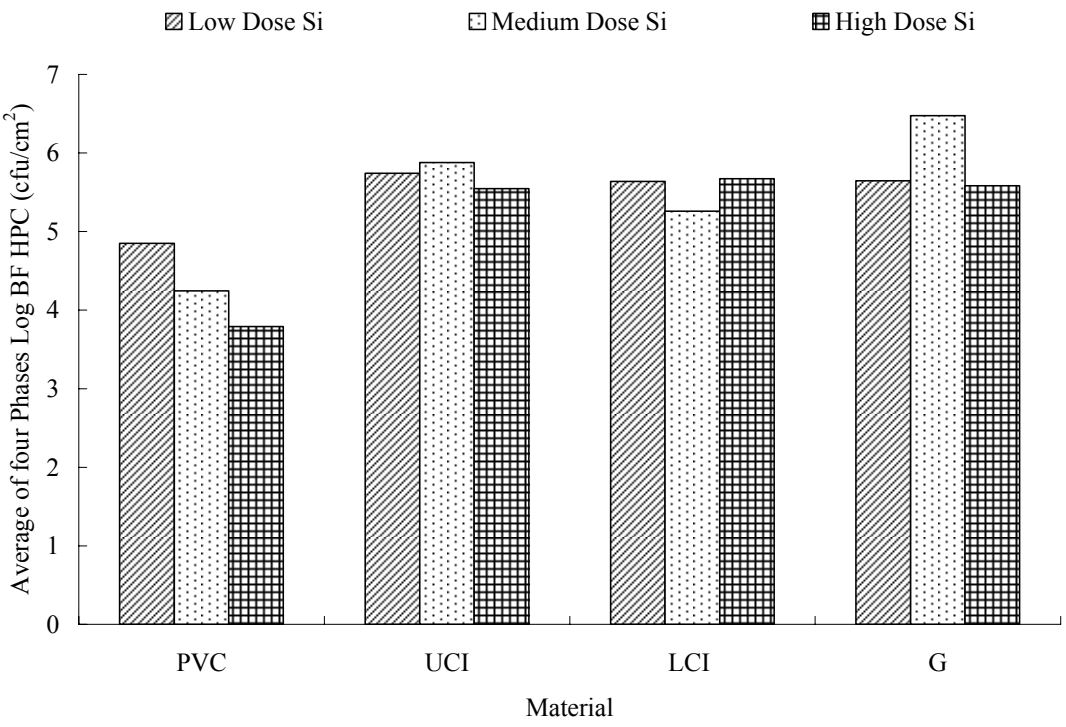

Figure 45 Biofilm density of four kinds of Materials on different doses Si inhibitor

Statistical Comparison of Biofilm densities between different PDSs

Since PDS 13-14 were the control pipes, PDS 1-9 (phosphate based inhibitors) and 10-12 (Si based inhibitors) were compared relative to the control PDSs. The data was also examined for a possible difference between phosphate based inhibitors PDSs and Si based inhibitor PDSs. The Normality Test failed in PDS 1-9 and PDS 10-12 data and the Kruskal-Wallis Test was used instead of instead of multiple t-test and Mann Whitney tests. Phosphate based inhibitor lines and Si based inhibitor lines were significantly different from the control lines but they are not different from each other. PDSs with phosphate inhibitor had 0.34 log greater median values for

biofilm HPC than the control PDSs. PDSs with silica inhibitor had 0.36 log greater median values than the control PDSs.

Biofilm HPC Model for All PDSs

The pooled model for Log BF HPC with data from all the PDSs for all study phases is shown in Equation 2. The coefficient matrix is listed in Table 45. 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 (p value less than 0.050) in all the comparisons and the Kruskal-Wallis Ranks Test was used. The medium of PVC biofilm density was a statistically significant lower number (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) corresponded to the importance of material in determining biofilm density. From both hypotheses tests, 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). 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 for galvanized steel, while the material impact on density was dominant. The same thing can be said for the other unlined metal, UCI.

$$LogBFHPC = a \times Temp^{b} \times TCl_{2-\inf}^{c} \quad \text{Equation 2}$$

Where: a = coefficients for different materials (PVC, LCI, UCI and G)
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)
$TCl_{2-\inf}$ = average bulk water influent total chlorine during incubation (mg/L)

Table 45 Coefficient matrix for All PDSs log BF HPC Model

	a	b	c
PVC	0.30	1.31	-0.94
UCI	2.68	0.25	-0.06
LCI	1.46	0.71	-0.62
G	2.95	0.20	0.0004

A sensitivity analysis on Equation 2 was conducted. PVC showed sensitivity to temperature and residual in the range of 4.2 and 8.4 logs, and LCI 3.2 and 5.9 logs respectively (Table 46). In contrast the unlined metals had approximately 1 log of variation due to temperature and 0.4 logs of variation or less due to residual. This was because most of the predicted BF HPC value came from the much larger coefficient "a" (see Table 45) 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, in the segregated models (PDS 1-9, 10-12, 13-14) the unlined metals showed slightly greater sensitivity to temperature and residual than PVC and LCI (data not shown). It seems to imply that 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 analysis of Equation 2 was much more informative.

Table 46 Sensitivity test for all PDSs for log BF HPC density (cfu/cm^2)

PVC

Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^{o}C)	23.1	29.7	10.4	5.6	4.0	1.4	4.2

TCl_{inf} (mg/L)	4.9	7.0	1.7	2.9	4.0	11.3	-8.4
			UCI				
Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^oC)	23.1	29.7	10.4	5.7	5.4	4.4	1.3
TCl_{inf} (mg/L)	4.9	7.0	1.7	5.3	5.4	5.7	-0.4
			LCI				
Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (°C)	23.1	29.7	10.4	6.1	5.1	2.9	3.2
TCl_{inf} (mg/L)	4.9	7.0	1.7	4.1	5.1	10.0	-5.9
			G				
Parameter	Project Water Parameter Values			Model Predicted HPC Values			Range
	avg	max	min	max	avg	min	(max-min)
Temp (^oC)	23.1	29.7	10.4	5.7	5.5	4.7	1.7
TCl_{inf} (mg/L)	4.9	7.0	1.7	5.5	5.5	5.5	< 0.01

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 46. The coefficient of determination R^2 quantifies the strength of the association of the predicted values to the actual values observed. The R^2 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. 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

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. As with the pooled PDS model for bulk effluent HPC, the low values were over predicted and some of the high values were under predicted.

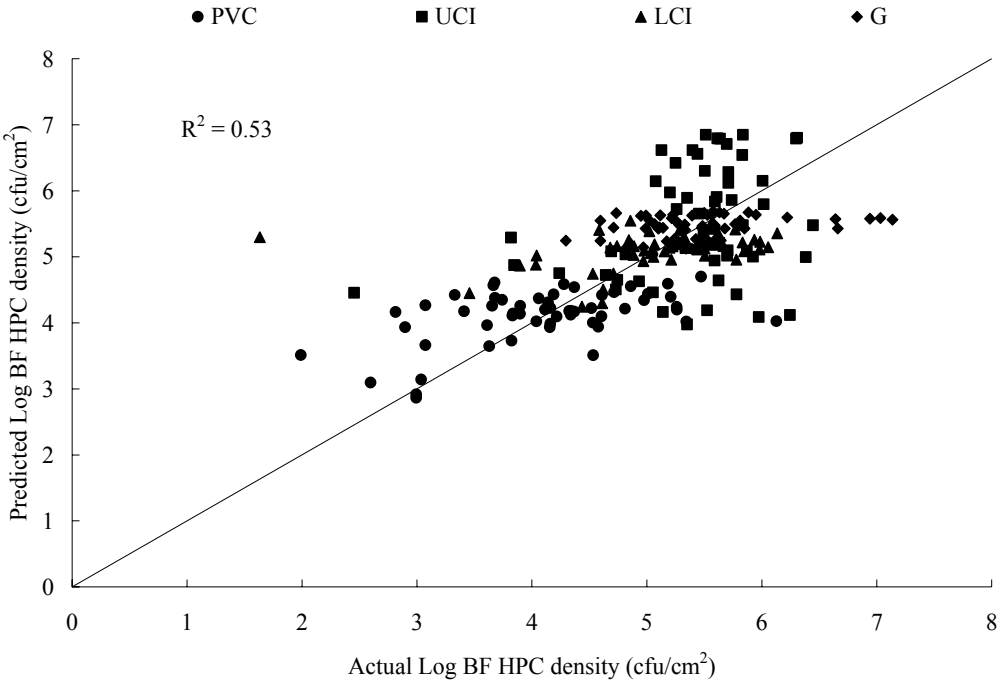

Figure 46 Predicted log BF HPC vs. actual project log BF HPC for all PDSs model

Comparison of Bulk HPC and Biofilm HPC 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 cm^2) than the average density of biofilm cells. The average log biofilm density was 5.67 cfu/cm^2 and the average log effluent bulk HPC was 3.44 cfu/ml. The empirical models of the PDSs for biofilm and bulk water

HPC arrived at solutions without either parameter remaining as an independent variable for the other.

Table 47 shows the sensitivity of BFHPC and Bulk HPC to temperature and residual terms in the corresponding regression models. If we exclude the galvanized material which was almost exclusively sensitive to temperature (according to the model) we see that overall temperature was more dominant for biofilms (a range of 33.1 to 99.7 % versus 27.7 to 71.1 % for bulk HPC), while residual influenced bulk HPC values more (a range of 28.9 to 72.3 % versus25.0 to 66.9 % for biofilms) This is probably because many of the bacteria in the biofilms will be protected from residual due to diffusion limitations as the biofilm gets deeper. Looking more closely at the data we can see that there were also significant differences in the variability associated with temperature versus residual for different material types in the biofilm models. Temperature dominated in both unlined materials (UCI and G). 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 5.89 cfu/cm^2 (5.71 cfu/cm^2 for UCI and 6.02 cfu/cm^2 for G), which was 0.66 log higher than that of PVC and LCI at 5.23 cfu/cm^2 (4.79 cfu/cm^{2}for PVC and 5.45 cfu/cm^2 for LCI), so there were thicker biofilm on the unlined materials than on the non-metallic and lined materials. A possible conclusion is that the sensitivity to residual may decrease as the biofilm density thickness increases. This is probably true but biofilm thickness was not measured, only density as cfu/cm^2.

For the bulk HPC model effluent residual had more impact than temperature in all cases except the pH lines. Looking at the overall impact of temperature and residual on both bulk and biofilm HPC it can be seen that residual had a greater impact than temperature for bulk HPC in all of the inhibitor PDSs, and for all the pipe materials except the unlined metals. In contrast

temperature was apparently dominant for the bulk HPC in the pH PDSs, and for the unlined metal pipe materials with respect to biofilm data.

The last column in Table 47 shows the ranges size relative to the maximum model predicted log BFHPC values. In the biofilm model the impact of temperature and residual on PVC and LCI was very significant. This impact was much smaller for the unlined metals, and for all the bulk HPC data being in general less than 40%. The difficulty is whether or not it is appropriate to assign a mechanistic explanation to this, or to view it instead as an artifact of the mathematical structure of the model obtained through regression. If we take the mechanistic approach a plausible explanation for the biofilm models is that for unlined metals the material effect is the dominant effect, while for LCI and PVC temperature and residual have a much higher impact since the biofilm is not as thick. For the bulk HPC samples the results suggest that HPC values in the PDSs were relatively constant and varied in a fairly narrow range throughout the conditions observed in this study. This was probably because a high but stable residual level was maintained throughout the study, so that more extreme conditions (e.g. low residual or residual depletion) were never encountered. In addition temperatures varied from warm to hot, but truly cold temperatures were never observed since the PDSs were located in Florida. This is probably why the two parameters did not affect the observed values more. Overall when comparing the two parameters it can be noted that residual was more dominant for bulk HPC and for the thinner biofilms of the LCI and PVC materials, while temperature was more important for the unlined metal biofilms.

In conclusion both temperature and residual have impacts on both bulk and biofilm HPC that are quite close in size when it is considered that bulk and biofilm HPC data varies by several orders of magnitude. For the most part temperature and residual account for anywhere from 25 to 75%

of the variability of the model calculated biofilm and bulk HPC values, with the sole exception of the temperature dominated galvanized pipe material. Residual had a somewhat greater impact than temperature for bulk HPC, and for the less dense biofilms on PVC and LCI. Temperature was more significant in the denser (and probably thicker) biofilms of the unlined metals UCI and G, probably because residual could not diffuse through the entire depth of these denser biofilms. Temperature also dominated in the pH PDSs but it is harder to determine a plausible explanation for this result. It is uncertain if this was an artifact due to the small amount of data for these two PDSs, or if in fact residual had a smaller impact on bulk HPC in the pH PDSs.

Table 47 Sensitivity percentages of temperature and residual in biofilm and bulk HPC models

BF HPC Model

	Term	Range	Term Range / Temp Range + TCl Rang	Range/Max
PVC	Temp (^{o}C)	4.18	33.1%	74.7%
	TCl_{inf} (mg/L)	-8.43	66.9%	74.4%
UCI	Temp (^{o}C)	1.32	75.0%	23.1%
	TCl_{inf} (mg/L)	-0.44	25.0%	7.7%
LCI	Temp (^{o}C)	3.19	35.0%	52.7%
	TCl_{inf} (mg/L)	-5.93	65.0%	59.3%
G	Temp (^{o}C)	1.06	99.7%	18.6%
	TCl_{inf} (mg/L)	0.00	0.3%	0.1%

Bulk HPC Model

Term	Range	Term Range / Temp Range + TCl Rang	Range/Max

BOP	Temp (^oC)	1.59	53.7%	43.5%
	TCl_{eff} (mg/L)	-1.37	46.3%	33.0%
OP	Temp (^oC)	0.76	43.0%	23.4%
	TCl_{eff} (mg/L)	-1.01	57.0%	27.3%
ZOP	Temp (^oC)	0.56	27.7%	17.8%
	TCl_{eff} (mg/L)	-1.46	72.3%	37.9%
Si	Temp (^oC)	1.14	36.9%	34.4%
	TCl_{eff} (mg/L)	-1.95	63.1%	46.3%
pH	Temp (^oC)	1.12	71.1%	36.5%
	TCl_{eff} (mg/L)	-0.46	28.9%	15.2%

Bulk HPC was affected consistently by influent residual and temperature, and was most sensitive to residual. Figure 47 shows the predicted bulk HPC using the pooled PDS model with temperature assumed to be constant at the average value for the study. BOP is shown because it had the highest predicted bulk HPC, while the pH_s PDS had the lowest. In Figure 47 at 2.8 log cfu/mL HPC and 0.75 mg/L residual is point A, which is a typical operational condition without inhibitor addition. The increase in HPC following the addition of 0.5 to 2.0 mg/L P-BOP at the same residual concentration is represented by B, which corresponds to 3.8 log, or a 1.0 log HPC increase. Instead it may be more desirable to increase the residual from 0.75 mg/L to 3.8 mg/L in order to maintain the original HPC level. This determination is shown in Figure 47 by the horizontal line from A to C and then tracing a vertical line from C down to the x axis.

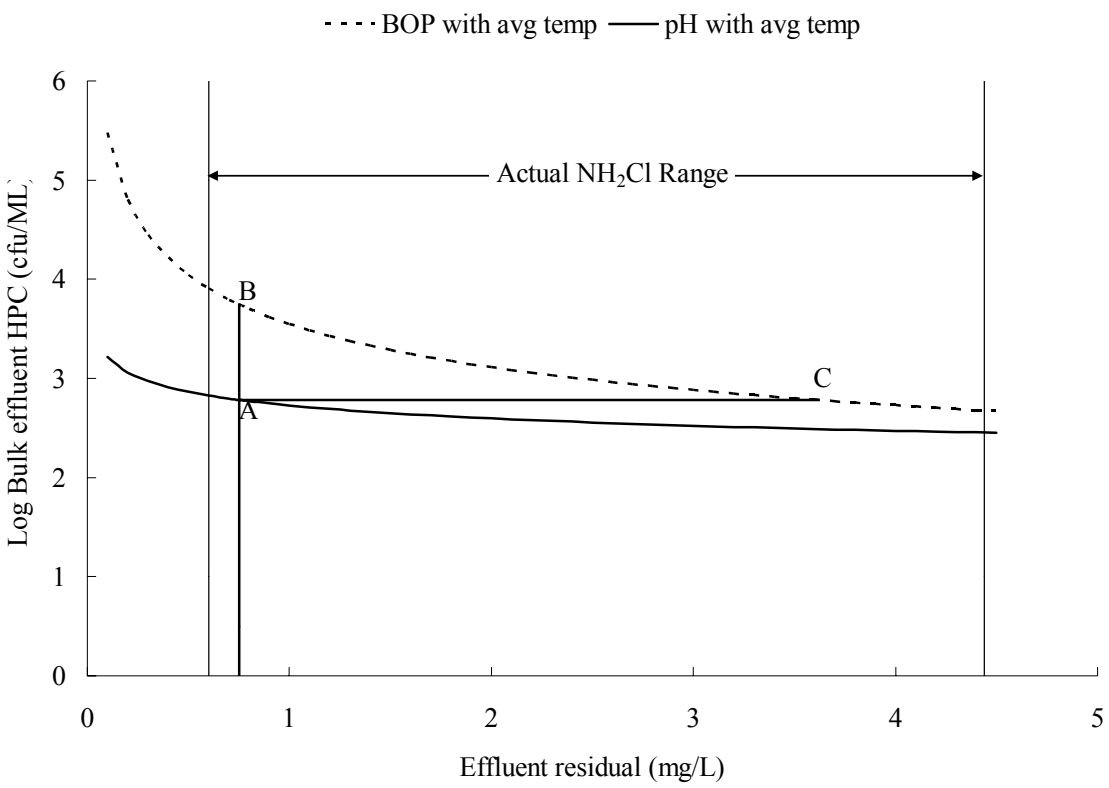

Figure 47 Log bulk effluent HPC versus effluent chloramine residual

BF HPC was affected by pipe material, effluent residual and temperature in addition to the relatively small increase due to inhibitor addition. It was most affected by material type, with PVC biofilm density consistently much lower than the other materials. Unlined metals consistently had the highest biofilm densities. After material, temperature was the dominant parameter affecting biofilm densities for the unlined metals (UCI and G). In contrast, for PVC and LCI, BF HPC had a similar dependence on residual and temperature, with residual being slightly more important than temperature. The lack of residual impact observed for the unlined metals was probably because of diffusion limitations of the chloramines residual when penetrating the denser UCI and G biofilms.

For biofilm HPC the model implied that the effect of material, temperature, and residual were more significant than the increase observed due to inhibitor addition. Unlined metals showed a

small but significant dependence on temperature, but little or no effect due to residual. This difference in behavior of the unlined metals versus PVC and LCI is illustrated in Figure 48 which shows the sensitivity of the PVC and UCI biofilm to residual. The data implies that the less dense PVC and LCI biofilms were sensitive to residual while the denser UCI and G biofilms were not. If this was due to diffusion limitations because of the thickness or density of the biofilm then flushing the biofilms should make them more sensitive to residual as the shear forces should remove some of the upper biofilm. In summary then residual should be a viable control strategy for lined pipe and PVC. For unlined metals flushing, pigging, and other strategies to make the biofilms less thick/dense should be used in conjunction with maintaining a significant residual.

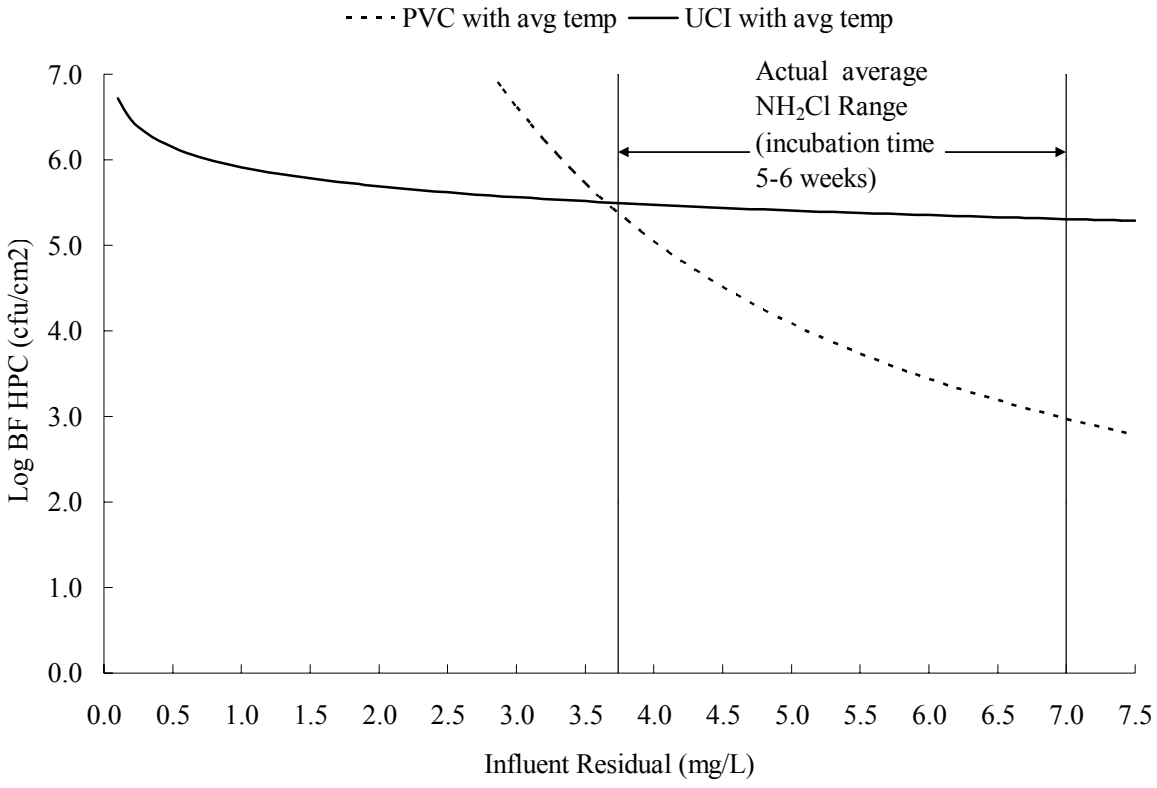

Figure 48 Details of predicted PDS log biofilm HPCs versus chloramine residual at average (23.6 °C) temperature

Conclusion

Inhibitor use increased both bulk and biofilm HPC but raising residual levels was able to compensate for this with the possible exception of biofilms on unlined metal. Unlined metals resulted in high biofilm density under all the conditions of this study. PVC consistently had the lowest biofilm density of all the materials used in this study. Elevated temperatures resulted in higher bulk and biofilm HPCs. Residual levels were the most important management tool for maintaining biostability in both the bulk and biofilm in this study.

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CHAPTER SEVEN: EVALUATION OF THE IMPACTS OF CORROSION INHIBITORS ON BULK AND BIOFILM HETEROTROPHIC BACTERIA

Introduction

Although not regulated it is generally accepted that heterotrophic plate count (HPC) levels in drinking water reflect basic microbial water quality (Rusin *et al.*, 1997). Heterotrophic bacteria include all bacteria that use organic nutrients for growth (Allen, 2004). The US Environmental Protection Agency (USEPA) has suggested that the heterotrophic bacterial counts in drinking water should not exceed 500 colony-forming units (cfu/ml), primarily because of the interference of coliform detection (USEPA, 1989). HPC counts below 500 cfu/mL can also be used a surrogate for disinfection residual requirement under surface water treatment regulations. As the result of bacterial regrowth, higher numbers are often found in the distribution system (Geldreich et al., 1985) and in the water treatment devices mounted at the household tap (Reasoner *et al.*, 1987). Analysis for HPC bacteria in water distribution systems can be helpful in determining changes in water quality both during storage and distribution. Levels of HPC bacteria may also be used to assess microbial growth on materials used in water distribution systems and for measuring bacterial after-growth in distributed water. Growth of bacteria in drinking water during distribution can lead to deterioration of water quality (taste, odor), violation of quality standards, and increased operating costs (Rice *et al.*, 1991; Charnock and KjØnnØ, 2000). Typically, in biologically unstable water, bacterial populations proliferate as a biofilm becomes attached to the pipe wall. In the biofilm, immobilized cells frequently are embedded in an organic polymer matrix of microbial origin (Charackilis and Marshall 1990). In general, the populations of suspended cells in distribution systems are orders of magnitude less than the population of biofilm cells (Camper 1996; Van der Wende, Characklis, and Smith 1989; Characklis 1988). Because suspended cells are

often considered to be introduced to the liquid phase from the biofilm the concentration of suspended cells might best be achieved by minimizing the number of biofilm cells (Ollos, Huck, and Slawson 2001). However, regression analysis suggests low or very low correlation between biofilm and suspended heterotrophic plate count (HPC) numbers (Ollos, Huck, and Slawson 1998). This study investigated the level of heterotrophic bacteria and the relationship between bulk water and biofilm bacteria. Biofilm heterotrophic plate count (HPC of detached and homogenized biofilm) was the parameter being used to quantify biofilm density in this study, and the biofilms are being quantified using four different materials, PVC, lined cast iron (LCI), unlined cast iron (UCI) and galvanized steel (G).

Materials and Methods

Distribution Networks

The experimental system for the project consisted of fourteen pilot distribution system (PDSs) being fed a blend of groundwater, surface water, and RO permeate from desalination. Four corrosion inhibitors were evaluated at three different inhibitor doses each (PDS 1 through 12). The inhibitors were blended ortho and polyphosphates, orthophosphates, silicates and zinc orthophosphate. The PDS systems can be described as follows:

The PDSs were identical and consisted of 4 pipes in series of PVC, lined cast iron, unlined cast iron and galvanized steel pipes.

The fourteen PDSs were divided into four sets of three each set of 3 for each inhibitor with the remaining two PDSs used as controls. Within each set of 3 the dosages were variable. PDS 13 and

14 were control systems with no inhibitor but operated at pHs (saturation with respect to calcium carbonate), and 0.3 pH unit above pHs.

All 14 of the PDSs received the same blend for a three month period. At the end of each three month period the blends for the PDS changed but the corrosion inhibitor and the dose of corrosion inhibitor were held constant.

Seasonal effects were evaluated by repeating the blend used for the first month phase (phases I) for the third 3 month phase (phases III).

The four inhibitors provided a wide spectrum of currently available corrosion control inhibitors. The inhibitor and dose were held constant for one year of investigation. The inhibitor doses are in Table 48.

Table 48 Inhibitor Dosage in Fourteen PDSs

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 SiO2
11	Silica	6 mg/L as SiO2
12	Silica	12 mg/L as SiO2
13	pHs	none
14	pHs+0.3	none

The three different blends used for investigation were similar to the anticipated actual finished water blends from (a) the Regional Surface Water Treatment Facility, (b) the Tmapa Bay Water (TBW) Desalination Facility and (c) the Cypress Creek Groundwater Treatment Facility. The blends used in this study were:

- 62 % Ground Water-27 % Surface Water-11 % Desal Water for Phase I and III
- 62 % Surface Water-27 % Ground Water-11 % Desal Water for Phase II
- 40 % Surface Water-40 % Ground Water-20 % Desal Water for Phase IV

Biofilms were cultivated on coupons located in a flow through cradle in parallel with (and receiving the same influent and inhibitor) the corresponding PDS. Bioassays were conducted on the bulk water from PDSs and coupons in the cradle as shown in Table 49. Pilot distribution system pipe coupons for biological sampling were approximately 3 cm in diameter with a small PVC peg made from PVC welding rod attached to what was the outer surface of the pipe. The material was drilled from aged or pristine pipe and then de-burred to give smooth edges. In each integrated pilot distribution system cradle, there were duplicate aged coupons of each of the four materials. The sequence of the coupons in these cradles from upstream to downstream was PVC, LCI, UCI and finally G coupons. The coupons were placed in this order to avoid transport of corrosion materials downstream to contaminate the less easily corroded materials such as PVC and LCI. Thus the two more easily corroded materials (i.e. UCI and G) were placed in the downstream locations. The aged pipe coupons were obtained from used pipe segments from actual member government (i.e. the communities receiving water from TBW) networks. Biofilms were generated on the coupons that were housed in the cradles and were harvested after 5-6 weeks of growth. The biofilms were assessed using biofilm HPC with units of cuf/cm^2. Duplicate coupons were placed in the same cradles as the sample coupons and tested parallel for quality assurance (QA). Replicates of four randomly picked sample coupons were examined in the laboratory.

Bulk water biological stability was assessed using HPCs. As one of the primary monitoring parameters of microbial water quality, bulk fluid HPCs are analyzed weekly for both influent and effluent of the 14 PDSs, 4 inhibitor tanks, three storage tanks and the blend tank. During each sampling event, six blind duplicate samples were tested with one from the inhibitor tanks, one

from the storage tanks, two from the PDS influents and two from the PDS effluents. At the same time, replicates of four randomly picked PDS samples were examined in the laboratory.

Table 49 Major Biological Parameters during the Study

	Weekly	6 Weeks
Cradle Coupons		
Cast Iron		
BFHPC		X
Galvanized Steel		
BFHPC		X
Lined Cast Iron		
BFHPC		X
PVC		
BFHPC		X
PDS Bulk		
HPC	X	

Water Analyses

HPC Measurement

HPC was measured with spreadplates on R2A agar. all plates were labled with sample number and location. Using a 10-100μL pipet, 0.1 mL sample was pipetted onto surface of predried agar plates. Using a sterile bent glass rob as spreader, inoculums were distributed over surface of the medium by rotating the dish on a turntable. Before spreading the plates, the glass

spreader was dipped into isopropyl alcohol and flamed. The spreader was touched to another sterile agar plate, and then placed in the middle of the sample and gently moved back and forth until the sample was spread across the entire glass rod. A turntable beneath the plate was spin to evenly spread the sample over the entire plate. After completion of the final plate, there was a15 minutes wait and the plates were inverted. Incubation was at 25 degrees Celsius for at least 48 hours, with triplicate plates for each dilution of a sample. The colonies were enumerated after incubation. There were two dilutions for each sample, including the blind duplicates. Four dilutions were used for lab replicate quality assurance (QA) samples.

Biofilm HPC Measurement

Biofilm HPC was measured the same way as bulk HPC except the biofilms were detached from the coupons and homogenized. Coupons colonized by biofilm were rinsed carefully with phosphate buffer solution (PBS) twice prior to detachment. Detachment from the coupon was accomplished with a sterile spatula (sterilized with 70% Ethanol and flamed) into 10 mL of sterile PBS. The sample was homogenized by using a tissue blender (Tissue Tearor™, Biospec products, Inc., Bartlesville, OK, USA) at 5000 rpm for 2 minutes. Between samples the blender top was cleaned in 10% bleach solution for 15 seconds and then in DI water for 15 seconds. The sample was then placed on an R2A agar plate after serial dilution as described in HPC measurement section. Incubation was at 25 degrees Celsius for at least 48 hours, with triplicate plates for each dilution of a sample. The colonies were enumerated after incubation. There were two dilutions for each sample, including the blind duplicates. Four dilutions were used for lab replicate QA samples.

Results and Discussion

Statistical Comparison between Control PDSs and Inhibitor PDSs

The experimental facilities allowed for side-by-side testing of fourteen separate PDS hybrid lines (each with four identical pipe materials) that received the same finished water blend. The individual hybrid PDSs received a different corrosion control strategy consisting of one of four inhibitors at a range of doses, and in two lines pH control without addition of an inhibitor.

Since PDS 13-14 were the control pipes which received no inhibitor, PDS 1-9 (phosphate based inhibitors) and 10-12 (Si based inhibitors) are compared with them. The statistics test by Sigmaplot® confirmed that the effluent HPC data of PDS 13-14 were not normally distributed (α value was 0.10) as shown in Table 50. The biofilm HPC data of PDS 1-9 and PDS 10-12 failed normality test (α value was 0.10) and the Kruskal-Wallis Ranks Test was used instead of instead of multiple t-test and Mann Whitney tests.

Table 50 log effluent HPC (cfu/mL) and log Biofilm density (cfu/cm^2) Biofilm HPC Data Normality Test

Bio Assay	Group Name	P value	Passed Normality Test?
Effluent HPC	PDS 1-9	0.177	YES
	PDS 10-12	>0.200	YES
	PDS 13-14	0.065	NO
BF HPC	PDS 1-9	<0.001	NO
	PDS 10-12	0.078	NO
	PDS 13-14	>0.200	YES

The Kruskal-Wallis Ranks Test showed that the difference of the two groups is greater than would be expected by chance; there was a statistically significant difference between effluent HPC of PDS 1-9 and PDS 13-14 (α value was 0.10) (Table 51). PDSs with phosphate inhibitor had 0.32 log greater median values for effluent HPC than the control PDSs (Table 52). There was also a statistically significant difference in biofilm density between PDS 1-9 and PDS 13-14. The PDSs with phosphate inhibitor had 0.34 log greater median values for biofilm HPC than the control PDSs. The distribution also showed that the lowest 25% of the values in PDS 1-9 were 0.44 log higher than the same value in the control PDSs. The lowest 75% of the observed values in PDS 1-9 were 0.38 log higher than the control PDSs. Thus through the entire distribution of the data the phosphate based inhibitors resulted in a statistically significant increase in biofilm density.

The Kruskal-Wallis Ranks Test confirmed that the differences between PDS 10-12 and PDS 13-14 of effluent HPC and biofilm HPC data were greater than would be expected by chance; there were statistically significant differences (α value was 0.10) between the Si based inhibitor and the control PDSs on both data sets as in Table 51. PDSs with silica inhibitor had 0.30 log greater median values than the control PDSs for effluent HPC and 0.36 log greater for biofilm HPC. The distributions also showed that the lowest 25% of the values in PDS 10-12 were both 0.34 log higher than the control PDSs for effluent HPC and biofilm HPC, and the lowest 75% of the values were 0.28 log and 0.55log higher respectively (Table 52). Again the addition of inhibitors corresponded to a significantly greater effluent HPC and biofilm HPC, and the increase with Si based inhibitors was at about the same level as was observed with phosphate based inhibitors. A possible mechanism to explain the increase in effluent HPC for the P-based inhibitors would be that P is a macro-nutrient which is known to be one of the major limiting nutrients in some full scale distribution systems (Sathasivan *et al.*, 1997; Sathasivan *et al.*, 1998). Supplemental

experiments showed that P17 and Nox (the bacteria used for assimilable organic carbon (AOC) standard methods 9217) growth was simulated by P in distilled water was as well as spiking with the inhibitors. The test confirmed that P was a nutrient but the increase with Si inhibitor may have been the result of another inorganic nutrients or trace organic in the inhibitor.

Table 51 Effluent HPC data statistical comparison (Kruskal-Wallis Test, p =<0.001)

Bio Assay	Comparison	Difference of Ranks	Statistical Critical Value	Significantly Different
Effluent HPC	PDS 1-9 vs PDS 13-14	143.09	48.02	Yes
	PDS 10-12 vs PDS 13-14	115.82	56.22	Yes
	PDS 1-9 vs PDS 10-12	27.21	41.27	No
BF HPC	PDS 1-9 vs PDS 13-14	29.29	26.86	Yes
	PDS 10-12 vs PDS 13-14	34.43	31.50	Yes
	PDS 1-9 vs PDS 10-12	5.13	23.09	No

Table 52 Comparison of Effluent HPC data

Bio Assay	Group	N	Missing	Median	25%	75%
Effluent HPC	PDS 1-9	468	6	3.14	2.76	3.52
	PDS 10-12	156	3	3.12	2.69	3.42
	PDS 13-14	104	0	2.82	2.35	3.15
BF HPC	PDS 1-9	144	0	5.26	4.65	PDS 1-9
	PDS 10-12	48	1	5.28	4.55	PDS 10-12
	PDS 13-14	32	0	4.92	4.21	PDS 13-14

Statistical Comparison between Inhibitors PDSs

The data (Table 51) were also examined for a possible difference between phosphate based inhibitors PDSs and Si based inhibitor PDSs. The Kruskal-Wallis Ranks Test showed that the difference of the two groups was not great enough to reject the possibility that the difference is due to random sampling variability. There was not a statistically significant difference between these two groups at a 90% confidence level. The median value of biofilm HPC for PDS 1-9 was only 0.02 log lower than PDS 10-12. At 25% level, the difference in the median value was 0.10 log and at the 75% level, it was 0.17 log difference. The biofilm density with phosphate-based inhibitors was about the same as Si based inhibitors and there was no statistically significant difference between the biofilm densities observed.

Statistical Comparison between Phosphate Based Inhibitor PDSs

Biofilm HPC data were not sufficient to statistically compare between phosphate based inhibitor PDSs and only effluent HPC were tested here. PDS 1-9 (phosphate based inhibitors) received three different corrosion control chemicals each at three different doses. PDS 1-3 received blended orthophosphate inhibitor, PDS 4-6 received orthophosphate, and PDS 7-9 used zinc orthophosphate. The differences between these phosphate based inhibitors were evaluated using statistical analysis. Statistical tests by Sigmaplot® confirmed that the HPC data of PDS 1-3, PDS 4-6 and PDS 7-9 were normally distributed (i.e. Normality Test Passed, p value was 0.017) and also they had equal variances (Equal Variance Test Passed, p value was 0.611). The Holm-Sidak method was used to compare all three sets of data. The statistical values are shown in

Table 53 and the results of the comparison are shown in Table 54.

Table 53 Log effluent HPC (cfu/mL) for phosphate based inhibitors – PDS 1-9

Group Name	N	Missing	Mean
BOP	156	2	3.25
OP	156	1	3.14
ZOP	156	3	3.06

Table 54 All pairwise multiple comparison of log effluent HPC (cfu/mL) for different phosphate based inhibitors (Holm-Sidak method)

	Difference of Means	t	Unadjusted p Value	Final Critical Level	Significant?
BOP vs. ZOP	0.19	2.76	0.006	0.017	Yes
BOP vs. OP	0.12	1.72	0.087	0.025	No
OP vs. ZOP	0.07	1.06	0.292	0.050	No

The differences in the mean values among the treatment groups are greater than would be expected by chance; there was a statistically significant difference (p value is 0.021). When performing the test, the p values of all comparisons were computed and ordered from smallest to largest. Each p value was then compared to a critical level that depends upon the significance level of the test, the rank of the p value, and the total number of comparisons made. A p value less than the critical level indicates there was a significant difference between the corresponding two groups. From Table 54 the mean effluent HPCs of BOP and ZOP were significantly different with BOP being higher by 0.19 log. The difference between BOP and OP was 0.12 log but this difference could not be shown to be significantly different (p value of 0.087 was greater than the

critical p level at 0.025). In a similar fashion the difference between the OP and ZOP PDSs was not significantly different with a p value equal to 0.292 compared to a critical value of 0.05.

Biofilm and Bulk HPC Comparison in Different Phases

The heterotrophic bacteria on both bulk and biofilm were shown and compared by percentage of total (bulk plus biofilm HPC in the distribution system) in different phases (Table 55). Phase I and Phase III used the same blend ratios of GW, SW and RO waters (62 %, 27 %, and 11 %) with different temperature (average temperature 20.9 and 25.2 °C). The total bacteria number increased 0.38 log in Phase I than in Phase III. However, the percentage of bulk HPC did not increase much (only by 1.2 %). It suggested that the higher temperature will increase the bacteria growth both in bulk and biofilm. Phase II and Phase III had close average temperature (25.9 and 25.2 °C) but different blend ratios of GW, SW and RO waters. As described before, in Phase II, surface water was 62 % and ground water was 27 %. RO water was kept as the same at 11 %.The total bacteria in Phase II increased 0.70 log than in Phase III. Also from Table 55, the percentage of bulk HPC decreased from 17.1 % (Phase III) to 11.6 % (Phase II). The data indicated that when the surface water ratio increased in the blend water, the heterotrophic bacteria in the distribution system increased greatly. Biofilm density was the main reason for this increasing.

Table 55 Biofilm and Bulk HPC Percentage in TBW II Phases

Phase	Average Temperature (°C)	Log Effluent HPC (cfu/mL)	Log BF HPC (cfu/cm^2)	Bulk HPC (%)	Biofilm HPC (%)
Phase I	20.9	9.64	10.29	18.3%	81.7%
Phase II	25.9	9.76	10.64	11.6%	88.4%

Phase III	25.2	9.23	9.91	17.1%	82.9%
Phase IV	20.1	9.13	9.74	19.7%	80.3%

The Relationship of Bulk HPC and Biofilm HPC

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 cm^2) than the average density of biofilm cells. The average biofilm density was 5.67 cfu/cm^2 and the average effluent bulk HPC was 3.44 cfu/ml. The empirical models of the PDSs for biofilm and bulk water HPC arrived at solutions without either parameter functioning as an independent variable for the other. Thus the results of the regression analysis suggested a poor correlation between biofilm and bulk HPC numbers. This poor relationship between the biofilm and bulk HPC can also be seen in Figure 49. The coefficient of determination R^2 is only equal to 0.01. Part of the reason for this may be because effluent HPC was influenced by all 4 pipe materials since the PDSs consisted of sections of each pipe material. The scatter plots for each material were separated in Figure 50, Figure 51, Figure 52, and Figure 53. The highest R square 0.21 were from galvanized steel material and the lowest one was only 0.01 from unlined cast iron.

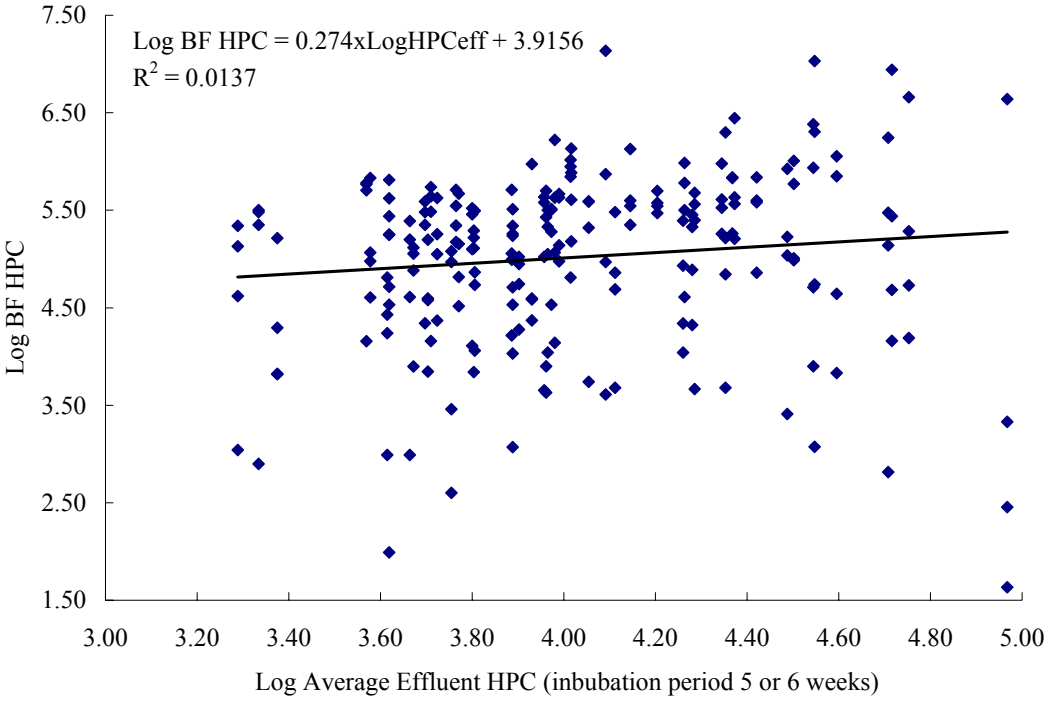

Figure 49 Scatter plot of log BF HPC and log average effluent HPC for all materials

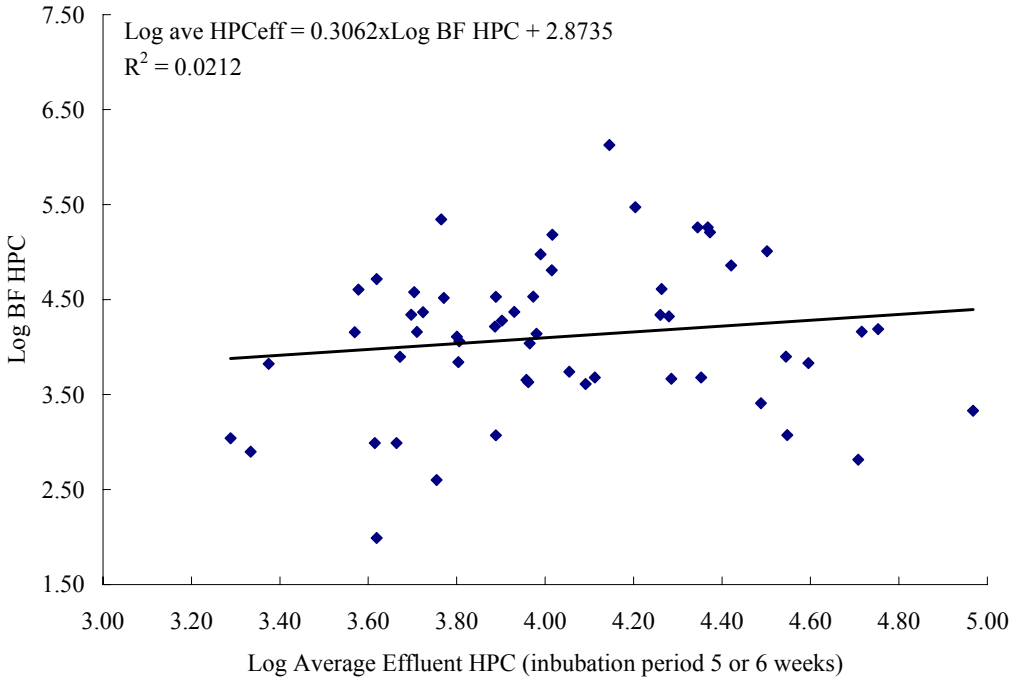

Figure 50 Scatter plot of log BF HPC and log average effluent HPC for PVC

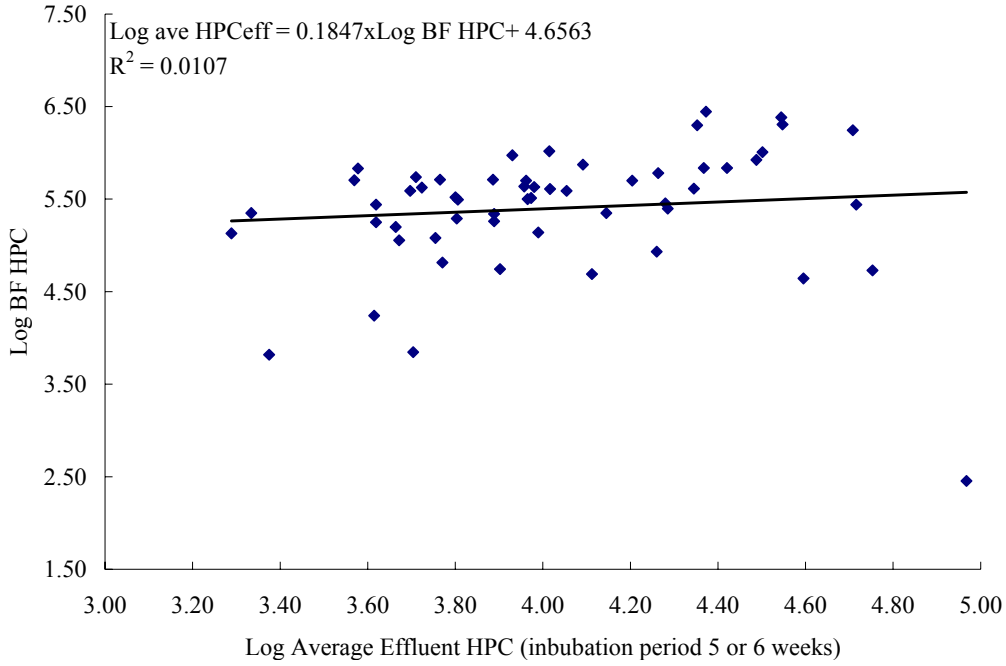

Figure 51 Scatter plot of log BF HPC and log average effluent HPC for UCI

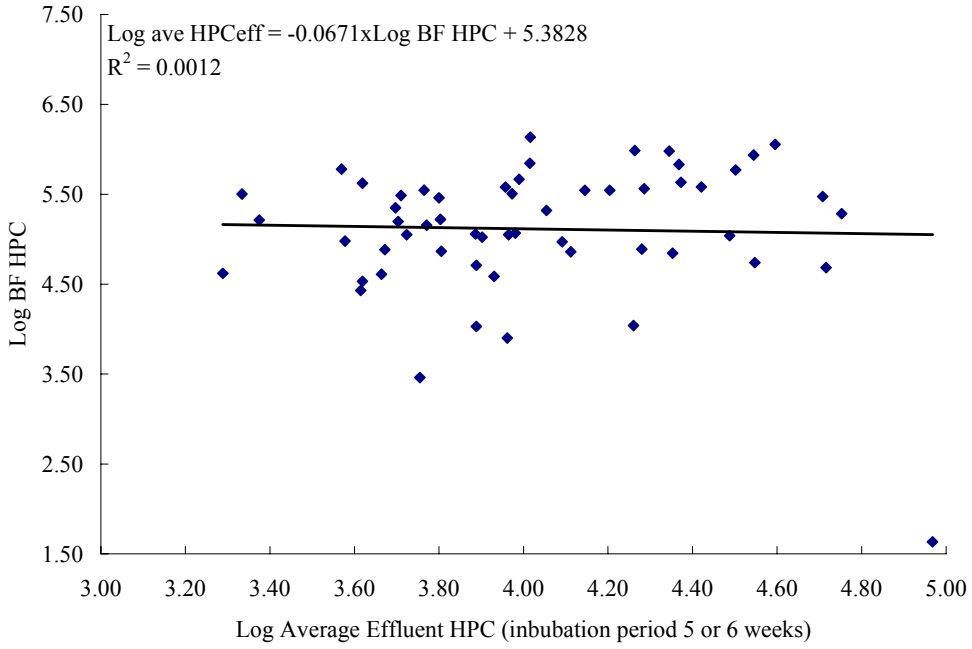

Figure 52 Scatter plot of log BF HPC and log average effluent HPC for LCI

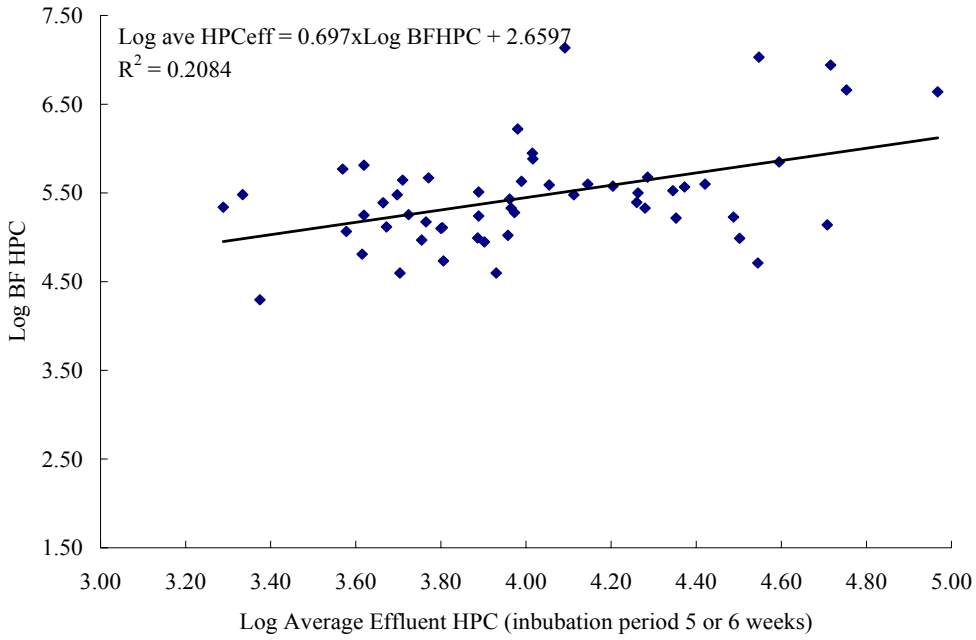

Figure 53 Scatter plot of log BF HPC and log average effluent HPC for G

Conclusion

In this study there was no direct correlation between BFHPC and bulk HPC. This was probably obscured in this study since a significant fraction of sloughed bacteria were likely inactivated by the high residual disinfectant in the bulk water (and thus were not quantified as being in the bulk water).

The results of the study indicate that addition of inhibitors can increase bulk HPC as much as 10 to 15% over an identical system using pH to control corrosion. This is because the addition of inorganic caused both the bulk and the biofilm HPC to proliferate. However if the percentage increases are no greater than 15% it is unlikely that this change would be significant for the bulk water microbial quality. However corrosion inhibitors might result in an increase in monitoring and maintenance requirements, particularly in dead ends, reaches with long HRTs, and possibly

storage facilities. In addition it is unknown what the effect of corrosion inhibitors are on coliform bacteria and opportunistic pathogens.

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CHAPTER EIGHT: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

All inhibitors increased bulk HPC and BFHPC significantly relative to the PDS controls (pH control with no inhibitor added). Phosphate based inhibitors of all types resulted in a bulk effluent HPC value 0.42 log (15.4%) greater than the control PDSs and this difference was statistically significant. The silica inhibitor resulted in 0.31 log (11.0%) greater median values than the control PDSs and this was also statistically significant. The lower bulk HPC level observed with silica inhibitors versus phosphate based inhibitors was significant (at a 93.5% confidence level). BFHPC was increased by 0.34 log (6.5 %) by phosphate based inhibitors, and 0.36 log (6.9%) by silica. Again both increases were statistically significant. However silica inhibitor biofilm density was only 0.02 log lower than P inhibitor PDSs, which was not statistically significant (p value = 0.579). On a percent basis the increase in biofilm density (6.5 to 6.9%) observed with inhibitor addition was significantly smaller than the increase in bulk HPC (11.0 to 15.4%), even though the log increase values were very similar in the biofilm (0.34 to 0.36 log) compared to the bulk HPC (0.31 to 0.42 log). The lower percentage implies the increase in biofilms with inhibitor addition would typically have no practical significance, while the increased bulk HPC levels would be more likely to have practical implications. For example if the increase was 15.4% a utility averaging 434 cfu/mL might increase to an average of 501 cfu/mL (The Surface Water Treatment Rule will except an HPC $\leq$ 500 cfu/mL in lieu of the required disinfectant residual of 0.2 mg/L free chlorine).

Bulk HPC was affected consistently by influent residual and temperature, and was most sensitive to residual. Sensitivity analysis for the bulk HPC model (all PDSs) showed that maintaining a chloramine residual at 2.6 mg/L instead of 1.1 mg/L would decrease bulk HPC by

anywhere from 0.5 to 0.9 log, which is greater than the increase in bulk HPC from the inhibitors at 0.31 to 0.42 log for Si and P based inhibitors respectively. This means that maintaining higher residual levels can counteract any increase due to inhibitors.

BFHPC was affected by pipe material, effluent residual and temperature in addition to the small increase due to inhibitor addition. It was most affected by material type, with PVC biofilm density being consistently much lower than other materials (0.66, 0.92, and 1.22 log lower than LCI, UCI, and G, respectively). Unlined metals consistently had the highest biofilm densities. After material, temperature was the dominant parameter affecting biofilm densities for the unlined metals (UCI and G). In contrast for PVC and LCI temperature and residual had a similar effect with residual being slightly more important than temperature. This was probably because of diffusion limitations of the chloramine residual when penetrating the denser UCI and G biofilms, while the PVC and LCI biofilms were less dense (and probably not as thick), allowing residual to diffuse through more of the depth of the biofilm.

Sensitivity analysis for the BFHPC model (all PDSs) showed that the less dense biofilms of PVC and LCI could be lowered by 1.7 or 1.3 log respectively by a change in residual from 3.2 to 4.7 mg/L (this was the range of our data for these materials, but it is probable that a 1.5 mg/L increase in residual over historical levels would mitigate any additional growth from the inhibitors).

These results imply that for PVC and LCI the additional biofilm resulting from inhibitor addition can easily be managed by maintaining residual. Furthermore a 6.5% and 6.9% increase in biofilm density is probably not a cause for practical concern in any case, and the real factor driving residual maintenance with inhibitor usage would be for the bulk water in areas susceptible to high chlorine demand.

Sensitivity analysis of the BFHPC data for the unlined metals shows that these denser biofilms were far less sensitive to residual levels than biofilms on PVC and LCI. This means that management options for these biofilms are likely to be more limited than those for biofilms on PVC and LCI. Since residual is not likely to be as effective in controlling biofilms on UCI and G other more labor intensive management strategies would need to be used (e.g. flushing, pigging, or looping). Temperature had a significant effect on both biofilm and bulk HPC levels but it is not practical to alter temperature for public drinking water distribution systems so temperature is not a management tool like residual. For many older utilities where many miles of the pipes are unlined metals the removal and replacement of unlined metal pipes is cost prohibitive, in particular where urban development exists above these pipes. However when unlined metal pipe is replaced it makes sense to replace it with PVC or a lined metal pipe. For new additions to existing distribution systems PVC or lined metals can be used. This would have advantages with respect to biostability and with respect to corrosion and non-biological water quality as well (e.g. red water, the lead and copper rule, etc.).

The only effect for inhibitor dosage in the study was for the silica inhibitor which may have resulted in a slight decrease in BFHPC with dosage, but this was not statistically significant. Phosphate inhibitor dosage didn't affect BFHPC values. As a result the significant increase in bulk and biofilm HPCs occurred at the lowest inhibitor dosages, and did not change much as dosage was increased. This observation fits well with the idea that the P and Si based inhibitors carried trace organic or inorganics which stimulated growth. If the low dosages of the inhibitors resulted in an excess of some trace nutrient (e.g. P, N, S, metal cations, etc.) then adding more of the nutrient (i.e. increasing the dosage) when it is already present in excess should have no effect. This hypothesis is consistent with the observed data.

Supplemental experiments measuring the growth response of P17 and NOx (the same organisms used in the AOC method) showed that, at least part of the time, phosphorous, all phosphate based inhibitors, and the silica inhibitor, could stimulate very significant increases in growth. These experiments, though not conducted for a large number of samples and at the end of the study, confirmed that P addition could stimulate growth, and in addition an unknown nutrient in the silica inhibitor (trace organic? inorganic?) could stimulate biological growth at least part of the time for the blend water in Phase IV. Multiple nutrient limitation (rather than biodegradable organic matter or BOM as the sole limiting nutrient) was observed in this study and, judging from the literature, may be the case for a significant fraction of water distribution systems.

Samples in the internal ports between pipe segments showed that bulk HPC changes through the different PDS pipe material segments were much more stable in Phase III (August 22nd. 2006 to November 14th. 2006) and Phase IV (November 28th. 2006 to February 12th. 2007) than in Phase II (May 10th.2006 to August 8th. 2006). This may have been due to the higher temperatures as Phase II was during the summer (the average temperature in Phase II, Phase III and Phase IV were 26.1 °C, 23.9 °C and 21.2 °C respectively).

Coliforms were detected in the biofilm with very low frequency (7 samples of total 252 samples, 2.8 %) and were less than 0.03 % of the biofilm in the highest observation of the study.

AOC did not change significantly as the bulk water passed through the PDSs.

The biological data showed that there was a statistically significant increase in both bulk water and biofilm bacterial densities (measured as HPC) with the addition of any of the four inhibitors.

Increases in biofilm density were about the same absolute level as increases in bulk HPC, with 0.34 log for P based inhibitors, and 0.36 log for Si inhibitors. However because biofilm

densities are much higher than bulk densities this represents a much smaller percent increase in the biofilm of 6.5% and 6.9% for the P and Si based inhibitors respectively.

Regression analysis of the biofilm data yielded a model showing that PVC and LCI biofilms, which were less dense than those of the unlined metals, could potentially be managed by increasing and/or maintaining residual when corrosion inhibitors or other chemical feeds are added during treatment. This also implies that the management options for biofilms on unlined metals are likely to be more limited than those for biofilms on PVC and LCI since residual is a relatively inexpensive and non-labor intensive solution.

Temperature had a significant effect on both biofilm and bulk HPC levels but it is not practical to alter temperature for public drinking water distribution systems so temperature is not a management tool like residual.

In this study there was no direct correlation between BFHPC and bulk HPC. This was probably obscured in this study since a significant fraction of sloughed bacteria were likely inactivated by the high residual disinfectant in the bulk water (and thus were not quantified as being in the bulk water).

The results of the study indicate that addition of inhibitors can increase bulk HPC as much as 10 to 15% over an identical system using pH to control corrosion. This is because the addition of inorganics caused both the bulk and the biofilm HPC to proliferate. However if the percentage increases are no greater than 15% it is unlikely that this change would be significant for the bulk water microbial quality. However corrosion inhibitors might result in an increase in monitoring and maintenance requirements, particularly in dead ends, reaches with long HRTs, and possibly storage facilities. In addition it is unknown what the effect of corrosion inhibitors are on coliform bacteria and opportunistic pathogens.

This study, like the one at Cypress Creek which preceded it, and an AWWARF grant in the 90s which monitored full scale distribution systems in Central and Southern Florida have all resulted in one consistent conclusions: the importance of residual maintenance in distribution systems with water quality varying over the range typical of North America.

An overview of all the biological data gathered indicates that it cannot be taken for granted that a chemical stream containing no organics will not stimulate growth. Acetate did stimulate growth, and so did the addition of phosphate salts, P based inhibitors, and Si based inhibitors. Phosphorus was identified as one of the nutrients stimulating growth, and there was also an unidentified nutrient in the Si based inhibitor.

Since corrosion inhibitors stimulated growth, it becomes important to know how they affect specific populations that have possible regulatory or health impacts. Research is needed to determine how coliform and opportunistic pathogens are affected by the additional nutrients in corrosion inhibitors (or any other inorganic chemical leaving the plant in the finished water). Changes in the pipe surfaces caused by the use of corrosion inhibitors also might favor one set of organisms over another.

The PDS data and the supplemental AOC experiments imply that for some waters carbon may not be the only limiting nutrient. As a consequence if AOC is being used to measure biostability then an inorganic growth control for any chemicals that are going to be added during treatment (as well as inorganic growth controls spiked with a phosphate salt), should be run in parallel with the acetate carbon growth control specified in Standard Methods. Trace organic impurities in the chemical feed could be quantified by NPDOC at high levels, or with AOC at low levels (if there is nothing inhibitory in the chemicals, most of which would need to be diluted to

run an AOC test. Also more than one nutrient may be limiting at the same time if our definition of a limiting nutrient is that, when added by itself, growth is greater than in a parallel control..

If we include autotrophic bacteria in our assessment of biostability, then ammonia and biodegradable organic nitrogen become additional limiting nutrients. This would be most relevant for systems using chloramines or in source waters with significant ammonia or other N species. However we cannot assume that inorganic compounds do not also fuel heterotrophic growth, even in the absence of significant autotrophic activity such as in this study. The number and identities of the limiting nutrient(s) is probably very sourcing water/plant specific.

Lined metal or PVC pipe should, ideally, replace unlined metal pipe whenever possible.

Recommendations for Future Research

The TBWII study that provided the data for this dissertation has several limitations. In particular the data was obtained for a range of temperatures and residual levels that do not cover all distribution systems.

TBW II took place at the Cypress Creek wellfield north of Tampa, Florida (close to Land 'O Lakes, Florida). The temperature range during the one-year project was from 10.4 to 29.7 °C. The biofilm and bulk HPC models were developed based on this temperature range. It is not reasonable to extrapolate these models outside this temperature range. Further research in places with cold temperatures, e.g. New York or Michigan would be necessary to develop new models or an extension of the existing model to cover a wider range of temperature.

Also in this project chloramines were used as the primary and secondary disinfectant. Chloramines can lead to nitrification under high temperature. In TBW II, nitrification happened during May, 2007. Two weeks of free chlorine burn were used to solve this problem. As a result

the present data set does not contain data under nitrifying conditions, or with a low chloramine residual. The range of influent residuals in the study was 1.7 mg/L to 7.0 mg/L. The impact of corrosion inhibitors on biostability when residuals levels drop below 1 mg/L is unknown, and the current model should not be extrapolated to low residual levels outside the range of the study. In addition the current model cannot be applied to systems using free chlorine as their secondary disinfectant and this also would be an avenue for future research.

Another area that warrants further investigation is the growth response for inorganics (whether with P17 and NOx or indigenous organisms). For example the nutrient provided in the Silica inhibitor was never identified. The inhibitor could be analyzed for trace organics, or for inorganic nutrients (P, N, S, etc.). It would also be important to evaluate other commonly used chemical feeds for organic and inorganic nutrients, and to determine from AOC or other growth responses whether or not a given distribution system matrix was inhibitory or not carbon limited. Potential inorganic nutrients that might affect biostability include P, N, and S forms, but also trace nutrients such as metal cations needed for enzymatic catalysis (e.g. Mg, Co, etc.). Screening can be done by injecting a mixture of inorganics and comparing the growth response to a parallel control. These additional experiments can, by process of elimination, determine which inorganics are limiting for a specific finished water and which will stimulate microbial activity.

It is unknown what the effect of corrosion inhibitors are on coliform bacteria and opportunistic pathogens based on the data in this study. Future research could focus on the corrosion inhibitors influence on coliform bacteria and opportunistic pathogens, or their impact on metabolic activities as well. It seems likely that inhibitors might influence population dynamics and composition as well as the observed impact on bulk and biofilm quantity observed in this study.

APPENDIX A: BIOLOGICAL DATA

Appendix A-1 PDS Influent HPC from January 30 to May 2, 2006

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
01/31/06	I	220	730	500	1250	573	2707	1227	200	147	313	347	23	560	63
02/08/06	I	467	260	367	567	353	1153		350	530	830	50	153	90	307
02/13/06	I		103	90	190	190	167	3	70	43	23	37	10	10	23
02/20/06	I	553	320	547	507	920	910	650	417	523	543	633	53	630	167
02/27/06	I	877	1057	597	147	1177	433	690	323	380	347		147	530	327
03/06/06	I	3927	2003	613	1083	1300	3127	913	797	817	347	1183	420	687	273
03/13/06	I	4000	9493	3547	3980	1307		5127		6907	2427	2880	2173	2547	1210
03/20/06	I	1753	3333	2000	1907	1403	1727	1267	463	2500	1170	1320	3343	533	183
03/27/06	I	3	540	733	197	160	670	227	323	760	510	2120	2773	203	50
04/03/06	I	7633	27733	21600	8987	444	407	3600	2600	2053	1513	1573	2570	242	89
04/10/06	I	14267	12100	1947	9000	2077	870	1260	300	10200	63	0	1520	323	115
04/17/06	I	580	21067	19333	6233	870	2457	5267	443	3067	3733	21233	14900	663	470
04/24/06	I	10800	31533	11000	24733	6667	13167	20133	12133	7767	16333	12533	14267	2380	1097
05/02/06	I	8400	17600	9233	22467	10600	3767	14600	1613	8933	15533	7100	10900	1423	1880

Appendix A-2 PDS Influent HPC from May 9 to August 16, 2006

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
05/10/06	II	9033	14867	11267	27667	7400	2173	12367	580	10267	11467	10033	6633	1300	2590
05/17/06	II	(a)													
05/24/06	II	6467	8733	2493	1380	1193	2540	7033	1260	10633	2393	1980	657	517	313
05/31/06	II	12667	24000	22033	20767	18733	10567	24300	14667	16800	6167	6733	6033	4900	1620
06/07/06	II	7367	5600	7867	12933	4200	8467	5333	1093	8067	6333	4567	19733	1127	717
06/15/06	II	3600	8867	6133	6767	1810	3500	3300	933	5700	3533	1987	6467	425	740
06/21/06	II	7000	7067	6300	9933	3767	3000	2180	993	1280	2253	1780	4500	1530	730
06/28/06	II	4133	6067	5700	10067	4300	11867	2320	1383	11867	5500	1100	1947	1127	970
07/05/06	II	2263	8467	3067	6033	1507	1487	1533	1773	6500	2367	5400	5933	1890	1187
07/12/06	II	2180	4033	5300	6100	2293	4067	2800	2440	6133	2193	3700	11800	1640	1107
07/19/06	II	2180	4100	2600	3500	1730	3733	10667	1427	5567	1940	4000	8867	1833	1245
07/26/06	II	1827	1840	2080	947	1957	460	5600	1250	2453	1367	4167	2833	2400	160
08/02/06	II	1313	2247	2950	1760	2333	633	1530	1260	2933	2480	2373	1320	2160	645
08/08/06	II	1073	2067	440	857	1240	495	1920	585	1173	937	2507	2720	700	450
08/16/06	II	(a)													

a Free chlorine burn this week; no samples collected.

Appendix A-3 PDS Influent HPC from August 20 to November 20, 2006

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
08/22/06	III	380	700	1580	380	1540	370	327	1027	1013	423	83	180	460	17
08/29/06	III	423	960	330	485	2293	573	310	780	207	297	233	197	57	377
09/05/06	III	887	563	2120	980	1197	1073	653	777	380	333	467	233	67	50
09/12/06	III	377	1030	1213	890	690	753	670	2167	500	0	433	233	267	100
09/19/06	III	210	510	2300	740	NC	NC	680	2833	390	250	940	330	220	NC
09/26/06	III	1033	653	767	733	3067	90	27	400	107	133	313	230	833	921
10/03/06	III	183	887	133	120	158	450	77	173	113	170	363	7	67	127
10/10/06	III	133	1240	130	23	104	2700	140	173	117	37	240	887	17	73
10/17/06	III	1085	2213	105	520	352	203	190	147	205	50	290	53	23	100
10/24/06	III	263	423	160	613	213	120	317	973	290	80	380	230	27	370
10/31/06	III	15	167	167	780	400	150	323	1020	215	73	160	110	80	173
11/06/06	III	327	385	243	180	150	180	343	285	163	197	117	77	107	40
11/14/06	III	460	365	643	363	233	103	77	1080	1107	533	140	220	47	203
11/20/06	III	(a)													

a Free chlorine burn this week; no samples collected.

Appendix A-4 PDS Influent HPC from November 28, 2006 to February 12, 2007.

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
11/28/06	IV	640	1223	70	1187	550	80	67	253	370	600	247	140	180	53
12/05/06	IV	67	40	800	907	807	200	507	100	157	277	250	457	1253	90
12/12/06	IV	140	200	400	1847	1147	830	2617	2487	2107	1980	633	2367	3033	2640
12/18/06	IV	550	290	270	553	63	157	7	200	3	77	587	330	50	160
12/25/06	IV	933	1197	2747	1840	27	7	1907	843	2143	1233	13	2053	23	747
12/31/06	IV	1013	763	1507	293	290	70	1467	7	3	2267	257	457	227	1660
01/09/07	IV	357	2867	3573	733	3407	2200	267	1533	53	1697	1027	807	23	NC
01/16/07	IV	6387	110	1667	67	80	103	5133	453	4460	143	80	193	5867	403
01/23/07	IV	93	1733	67	277	843	547	97	160	57	177	1460	1537	733	50
01/30/07	IV	130	437	173	167	177	43	390	187	247	50	160	83	103	107
02/06/07	IV	190	313	633	920	1467	87	510	467	297	77	70	980	60	1220
02/12/07	IV	197	560	183	317	393	517	620	593	530	540	370	553	150	457

Appendix A-5 Summary of Phase I and II Influent HPC.

	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH
Phase I Summary														
Average	411	9134	5151	5803	2003	2428	4228	1541	3188	3120	3924	3804	773	447
Minimum	3	103	90	147	160	167	3	70	43	23	0	10	10	23
Maximum	142	3153	2160	2473	1060	1316	2013	1213	1020	1633	2123	1490	2547	1880
Std Dev	470	1110	7325	8173	2962	3425	6189	3258	3632	5525	6306	5368	795	554
Count	14	14	14	14	14	14	14	14	14	14	14	14	14	14
Phase II Summary														
Average	470	7535	6018	8362	4036	4076	6222	2280	6875	3764	3871	6111	1658	959
Minimum	107	1840	440	857	1193	460	1530	580	1173	937	1100	657	425	160
Maximum	126	2400	2203	2766	1873	1186	2430	1466	1680	1146	1003	1973	4900	2590
Std Dev	354	6120	5605	8130	4746	3809	6458	3753	4593	2916	2456	5171	1149	634
Count	13	13	13	13	13	13	13	13	13	13	13	13	13	13

Appendix A-6 Summary of Phase III and IV Influent HPC

	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH
Phase III Summary														
Average	444	777	761	524	866	564	318	910	370	198	320	230	175	213
Minimum	15	167	105	23	104	90	27	147	107	0	83	7	17	17
Maximum	108	2213	2300	980	3067	2700	680	2833	1107	533	940	887	833	921
Std Dev	343	528	790	301	971	738	227	801	329	162	223	217	235	253
Count	13	13	13	13	12	12	13	13	13	13	13	13	13	12
Phase IV Summary														
Average	891	811	1008	759	771	403	1132	607	869	760	429	830	975	690
Minimum	67	40	67	67	27	7	7	7	3	50	13	83	23	50
Maximum	638	2867	3573	1847	3407	2200	5133	2487	4460	2267	1460	2367	5867	2640
Std Dev	176	830	1149	611	948	621	1500	725	1364	816	435	764	1769	834
Count	12	12	12	12	12	12	12	12	12	12	12	12	12	11

Appendix A-7 PDS Effluent HPC from January 30 to May 2, 2006

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
01/31/06	I	220	730	500	1250	573	2707	1227	200	147	313	347	23	560	63
02/08/06	I	467	260	367	567	353	1153		350	530	830	50	153	90	307
02/13/06	I		103	90	190	190	167	3	70	43	23	37	10	10	23
02/20/06	I	553	320	547	507	920	910	650	417	523	543	633	53	630	167
02/27/06	I	877	1057	597	147	1177	433	690	323	380	347		147	530	327
03/06/06	I	3927	2003	613	1083	1300	3127	913	797	817	347	1183	420	687	273
03/13/06	I	4000	9493	3547	3980	1307		5127		6907	2427	2880	2173	2547	1210
03/20/06	I	1753	3333	2000	1907	1403	1727	1267	463	2500	1170	1320	3343	533	183
03/27/06	I	3	540	733	197	160	670	227	323	760	510	2120	2773	203	50
04/03/06	I	7633	27733	21600	8987	444	407	3600	2600	2053	1513	1573	2570	242	89
04/10/06	I	14267	12100	1947	9000	2077	870	1260	300	10200	63	0	1520	323	115
04/17/06	I	580	21067	19333	6233	870	2457	5267	443	3067	3733	21233	14900	663	470
04/24/06	I	10800	31533	11000	24733	6667	13167	20133	12133	7767	16333	12533	14267	2380	1097
05/02/06	I	8400	17600	9233	22467	10600	3767	14600	1613	8933	15533	7100	10900	1423	1880

Appendix A-8 PDS Effluent HPC from May 9 to August 16, 2006

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
05/10/06	II	9033	14867	11267	27667	7400	2173	12367	580	10267	11467	10033	6633	1300	2590
05/16/06	II	(a)													
05/24/06	II	6467	8733	2493	1380	1193	2540	7033	1260	10633	2393	1980	657	517	313
05/31/06	II	12667	24000	22033	20767	18733	10567	24300	14667	16800	6167	6733	6033	4900	1620
06/07/06	II	7367	5600	7867	12933	4200	8467	5333	1093	8067	6333	4567	19733	1127	717
06/15/06	II	3600	8867	6133	6767	1810	3500	3300	933	5700	3533	1987	6467	425	740
06/21/06	II	7000	7067	6300	9933	3767	3000	2180	993	1280	2253	1780	4500	1530	730
06/28/06	II	4133	6067	5700	10067	4300	11867	2320	1383	11867	5500	1100	1947	1127	970
07/05/06	II	2263	8467	3067	6033	1507	1487	1533	1773	6500	2367	5400	5933	1890	1187
07/12/06	II	2180	4033	5300	6100	2293	4067	2800	2440	6133	2193	3700	11800	1640	1107
07/19/06	II	2180	4100	2600	3500	1730	3733	10667	1427	5567	1940	4000	8867	1833	1245
07/26/06	II	1827	1840	2080	947	1957	460	5600	1250	2453	1367	4167	2833	2400	160
08/02/06	II	1313	2247	2950	1760	2333	633	1530	1260	2933	2480	2373	1320	2160	645
08/08/06	II	1073	2067	440	857	1240	495	1920	585	1173	937	2507	2720	700	450
08/16/06	II	(a)													

a Free chlorine burn this week; no samples collected.

Appendix A-9 PDS Effluent HPC from August 20 to November 20, 2006

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
08/22/06	III	1240	2627	2933	2467	1187	1040	1893	380	847	767	827	367	130	107
08/29/06	III	2453	5173	7653	2467	480	1680	2420	1460	1880	867	753	330	2253	827
09/05/06	III	1647	3147	2400	4253	1073	567	1430	1033	893	625	443	1193	2690	497
09/12/06	III	1067	1587	6700	5507	960	757	830	1613	3683	1473	603	1453	1485	170
09/19/06	III	340	2573	3267	4040	1000	137	1280	1320	205	1720	NC	1067	210	887
09/26/06	III	513	1033	1800	2533	1757	1200	1000	c.	960	83	765	93	515	1760
10/03/06	III	1480	2220	3833	2400	213	137	260	553	3667	2720	2553	723	477	927
10/10/06	III	947	285	1000	440	2103	1117	325	240	1133	2627	1375	510	63	580
10/17/06	III	1320	1200	763	1440	1373	263	330	467	1900	1393	893	960	120	987
10/24/06	III	1293	2140	2320	3653	2373	2027	2453	1707	10267	2040	2433	1160	1300	2307
10/31/06	III	253	850	600	1133	700	617	413	1047	2087	617	900	357	310	457
11/06/06	III	1387	1533	1880	1133	740	425	310	1260	727	1087	2973	1320	427	393
11/14/06	III	1093	327	620	867	1013	667	447	1840	630	77	343	217	247	97
11/20/06	III	(a)													

a Free chlorine burn this week; no samples collected.

Appendix A-10 PDS Effluent HPC from November 28, 2006 to February 12, 2007.

Date	Phase	PDS 1 cfu/ml	PDS 2 cfu/ml	PDS 3 cfu/ml	PDS 4 cfu/ml	PDS 5 cfu/ml	PDS 6 cfu/ml	PDS 7 cfu/ml	PDS 8 cfu/ml	PDS 9 cfu/ml	PDS 10 cfu/ml	PDS 11 cfu/ml	PDS 12 cfu/ml	PDS 13 cfu/ml	PDS 14 cfu/ml
11/28/06	IV	350	167	473	257	217	313	307	117	177	450	130	573	70	97
12/05/06	IV	407	370	517	660	33	133	1733	800	110	300	937	1643	2667	1220
12/12/06	IV	2500	2907	3360	5533	627	2960	1867	213	3387	2933	2493	1213	1947	2733
12/18/06	IV	1843	1333	600	1160	560	713	3680	530	1150	487	497	203	493	2360
12/25/06	IV	1080	2560	327	1187	2240	110	583	600	273	587	2040	2267	180	1840
12/31/06	IV	103	1773	327	420	230	457	790	987	23	110	297	1867	927	750
01/09/07	IV	30	2893	3060	10	1613	1580	2373	413	1080	113	33	5180	77	780
01/16/07	IV	800	4240	4900	3173	4120	2667	1627	1973	110	760	760	437	447	2107
01/23/07	IV	1030	563	510	1360	1320	3067	1587	303	323	493	267	1527	1423	207
01/30/07	IV	1193	NC	1403	463	1573	1380	887	477	660	270	273	310	127	187
02/06/07	IV	333	2787	450	2153	487	1493	907	493	203	2387	707	1443	1373	1493
02/12/07	IV	803	603	473	587	517	547	677	537	647	703	640	403	147	823

Appendix A-11 Summary of Phase I and II Effluent HPC.

	PDS 1	PDS 2	PDS 3	PDS 4	PDS 5	PDS 6	PDS 7	PDS 8	PDS 9	PDS 10	PDS 11	PDS 12	PDS 13	PDS 14
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
Phase I														
Average	5151	5803	2003	2428	4228	1541	3188	3120	3924	3804	773	447	146	164
Minimum	90	147	160	167	3	70	43	23	0	10	10	23	119	157
Maximum	21600	24733	10600	13167	20133	12133	10200	16333	21233	14900	2547	1880	168	169
Std Dev	7325	8173	2962	3425	6189	3258	3632	5525	6306	5368	795	554	16.6	3.9
Count	14	14	14	14	14	14	14	14	14	14	14	14	14	14
Phase II														
Average	6018	8362	4036	4076	6222	2280	6875	3764	3871	6111	1658	959	92	106
Minimum	440	857	1193	460	1530	580	1173	937	1100	657	425	160	84	98
Maximum	22033	27667	18733	11867	24300	14667	16800	11467	10033	19733	4900	2590	99	114
Std Dev	5605	8130	4746	3809	6458	3753	4593	2916	2456	5171	1149	634	4.7	4.6
Count	13	13	13	13	13	13	13	13	13	13	13	13	9	10

Appendix A-12 Summary of Phase III and IV Effluent HPC.

	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS 9 pH	PDS pH	PDS pH	PDS pH	PDS pH	PDS pH
Phase III														
Average	1156	1900	2752	2487	1152	818	1030	1077	2221	1238	1239	750	787	769
Minimum	253	285	600	440	213	137	260	240	205	77	343	93	63	97
Maximum	2453	5173	7653	5507	2373	2027	2453	1840	10267	2720	2973	1453	2690	2307
Std Dev	583	1325	2222	1517	619	578	805	551	2653	862	899	464	870	648
Count	13	13	13	13	13	13	13	12	13	13	12	13	13	13
Phase IV														
Average	873	1836	1367	1414	1128	1285	1418	620	679	799	756	1422	823	1216
Minimum	30	167	327	10	33	110	307	117	23	110	33	203	70	97
Maximum	2500	4240	4900	5533	4120	3067	3680	1973	3387	2933	2493	5180	2667	2733
Std Dev	730	1334	1537	1572	1162	1097	945	487	933	900	762	1368	856	892
Count	12	11	12	12	12	12	12	12	12	12	12	12	12	12

Appendix A-13 PDS BF HPC Density of Different Material Coupons from January 30 to May 1, 2006

Date	Phase	Material	PDS 1 cfu/cm^2	PDS 2 cfu/cm^2	PDS 3 cfu/cm^2	PDS 4 cfu/cm^2	PDS 5 cfu/cm^2	PDS 6 cfu/cm^2	PDS 7 cfu/cm^2	PDS 8 cfu/cm^2	PDS 9 cfu/cm^2	PDS 10 cfu/cm^2	PDS 11 cfu/cm^2	PDS 12 cfu/cm^2	PDS 13 cfu/cm^2	PDS 14 cfu/cm^2
03/13/06	I	PVC		1.34E+06			2.20E+05			7.90E+02			1.44E+04		3.78E+04	6.65E+03
	I	LCI		3.50E+05			3.52E+05			3.17E+05			3.06E+05		1.58E+05	1.63E+05
	I	UCI		2.24E+05			5.13E+05			2.24E+05			5.46E+05		7.01E+03	6.58E+03
	I	GAL		3.98E+05			1.49E+05			3.03E+05			4.40E+05		3.95E+04	1.97E+04
05/01/06	I	PVC	7.92E+03		1.55E+04	6.52E+02		4.63E+03	1.02E+05		7.24E+04	1.82E+05		6.77E+03		
	I	LCI	2.42E+06		4.84E+04	1.76E+06		2.49E+05	1.02E+06		6.87E+05	6.87E+05		4.40E+04		
	I	UCI	8.62E+05		1.92E+05	2.98E+05		3.65E+05	1.76E+05		3.82E+05	6.79E+05		1.14E+06		
	I	GAL	5.13E+04		4.57E+06	9.74E+04		4.77E+05	9.74E+04		3.98E+05	TNTC		7.06E+05		

TMTN: too many to numerated.

Appendix A-14 PDS BF HPC Density of Different Material Coupons from May 9 to August 16, 2006

Date	Phase	Material	PDS 1 cfu/cm^2	PDS 2 cfu/cm^2	PDS 3 cfu/cm^2	PDS 4 cfu/cm^2	PDS 5 cfu/cm^2	PDS 6 cfu/cm^2	PDS 7 cfu/cm^2	PDS 8 cfu/cm^2	PDS 9 cfu/cm^2	PDS 10 cfu/cm^2	PDS 11 cfu/cm^2	PDS 12 cfu/cm^2	PDS 13 cfu/cm^2	PDS 14 cfu/cm^2
06/28/06	II	PVC		2.13E+03			1.45E+04			5.29E+03			1.18E+03		4.08E+03	1.38E+04
	II	LCI		2.84E+05			2.16E+05			1.99E+06			2.03E+06		7.44E+05	4.26E+05
	II	UCI		4.29E+04			4.82E+04			6.98E+04			5.49E+04		5.84E+04	1.17E+05
	II	GAL		5.42E+06			8.74E+06			1.65E+05			1.07E+07		1.37E+07	1.66E+06
08/02/06	II	PVC	9.48E+04		2.96E+05	4.08E+04		1.52E+05	1.82E+05		1.61E+05	6.45E+04		2.30E+03		
	II	LCI	5.45E+04		5.00E+05	6.03E+05		4.05E+05	4.09E+05		2.78E+06	1.04E+06		8.40E+05		
	II	UCI	4.64E+05		3.50E+05	9.68E+05		1.36E+06	1.47E+06		4.30E+05	7.00E+05		1.09E+05		
	II	GAL	4.28E+05		3.77E+05	3.44E+05		7.65E+05	3.36E+05		3.69E+05	8.89E+05		1.69E+05		

Appendix A-15 PDS BF HPC Density of Different Material Coupons from August 20 to November 20, 2006

Date	Phase	Material	PDS 1 cfu/cm^2	PDS 2 cfu/cm^2	PDS 3 cfu/cm^2	PDS 4 cfu/cm^2	PDS 5 cfu/cm^2	PDS 6 cfu/cm^2	PDS 7 cfu/cm^2	PDS 8 cfu/cm^2	PDS 9 cfu/cm^2	PDS 10 cfu/cm^2	PDS 11 cfu/cm^2	PDS 12 cfu/cm^2	PDS 13 cfu/cm^2	PDS 14 cfu/cm^2
09/26/06	III	PVC		1.10E+04			8.33E+03			4.50E+03			1.67E+04		9.60E+03	4.00E+03
	III	LCI		4.33E+04			2.60E+05			1.58E+05			3.31E+04		2.80E+04	5.75E+04
	III	UCI		4.50E+03			9.80E+04			6.60E+04			9.60E+04		7.10E+04	4.97E+04
	III	GAL		1.43E+05			6.13E+04			2.87E+04			1.84E+04		5.10E+04	8.50E+04
11/06/06	III	PVC	2.34E+04		2.34E+04	2.96E+03		5.19E+04	4.03E+04		1.61E+04	3.40E+04		1.43E+04	2.34E+04	
	III	LCI	1.47E+05		3.61E+04	3.80E+05		4.20E+05	9.51E+04		7.75E+04	3.20E+05		6.02E+05	1.47E+05	
	III	UCI	4.21E+05		9.41E+05	4.33E+05		2.57E+05	8.74E+05		2.84E+05	3.25E+05		5.05E+05	4.21E+05	
	III	GAL	1.80E+05		3.95E+04	1.05E+05		6.49E+05	1.17E+05		2.13E+05	1.94E+05		4.76E+05	1.80E+05	

Appendix A-16 PDS BF HPC Density of Different Material Coupons from November 27, 2006 to February 12, 2007.

Date	Phase	Material	PDS 1 cfu/cm^2	PDS 2 cfu/cm^2	PDS 3 cfu/cm^2	PDS 4 cfu/cm^2	PDS 5 cfu/cm^2	PDS 6 cfu/cm^2	PDS 7 cfu/cm^2	PDS 8 cfu/cm^2	PDS 9 cfu/cm^2	PDS 10 cfu/cm^2	PDS 11 cfu/cm^2	PDS 12 cfu/cm^2	PDS 13 cfu/cm^2	PDS 14 cfu/cm^2
01/09/07	IV	PVC		1.30E+04			1.09E+04			5.53E+03			2.19E+04		4.74E+03	6.85E+03
	IV	LCI		2.88E+05			1.11E+05			2.11E+05			2.23E+05		7.20E+04	1.67E+05
	IV	UCI		3.33E+05			3.20E+05			3.91E+05			3.89E+05		4.89E+04	1.94E+05
	IV	GAL		1.26E+05			2.15E+05			3.90E+05			3.05E+05		2.99E+05	1.29E+05
02/13/07	IV	PVC	9.87E+01		1.18E+03	3.42E+04		4.25E+03	3.95E+02		1.09E+03	9.87E+02		9.87E+02		
	IV	LCI	3.36E+04		1.08E+04	1.81E+05		7.86E+03	2.87E+03		4.17E+04	4.08E+04		2.72E+04		
	IV	UCI	2.72E+05		2.17E+05	5.11E+04		4.97E+05	1.19E+05		1.34E+05	1.59E+05		1.73E+04		
	IV	GAL	1.77E+05		3.27E+05	1.73E+05		2.68E+05	9.39E+04		2.19E+05	2.46E+05		6.46E+04		

Appendix A-17 PDS BF TC E/Coli of Different Material Coupons from January 30 to August 16, 2006

Date	Phase	Material	PDS 1 MPN / cm^2	PDS 2 MPN / cm^2	PDS 3 MPN / cm^2	PDS 4 MPN / cm^2	PDS 5 MPN / cm^2	PDS 6 MPN / cm^2	PDS 7 MPN / cm^2	PDS 8 MPN / cm^2	PDS 9 MPN / cm^2	PDS 10 MPN / cm^2	PDS 11 MPN / cm^2	PDS 12 MPN / cm^2	PDS 13 MPN / cm^2	PDS 14 MPN / cm^2
03/13/0	I	PVC		ND			ND			ND			ND		ND	ND
	I	LCI		ND			ND			ND			ND		ND	ND
	I	UCI		ND			ND			ND			ND		ND	ND
	I	GAL		ND			ND			ND			ND		ND	ND
05/01/0	I	PVC	ND		ND	ND		ND	ND		ND	ND		ND		
	I	LCI	ND		ND	ND		ND	ND		ND	ND		ND		
	I	UCI	ND		ND	ND		ND	ND		ND	ND		ND		
	I	GAL	ND		ND	ND		ND	ND		ND	ND		ND		
06/28/0	II	PVC		ND			ND			ND			ND		ND	ND
	II	LCI		ND			ND			ND			ND		ND	ND
	II	UCI		ND			ND			ND			ND		ND	ND
	II	GAL		ND			ND			ND			ND		ND	ND
08/02/0	II	PVC	ND		ND	ND		ND	ND		ND	ND		ND		
	II	LCI	ND		ND	ND		ND	ND		ND	ND		ND		
	II	UCI	ND		ND	ND		ND	ND		ND	ND		ND		
	II	GAL	ND		ND	ND		ND	ND		ND	ND		ND		

Appendix A-18 PDS BF TC E/Coli of Different Material Coupons from August 20, 2006 to February 13, 2007

Date	Phase	Material	PDS 1 MPN / cm^2	PDS 2 MPN / cm^2	PDS 3 MPN / cm^2	PDS 4 MPN / cm^2	PDS 5 MPN / cm^2	PDS 6 MPN / cm^2	PDS 7 MPN / cm^2	PDS 8 MPN / cm^2	PDS 9 MPN / cm^2	PDS 10 MPN / cm^2	PDS 11 MPN / cm^2	PDS 12 MPN / cm^2	PDS 13 MPN / cm^2	PDS 14 MPN / cm^2
09/26/06	III	PVC		ND			ND			ND			ND		ND	ND
	III	LCI		ND			ND			ND			ND		ND	ND
	III	UCI		ND			ND			ND			ND		ND	ND
	III	GAL		ND			ND			ND			ND		ND	ND
11/05/06	III	PVC	ND		ND	1.00		ND	ND		ND	ND		ND	ND	
	III	LCI	ND		ND	1.00		ND	ND		ND	ND		ND	ND	
	III	UCI	ND		ND	1.00		ND	ND		ND	ND		ND	ND	
	III	GAL	ND		ND	ND		ND	ND		ND	ND		ND	ND	
01/09/07	IV	PVC		ND			ND			ND			ND		ND	ND
	IV	LCI		ND			ND			ND			ND		ND	ND
	IV	UCI		ND			ND			ND			920.		ND	ND
	IV	GAL		ND			ND			ND			ND		ND	6.30
02/13/07	IV	PVC	ND		ND	ND		ND	ND		ND	ND		ND		
	IV	LCI	ND		ND	ND		ND	ND		ND	ND		ND		
	IV	UCI	ND		ND	ND		ND	ND		ND	ND		ND		
	IV	GAL	ND		ND	ND		ND	ND		ND	ND		ND		

ND – no positive wells, i.e. none detected
MPN – most probable numbers
*All coupons had roughly 6 cm^2 surface area, making the effective detection limit 1 MPN/6 cm^2 or 0.19 MPN/cm^2

Appendix A-19 PDS AOC from January 30, 2006 to February 12, 2007

Date	Phase	Port	PDS 1 µl/L	PDS 2 µl/L	PDS 3 µl/L	PDS 4 µl/L	PDS 5 µl/L	PDS 6 µl/L	PDS 7 µl/L	PDS 8 µl/L	PDS 9 µl/L	PDS 10 µl/L	PDS 11 µl/L	PDS 12 µl/L	PDS 13 µl/L	PDS 14 µl/L
01/31/06	I	Influent		179			159			214			202		161	139
	I	Effluent		126			185			128			144		149	167
07/18/06	II	Influent		120			138			146			151		148	150
	II	Effluent		177			141			179			119		124	121
10/17/06	III	Influent		102			0			20			7		0	63
	III	Effluent		154			139			121			119		110	171
12/16/06	IV	Influent		11			2.5			0			0		25	0
	IV	Effluent		72			62			72			83		62	94

APPENDIX B: AOC POOLED QC DATA DISTRIBUTION FIT TESTS

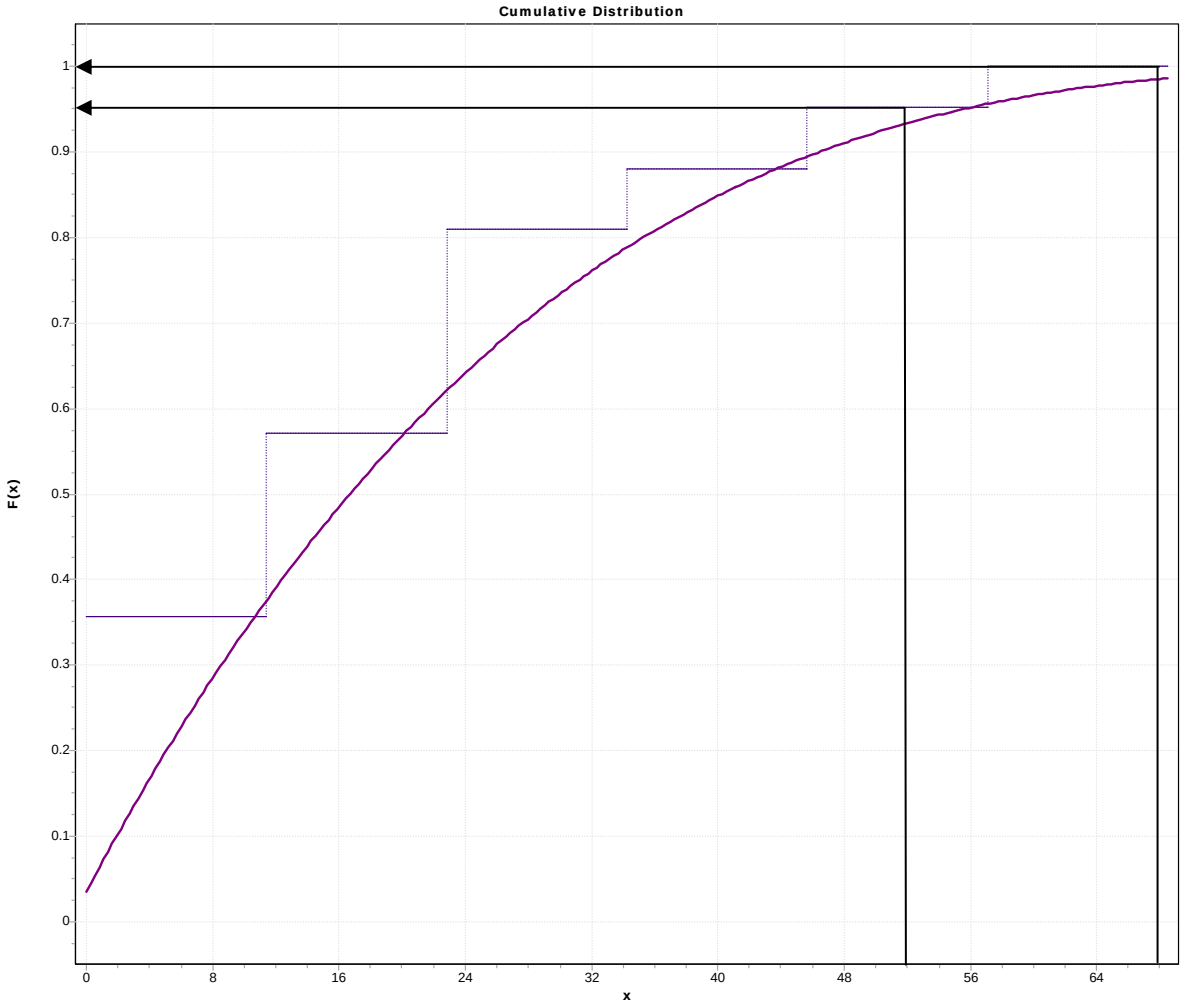
Since there were only 24 duplicate and 17 replicate data points it was very unlikely that any difference between the replicates and duplicates would be statistically significant. Unlike the other biological parameters the AOC QC replicates and duplicates were analyzed together (i.e. pooled QC data) throughout the study. In addition relative percent difference (RPD) was used to build the QC chart instead of log transformed range. The pooled AOC QC data were tested and they fit the Wakeby probability density distribution best as shown in Appendix B- 1.

The actual warning limit and control limit according to NIST formulas were 52% and 68%. However, from Figure 29, 4.9 % instead of 5 % from NIST of the observations would exceed the warning limit and there was no observation that exceeded the control limit as shown in figure Appendix B- 2.

There were 42 observations in the whole AOC QC chart. 4 of them (9.52%) of the observations exceeded the NIST warning limit. 1 of them (2.38%) exceeded the NIST control limit. Between the control limit and warning limit, there were 3 observations (7.14 %).

Appendix B- 1 AOC pooled QC data probability density distribution fit Wakeby distribution

The UWL and UCL from the cumulative distribution function were 50% and 67%, which were 13.04 % and 19.30 % lower than the actual limits. This result suggested when there was a QC data set with small number of observations, the NIST formulas were less stringent rather than more stringent as they were with the bulk HPC data for example. This result may also be affected by the distribution of the data which is different for AOC data and bulk HPC data.



Appendix B- 2 AOC pooled QC data cumulative distribution function plot

APPENDIX C: BIOFILM TC/ E. COLI

Biofilm density was quantified almost exclusively by biofilm HPC during the study as biofilm coliform were only observed in Phase III and Phase IV samples, and a positive E. coli was never observed. The coupon material and the density of the coliforms observed in Phase III and Phase IV are listed in Appendix C- 1. The highest coliform density observed was 153.47 MPN/cm^2 and the minimum was 0.17 MPN/cm^2. From the table, PDS 4 had coliform growth on all four kinds of material coupons. In all cases the density of coliforms was a very small fraction of the overall biofilm, being far less than 0.05 % in all cases.

Appendix C- 1 Observed coliform in TBW II Phase III and Phase IV

PDS	Material	Most Probable Density on Coupon (MPN/cm^2)	BF HPC Density (MPN/cm2)	Percentage
Phase III				
4	PCV (duplicate sample)	0.2	4.25E+03	0.0047%
	LCI	0.17	2.44E+03	0.0070%
	UCI	0.2	2.16E+05	0.0001%
	GAL	1.03	1.36E+05	0.0008%
6	G	0.22	1.38E+05	0.0002%
Phase IV				
11	UCI	153.47	4.27E+05	0.0360%
14	G	1.1	1.29E+05	0.0009%

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