ENGINEERED BIOFILTRATION FOR ENHANCED HYDRAULIC AND WATER TREATMENT PERFORMANCE

By

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To Courtney and Marley, I love you both. This is yours more than it is mine.
I know I have a lot of making up to do!

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AAS	atomic adsorption spectrophotometer
AOC	assimilable organic carbon
AP	Associated Press
ATP	adenosine triphosphate
BDOC	biologically degradable organic carbon
BOM	biodegradable organic matter
C	carbon
CFU	colony forming units
City	City of Arlington, Texas
C:N:P	bioavailable carbon:ammonium-N:orthophosphate-P
CT	contact time
CV	crystal violet
°C	degree Celsius
DBP	disinfection by-product
DBPFP	disinfection by-product formation potential
DEET	N,N-Diethyl-meta-tolumide
DI	deionized water
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DOC	dissolved organic carbon
DPD	N,N-Diethyl-p-Phenylenediamine
DSA	deposit and surface analysis
DWU	Dallas Water Utilities
EBCT	empty bed contact time

EDCs	endocrine disruptoring compounds
EDS	energy dispersive spectroscopy
EPS	extracellular polymeric substances
ESI	electrospray ionization
FDA	Food and Drug Administration
Fe	iron
FISH	fluorescence in situ hybridization
FTIR	Fourier transform infrared
ft	feet or foot
GAC	granular activated carbon
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GHz	gigahertz
gpd	gallons per day
gpm/ft^2	gallons per minute per square foot
HLR	hydraulic loading rate
HMI	human machine interface
$HO^{\cdot}$	hydroxyl radicals
HPCs	heterotrophic plate counts
HPLC	high performance liquid chromograph
hr	hour(s)
ID	inner diameter
ISE	ion-selective electrode
JKWTP	John F. Kubala Water Treatment Plant
LB	Luria -Bertani

L/d	(filter) length-to-diameter (particle of media)
L/min	liters per minute
LC/MS	liquid chromatography/mass spectrometry
LC/MS/MS	liquid chromatography/quadrupole mass spectrometry
LOD	limit of detection
MCL	maximum contaminant level
µg/L	micrograms per liter
µg/mL	micrograms per milliliter
mg	milligrams
MG	million gallons
mg/L	milligrams per liter
mgd	million gallons per day
MIB	2-methylisoborneol
min	minute(s)
mL	milliliter
mL/min	milliliter per minute
mm	millimeter
mM	millimolar
Mn	manganese
MRLs	minimum reporting levels
mRNA	messenger ribonucleic acid
MWH	Montgomery-Watson Harza
ng/L	nanograms per liter
NH_4-N	ammonia-nitrogen
nm	nanometer

NOM	natural organic matter
NSF	National Sanitation Foundation
NTU	Nephelometric Turbidity Units
O_3	ozone
ORD	Office of Research and Development
PO_4-P	orthophosphate-phosphorus
PAC	powdered activated carbon
PBS	phosphate-buffered saline
PBSWTP	Pierce Burch South Water Treatment Plant
PCR	polymerase chain reaction
PLC	programmable logic controler
PVC	polyvinyl chloride
qPCR	quantitative polymerase chain reaction
RLU	relative light units
RNA	ribonucleic acid
RPM	revolutions per minute
rRNA	ribosomal ribonucleic acid
RSD	relative standard deviation
RT-PCR	reverse transcription polymerase chain reaction
SCADA	supervisory control and data acquisition
SDSDBP	simulated distribution system disinfection by-product
SEM	scanning electron microscopy
SI	Sørensen index
SM	standard method
SWI	Shannon-Weaver Index

T&O	taste and odor
TCCP	tris (2-chloroisopropyl) phosphate
TCEP	tris (2-charboxyethyl) phosphine
TCPP	tris (2-chlorophropyl) phosphate
TEM	transmission electron microscopy
TOC	total organic carbon
T-RFLP	terminal restriction fragment length polymorphism
TRWD	Tarrant Regional Water District
U.S.	United States
UFRV	unit filter run volume
UHPLC	ultra high-pressure liquid chromotograph
USEPA	United States Environmental Protection Agency
V	volt
VFDs	variable frequency drives
WTP	water treatment plant

Abstract of Dissertation Presented to the Graduate School
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ENGINEERED BIOFILTRATION FOR ENHANCED HYDRAULIC AND WATER TREATMENT PERFORMANCE

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Currently, biofiltration is largely operated as a passive process in the water treatment industry. Particle removal and headloss drive the design and operation of conventional filtration and biofiltration. Thus, biofilter design parameters are typically limited to media configuration, backwash strategy, and loading rate. The removal of dissolved organic and inorganic contaminants is an anticipated benefit of biofiltration. However, common design and operational practice does not seek to enhance the biological activity responsible for those mechanisms. Indeed, in an effort to improve filter productivity and minimize headloss, many utilities employ chlorinated backwashes and other biomass control strategies. However, these are often to the detriment of biological activity and may be ineffective at removing a primary foulant of biofilters – extracellular polymeric substances (EPS).

This study focused on identifying enhancement strategies to improve both water quality and hydraulic performance of drinking water biofilters by increasing microbial activity while decreasing biological fouling. These strategies included biofilter substrate-, nutrient-, and oxidant-enhancement. Of the strategies tested, nutrient enhancement and

oxidant-enhancement showed the most promise for drinking water biofilter applications. The nutrient enhancement strategy is elegant in its simplicity: operate a given biofiltration process so that an approximate bioavailable carbon: ammonia-nitrogen:orthophosphate-phosphorus molar ratio of 100:10:1 is maintained. Achieving a nutrient balance decreased terminal headloss by ~15 percent relative to the control, possibly the result of reduced EPS formation. Nutrient enhancement also sustainably decreased contaminant breakthrough relative to the control biofilter, including 2-methylisoborneol (MIB), manganese (Mn), and dissolved organic carbon (DOC). A preliminary evaluation of the oxidant-enhancement strategy was implemented by providing a 1 mg/L dose of hydrogen peroxide to the biofilter feed over a 2-week test period. The objective was to enhance the oxidative action and response of biofilter microorganisms and to promote the oxidation of inactive biomass. The filter demonstrated ~15 percent removal of filter feed DOC (7 percent less breakthrough than control), and removal of Mn and MIB to non-detect levels. The oxidant enhancement strategy also decreased terminal headloss to a mean 2.2 feet, or ~60% of the control. Both strategies showed enhanced water treatment performance without compromising filter productivity or particulate removal performance.

CHAPTER 1
INTRODUCTION AND OBJECTIVES

Problem Statement

The City of Arlington, Texas (City) owns and operates two ozone/biofiltration drinking water treatment facilities, the Pierce-Burch South Water Treatment Plant (PBSWTP) and the John F. Kubala Water (JKWTP). Both facilities receive water from the Tarrant Regional Water District system (TRWD). The TRWD system pumps water from the Richland Chambers, Benbrook, and Cedar Creek reservoirs. Water quality is similar among the reservoirs, and changes in blending ratios have some impact on facility performance. The JKWTP receives water directly from the TRWD system. However, Lake Arlington is used as terminal storage for TRWD water before it is pumped to the PBSWTP. Lake Arlington contributes additional seasonal loads of taste and odor (T&O) and manganese (Mn) to the PBSWTP.

The City implemented ozone/biofiltration processes at these facilities in 2001 to remove tastes and odors (T&O), iron (Fe), manganese(Mn), turbidity, and to minimize disinfection by product formation potential (DBPFP), distribution system chloramine demand, and regrowth potential. Currently, the PBSWTP has a production capacity of 72 million gallons per day (mgd) with a process train that includes coagulation, flocculation, sedimentation, biofiltration (40 inches of granular activated carbon [GAC], 6 inches of sand, and Leopold IMS© cap) [1], and final disinfection. Disinfection residual consists of a chlorination step to achieve free chlorine contact time followed by downstream ammonia addition to produce chloramines for distribution. Primary

[1] The PBSWTP filter design was altered in 2009, during this study, to replace the Leopold IMS© caps with gravel over the underdrains.

disinfection is obtained through the intermediate ozonation process. The City practices a non-chlorinated/chloraminated backwash (BW) at both the JKWTP and PBSWTP. Backwash wastewater (BWW) is conveyed to onsite lagoons.The JKWTP has a capacity of 97.5 mgd and includes the same treatment process scheme as the PBSWTP plant, with the exception that JKWTP biofilters contain 48 inches of GAC, 8 inches of sand, and 12 inches of gravel over the underdrain. Figure 1-1 provides a conceptual process schematic for the JKWTP and PBSWTP.

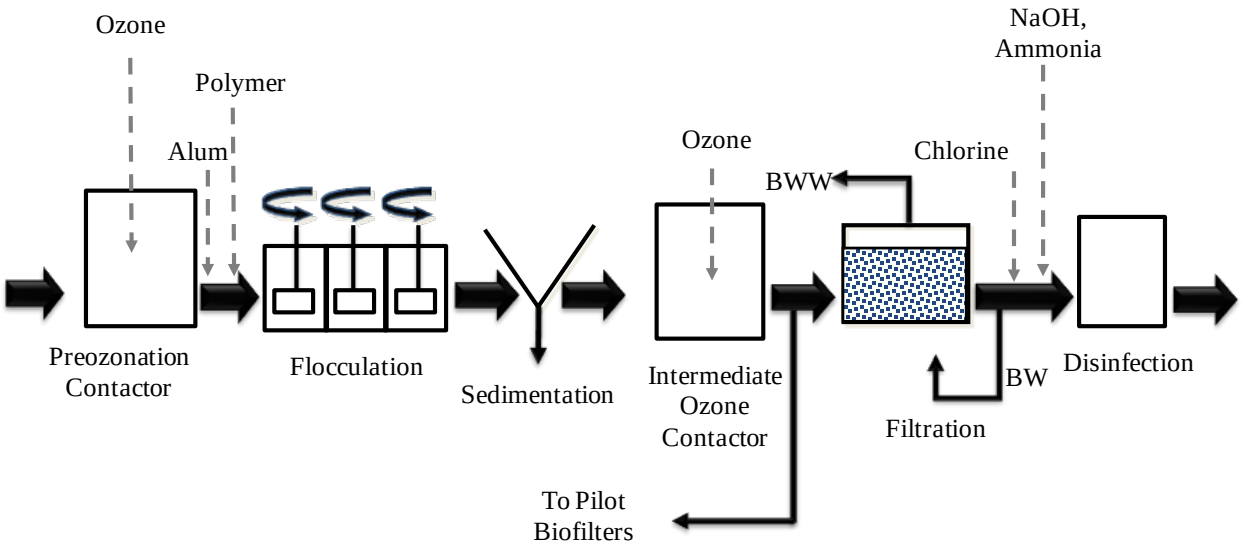

Figure 1-1. Conceptual process schematic for the JKWTP and PBSWTP

While the PBSWTP and JKWTP have performed well for many years, recently observed biofilter hydraulic and water treatment performance disruptions led the City to evaluate their biofiltration systems to identify potential enhancement strategies. Specific performance disruptions included underdrain clogging, increased chloramines residual decay rates (i.e., organic carbon breakthrough from the biofilters, specific to PBSWTP), decreased Mn removal efficiency, and decreased T&O removal efficiency resulting in odor complaints. In addition, a 2007 survey of JKWTP and PBSWTP raw and finished

waters identified low levels (ng/L) of pharmaceuticals and endocrine disrupting compounds. Through one sampling effort, the City analyzed both raw and treated water for both the JKWTP and PBSWTP. The City determined that their treatment processes removed all but one identified pharmaceutical present in the raw water to below parts per trillion. Therefore, the City desired to determine if enhancing biofiltration would further remove this compound and other potential compounds through participation in this study. Detailed discussions on the PBSWTP and JKWTP hydraulic and water treatment performance upsets are provided in the subsequent sections.

Hydraulic Performance

The PBSWTP and JKWTP biofilters were designed as rising level/constant rate, inter-filter backwashing filters, with a Leopold universal air/water underdrain and IMS® cap. The inter-filter backwash uses the effluent and discharge head from filters in production mode for the backwash process. Although both facilities produce exceptional filtered water quality (<0.08 Nephelometric Turbidity Units [NTU] effluent), filter productivity has declined through the years. As shown in Figure 1-2, the average unit filter run volume (UFRV) for JKWTP decreased by approximately 50% from January 2001 to May 2005. The largest observed decrease in productivity occurred between 2001 and 2002, coinciding with the ozone/biofiltration system going online.

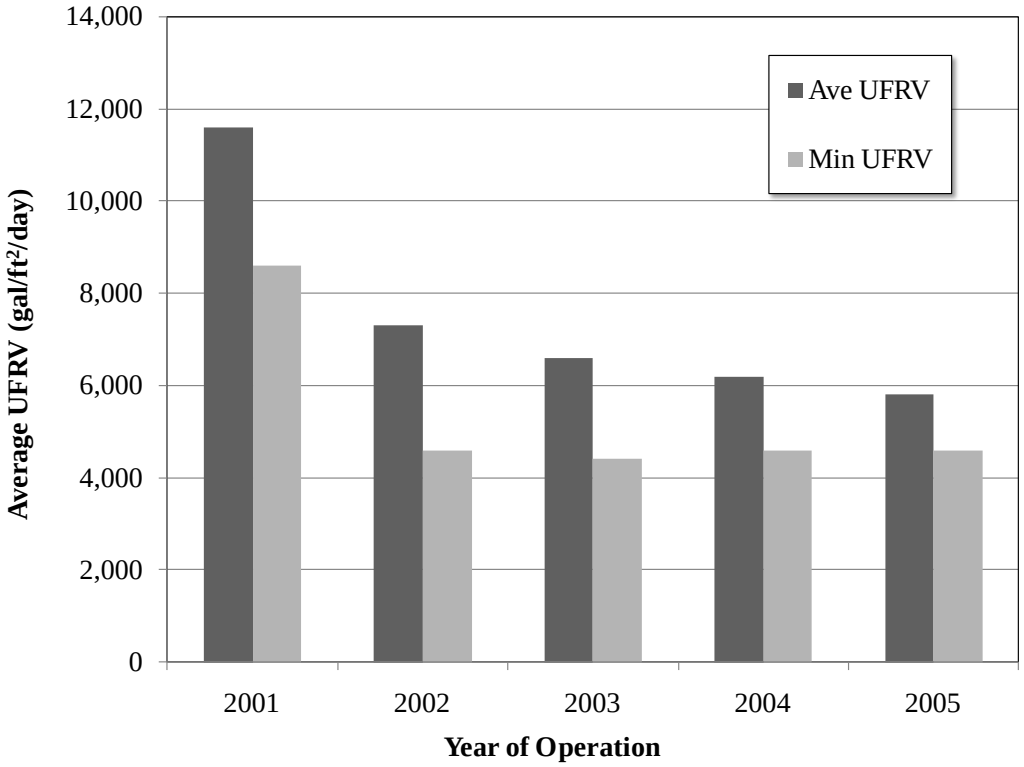

Figure 1-2. Historical UFRVs for the JKWTP

It was determined that filter media mud ball formation and underdrain cap fouling were contributing factors for decreased filter productivity. These factors also limit the efficacy of the backwash system, creating a self-exacerbating condition. Clean-bed headloss has increased through the years, resulting in higher water elevations within the filters at the start of filter runs. Table 1-1 summarizes an analysis of six selected JKWTP biofilter runs for three biofilters operated from May 2006 through September 2006. There is a total of eight feet of head available for filtration.

The average biofilter clean bed headloss ranged from 5.3 to 7.9 feet, leaving 2.7 to 0.1 feet of head available for filtration. Based on media characteristics, bed depth, and loading rates, the calculated expected clean bed headloss for the media configuration at the JKWTP is 0.9 feet (calculation provided in Table 1-1 notes). The discrepancy

between actual and expected clean bed headloss reflects considerable fouling of the media and porous plate in the underdrain. Indeed, at both the JKWTP and the PBSWTP, headloss accumulation across the biofilters has led to underdrain failures, including blown out mastic seals and stripped IMS® cap anchoring screws. Figures 1-3 and 1-4 provide photographs of failed IMS® caps removed from the JKWTP. Compromised IMS® caps create short-circuiting through the biofilters, which diminishes treatment performance and backwash effectiveness. Consequently, additional particles and biomass accumulate, exacerbating the original short-circuiting problem.

The City of Arlington had the failed JKWTP IMS® Caps autopsied to determine cause of failure. The autopsies were performed by Cyrus Rice Water Consultants (Pittsburgh, Pa.) and included microbial analyses and scanning electron microscopy (SEM) coupled with an energy dispersive x-ray. The results of these tests suggested that the primary foulant was microorganisms and associated biological materials. It was believed that the presence of excess biological materials in the caps was accelerating the collection and entrapment of inorganic foulants as well. During operation of the pilot study discussed in this dissertation, Arlington elected to remove all biofilter IMS® caps and replaced them with gravel underdrains.

Table 1-1. Summary of calculated hydraulic parameters for biofilters*

Filter no.	Average clean bed headloss (ft) †,‡	Resulting available head for production (ft) †,‡
1	5.3	2.1
5	7.9	0.1
9	5.7	2.9

* Data collected for JKWTP Expansion II Project

† Calculated clean bed headloss (Darcy's Equation) = 0.5 ft (GAC) + 0.3 ft. (sand) + 0.1 underdrain = 0.9 ft.

‡ Average of six different filter runs from May 15, June 14, July 15, August 15, and September 15, 2006, using data from instrument readings for water level in filter boxes, backwash weir, and elevations from record drawings.

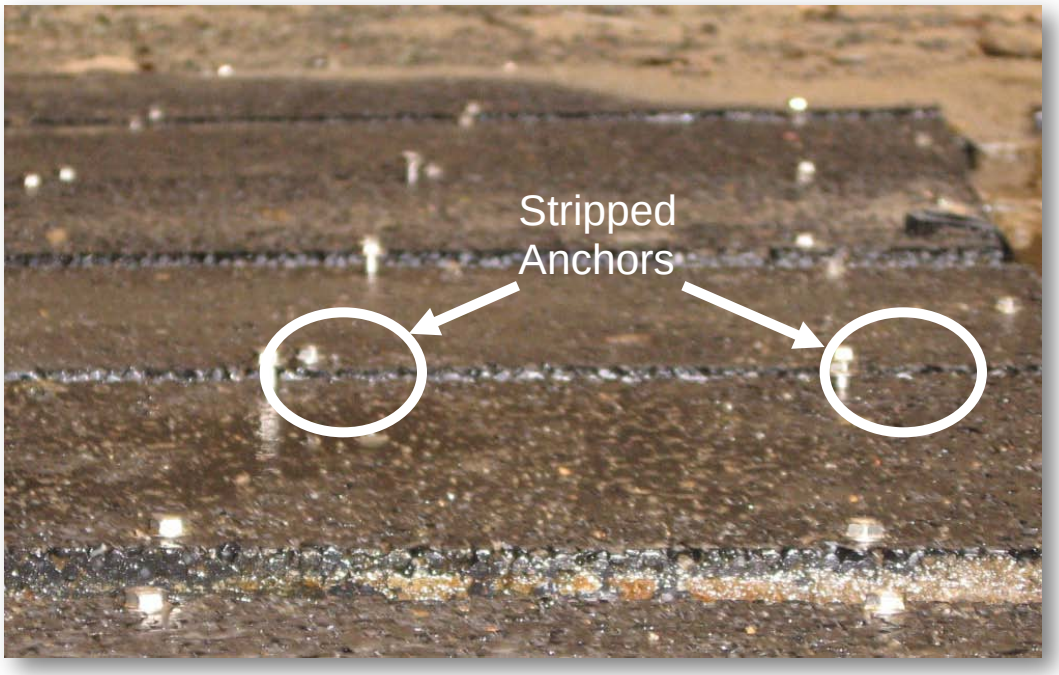

Figure 1-3. Failed IMS® cap removed from a JKWTP biofilter with stripped anchoring screw

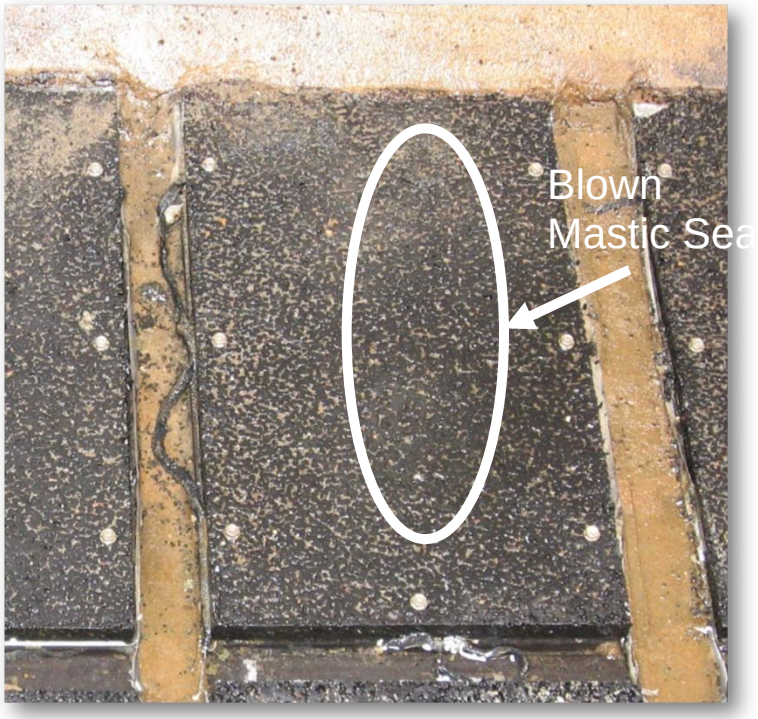

Figure 1-4. Photograph of failed IMS® cap removed from a JKWTP biofilter with blown mastic seal

Water Treatment Performance Concerns

The PBSWTP and JKWTP provide regulatory-compliant, high-quality finished waters. However, occasional process upsets and seasonal loading events have negatively affected effluent aesthetics and stability. Contaminant breakthrough has included Mn and T&O compounds. Unstable chloramine levels also have been observed at various locations in the distribution system.

Manganese. JKWTP and PBSWTP effluent Mn concentrations rarely exceed the secondary maximum contaminant level (MCL) of 0.05 mg/L. The ozonation/biofiltration process historically has performed well, removing large fractions (>85%) of soluble Mn via oxidation/filtration. However, seasonally high loads at the PBSWTP and extended periods of low-level breakthrough have led to an accumulation of Mn precipitates in the distribution system. Small decreases in distribution system redox potential have caused dissolution of the particulate Mn, resulting in colored-water episodes. Distribution system redox can be impacted by decreasing chloramines residual and/or decreasing dissolved oxygen (DO) caused by microbial regrowth. The City received approximately 47 customer complaints related to colored tap water from January 2008 through October 2009.

Tastes and odors. The impounded reservoirs that provide raw water to the PBSWTP and the JKWTP experience seasonal blue-green algae blooms. These blooms produce metabolic by-products that impart T&O to the water source. Two of the most prevalent T&O-causing blue-green algae metabolites detected in PBSWTP and JKWTP finished waters are MIB and trans-1,10-dimethyl-trans-9-decalol (geosmin). Although the ozone/biofiltration process may remove 80 to 90% of these compounds, finished water concentrations above the commonly accepted odor threshold

concentration (OTC) of 10 ng/L may still lead to customer complaints. The City received approximately 63 customer complaints related to T&O from January 2008 through October 2009.

Chloramine instability. The City has observed chloramine instability in some parts of the distribution system. Chloramine instability in ozone/biofiltration effluent may have multiple causes, including the breakthrough of particulates, organic carbon, or microbial cells.

Pharmaceuticals and pesticides. A quick scan of any newspaper across the United States (U.S.) in early March 2008 reveals the potential impact that pharmaceuticals may have on the drinking water industry. On March 9, 2008, the Associated Press (AP) released a report indicating that their investigative team discovered the presence of pharmaceuticals in the drinking water supplies of at least 41-million Americans in 24 major metropolitan areas across the country. The City had proactively sampled its raw and finished waters in 2006 to characterize the presence of pharmaceuticals and pesticides. The results from this characterization were disclosed to the AP and released as part of the March 9 report. While detected concentrations were very low and health implications are far from well understood, the concern about drugs in drinking water is now ever-present in the minds of utilities, consumers, regulators, and drinking water professionals in general. Congress has already called on the EPA to establish a national task force to study this issue further. In other words, while regulations covering many of these compounds are not on the immediate horizon, utilities must begin to better understand the presence and removal of pharmaceuticals from drinking water supplies. Many pesticides are currently regulated under the Safe

Drinking Water Act and have maximum contaminant levels (MCLs) that are included in the Primary Drinking Water Standards. However, the mere presence of these compounds at detectable concentrations in drinking water supplies may also promote negative media and public attention. Trace levels of pharmaceuticals and pesticides were detected in the City's source and finished water supplies in the 2006 study. Table 1-2 provides a summary of the results from that survey.

Although none of the current water quality concerns threatens compliance, the City sought to improve effluent water aesthetics and stability.

Table 1-2. Summary of pharmaceuticals and pesticides measured in the City of Arlington's raw and finished waters

	JKWTP		PBSWTP	
Contaminant	Raw water (ng/L)	Finished water (ng/L)	Raw water (ng/L)	Finished water (ng/L)
Sulfamethoxazole	1.4	<0.25	0.62	<0.25
Meprobamate	3.5	1.4	2.6	1.0
Dilantin	1.9	<1.0	1.0	<1.0
Carbamazepine	1.6	<0.50	1.8	<0.50
Naproxen	0.85	<0.50	<0.50	<0.50
Estrone	0.63	<0.20	0.28	<0.20
Estradiol	<0.50	<0.50	17	<0.50
Atrazine	780	240	460	220
DEET†	35	<25	<25	<25
TCEP‡	57	<0.50	<0.50	<0.50
TCPP§ (Fyrol PCF)	71	<0.50	<0.50	<0.50
Nonylphenol	72	<0.50	83	<0.50

* Samples collected and sent for analyses on October 30, 2006 and analyzed at the Southern Nevada Water Authority by Shane Snyder.
† N,N-Diethyl-meta-tolumide.
‡ tris (2-charboxyethyl) phosphine.
§ tris (2-chloropropyl) phosphate.

Hypothesis and Objectives

In conventional practice, turbidity removal and headloss drive the design and operation of both conventional filtration and biofiltration for drinking water treatment in surface water applications. Thus, biofilter design parameters are often limited to media configuration, loading rate, and backwash strategy. While these parameters can significantly impact biofilter performance, their influence on improved biological activity is largely passive. The biological removal of dissolved organic and inorganic contaminants is an anticipated benefit of biofiltration. However, common design and operational practice does not seek to enhance the biological activity responsible for those mechanisms. Furthermore, current biofilter operational practices primarily focus on biofilm control to maintain hydraulic performance, often to the detriment of biological activity (and optimal water treatment).

The hypothesis of this work is both water treatment and hydraulic performance of a biofilter can be improved by modifying influent conditions for enhanced biological activity. The purpose of this research was to identify strategies to enhance the biological activity in a biofilter without compromising productivity or particulate removal performance. Specific objectives included:

- Evaluate potential biofilter enhancement strategies comprised of dosing low levels of common drinking water treatment chemicals at a feed point just upstream of a biofiltration process. These chemicals were added to provide substrate, nutrient, and/or oxidant optimization of the biofilter process influent.
- Investigate biological drinking water treatment process fundamentals (e.g., microbial ecology, bacterial metabolism, and contaminant removal mechanisms) to understand how

DOC, MIB, geosmin, and Mn can be removed effectively in a single treatment step

Biological clogging (filter headloss) can be minimized

The ultimate goal of this work is to shift an industry-accepted paradigm so that the design and operation of biofilters are driven not only by filtration but also by biological treatment objectives.

Approach

The research study included ten months of biofiltration enhancement pilot-scale testing at the JKWTP to evaluate methods for restoring and enhancing the performance of the City's ozone/biofiltration process. This evaluation entailed both a characterization and evaluation of biological activity in the filters and an examination of potential enhancement strategies. The premise of this work is that small changes in filter feed conditions could greatly improve the health and activity of the microbial community in the biofilters, consequently enhancing performance. The strategies tested were selected based on previously published literature and industry experience. The following studies were performed to meet research objectives:

1. Characterize the baseline performance of the JKWTP operating under existing conditions. This included an assessment of the system's ability to meet the City's current treatment objectives.
2. Evaluate primary substrate augmentation enhancement strategies.
3. Evaluate nutrient augmentation enhancement strategies.
4. Evaluate peroxide supplementation for augmenting the oxidative action and response of the biofiltration process.
5. Identify and track the microbial communities active present in the biofilters.
6. Develop full-scale design and operating parameters for implementation of the recommended modified biofiltration process at the City's WTPs and estimate the associated capital and production costs

CHAPTER 2
LITERATURE REVIEW

The purpose of this chapter is to provide a summary of both conventional and state-of-the-art understanding with respect to biofiltration fundamentals, contaminant applicability, biofilter design, operations, and monitoring. The basis for the tested enhancement strategies is also discussed.

Historical Background

Perhaps the first recognized commercial use of filtration for water treatment occurred in 1804 at a bleachery in Paisley, Scotland (Huisman and Wood, 1974). The treatment system was an experimental slow sand filter, designed and built by John Gibb. Surplus water produced by the filters was sold to the public at a halfpenny per gallon (Baker, 1948). These early slow sand filters could effectively remove turbidity and pathogenic organisms. Furthermore, these slow sand filters were not only the first effective municipal water treatment system, they were also likely the first example of drinking water biological treatment. The design offered indigenous microorganisms ample surface area and detention time to degrade nutrients and organic compounds present in the source water. The success of this system to improve water aesthetics and public health lead to metropolitan use of slow sand filters across Europe and the United States by the late nineteenth century (Johnson, 1914; Huisman and Wood, 1974). However, the loading rates of slow sand filters were low, typically 0.04 to 0.08 gallons per minute per square foot (gpm/ft^2). Thus, large areas of land were required to accommodate the facilities for even modest urban communities. Reliable slow sand filter treatment also required low turbidity influent (<10 NTU) and high operator effort for media cleaning and replacement. As populations and water demands increased, the

space, labor, and source water requirements for slow sand filters became unfeasible in many applications. In 1896, the first rapid rate sand filters were installed in the United States, utilizing an expanded-bed backwash with water and air supply (Baker, 1948). Rapid rate sand filters provided filtration at rates from 2 to >10 gpm/ft^2, requiring significantly less space than earlier filtration technologies. In addition, rapid rate filters provided better performance for treating water with high, or flashy, influent turbidity (EPA, 1990). Coincidentally, implementation of rapid rate filtration coincided with the widespread use of chlorine as a primary disinfectant for cholera and typhoid fever control (Leal, 1909). By 1925, there were approximately 18 million U.S. residents served by utilities employing rapid rate sand filters, and only 5 million served those with slow sand filtration (Gillespie, 1925). Thus, most drinking water filters in the U.S. moved away from biological treatment to conventional filtration by the early twentieth century.

Today, suppressed-biological (chlorine present) rapid rate granular media filtration remains the most common system to remove particulate matter in water treatment systems. Indeed, biological drinking water treatment was limited in the United States until recently (Urfer et al., 1997), likely due to public perception and resistance to the intentional use of microorganisms within water treatment processes (Evans, 2009). However, new regulatory and technological developments are driving more utilities to consider the use of biological processes to treat their drinking water. These developments include (1) the promulgation of regulations further limiting the formation of DBPs; (2) the emergence of ozone for taste, odor, and color control; (3) the increased awareness that biologically-stable water must be produced in the treatment plant to avoid the negative impacts of biological activity in the distribution system (e.g.,

disinfectant demand, aesthetic upsets, and corrosion); and (4) the push for green technologies.

Configurations of Biological Treamtent

There are numerous forms and configurations of biological treatment processes for drinking water (Rittmann and McCarty, 2001). A small number of drinking water biological treatment technologies operate as suspended growth systems, in which free-floating bacteria and other microorganisms are hydraulically maintained within a reactor. However, most are operated as biofilm systems, including fixed-bed biofilm systems, fluidized-bed biofilm systems, and hollow-fiber membrane biofilm reactors (Brown, 2007a). Among the various biological treatment configurations used for drinking water applications, fixed-bed biofilm reactors are the most common. This process includes a biogrowth support medium such as sand, anthracite, and/or GAC on which microbial communities attach and grow. The most common fixed-bed biofilm processes are positioned just upstream of final disinfection and serve not only as bioreactors to oxidize organic and inorganic contaminants but also as filters to remove particles, thus the name “biofilter.” In typical configurations, biofilters are fed post-coagulated/flocculated settled water (with or without ozonation). The biofilters serve as the final step to remove remaining suspended particles and unsettled floc. Therefore, the biological action of these systems must not compromise their ability to meet filtration design criteria (i.e., turbidity limits and unit filter run volumes).

The following sections provide additional detail on the contaminant treatability, design parameters, conventional and enhanced operational procedures, and monitoring techniques for biofilters in water treatment applications.

Contaminant Treatability

Overview

Biofiltration can be used to treat a wide range of organic and inorganic contaminants in surface water and groundwater. The following sections provide a general description of the current state of knowledge on the effectiveness of drinking water biofiltration for the contaminants tracked during this study.

Natural Organic Matter

Nearly all surface water utilities and many groundwater utilities are affected by organic carbon, which can (1) cause organic or biological fouling within any unit process, (2) exert an oxidant demand, (3) serve as a DBP precursor, and (4) cause biological regrowth in distribution systems (LeChevallier et al., 1992; Escobar et al., 2001) recommended that distributed assimilable organic carbon (AOC) concentrations be less than 100 µg/L to limit regrowth. Numerous studies have investigated the design and operation of biological filters to reduce organic carbon concentrations. Biofilter organic carbon removal efficiencies have been reported to vary between 5 and 75%, with typical removals of 10 to 20% (Bouwer and Crowe, 1990, Raymond et al., 1995; Urfer et al., 1997). Organic carbon removal can be correlated to biofilter activity, as measured by dissolved oxygen (DO) uptake, adenosine triphosphate (ATP), and/or phospholipid fatty acids, which may be affected by feed water source and characteristics, operating temperature, backwash protocol, disinfectant application, and ozone dose (Huck et al., 2000; Liu et al., 2001; Westerhoff et al., 2005; Wert et al., 2008).

MIB and Geosmin

An increase in blooms of cyanobacteria (blue-green algae) in freshwaters has been a global phenomenon in recent years. Blue-green algae blooms occur on every continent, excluding Antarctica, and in over 30 states in the U.S. (Carmichael, 2001). These blooms are responsible for producing algal metabolites that can impart tastes, odors, and toxicity to surface waters. In areas that rely heavily on surface water for drinking water supply, these metabolites may limit a critical resource by degrading water aesthetics and safety. The presence of objectionable T&O compounds in surface water supplies is a growing problem facing drinking water utilities across the U.S. and worldwide. Two of the most common surface water T&O-causing compounds, MIB and geosmin, are metabolites of blue-green algae. MIB and geosmin have an earthy/musty T&O that can be detected by human senses at concentrations as low as 10 ng/L. Though MIB and geosmin are not regulated, the importance of their removal from drinking water sources is critical for maintaining consumer confidence. Mallevialle and Suffet (1987) suggested that the majority of consumers judge the safety of their water by appearance, taste, and smell. Therefore, utilities must remove T&O compounds, though they are under no regulatory pressure to do so. Suffet et al. (1995) also noted that 128 of 800 utilities surveyed in the U.S. experience serious T&O problems and that, on average, U.S. utilities spend approximately 4.5% of their total treatment budget on T&O control.

Conventional approaches for mitigating blue-green algae metabolite impacts on drinking water include (1) blue-green algae population control through reservoir management, and (2) metabolite removal at the water treatment facility. Reservoir management (e.g., copper dosing) has shown some success at reducing algal

populations and the resulting production of blue-green algae metabolites. However, the growth of copper-resistant blue-green algae strains and increased nutrient loading to surface waters limit the effectiveness of this approach. Furthermore, conventional treatment methods, such as pre- and post-chlorination, coagulation, sedimentation, and filtration, are marginally effective at reducing algal metabolite concentrations. Powdered activated carbon (PAC) is often supplemented to these processes to achieve adequate removal, though its use can be cost-prohibitive over lengthy or intense algal events. Recently, many utilities have begun to employ the combination of ozone and biofiltration as a strategy to mitigate MIB, geosmin, and other T&O compounds. In this process, ozonation typically achieves 30 to 75% removal of MIB and 40 to 60% removal of geosmin at dosages of 1.5 mg/L (Lundgren et al.; 1998; Nerenberg et al., 2000; Westerhoff et al., 2005). Biofilters operated downstream of ozonation typically achieve an additional 50 to 80% of the remaining MIB and geosmin (Nerenberg et al., 2000; Westerhoff et al., 2005; Elhadi et al., 2006).

MIB is similar in structure to the bicyclic monoterpene, camphor. Camphor is metabolized by pathways that involve the sequential cleavage of each ring by formation of unstable lactones (Trudgill, 1984). MIB biodegradation probably follows a similar pathway, i.e., ring cleavage following formation of each lactone. However, isoborneol (MIB without the methyl group bonded to the same carbon as the hydroxyl group) was degraded at a faster rate than MIB and was unable to enrich microbial populations for MIB degradation (Izaguirre et al., 1988a,1988b). The tertiary alcohol of MIB may be responsible for the slower kinetics by making it more difficult for enzymes to oxidize the alicyclic ring to the alicyclic ketone prior to lactone formation. Despite relative

recalcitrance, microbial isolates capable of degrading MIB to levels below the odor threshold concentration (OTC) have been identified. Previously isolated MIB-degrading bacteria include *Pseudomonas* sp., *Flavobacterium* sp., and *Bacillus subtilis* (Ishida and Miyaji, 1992; Egashira et al., 1992; Lauderdale, 2004).

Manganese Removal

The presence of Mn in drinking water creates aesthetic challenges for utilities. Mn may produce brown-, black-, or purple-colored water events, depending on the concentration and oxidative reduction potential (ORP). A 2001 study summarized the available utility Mn occurrence data, indicating that 20% and 4.4% of drinking water systems treating groundwater and surface water, respectively, had raw water Mn levels above 0.05 mg/L (WaterRF, 2001). Effluent Mn concentrations less than the secondary MCL may also present concerns for utilities as low-level breakthrough can lead to an accumulation of Mn precipitates in the distribution system. Small decreases in distribution system ORP may cause dissolution (resuspension) of the particulate Mn, resulting in colored water episodes (Kohl and Medlar, 2006). Distribution system ORP can be impacted by changing disinfectant residual and/or by biofilm-induced DO fluctuations.

Particulate Mn (Mn^{+4}) is easily managed in most WTPs using sedimentation and filtration processes (Knocke et al., 1990). However, soluble Mn (most commonly Mn^{+2} in surficial waters) is much more difficult to treat. The conventional approach for soluble Mn treatment is the continuous application of free chlorine to increase the ORP sufficiently to oxidize Mn^{+2} to Mn^{+4}. This change in oxidation state precipitates the soluble Mn, which then plates on the filter media. This strategy requires continuous maintenance of the applied chemical oxidant, as the particles formed are only stable at

the ORP at which they were formed. Interruption of oxidant application leads to the reduction of Mn^{+4} to Mn^{+2} and the release of soluble Mn in the finished water. Recently, DBP concerns have forced utilities to consider alternative oxidants such as ozone. Ozone oxidation of Mn may lead to the formation of colloidal Mn, which may be too small to be effectively removed during filtration (Kohl and Medlar, 2006). By adjusting the ozone dose, it is possible to improve the rate and extent of particulate Mn formation, thereby minimizing colloidal Mn release to the distribution system. However, the optimal dose is controlled by multiple factors including ozone demand and water temperature.

An alternative Mn treatment technology is biofiltration. Biofilters take advantage of the Fe- and Mn-oxidizing ability of certain autotrophic bacteria (Kohl et al., 2006). The bacteria (e.g., *Metallogenium* sp*., Burkholderia* sp*. Pseudomonas* spp*., Bacillus* spp*.*) oxidize soluble Mn^{+2} to Mn^{+4}, which readily precipitates as MnO_2 under most filter conditions. Mn oxidation may be driven through oxidase enzymes (intra- and/or extracellular) or be promoted by metabolic by products that sufficiently elevate pH or ORP (Diem and Stumm, 1984; Nealson 1992).

The biologically-oxidized Mn becomes integrated (adsorbed) within the biofilm-filter media matrix. Surficial charges within the matrix maintain Mn stability, preventing desorption (Sahabi et al., 2009). Excess biofilm and sloughed microbial aggregates containing Mn are removed from the filter media during backwash procedures. Drinking water biofiltration for Mn removal has been practiced for decades in the U.S. and Europe (WaterRF, 2001). Despite success, there are process challenges during facility startup and operational upsets. Indeed, non-acclimated or disrupted systems may take months before desired steady-state Mn removal performance is achieved. The potential

rapid release of Mn from filter media matrices following an ORP shift remains a significant concern for utilities considering conversion of existing chlorine-treated filters to a biological mode.

Pharmaceuticals and Pesticides

A quick scan of any newspaper across the U.S. in early March 2008 revealed the potential impact that pharmaceuticals and pesticides may have on the drinking water industry. On March 9, 2008, the AP released a report indicating that their investigative team discovered the presence of pharmaceutical drugs in the drinking water supplies of at least 41 million Americans, in 24 major metropolitan areas across the country (AP, 2008). While detected concentrations were very low and health implications are far from well understood (Snyder et al., 2003; Snyder et al., 2005; Snyder et al., 2007, the concern about drugs in drinking water persists with utilities, consumers, regulators, and drinking water professionals in general. Congress has already called on the EPA to establish a national taskforce to study this issue further. While regulations covering pharmaceuticals and pesticides are not on the immediate horizon, utilities must begin to better understand the presence and removal of pharmaceuticals and pesticides from drinking water supplies.

The literature suggests that ozone-biofiltration may effectively remove some trace organic compounds (Snyder et al., 2003; Snyder et al., 2005; Al-Rifai et al., 2007; Guay et al., 2007; Snyder et al., 2007). Generally, ozonation alone may effectively treat compounds containing amine groups, phenolic groups, and unsaturated carbon structures. However, many other trace compounds are not removed effectively by ozonation. Snyder et al. (2007) summarized the removal of selected trace organics with ozone. Table 2-1 presents the summarized results.

Table 2-1. Summary of minimum % removal of selected pharmaceuticals and pesticides by ozonation at drinking water dosages (Snyder et al., 2007)

>95% removal	>80% removal	50-80% removal	20-50% removal	<20% removal
Acetaminophen	Androstenedione	DEET	Atrazine	TCEP
Carbamazepine	Caffeine	Diazepam	Iopromide	
Diclofenac	Pentoxifylline	Dilantin	Meprobamate	
Erythromycin-H_20	Progesterone	Ibuprofen		
Estradiol	Testosterone			
Estriol				
Estrone				
Ethynylestradiol				
Fluoxetine				
Gemfobrozil				
Hydrocodone				
Naproxen				
Oxybenzone				
Sulfamethoxazole				
Triclosan				
Trimethoprim				

Biofiltration is an effective post treatment for ozonation, as many pharmaceuticals and pesticides may be removed through biotransformation (Fuerhacker et al., 2001; Snyder et al., 2003; Snyder et al., 2005; Snyder et al., 2007; Al-Rifai et al., 2007; Guay et al., 2007). Snyder et al. (2007) summarized the removal of trace organics from ozone-biofiltration pilot testing. In general, the removals of the tested trace organic compounds were low. Table 2-2 presents a summary of the results.

Table 2-2. Summary of % removal of selected pharmaceuticals and pesticides by pilot biofilters (with ozone pretreatment) (Snyder et al., 2007)

>95% removal	>80% removal	50-80% removal	20-50% removal	<20% removal
Fluoxetine	Benzo[a]pyrene	Progesterone	Androstenedione	Acetaminophen
	DDT		Erythromycin-H_20	Atrazine*
			Fluorene	Caffeine
			Testosterone	Carbamazepine
			Triclosan	DEET
			Trimethoprim	Diazepam
				Diclofenac
				Dilantin
				Estradiol
				Estriol

*Contaminants detected (<1 µg/L) in City of Arlington finished waters

Convention Biofiltration Design Considerations

Flow Equalization

In general, water treatment processes operate the most effectively under uniform, steady hydraulic conditions. Research has shown that transient hydraulic and biodegradable organic matter (BOM) loading episodes can negatively impact biofiltration performance (Manem and Rittmann, 1992; Ahmad et al; 1998). Therefore, biofilters should be operated in a constant rate mode, and adding flow equalization capabilities upstream of biofilters might be considered if a given system often experiences wide swings in demand. Some degree of flow equalization can also be achieved by designing redundancy into a biofilter process. Filters can be taken in and out of service as necessary to buffer changes in hydraulic conditions caused by backwashing events, changes in demand, or filter repair. However, redundancy must be balanced by the resulting biofilter downtime schedule, as downtime can have a deleterious effect on biological activity (Niquette et al., 1998; Vokes, 2007). Other design features that can help minimize flow changes include: (1) increasing the size of filter-to-waste piping to allow operation at the capacity of an on-line filter, (2) installing automated controls and a filter-to-waste modulating valve to allow for a smooth transition when a filter is taken out of service for backwashing, and (3) adding a flow control valve to the filter waste washwater line.

Pre-treatment

Coagulation, flocculation, and sedimentation processes can greatly decrease the particle loading rate to biofilters, thereby increasing biofilter run times. Coagulant or filter aid polymer can also be added just upstream of a biofilter to minimize filtration turbidity removal ripening periods. Water utilities commonly couple biological filtration with

ozonation for improved water treatment performance (Krasner et al., 1993; Price et al., 1993; Westerhoff et al., 2005; Emelko et al., 2006; Wert et al., 2008). After ozone is dosed to the process, it immediately creates both molecular ozone (O_3) and hydroxyl radicals (*HO), both of which are strong oxidants. The O_3 and *HO oxidize complex, recalcitrant organic matter into lower molecular weight organic matter, rendering it more labile. Furthermore, the increased concentration of BOM from ozonation increases biomass in the filter, which can improve the degradation of trace organic contaminants (Dewaters and DiGiano, 1990; LeChevallier et al., 1992; Rittmann, 1995). The increase in BOM across an ozonation pretreatment step is a function of the ozone to TOC ratio (Juhna and Melin, 2006). Chlorination can also be used to increase BOM upstream of biofiltration, but a dechlorination step must be included to minimize the impact to the microbial community in the biofilters (LeChevallier et al., 1992; Zappia et al., 2007). Unlike free chlorine, ozone residual is consumed within a few minutes after application and poses little risk of microbial inactivation in the biofilter.

Media Selection

Biofilter granular media serves as both the particle screening mechanism and the biogrowth support structure. Biofilters are typically dual media with sand as the base layer and anthracite or GAC as the top layer. Anthracite/GAC selection drivers include cost, need for adsorptive capacity, and fluidization characteristics (Najm et al., 2005).

Another important consideration is the media's capacity to support biological activity. Media size selection plays an important role in the available surface area for biological attachment, which has implications for improving BOM degradation (Zhang and Huck, 1996). However, improved BOM degradation must be balanced with filter hydraulic performance when considering media size. Media type will also affect

available surface area. GAC has been shown to house three to eight times more biomass than does anthracite, which is likely due to the increased surface area available for microbial attachment (LeChevallier et al., 1992; Wang et al., 1995). It has been demonstrated that temperature, chlorinated backwash water, and transient hydraulic conditions can be detrimental to biofiltration performance (Manem and Rittman, 1992; Miltner et al., 1995; Ahmad et al., 1998; Moll et al., 1999; Andersson et al., 2001; Emelko et al., 2006). GAC may provide more robustness over anthracite under these challenging conditions due to high levels of biological activity (Emelko et al., 2006; Liu et al., 2001; Wang et al., 1995). Media hardness must also be considered when selecting media, as attrition is a larger concern for GAC than for anthracite.

Backwash System

An effective backwash system is critical to the successful hydraulic and water treatment performance of any biofilter. Therefore, careful consideration must be given to its design. Some studies have observed a reduction in BOM removal in biofilters over filter run time, suggesting that filter run time (backwash interval) may play an important role in biological treatment performance (Ahmad et al., 1998). Application of chlorine and chloramines to biofilter backwash has been shown to have detrimental effects to biofilter BOM and soluble Mn removal and should be avoided for optimal biofilter performance (Moll et al., 1999; Liu et al., 2001; Vokes, 2007). Chlorine application solubilizes Mn matrices present on the filter, as well as biofilm containing absorbed manganese. Other backwash design factors include source water, backwashing duration, backwashing rate, hydraulic loading rate, backwash wastewater handling, air scour, and surface wash.

Conventional Biofiltration Operation Considerations

Loading Rate and Contact Time

Together with the L/d (length [depth] of media/media particle diameter) criterion, hydraulic loading rate (HLR - flow rate applied per unit filter area) is a key design parameter for sizing conventional granular media filters. Excellent particulate filter effluent water quality can be achieved even at high HLRs (10 gpm/ft^2), though headloss may become a constraint (AWWA, 1999). The HLR and filter bed depth determine the empty-bed contact time (EBCT), which is considered a vital criterion for effective biofiltration (DeWaters and Digiano, 1990; LeChevallier et al., 1992; Zhang and Huck, 1996b; Urfer et al., 1997; Carlson and Amy, 1998; Huck et al., 2000; Westerhoff et al., 2005). The selection of a design EBCT is dependent on hydraulic constraints and ultimate water quality objectives. Studies have shown that 90% of biofilter influent BOM may be removed with a design EBCT of 10-20 min (Provost et al., 1995). EBCT is particularly important for biofilter applications targeting trace organic compounds, such as MIB, geosmin, endocrine disruptors, and pharmaceutically active compounds, which are more recalcitrant to biodegradation than is typical BOM (Westerhoff et al., 2005; Brown, 2006; Lim et al., 2008).

Acclimation Procedures

One consideration for any biological process is the amount of time required to reach steady-state biological activity. This is a concern both during the initial start-up and after any process upsets or downtime, as significant breakthrough of organic compounds may occur. Liu et al. (2001) found that 20 to 40 days was required to reach steady state biofilter removal of easily degradable BOM at 20°C. If GAC is used as biofilter support media, most of the initial DOC removal occurs through physical

adsorption while the bacteria in the associated biofilm are acclimating. This synergism may last approximately 2 to 3 months until the GAC is exhausted and/or the biological activity has reached steady state (Servais et al., 1994). The elimination of chlorine in the backwash water may decrease acclimation time and improve the general robustness of biofilter, particularly at low temperatures. When targeting trace organic compounds such as MIB and geosmin, acclimation periods can be several months (Westerhoff et al., 2005; Chae et al., 2006). This suggests that acclimation may promote an increase in the microbial populations involved in trace organic degradation. Seeding virgin biofilters with biological media that have already been acclimated to the biodegradation of MIB and geosmin may reduce start-up times (Westerhoff et al., 2005), although it remains unknown whether the acclimation is to MIB and geosmin (secondary substrates) or to the background NOM (primary substrates). Nutrient and/or primary substrate addition also have the potential to decrease biological acclimation time due to increased rates of cell growth (Hozalski and Bouwer, 2001; Lauderdale and Brown, 2007). Lastly, minimizing biofilter downtime is important in maintaining sufficient biological activity (Niquette et al., 1998; Vokes, 2007). Thus, an appropriate cycling of “stand-by” and “duty” biofilters must be established.

Backwash Protocol

A common practice to control growth in biofilters is backwashing. Numerous backwashing criteria can be considered, including frequency, air scour, surface wash, backwash rate, duration, % bed expansion, addition of chlorine, and pulsing. Removing bound organic/inorganic substances helps maintain uniform hydraulic conditions and controls the headloss across the biofilter bed. Backwashing removes considerable BOM and inactive microorganisms from the biofilter (Brown, 2006) and decreases the local

nutrient concentration around the microbial biofilm by using post-filtered water. Therefore, backwashing exposes the biofilm to a potential adverse environmental condition with limited nutrient availability (Simpson, 2008). Although a fraction of the active bacterial biomass fixed on GAC may also be eliminated during backwashing (Dussert and Van Stone, 1994; Kim and Logan, 2000), the biofilm is generally very resistant to the high shear stress rates that are directed onto the GAC surface (Scholz and Martin, 1997). However, over-aggressive backwashing (intensity or frequency) can leave insufficient biological activity to meet treatment objectives, can lead to BOM breakthrough, and can hamper the stability of final disinfectants. Substantial research has been conducted to evaluate the impacts of various backwashing criteria on biological treatment performance (Miltner et al., 1995; Wang et al., 1995; Urfer et al., 1997; Emelko et al., 2006; Huck et al., 1998; Ahmad et al., 1998; Choi et al., 2007; Vokes, 2007; Brown, 2007b; Marda et al., 2008).

In the water treatment industry, biofilter backwash frequency may range from less than 12 hours to more than 48 hours. Backwash episodes are typically triggered by high headloss across the biofilter, biofilter run times, or turbidity breakthrough. As stated previously, biofilm growth may also be controlled through application of chlorine/chloramines to backwash water. However, this practice may inhibit optimal biological treatment performance through the inactivation of viable organisms. Indeed, the presence of chlorine/chloramines in biofilters may result in both a decrease in net biological activity and a shift in microbial communities to organisms that may disrupt distribution stability. Norton and LeChevallier (2000) found an increased occurrence of gram-positive organisms in biofilters regularly conditioned with chlorine application.

Gram-positive organisms have a thick peptidoglycan layer in the cell wall (relative to gram-negative bacteria) that may increase resistance to final disinfection (Norton and LeChevallier, 2000; Madigan et al., 2009). In addition, the application of chloramines may select for chlorine-resistant nitrifying bacteria (Eichler et al., 2006).

Engineered Biofiltration Operational Considerations

Primary Substrate Augmentation

By mediating the transfer of electrons between electron donor substrates and electron acceptor substrates, microorganisms gain the energy they need to grow and maintain cell metabolism. Primary substrates provide energy during cell metabolism. As described by Monod in the 1940s, the rate of cell synthesis is proportional to the concentration of the rate-limiting primary substrate (i.e., concentration of limiting electron donor or electron acceptor), the cell yield, and the maximum specific primary substrate utilization rate. Equation 2-1 below further describes Monod's equation.

$$\mu = \hat{\mu}\frac{S}{K_s + S} \qquad (2\text{-}1)$$

Where:

μ: specific growth rate

$\hat{\mu}$: maximum growth rate

S: concentration of limiting substrate (electron donor or acceptor)

K_s: half-saturation coefficient for the limiting substrate (electron donor or acceptor)

The minimum concentration of primary substrate that can support steady-state biomass is known as S_{min}. When the rate-limiting primary substrate concentration equals S_{min}, the rate of cell synthesis equals the rate of cell decay. Any substrate that is present below its S_{min} concentration is known as a secondary substrate. Though

secondary substrates can be biodegraded, bacteria gain little to no energy in doing so, which means that a primary substrate must be biodegraded simultaneously if any biodegradation of the secondary substrate is to be achieved (Kobayashi and Rittmann, 1982; Stratton et al., 1983; Namkung and Rittmann, 1987a, 1987b). The rate of secondary substrate degradation is proportional to the concentration of active biomass present, which is a function of the concentration of primary substrate, and other variables. The kinetics of secondary substrate degradation is driven by Equation 2-2:

$$r_{ut} = -\frac{\hat{q}_s S_s}{K_s + S_s} X \qquad (2\text{-}2)$$

Where:

r_{ut} : the rate of utilization of a secondary substrate ($mg_{(secondary\ substrate)}\ cm^{-3}h^{-1}$)

S_s : Concentration of the secondary substrate ($mg_s\ cm^{-3}$)

$\hat{q}_s$: maximum specific rate of secondary substrate utilization ($mgs\ mg_x^{-1}h^{-1}$)

K_s : half-maximum rate constant for the secondary substrate ($mg_s\ cm^{-3}$)

X : concentration of active bacteria ($mg_/\ cm^{-3}$)

Because trace organic compounds like MIB, geosmin, endocrine disruptors (EDCs), and pharmaceutically active compounds are present at ng/L or µg/L concentrations in most natural waters, they likely are biodegraded as secondary substrates. Thus, biological treatment processes designed to biodegrade these compounds require the presence of a primary substrate (Lauderdale et al., 2007). Increasing the concentration of primary substrate may increase the rate of trace organic compound degradation (Lim et al., 2008), decrease bioacclimation time requirements (where bioacclimation is the time required after startup for a biofilter to reach steady-state treatment performance) and enhance the robustness of the system during process

upsets (Lauderdale and Brown, 2007). It can also enhance the removal rate of slowly degradable organic material (Hozalski and Bouwer, 2001). Primary substrate augmentation is most commonly conducted in biofilters with ozone pretreatment. Ozonation breaks large natural organic molecules into smaller, more readily biodegradable organic molecules (Becker and O'Melia, 1996), thereby increasing the concentration of primary substrate. Thus, adjusting the ozone dose may improve the rate of both primary substrate (i.e., macro-organic carbon that contributes to biologically degradable organic carbon [BDOC], DBP formation potential, and chloramine demand) and secondary substrate (e.g., MIB, geosmin, EDC removal) degradation.

Nutrient Augmentation

The role of EPS in microbial systems

Nutrients play an important role in the microbial production and secretion of EPS (Liu et al., 2006), which form an adhesive gelatinous matrix comprising mostly insoluble exopolysaccharides. EPS may also consist of proteins, nucleic acids, lipids, and humic substances (Flemming et al., 2000). The presence of EPS can be observed through microscopy as a rough, matted material or as filamentous strands (Flemming and Wingender, 2001). EPS is an essential component of biofilms, as it promotes surface attachment and maintains structural stability. The EPS matrix creates the biofilm (or flocculant) that provides a competitive advantage to all organisms present. EPS in biofilm drives the surface charge, hydrophobicity, and noncovalent interactions of microbial cells (Flemming et al., 2000). Bacteria held within a biofilm may establish and maintain their position over a long period of time, compared to those that are planktonic. Biofilm EPS also provides bacteria with protection from xenobiotics, high/low pH, oxidants, shear, and other forms of stress for the associated microbial community

(Sutherland, 2001; Mauclaire et al., 2004; Wang et al., 2008). Indeed, the stimulation of expression of EPS in biofilms is affected greatly by these environmental parameters and the availability of nutrients (Sutherland 2001; Liu et al., 2006; Fang et al., 2009).Studies have shown that the amount of EPS synthesis within a biofilm is dependent on the (1) availability of carbon substrates, and (2) balance between those substrates and other nutrients, such as nitrogen (ammonia) and phosphorus (ortho-phosphate) (Sutherland, 1977; Sutherland, 2001; Priester, et al., 2006; Fang et al., 2009). Indeed, the production of EPS serves as a competitive advantage in low nutrient conditions as it may help sequester nutrients and other factors necessary for microbial growth (Decho, 1990).

EPS-related challenges in water treatment

EPS may be the primary contributor to biofilter clogging. In a study by Mauclaire et al. (2004), biological material reduced the pore space of a clogged biofilter by 7 to 13%, whereas pore space reduction due to particle deposition was not greater than 7%. The volume of microbial cells did not exceed 0.2% of the pore volume, indicating that extracellular biological materials play a significantly larger role in filter clogging than do the microbial cells themselves. In the Mauclaire et al. (2004) study, filter clogging was defined as a condition where hydraulic conductivity is reduced by a factor of 4. Other studies have suggested that the proportion of EPS in biofilms can be as high as 90% of the total organic matter (Christensen and Characklis, 1990; Nielsen et al., 1997). EPS also accelerates the sequestration of minerals into the biofilm, which may also adversely affect the biofilter production and further increase clogging (Ali et al., 1985; Rinck-Pfeiffer et al., 2000). One study has shown that calcium and other mineral precipitation can occur preferentially in EPS matrices, via proton exchange and/or sequestion of suspended precipitates and subsequent crystallization of dissolved

species, leading to deposits with remarkable mechanical stability (Flemming and Wingender, 2001). EPS may also cause microbial influenced corrosion (Flemming and Wingender, 2001b). The interaction and sequestration of Fe and other metals in EPS present in distribution pipes can increase the kinetics of metal corrosion (Beech et al., 1997). Indeed, high levels of EPS have been found on copper surfaces creating significant corrosion; it is possible that excess amounts of EPS provide microorganisms a protective mechanism against the toxicity of copper (Flemming and Wingender, 2001b).

Nutrient limitations can be determined based on the relative concentrations of carbon and nutrients in the biofilter feed. The empirical formula for a bacterial cell is $C_{55}H_{77}O_{22}N_{11}P$ (Metcalf and Eddy, 2002), indicating that a stoichiometric C:N:P molar ratio of approximately 55:11:1 is required to avoid a nutrient-limited condition. However, nitrogen and phosphorus are more conservative than carbon substrate in an aerobic biological system, which may oxidize to carbon dioxide. In environmental systems, C:N:P ratios as low as 100:10:1 have been shown to eliminate microbial stress and promote optimal growth (USEPA, 1991; LeChevallier et al., 1991). The 100:10:1 C:N:P stoichiometric ratio is equivalent to a concentration ratio of 1 mg/L bioavailable carbon substrate: 0.117 mg/L NH_4-N: 0.026 mg/L PO_4-P. Nitrate (NO_3^--N) may also serve as an inorganic source of nitrogen in aerobic environments (Madigan et al., 2009). However, NH_4-N can be incorporated directly into amino acids by most bacteria, without requiring intracellular reduction (Fenchel et al., 2000). Nutrient limitations are an important consideration for most drinking water biofilters due to the common pretreatment processes of coagulation and sedimentation. Common coagulants such as aluminum

phosphate, polyaluminum phosphate, and ferric chloride are extremely effective at removing phosphorus (Tchobanoglous et al., 1991, WEF, 1998). Biofilters downstream of these processes will likely receive feed waters that have phosphorus concentrations below 0.01 mg/L (Nishijima et al., 1997; WEF, 1998), thereby creating a phosphorus-limited condition if the concentration of biodegradable organic substrates is greater than 0.38 mg/L.

Nutrient supplementation may have a two-fold benefit to water utilities operating biofilters under nutrient limited conditions: (1) it may enhance biofilter biological activity, both increased biomass and substrate utilization, which could lead to improved water treatment performance, and (2) it may inhibit EPS production (Nishijima et al., 1997; Sang et al., 2003; Ryu et al., 2004; Brown et al., 2009; Li et al., 2009), which may improve biofilter hydraulic performance. The transition from a phosphorus/nitrogen limitation to a biodegradable carbon limitation is believed to be the driver behind these benefits. As the carbon substrate becomes limited with nutrient supplementation, microbial cells may utilize more carbon for cell synthesis and energy production instead of producing the EPS (Mauclaire et al., 2004). Studies have shown that nutrient supplementation can increase microbial concentrations and may enhance the ability of microorganisms to degrade contaminants (Juhna and Rubulis, 2004). Furthermore, the decreased biofilter EPS creates a biofilm structure that may be more susceptible for removal through filter backwashing.

Oxidant Augmentation with Peroxide

Another approach for enhancing biological activity and performance in a biofilter is through oxidant augmentation with peroxide. Hydrogen peroxide provides microorganisms with an additional source of dissolved oxygen and induces certain

microbial communities in the bioreactor to express peroxidase-family oxidoreductase enzymes such as peroxidase that catalyze the oxidation of organics present in the biofilter feed (Pardieck et al., 1992). Indeed, a wide variety carbon substrates are oxidized in microbial cells by peroxidase family oxidoreductase enzymes (Schumb et al., 1955). Peroxidase-catalyzed transformation of aromatic compounds, such as phenols, amines, and polyphenols, from wastewaters was first reported by Klibanov et al. (1980) and has since received much attention. When peroxidase and hydrogen peroxide are added to water containing these compounds, the enzyme undergoes a catalytic cycle that leads to the release of free radicals. These radicals undergo various reactions. Free radicals may participate in the oxidation of recalcitrant organic compounds. Auriol et al. (2007) demonstrated that four endocrine-disrupting compounds can be oxidized using horseradish peroxidase. Based on this evidence, the peroxidase enzymes naturally present within microbial systems may catalyze reactions between peroxide and organic substrates forming free radicals that lead to the removal of TOC, MIB, geosmin, and EDCs. Furthermore, compounds that are not readily available substrates to the enzyme may be oxidized through radical transfer.

In addition, studies suggest that low dosages of hydrogen peroxide (<1 mg/L) may effectively oxidize and remove inactive biomass and EPS, while preserving biological activity (Christensen et al., 1990; Neyens et al., 2002). EPS oxidative reductive depolymerization results in cleavage of linkages in the polymeric backbone (Christensen et al., 1990). This reaction involves a series of free radical reactions, which ultimately lead the cleavage of polymer chains and efficient removal. The mechanism for this action may be driven through Fenton’s reaction (ferrous catalysts). However,

Fenton's reaction may be limited in most drinking water biofilter applications, as efficiency drops significantly (first order, k = >1 min^{-1}) at pH higher than 6. The drop in efficiency at higher pH is attributed to the transition of Fe from a hydrated ferrous ion to a colloidal ferric species. Colloidal ferric catalytically decomposes the hydrogen peroxide into oxygen and water, without forming hydroxyl radicals. Fenton's reaction is provided in Equation 2-3

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^*OH + OH^- \qquad (2\text{-}3)$$

EPS may also be degraded through direct oxidation by hydrogen peroxide or by free radicals produced through oxdioreductive enzyme activation. Direct oxidation of polysaccharides by peroxide may occur at conditions feasible in drinking water biofilters (neutral pH, 30°C, no catalyst, low hydrogen peroxide concentrations) (Miller, 1986).

One of the major considerations for using hydrogen peroxide as a catalyst for enzyme stimulation is its inherent toxicity toward microorganisms. Hydrogen peroxide concentrations in the 10 to 1000 mg/L range have been found to inhibit bacterial growth at room temperatures, while higher concentrations destroy the organisms (Schumb et al., 1955). However, a study by Urfer and Huck (1999) showed that hydrogen peroxide residuals of less than 1 mg/L did not lead to an appreciable inhibition of substrate utilization in a biological filter. The oxidoreductase enzymes present in some bacteria serve as intracellular defense systems against free radicals. Peroxides may operate within a biofilm, degrading EPS, while sparing active microorganisms that can express these enzymes (Christensen et al., 1990).

Therefore, hydrogen peroxide supplementation may be an effective approach to improve water treatment and hydraulic performance in a biofilter.

Monitoring Techniques

Tracking the operation and health of a given biofiltration process enables operators to recognize and anticipate performance problems and subsequently implement the necessary corrective actions. There are numerous monitoring tools available, and they can be divided into those that provide feedback in real-time and those that require more time to provide feedback.

Real-Time Monitoring Tools

Water quality analyses

In-line turbidimeters can be used to (1) demonstrate compliance with turbidity regulations, (2) help identify appropriate run times, backwash strategies, and ripening periods, and (3) reveal hydraulic anomalies such as short-circuiting. In-line particle counters can be added to characterize particle breakthrough as a function of size (Goldgrabe et al., 1993). DO can be measured in-line, and since bacteria couple the oxidation of BOM with the reduction of DO, it can be used as an indirect indicator of biological activity (Huck et al., 2000). A change in the DO consumption across the biofilter may indicate that (1) the BOM loading has changed, (2) activity in the microbial community has changed, and/or (3) biofilter hydraulics have been altered. UV_{254} (UV light absorbance at a wavelength of 254 nm) can also be measured in-line or using a bench-top spectrophotometer and can serve as a surrogate for TOC concentration (Howe and Clark, 2002). Thus, to some extent, organic carbon removal and breakthrough can be monitored in near-real-time. A correlation must be developed between UV_{254} and TOC concentration at a given site for this tool to provide meaningful data.

Headloss tracking

As particles accumulate and bacteria grow over the course of a run, headloss builds across a biofilter, which can be measured using pressure transmitters or a piezometric method. Changes in headloss trends within a single run or over long operational periods may indicate (1) a change in particle or BOM loading, (2) deficiencies in the backwash protocol, (3) short-circuiting or other hydraulic anomalies, (4) changes in the effective size of the granular media (e.g., caused by abrasion), or (5) changes in microbial activity. Therefore, tracking of headloss trends can reveal significant insights into the overall effectiveness and microbial health of a biofiltration process and can be used to anticipate changes in treatment performance.

ATP analysis

Being able to accurately quantify biological activity is critical for characterizing the health of any biofilter process. One means of achieving a real-time assessment of biological activity is by measuring the quantity of ATP present in the biofilm. As the primary energy carrier for all living organisms, ATP provides an indication of biomass growth rate, substrate removal rate, and physiological state of the bacteria (Nouvion et al., 1987). Active biomass concentration generally matches BOM removal (Liu et al., 2001). Most biological activity measurements (see below) require hours to days for analysis. On the other hand, methods have been developed for ATP measurements that require only a few minutes (LuminUltra, 2008). The method is simple, fast (15 minutes), and reproducible. ATP is measured using the firefly luciferase assay, wherein a sample containing ATP is introduced to a mixture containing luciferins and the enzyme luciferase. The luciferase catalyzes a reaction between ATP, DO, and luciferins in the sample to produce light that can be detected in a luminometer as Relative Light Units

(RLU). The RLU produced correlates with the quantity of ATP in the sample based on the following relationship:

$$ATP + O_2 + luciferin \xrightarrow[luciferase]{Mg++} AMP + PPi + oxyluciferin + light \quad (2\text{-}2)$$

Where:

ATP = Adenosine Triphosphate

AMP = Adenosine Monophosphate

PPi = Pyrophosphate

Mg^{2+} = Magnesium ion

As shown in Equation 2-2, chemical energy produced from the breakdown of ATP is converted into light. Each molecule of ATP consumed in the reaction produces one photon of light (Greer and Szalay, 2002).

Non-Real-Time Monitoring Tools

Water quality analyses

A variety of water quality parameters can be routinely monitored as grab samples across a biofiltration process to assess microbial health and performance, including TOC, DOC, assimilable organic carbon (AOC), BDOC, and heterotrophic plate counts (HPCs). Simulated distribution system disinfection by-product (SDSDBP) tests and DBPFP tests can also be used to reveal organic carbon breakthrough from biofilters. All these analyses are typically performed in a laboratory and require hours to days for processing.

Annular reactor study

Distribution system biological regrowth potential, which can be assessed indirectly by DOC, BDOC, and AOC data, can be measured directly using bench-top annular

reactors (Volk and LeChevallier, 1999). Annular reactors consist of a rotor inside a stationary outer cylinder that can simulate detention times, shear stresses, and water velocities typical of drinking water distribution systems. The reactors allow the collection of both water samples and coupons (made of distribution system pipe material) from which biofilm growth may be determined and analyzed.

Filter coring

Depthwise cores of a given biofilter can be extracted using any number of filter coring devices. Analyses of filter coring data can provide abundant biofilter system health and performance data. Visual observation of a core will detect clumping or mudballing, indicating a deficiency in the backwash process. Media samples can then be taken for microbial community analyses, which can be monitored over time and correlated to system performance. Floc retention profiles can be developed for each cored biofilter, before and after backwashing, to reveal the distribution of particles and biomass across a given bed and assess backwash effectiveness. Sieve analysis of a biofilter core will reveal media intermixing, attrition/degradation, and will provide accurate L/d values for comparison against the design criteria. Lastly, SEM microscopic examination of biofilter core sections can reveal key information related to elemental (e.g., Fe and Mn) and cell morphologies and distribution.

Biological activity analyses

Quantifying and tracking biological activity can be used not only to assess the health of a biofilter process but also to anticipate and correct performance deficiencies before they become substantial. Non-real-time biological activity measurement methods include phospholipids analysis to quantify and characterize active cell biomass (Liu et al., 2000), tetrazolium reduction assays to evaluate cellular activity, via colorimetric

measurement of reduced tetrazolium(Fonseca et al., 2001), and nucleic acid based activity methods, including the determination of RNA/DNA ratios (Chícharo and Chícharo, 2008), quantification of precursor rRNA levels (Oerther, 2000), and evaluation of transcriptional activity by quantifying messenger RNA (mRNA) levels for housekeeping genes using reverse transcription quantitative polymerase chain reaction (RT-qPCR) (Nielsen and Boye, 2005). All of these methods require specialized instrumentation, are typically performed in a laboratory, and require hours to days for processing

Microbial community analysis

To better understand how microorganisms contribute to contaminant removal, it is important to identify the key microbial populations present in biofilters and link microbial population dynamics to operational performance measures. Microbial community composition can be characterized by constructing clone libraries of the small subunit rRNA genes or selected functional genes in biomass samples, DNA sequencing, and phylogenetic analyses (Briones et al., 2007). Microbial population dynamics and quantification of population abundance in different niches in biofilters can be determined using real-time qPCR.

Microscopy

Biofilter cores can be analyzed using SEM and transmission electron microscopy (TEM). SEM can be performed under low voltage, low vacuum conditions to minimize sample disruption. SEM provides surface images down to the 0.5-µm scale and can be used to assess biofilm structure, identify the presence of protozoa, and assess the elemental composition of filter-associated particles. TEM, which provides images down to the 20-nm scale, can evaluate sectional layers of biofilm, identify the presence of

viruses, and accurately quantify biofilm thickness - a key input parameter to most biofilm models. To determine the spatial distribution of specific bacterial populations, fluorescence in situ hybridization (FISH) targeting the small subunit rRNA can be used in combination with confocal laser scanning microscopy, to obtain information on possible niche differentiation of microbial populations (Amann and Fuchs, 2008).

Post-treatment analysis

In addition to the water quality analyses discussed above, it is important to evaluate the potential of microbial contamination of finished drinking water. Therefore, the levels of bacteria in biofilter effluents, including soon after backwashing, need to be determined. HPCs are commonly used for this, given the USEPA Surface Water Treatment Rule for finished drinking water (i.e., less than 500 colony forming units (CFU)/mL). Biofilter effluents have may have HPC counts comparable to those in surface water sources (5×10^6 in a Mississippi River sample (Norton and LeChevallier, 2000). Therefore, it is important to study inactivation kinetics of mixed communities of microorganisms present in biofilter effluents to better assess disinfection methods. With a few exceptions (Pernitsky et al., 1995), most research efforts dealing with inactivation kinetics have used pure cultures of microorganisms and many of these studies are performed at conditions dissimilar to those commonly found in the drinking water field, such as the study performed by Berry et al. (2008). It will also be important to expand inactivation kinetics analyses beyond simple HPC based evaluations (Pernitsky et al., 1995) for evaluation of biological safety.

CHAPTER 3
MATERIALS AND METHODS

Background

The purpose of this chapter is to provide a summary of the materials and methods that were followed to perform this work. The chapter includes 1) a broad overview of the pilot system and support equipment that were employed to evaluate the baseline biofilter performance and various enhancement strategies, 2) the followed experimental design, and 3) the analytical methods that were used for the hydraulic, water quality, and microbial characterizations.

Pilot Biofiltration System

Pilot Biofiltration System

The pilot skid (Intuitech, Salt Lake City, Utah) included four parallel 6-indiameter biofilters. The biofilters were operated as a closed (pressurized) system. Each biofilter contained the same sample media configuration as the full-scale filters (40 in of GAC on top of 8 in of sand). Three pilot biofilters contained Norit® GAC 820 (effective size 1.1 mm) (Marshall, TX) and sand (effective size 0.55 mm) media from the full-scale biofilters at the JKWTP. These media were collected from a full-scale biofilter (Filter No. 2) using shovels, with an attempt to obtain a homogenized sample of each medium. The remaining pilot biofilter media configuration included 40 in of virgin MeadWestvaco Bionuchar GAC (Richmond, VA) and 8 in of sand (effective size 0.55 mm). The media in each pilot biofilter was supported by a Leopold IMS® cap and S-type underdrain system (Zelienople, PA) Each biofilter had an independent influent pump with automatic flow control. A polyethylene 150-gallon effluent break tank served as a backwash water supply. The backwash system also included a dedicated pump and air scour system.

Pilot instrumentation included an inline effluent turbidimeter (1720C, HACH, Loveland, CO), flow transmitter (3-2551-PO-12, GF Signet, El Monte, CA), and piezometric sensor for each biofilters to measure headloss. Pilot equipment and instrumentation were monitored and controlled by an HMI (Human Machine Interface) that communicated with a small programmable logic controller (PLC) in the control panel. Other features included automatic data logging of key parameters (flow, turbidity, and headloss), remote monitoring, and control using a standard web browser, and email and text message alarm notifications.

General Process and Control

A portion of the full-scale biofilter feed water (post-intermediate ozone) was pumped to a 525-gallon break tank located in the chemical room at the JKWTP. Flow from the break tank was then pumped to the pilot skid by the four independent feed pumps. The four biofilters were operated under full-scale average loading conditions (4.5 gpm/ft^2), except during selected optimization and robustness tests. Biofilter effluent was gravity fed to the effluent break tank before being discharged to the sanitary sewer. The pilot was operated in an automated mode. Parameters such as loading rate, backwashing protocol, and backwash triggers were controlled and selected through the PLC. Backwashing was initiated by the operator in the manual mode, or on runtime, headloss, or effluent turbidity triggers (Table 3-1) in the automatic mode. Only one filter could be backwashed at a time. Figure 3-1 provides a conceptual process flow schematic of the pilot biofiltration system.

Supplemental Chemical Dosing

Three chemical dosing modules were used during enhancement studies to provide supplemental chemicals to pilot biofilters. Each module consisted of a diaphragm

metering feed pump (Grundfos City, PA) and 10-L tank. Chemical dosing modules could be flow paced to individual pilot biofilters.

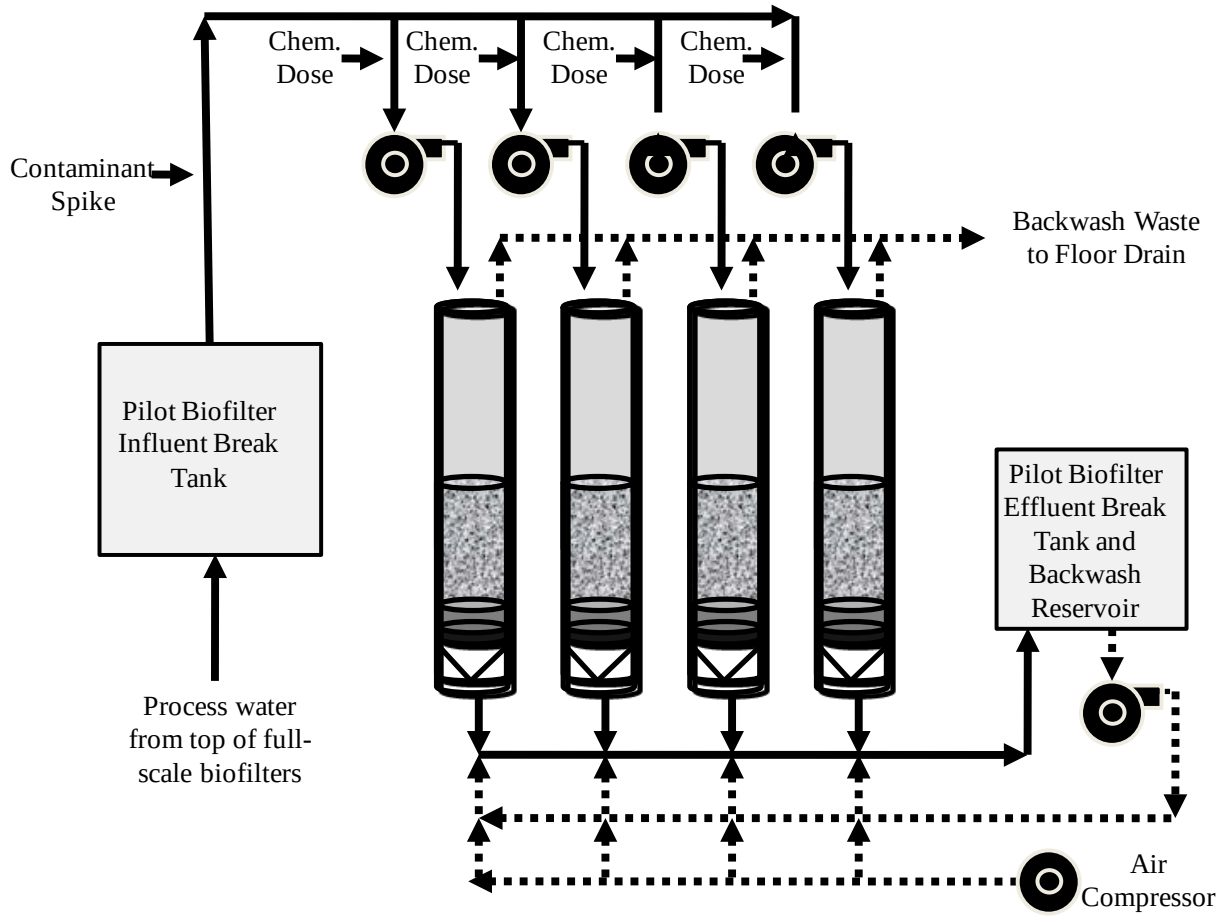

Figure 3-1. Process flow schematic for pilot biofiltration system

Contaminant Spiking

A peristaltic pump (Masterflex, Vernon Hills, IL) was used to spike contaminants from a 40-L chemical tank to a feed point upstream of the biofiltration skid. A static mixer was installed downstream of the injection point to promote mixing before flow was diverted to individual pilot biofilters.

Biofilter Augmentation and Contaminant Feed Chemicals

Caffeine and carbamazepine as neat chemicals and MIB and geosmin stock solutions in methanol were purchased from Sigma Chemical Company (St. Louis, MO).

All other chemicals used for contaminant spiking were reagent grade or better. Phosphorus supplementation was performed using National Sanitation Foundation International (NSF) certified 83% phosphoric acid. Ammonia supplementation was performed using reagent grade ammonium chloride. The supplemental substrates used in the substrate enhanced biofiltration studies included NSF-certified glacial acetic acid, beverage grade 95% ethanol, Food and Drug Administration (FDA) certified food grade molasses, and a high grade glycerin product, MicroCglycerin™ from Environmental Operating Solutions (Bourne, MA). Food grade, 3% hydrogen peroxide was used for the oxidant enhancement studies. A Water & Power Technologies reverse osmosis system (Arlington, TX) was used to deionize all water used for stock solutions of contaminants and supplements. Stock solutions were made to provide approximately 1 week of chemical feed before tank switch out (under average flow and dosage conditions). The target stock concentrations for hydrogen peroxide and all C substrates was 0.13% (w/v). The stock concentrations for PO_4-P and NH_4-N were 0.0025% (w/v) and 0.014% (w/v), respectively.

Backwash Protocol

All biofilters were operated at an 18-hour filter run interval, and a uniform backwash strategy was used. Maintaining a consistent run time and backwash strategy allowed meaningful hydraulic performance comparisons between operational conditions. The pilot biofilter run time and backwash strategies were modified from current full-scale operation to obtain consistent hydraulic performance. The full-scale biofilter backwashing protocols led to excessive media attrition when implemented on the pilot biofilters. The effect was believed to be inherent to hydraulic and mechanical limitations of the scaled-down pilot design. A backwash strategy was found to provide

acceptable clean-bed headloss, consistent headloss profiles, and limited media attrition for both control and enhanced test conditions. Attrition was limited through by extending the air-scour step to provide additional particle/media collisions for mudball destruction. Table 3-1 provides a summary of backwash protocols for the pilot- and full-scale biofilters.

Table 3-1. Backwash protocols

	Pilot-scale biofilters*	Full-scale biofilters
Backwash triggers		
Filter run time (hr)	18	24†
Headloss (ft)	13.5‡	18
Turbidity (NTU)	1.00	1.00
Backwash parameters		
Air Scour		
Rate	3 scfm	3 scfm
Duration (min)	10	5
Air scour/low rate combined backwash		
Duration (min)	3	~1
High rate backwash		
Rate (gpm/ft^2)	30.5	18
Duration (min)	8	20
Low rate backwash		
Rate (gpm/ft^2)	10	6
Duration (min)	5	5

* Pilot backwash protocol was developed iteratively over first two weeks of pilot testing to minimize clean-bed headloss, media attrition, backwash duration, and wastewater production.
† Plant staff manually initiates backwash every 18 to 24 hours if other conditions are not met first.
‡ Pilot headloss is limited by influent feed pump capacity.

Experimental Design

Research Testing Plan

The research-testing plan included multiple studies to characterize the hydraulic and water treatment performance at the JKWTP (full-scale and pilot-scale biofilters under control conditions) and to identify and evaluate potential improvements through modifications of the biofiltration process at the pilot scale. The research studies included:

- Baseline Characterization
- Substrate- Enhancement Evaluation
- Nutrient-Enhancement Evaluation
- Peroxide Enhancement Evaluation
- Microbial Tracking
- Full-Scale Process Integration Assessment and Economic Evaluation.

Operational Setup

Multiple research studies were conducted simultaneously as the four pilot biofilters were operated in parallel. A summary of the operational objectives targeted by each pilot biofilter is provided in Table 3-1.

Sampling Plan

Water quality and biofilter media samples were collected from pilot and full-scale systems during each study. The sampling plan provided in Table 3-2 provides an approximate summary of the samples taken at each process point for various analyses.

Baseline Biofiltration Characterization

The objective of this study was to characterize baseline water treatment performance, confirming similitude between pilot- and full-scale treatments, and to provide control data for parallel tests occurring with other pilot filters. Pilot system similitude to full-scale performance was characterized by operating the pilot under full-scale conditions and monitoring hydraulic performance and effluent water quality. The baseline performance characterization study was conducted by operating the pilot control biofilter under full-scale operational conditions at steady state for five months. The control was operated continuously with no carbon, nutrient, or hydrogen peroxide supplementation.

Biofiltration Substrate- Enhancement Evaluation

The objective of this study was to evaluate various primary substrate augmentation strategies for enhancing biofiltration performance. This study evaluated four substrates for improved biodegradation of organic carbon (trace organic compounds and background DOC). The substrates tested included acetic acid, molasses, MicroC© (a proprietary glycerin-based product), and ethanol. The substrates were tested individually through four successive 1- to 2-month phases, each dosed at 1 mg/L as carbon (C). Biofiltration substrate-enhancement was evaluated by monitoring changes in biofilm appearance, hydraulic and water treatment performance, and microbial activity.

Table 3-2. Pilot biofilter operational parameters

Parameter	Biofilter 1	Biofilter 2	Biofilter 3	Biofilter 4
Pilot biofilter operational objective (duration)	Study 2: substrate enhancement (6 months) Study 3: nutrient enhancement (1 month) Study 4: Peroxide enhancement (2 weeks)	Studies 2 and 3: substrate and nutrient enhancement (9 months)	Study 1: Full-scale simulation for baseline characterization with different GAC support media (9 months)	Study 1: Full-scale simulation for baseline characterization (5 months steady state, 2 months robustness testing) Validation of Study 3: nutrient enhancement (2 weeks)
Media configuration	8-inches sand 40-inches bituminous-based GAC obtained from JKWTP biofilter	8-inches sand 40-inches bituminous-based GAC obtained from JKWTP biofilter	8-inches sand 40-inches “virgin” wood-based based GAC	8-inches sand 40-inches bituminous-based GAC obtained from JKWTP biofilter

Table 3-3. Approximate sampling schedule for routine analyses

Parameter	Sampling location	Sampling frequency[*,†]
Turbidity[‡]	Full-scale biofilter influent	7/week
	Full-scale biofilter effluent	7/week
	Pilot biofilter influent	1/ week
	Pilot BF effluent	Continuously (every 5 min/biofilter)
pH[‡]	Full scale biofilter influent	7/week
	Full-scale biofilter effluent	7/week
	Pilot biofilter influent	1/week/biofilter
	Pilot biofilter effluent	1/week/biofilter
Temperature[‡]	Full-scale raw water	7/week
Dissolved oxygen	Full-scale biofilter influent	1/month
	Full-scale biofilter effluent	1/month
	Pilot biofilter influent	1/week/biofilter
	Pilot biofilter effluent	1/week/biofilter
Ozone residual[‡]	Full-scale ozone contactor cell 8	7/week
	Full-scale biofilter influent	7/week
Total coliforms, fecal coliforms,	Full-scale biofilter effluent	3/study
heterotrophic plate count	Pilot biofilter effluent	3/study
DOC	Raw water	1/week
	Full-scale filter influent	1/week
	Pilot biofilter influent	2/week
	Pilot biofilter effluent	2/week/biofilter
Ortho-phosphate and ammonia-	Full-scale biofilter influent	1/week
nitrogen	Full-Scale biofilter effluent	1/week
	Pilot biofilter influent	2/week
	Pilot biofilter effluent	2/week/biofilter
Iron and manganese	Raw water	1/week
	Ozone influent	1/week
	Pilot biofilter influent	2/week
	Pilot biofilter effluent	2/week/biofilter
MIB and geosmin	Raw water	1/week
	Ozone influent	1/week
	Pilot biofilter influent	2/week
	Pilot biofilter effluent	2/week/biofilter
Pesticide and pharmaceutical	Raw water	4/study
suite	Ozone influent	4/study
	Full-scale biofilter influent	4/study
	Full-scale biofilter effluent	4/study
	Pilot biofilter influent	1/month
	Pilot biofilter effluent	1/month/biofilter
Adenosine triphosphate	Full-scale biofilter GAC	4/study
	Pilot biofilter GAC	1/month/biofilter
SEM and other microbial	Full-scale biofilter GAC	5/study
tracking assays	Pilot biofilter GAC	1/month/biofilter

* The sample frequency represents an average minimum collection frequency. Samples were taken with much higher frequencies for many analytes during high sensitivity testing.

† Full-scale biofilter influent was equivalent to pilot biofilter influent when operated under control conditions. In these instances, only one sample was collected and analyzed for the full-scale sample location.

‡ Turbidity, pH, temperature, and ozone residual measurements were performed at least daily by JKWTP plant operators for all full-scale treatment sample locations.

Biofiltration Nutrient- Enhancement Evaluation

The objective of this study was to evaluate various nutrient augmentation strategies for enhancing biofiltration performance. Phosphoric acid and/or ammonia were dosed to pilot biological filters to achieve a target molar ratio of bioavailble C:N:P of 100:10:1, where bioavailble C was determined iteratively by the amount of DOC removed in the biofilter. The nutrient enhancement strategy was evaluated by monitoring changes in biofilm appearance, hydraulic and water treatment performance, and microbial activity.

Oxidant- Enhancement Evaluation

The objective of this study was to evaluate peroxide supplementation for augmenting the oxidative action and response of the biofiltration process. Many microorganisms express a class of enzymes, known as oxidoreductases, when exposed to hydrogen peroxide. Preliminary testing of this strategy was conducted by dosing hydrogen peroxide to a pilot biofilter (with no other nutrient of carbon supplementation) at 1 mg/L to evaluate the peroxide enhancement strategy. This condition was operated continuously for two weeks. The hydrogen peroxide enhancement strategy was evaluated by monitoring changes in biofilm appearance, hydraulic and water treatment performance, and microbial activity.

Microbial Tracking

The objective of this study was to correlate the microbial ecology and activity in the biofilters to hydraulic and treatment performance. Media samples were collected from the pilot and full-scale biofilters after each process change and analyzed for microbial activity, speciation, and morphology. The microbial tracking study included the following

analyses: SEM, ATP quantification, biofilm morphological characterization, T-RFLP, and clone libraries.

Full-Scale Process Integration Assessment and Economic Evaluation

A full-scale process integration assessment was performed for the nutrient enhancement strategy, as it was the most effective and best characterized enhancement strategy. Capital and operation cost estimates were also developed for integrated enhancement strategies, including the estimated cost savings that may be realized during operation.

Data Analyses

General

All collected data sets were described by determining mean, standard deviation, maximum, and minimum values. The error bars presented in all figures in this dissertation represent the standard deviation of the data set. This standard deviation accounts for operational variability (i.e., feed water conditions) and sampling/analytical error for that data set. All non-detects were accounted for as 50% of the detection limit in all statistical analyses.

Paired T-Test

The paired t-test was the statistical method used to determine if there were statistical differences between sets of collected data from the full-scale biofilter, pilot biofilter control, and various pilot enhanced biofilters. The paired t-test is a variation of the standard t-test and is used to compare two treatment methods where experiments are performed in pairs and the differences are of interest. Since sample collection was performed in pairs in the pilot biofilter studies and the differences in the collected data sets are of interest, the paired t-test was appropriate to use. For the purposes of this

study, two means with a paired t-test $p \leq 0.05$ were considered to have a statistically significant difference. All paired t-tests were calculated with two tails (two sided p value).

Analytical Methods

General Water Quality Parameters

Water quality data were collected and analyzed using in-line pilot instrumentation, field equipment, and laboratory equipment. Turbidity, pH, DO, temperature, free and total chlorine, ozone residual, and hydrogen peroxide analyses were performed onsite at the JKWTP. The City performed all laboratory analyses except for N-nitrosodimethylamine and haloacetic acids, which were performed by Montgomery-Watson Harza (MWH) Laboratories (Monrovia, CA).

Sample collection for laboratory analyses

Aqueous samples were collected from the pilot and full-scale system in bottles provided by the laboratory. Full-scale biofilter samples were collected from JKWTP Filter No. 2. Samples were either delivered same day to the City laboratory, shipped overnight in a cooler packed with ice to MWH, or delivered twice weekly to the Dallas Water Utility Central Wastewater Plant Water Quality Lab (MIB and geosmin). Replicate measurements were performed for each sample set, and replicate samples were collected monthly.

Turbidity

In-line nephelometers were used to perform continuous turbidity measurement for the pilot (Hach 1720E, Loveland, CO) and full-scale biological filters (Hach 1720D, Loveland, Colorado). A desktop nephelometer was used to measure raw and settled water turbidities (Hach 2100N, Loveland, CO). Formazin standards were used for instrument calibration in accordance to manufacturer's protocols.

pH

pH measurements were performed using an Orion pH Electrode (Thermo Fischer Scientific Inc., Waltham, MA) per the manufacturer's protocol. A 3-point calibration of the pH electrode was performed daily. Slope limits were between 92 to 102%.

Dissolved oxygen and temperature

DO and temperature were measured on site using a YSI 55 dissolved oxygen DO probe (Yellow Springs, OH). Measurements and calibration were conducted per the manufacturer's protocol.

Free and total chlorine

Free and total chlorine were measured on site using a Hach DR890 colorimeter (Loveland, CO). Free chlorine was analyzed using the EPA N,N-Diethyl-p-Phenylenediamine (DPD) Method 8021. Total chlorine was analyzed using the EPA DPD method 8167.

Hydrogen peroxide

Hydrogen peroxide was measured on site using a CHEMets Colorimetric Hydrogen Peroxide Test Kit (Chemtech International, Media, PA) according to the manufacturer's instructions. The colorimetric test is based upon the ferric thiocyanate method.

Ozone residual

Ozone residual was measured in pilot influent. All measurements were collected using a Hach DR890 colorimeter (Loveland, CO), following EPA Indigo Method 8311.

Total and dissolved organic carbon

The City laboratory performed TOC and DOC measurements in accordance with the Standard Methods for Examination of Water and Wastewater 21st Edition (2005),

Standard Method (SM) 5130B (2005). A Shimadzu TOC analyzer (Kyoto, Japan) was used for the analyses.

UV_{254}

The City laboratory performed UV_{254} measurements in accordance with SM 5910B (2005). UV_{254} measurements were performed using a Bausch & Lomb spectrophotometer (Rochester, NY).

Iron

The City laboratory performed total Fe measurements in accordance with SM 3111B (2005). A Varian atomic adsorption spectrophotometer (AAS) (Palo Alto, CA) was used to perform these analyses. All measurements were of total Fe.

Manganese

The City laboratory performed total Mn measurements in accordance with SM 3111B (2005). A Varian AAS (Palo Alto, CA) was used to perform these analyses. All measurements were of total Mn.

Nitrates, nitrites

The City laboratory performed nitrate and nitrate measurements in accordance with EPA Method 300.0. A Dionex ion chromatograph (Sunnyvale, CA) was used to analyze nitrates and nitrites.

Ammonia

The City laboratory performed ammonia measurements with a Thermo Electron Corporation ammonium ion-selective electrode (ISE). All measurements were performed following the manufacturer's protocol.

Orthophosphate

The City laboratory performed orthophosphate measurements in accordance with EPA Method 300.0. A Dionex ion chromatograph (Sunnyvale, CA) was used tor all orthophosphate measurements.

Regulated microbial parameters

The City laboratory performed all analyses for aqueous microbial parameters. Measurements for heterotrophic plate counts, total coliforms, and fecal coliforms were performed in accordance with SM 9215 (1998), 9222 (1998), and 9221 (1998), respectively.

Disinfection by-products

The City laboratory performed total trihalomethane measurements in accordance with EPA Method 501.1, using a gas chromatography (GC). MWH laboratories performed haloacetic acid measurements following SM 6251B (1998).

Tastes and odors

MIB and geosmin analyses were performed by the Dallas Water Utilities (DWU) analytical laboratory. The protocol for analyses followed SM 6040 D, Odor Causing Compounds MIB, geosmin by gas chromatography/mass spectrometry (GC/MS) (2005). The limit of detection (LOD) for both MIB and geosmin was 0.4 ng/L.

Pesticides and Pharmaceuticals

All pesticide and pharmaceutical analyses were performed at the University of Colorado-Boulder. The compounds included in the initial screening and continued monitoring are provided in Table 3-4 with their respective LODs. The methods described herein were derived from Thurman et al. (2006).

Sample collection

Aqueous samples were collected in 1-L amber glass bottles. Full-scale biofilter effluent samples were collected from JKWTP Filter No. 2. Samples were stored on ice and shipped overnight to the University of Colorado-Boulder for pesticide and pharmaceutical analyses.

Table 3-4. Limit of detection for screened pharmaceuticals and pesticides assuming 100% recovery by solid phase extraction. Individual water-sample matrices may vary

Contaminant	LOD (ng/L)
Pharmaceuticals	
1,7-Dimethylxanthine	100
Acetaminophen	50
Albuterol (Salbutamol)	10
Ampicillin	10
Azithromycin	10
Caffeine	10
Carbamazepine	5
Cefotaxime	10
Cimetidine	10
Ciprofloxacin	5
Clarithromycin	10
Cloxacillin	50
Cotinine	10
Dehydronifedipine	2
Diclofenac	20
Digoxigenin	20
Digoxin	10
Diltiazem	5
Diphenhydramine	2
Enrofloxacin	5
Erythromycin	5
Flumequine	10
Fluoxetine	10
Gemfibrozil	10
Ibuprofen	50
Lincomycin	5
Lomefloxacin	5

Table 3-4. Continued

Contaminant	LOD (ng/L)
Metformin	50
Miconazole	5
Naproxen	50
Norfloxacin	5
Ofloxacin	5
Oxacillin	20
Oxolinic Acid	20
Ranitidine	10
Roxithromycin	5
Sarafloxacin	5
Sulfachloropyridazine	20
Sulfadiazine	50
Sulfadimethoxine	5
Sulfamerazine	20
Sulfamethazine	5
Sulfamethizole	20
Sulfamethoxazole	10
Sulfanilamide	50
Thiabendazole	5
Triclocarban	20
Trimethoprim	5
Tylosin	10
Viginiamycin	20
Warfarin	10
Sulfadimethoxine	5
Hormones	
Estrone	1
Estradiol	1
Ethinylestradiol	1
Cholesterol	1
Coprastanol	1
Pesticides	
Acetamiprid	3
Acetochlor	2
Alachlor	3
Aldicarb	5
Aldicarb sulfone	3
Aldicarb sulfoxide	4
Azoxystrobin	0.1

Table 3-4. Continued

Contaminant	LOD (ng/L)
Atrazine	1
Benalaxyl	0.04
Bendiocarb	5
Bensultap	281
Bromoxynil	20
Bromuconazole	0.3
Buprofezin	0.6
Butylate	3
Captan	15
Carbaryl	3
Carbendazim	0.8
Carbofuran	4
Cartap	15
Chlorfenvinphos	0.2
Chlorpyrifos methyl	30
Cyanazine	2
Cyproconazole	1
Cyromazine	9
Deethylatrazine	2
Deethylterbuthylazine	1.5
Deisopropylatrazine	2
Diazinon	0.05
Dichlorvos	0.5
Difeconazole	0.5
Difenoxuron	0.4
Diflubenzuron	12
Dimethenamide	1

Table 3-4. Continued

Dimethoate	1.5
Dimethomorph	4
Diuron	0.6
Ethiofencarb	4
Fenamiphos	0.1
Fenuron	10
Flufenacet	3
Flufenoxuron	6
Fluoroacetamide	80
Fluoroxypyr	45
Hexaflumuron	8

Table 3-4. Continued

Contaminant	LOD (ng/L)
Hydroxyatrazine	0.4
Imazalil	0.3
Imazapyr	5
Imazaquin	0.7
Imidacloprid	2
Ioxynil	15
Iprodione	4
Irgarol 1051	0.1
Irgarol metabolite	0.5
Isoproturon	0.7
Lenacil	9
Lufenuron	9
Malathion	1.5
Mebendazole	0.8
Metalaxyl	0.2
Metamitron	3
Methidathion	15
Methiocarb	0.7
Methiocarb sulfone	9
Methomyl	2
Metolachlor	0.8
Metolcarb	12
Metribuzin	0.6
Molinate	1.5
Monuron	0.7
Nicosulfuron	0.8
Nitenpyram	0.2
Oxadixyl	14
Parathion ethyl	17
Pendimethalin	11
Phosmet	0.9
Prochloraz	0.7
Profenofos	1
Promecarb	3
Prometon	1
Prometryn	0.3
Propachlor	0.5
Propanil	0.7
Propiconazole	0.3

Table 3-4. Continued

Contaminant	LOD (ng/L)
Prosulfocarb	2
Simazine	0.4
Spinosad A	0.9
Spinosad D	6
Spiromesifen	120
Spiroxamine	8

Chemicals and reagents

Analytical standards were purchased from Sigma-Aldrich (St. Louis, MO) and Supelco (Billerica, PA) Individual stock solutions (1000 µg/mL) were prepared in pure methanol and stored at -18ºC. HPLC-grade acetonitrile and methanol were obtained from Burdick & Jakson (Muskegon, MI). Formic acid was obtained from Sigma-Aldrich. A Milli-Q-Plus ultra-pure water system from Millipore (Billerica, MA) was used throughout the study to obtain the HPLC-grade water used during the analyses.

Sample preparation (solid phase extraction)

An off-line solid phase extraction (SPE) was used for the pre-concentration of the water samples. All the extraction experiments were performed using an automated sample preparation with extraction column system (GX-271 ASPEC, Gilson, Inc., Middleton, WI) fitted with a 25-mL syringe pump for dispensing the water samples through the SPE cartridges. Disposable cartridge columns packed with 500 mg of Oasis HLB sorbent were used. The cartridges were conditioned with 4 mL of methanol followed by 6 mL of HPLC water at a flow rate of 1 mL/min. The water samples (200 mL) were loaded at a flow rate of 10 mL/min. Elution of the analytes from the cartridge was carried out with 5 mL of methanol. The solvent was evaporated to 0.5-mL with a stream of nitrogen at a temperature of 45ºC in a water bath using a Turbovap

concentration workstation (Caliper Life Sciences, Palo Alto, CA). The extracts were analyzed by liquid chromatography/mass spectrometry (LC/MS).

LC/TOF-MS analyses of pesticides and pharmaceuticals

The separation of pesticides and pharmaceuticals was carried out using a high performance liquid chromatography (HPLC) system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1200, Agilent Technologies, Santa Clara, CA) equipped with a reversed phase C8 analytical column of 150 mm x 4.6 mm and 5 µm particle size (Zorbax Eclipse XDB-C8). Column temperature was maintained at 25ºC. The injected sample volume was 50 µL. Mobile phases A and B were acetonitrile and water with 0.1% formic acid, respectively. The optimized chromatographic method held the initial mobile phase composition (10% A) constant for 5 min, followed by a linear gradient to 100% A after 30 min. The flow-rate used was 0.6 mL/min. A 10-min post-run time was used after each analysis. This HPLC system was connected to a time-of-flight mass spectrometer Agilent 6220 MSD TOF equipped with a dual electrospray interface operating in positive ion mode, using the following operation parameters: capillary voltage: 4000 V; nebulizer pressure: 45 psig; drying gas: 9 L/min; gas temperature: 300ºC; fragmentor voltage: 190 V; skimmer voltage: 60 V; octopole RF: 250 V. LC/MS accurate mass spectra were recorded across the range 50-1000 m/z at 4GHz. The data recorded were processed with MassHunter software. Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a dual-nebulizer electrospray ionization ESI source that introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution (calibrant solution A, Agilent Technologies, Santa Clara, CA), which contains the internal reference masses

(purine ($C_5H_4N_4$ at m/z 121.0509 and HP-921 [hexakis-(1H,1H,3H-tetrafluoro-pentoxy)phosphazene] ($C_{18}H_{18}O_6N_3P_3F_{24}$) at m/z 922.0098. The instrument provided a typical mass resolving power of 15000±500 (m/z 922). Quantification was carried out using external standard curves.

LC/MS-MS analyses of pharmaceuticals at low level (ppt concentration)

A special method for the detection of two pharmaceuticals (meprobamate and carbamazepine) at low levels of concentration was developed. For this purpose, an LC/MS/MS triple quadrupole Agilent Model 6460 with Jet Stream technology was used. Three different transitions (one for quantitation and two for confirmation) for each compound were used for quantification and qualification of each compound. Fifteen microliters of sample extract were analyzed for each sample. Chromatography consisted of UHPLC, ultra high-pressure liquid chromatography, using the Agilent Infinity system 1290 Model (Agilent Technologies, Inc., Santa Clara, CA). The mobile phases were 0.1% formic acid and acetonitrile. The column was the Zorbax Eclipse C18 2.1 mm x 50 mm with 1.8 micron packing. The limits of detection with this method were 0.1 ng/L for both compounds. The relative standard deviation for this method is 6%.

Microbial Tracking. ATP analyses were performed on site. The SEM work was performed at the United States Environmental Protection Agency Office of Research and Development (USEPA ORD, Cincinnati, OH). All other microbial tracking analyses were performed at the University of Texas-Austin, including filter media HPC, EPS quantification, biofilm formation potential, terminal restriction length fragment polymorphism (T-RFLP), and clone libraries.

Biofilter Media Microbial Characterization and Analyses

Sample collection for ATP analyses

GAC media samples were collected from pilot-scale biofilters at the beginning and end of a filter run for ATP analyses. Prior to sample collection the pilot filters were taken offline and partially drained. The top 6 inches of media were then homogenized via stirring. Approximately 50 grams of GAC were then removed from the top of a given biofilter. GAC obtained from the full-scale biofilters was used to replace the removed sample.

ATP analyses

The ATP analyses were conducted using the LuminUltra (NB, CA) Deposit and Surface Analysis (DSA) test kit. The DSA test kit is based on the premise that ATP "is a direct and interference-free indicator of total" active biomass (Luminultra, 2008).

Collected biofilter media was decanted and measured into 1.1 mL test samples. The samples were then weighed for reference and analyzed following the DSA manufacturer's protocols. RLU were measured using a Kikkoman C-100 LumiTester (Tokyo, Japan). Triplicate samples were run for each sample set.

Sample collection for other microbial tracking analyses

GAC media samples were collected from pilot-scale and full-scale biofilters at the beginning of a filter run for analyses. Full-scale biofilter GAC media samples were collected from JKWTP Filter No. 2. Prior to sample collection, the pilot biofilters were taken offline and partially drained. The top 6 inches of media were then homogenized via stirring. Approximately 100 grams of GAC were then removed from the top of the biofilter. Samples of GAC were then placed in 60-mL amber glass vials, which were then filled with pilot filter feed water. The vials were stored on ice and shipped overnight

to the USEPA ORD for SEM analyses and to the University of Texas-Austin for all other microbial tracking analyses.

Scanning electron microscopy

Upon receipt, samples were logged and stored at 4°C. Prior to analyses, samples were portioned into processing containers and rinsed in deionized water (DI) for 30 seconds to remove extra debris. Samples were then fixed with 2.5% gluteraldehyde, 4% paraformaldehyde solution in a 0.1M cacodylate buffer that was adjusted to pH 7.3. Samples were allowed to fix for 6 hours and then washed twice for 15 minutes with the cacodylate buffer. Samples were then washed again in DI water for an additional 15 minutes. Next, the samples were postfixed for an additional hour in a 1% osmium tetroxide solution. The postfixed samples were then washed three additional times for 15 minutes each. After osmium fixation the samples were dried using a dilution series of ethanol (25, 50, 75, 95, 100, 100) for 30 minutes each. Samples were then chemically dried using two exchanges of propylene oxide (30 minutes each). Excess propylene oxide was removed and granules were air dried in a chemical hood for an hour on blotter paper then transferred to a desiccation jar. Prior to imaging, several granules were mounted on aluminum SEM stubs and sputter coated with gold-palladium for 90 seconds. Biofilms were imaged at 15kV under high vacuum using the JEOL JEM6490LV scanning electron microscope (EPA, Cincinnati, OH). Multiple sets were examined to ensure continuity of imaging in the samples. In all, SEM was performed on 32 samples, with typically 25 to 30 media granules per sample.

Biofilter media heterotrophic plate count

Upon receipt, samples were logged and stored at 4°C. Two grams of sample were suspended in 10 mL of phosphate-buffered saline (PBS), submerged in a sonicator bath

for one minute, put on ice for one minute, and vortexed vigorously for five seconds. This procedure was repeated five times to dislodge the biofilm from the activated carbon. Ten-fold serial dilutions were prepared through the 10^{-6} dilution. The dilutions were plated in triplicate on R2A agar and incubated at 30ºC for 24 hours.

Crystal violet (CV) assay (biofilm formation potential)

The biofilm formation capacity of filter media biofilms was evaluated using the crystal violet (CV) assay as described by O'Toole and Kolter (1998). The CV assay results are heavily dependent on the initial cell concentration of the inoculums. Therefore, the biofilm formation capacity of the filter samples was compared among inocula with similar cell concentrations (as determined by CFU counts).

One µL from each dilution prepared for CFU counts was taken to seed 100 µL of R2A broth on a 96-well microtitre plate. This was done in triplicate for each dilution. These cultures (three per dilution of biologically active carbon sample) were allowed to grow statically at 30ºC for 24 hours. After incubation, the medium was poured off and the remaining biofilm was stained with CV. The plates were rinsed with water and dried, and then 200 µl of 96% ethanol was used to solubilize the crystal violet in each well. Absorbance was measured at 600nm using a spectrophotometer. Higher absorbance measurements indicated greater biofilm formation

Phenol-sulfuric acid assay (EPS quantification)

This assay was performed following the method of Dubois (1956). Two grams of sample were suspended in 10 mL of PBS, submerged in a sonicator bath for one minute, put on ice for one minute, and vortexed vigorously for five seconds. This procedure was repeated five times to dislodge the biofilm from the activated carbon. Eight milliliters of the suspension were transferred to a clean tube and centrifuged at

10,000 RPM at 4ºC. The resulting supernatant was then transferred to another test tube, and the pellet was resuspended in a buffer (10 mM Tris/HCl, pH 8, 10 mM EDTA, 2.5% NaCl) and incubated for 8 hours at room temperature. To measure free EPS, 2 mL of the supernatant was transferred to a tube where it was mixed with 50% phenol solution and 5 mL of concentrated sulfuric acid. A yellow color was developed, and absorbance was read at 480 nm. To measure bound EPS, the resuspended pellet was centrifuged and processed as described above for the supernatant. A glucose calibration curve was constructed for data analyses.

Terminal restriction fragment length polymorphism

DNA was extracted in triplicate from each sample using the MoBio UltraClean® Microbial DNA Isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Approximately two hundred milligrams of biologically active carbon was used per extraction.

DNA from the triplicate extractions was combined and used for polymerase chain reaction (PCR). DNA was amplified in duplicate reactions using 8F (FAM-labeled at the 5' end) and 1492R targeting the 16S rRNA gene. Each 50-µL PCR reaction contained 1.25 U Taq DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 0.4 µM of each primer, and 100 ng of template DNA. The reactions were run under the following amplication conditions: denaturation at 94^{o}C for 3 min, followed by 20 cycles of denaturation at 94^{o}C for 30 s, annealing at 52^{o}C for 30 s, and extension at 72^{o}C for 1 min, and a final extension at 72ºC for 7 min. Amplicon from the duplicate reactions was combined, and 1 µL of amplicon was added to duplicate nested PCR reactions. These nested PCR reactions, with primers 8F (FAM-labeled at the 5' end) and 926R, were performed to increase the concentration and

specificity of the amplicon; with the exception that 0.5 µM of each primer was added to the reactions, the reaction conditions and thermal cycling parameters were the same as aforementioned. Amplicon from the duplicate nested PCR reactions was combined and visualized on a 1% agarose gel stained with ethidium bromide.

The amplicon (1 µg for each sample) was treated with the Klenow enzyme as described in Egert and Friedrich (2005) and purified with the MoBio Ultraclean PCR clean-up kit (MoBio Laboratories, Inc., Carlsbad, CA). One hundred nanograms of purified amplicon was digested with 40 U of HhaI in a 20-µL reaction at 37ºC for 3 h and purified with a centrifugal filter (YM-30, Millipore Corp., Billerica, MA.). The digested sample was sent to the University of Texas at Austin Institute for Cellular and Molecular Biology core facility for fragment analysis on an ABI 3130 DNA analyzer. The electropherograms were processed using GeneMarker® 1.70 (SoftGenetics, LLC, State College, PA); bands greater than 60 bases in length and greater than the intensity threshold of 40 were included in the analysis. The diversity of each sample was determined by the Shannon-Weaver index (SWI) (Wani et al., 2006). Community similarity between samples was assessed with the Sørenson index (SI) using ±0.5bp comparisons (Wawrik et al., 2005).

Clone libraries

DNA was extracted, and the 16S rRNA gene was amplified as described for T-RFLP, with the exception that non-labeled 8F was used for PCR. The cloning reactions were carried out using a TOPO TA Cloning Kit with OneShot Top 10 chemically competent cells (Invitrogen™, Carlsbad, CA) following the manufacturer's instructions. The transformed *E.coli* were transferred to Luria-Bertani (LB) plates containing a 50 µg/mL kanamycin and then incubated at 37ºC overnight. Colonies were selected

randomly and used to inoculate a 96-well microplate for each sample. The 96-microplates were incubated at 37ºC for 3 days with shaking at 200 rpm. Plasmids were purified with the QIAprep 96 Turbo Miniprep Kit (Qiagen Inc., Valencia, CA). The purified plasmids were sent to the University of Texas at Austin Institute for Cellular and Molecular Biology core facility and sequenced with the T7 primer. For sequence analyses, the PCR primer site was identified using Geneious Pro 4.8.5 (Biomatters Ltd., Auckland, New Zealand), and the vector sequence was removed. The sequences were submitted to BLAST (blastn and megablast queries) at http://blast.ncbi.nlm.nih.gov/Blast.cgi to identify library sequences most closely resembling the query sequence.

Summary of Responsible Parties for Analytical Work Performed

Table 3-5. Responsible Parties for Analytical Work Performed

Analytical Parameter	Responsible Party for Analysis Performance
Temperature	On-site, Chance Lauderdale
Dissolved Oxygen	On-site, Chance Lauderdale
pH	On-site, Chance Lauderdale
UV254	On-site, Chance Lauderdale
Dissolved Organic Carbon	Arlington Water Utilities Analytical Laboratory
Ortho-phosphate	Arlington Water Utilities Analytical Laboratory
Ammonia-Nitrogen	Arlington Water Utilities Analytical Laboratory
Manganese	Arlington Water Utilities Analytical Laboratory
Iron	Arlington Water Utilities Analytical Laboratory
TTHM & HAA	Arlington Water Utilities Analytical Laboratory
MIB	Dallas Water Utilities Analytical Laboratory
Geosmin	Dallas Water Utilities Analytical Laboratory
Color	Arlington Water Utilities Analytical Laboratory
Total organic carbon	Arlington Water Utilities Analytical Laboratory
Selected EDCs	Univerisity of Colorado-Boulder
Biofilter Effluent HPC (R2A)	Arlington Water Utilities Analytical Laboratory
Total and Fecal Coliforms	Arlington Water Utilities Analytical Laboratory
Scanning Electron Microscopy	United States Environmental Protection Agency Office of Research and Development
Biofilter Media HPC	University of Texas-Austin
EPS	University of Texas-Austin
Biofilm Formation Potential	University of Texas-Austin
ATP	On-site, Chance Lauderdale
Genetic analyses	University of Texas-Austin

CHAPTER 4
BASELINE CHARACTERIZATION AND CONTROL STUDIES

Objectives

The objective of this study was to characterize the hydraulic and water treatment performance of the pilot biofilter operated under baseline conditions. This allowed for the confirmation of similitude between pilot- and full-scale biofiltration performance and provided control data for parallel tests occurring with other pilot biofilters operating under various enhancement strategies. Similitude was characterized by operating a control biofilter under full-scale operational conditions (i.e., no enhancement strategies were used for the control biofilter) for approximately seven months. The seven months of operation included approximately one month of acclimation, five months of steady state operation, and one month of robustness testing. Contaminant loading (Mn, MIB, atrazine) was also performed to assess biofilter baseline performance, as full-scale influent concentrations sufficient for sustained removal characterization were not observed during the study. Monitored water quality parameters included turbidity, TOC, DOC, Fe, Mn, NH_4-N, PO_4-P, MIB, geosmin, and a suite of over 150 pharmaceuticals and pesticides.

Biofilter Configuration

All pilot biofilters were configured and operated under conditions equivalent to the full-scale biofiltration facility; including filter feed loading rate (4.5 gpm/ft^2), media configuration (8 inches of sand, 40 inches of bituminous GAC obtained from full-scale filters, 6 years in previous operation). The pilot biofilters were operated without a filter-to-waste step, as the full-scale biofilters do not use filter-to-waste. Contaminant spiking

was performed on all pilot biofilters after the first month of operation and continued intermittently under a managed experimental plan through the remainder of the study.

Biofilter Backwash Strategy Development

The initial pilot biofilter backwash protocol was developed to mirror the full-scale biofilter backwash protocol However, mudballs, media attrition, and increasing clean-bed headloss were observed during the first two weeks of operation, and a more aggressive pilot backwash protocol was implemented. The backwash duration and intensity were increased over current full-scale operation, and pilot biofilter run times were limited to 18 hours for the remainder of the study. These modifications provided consistent clean bed headloss and biofilter hydraulic profiles. In addition, 18-hour pilot biofilter run times provided straightforward hydraulic performance comparisons between test conditions, through the observation of terminal headloss. Table 3-1 provides a summary of the full-scale and modified pilot backwash strategies.

Wood Based Gac Media Evaluation

A pilot biofilter (Biofilter 3) was operated under baseline conditions for the duration of the study to evaluate a wood-based GAC as biofilter support media. Unlike the bituminous GAC used in the other three biofilters, the wood-based GAC in Biofilter 3 was virgin media at the start of the study. Complete breakthrough of DOC or the tracked contaminants was not observed during the study. The adsorption capacity of the virgin GAC was not fully characterized, and therefore Biofilter 3 results were not comparable to the results of the other three biofilters. In addition, the mechanisms for contaminant removal (e.g., biological transformation versus adsorption) were not evaluated. Therefore, the extent of biological activity and its effects on performance remain unknown. The results of the wood-based GAC media evaluation do not support the

project hypothesis nor project objectives, thus they are not discussed further in the dissertation. Any future comparative evaluations of biofilters composed of virgin GAC support media should include pre-exhaustion step (via high contaminant and/or NOM loading until complete breakthrough is observed).

Hydraulic Characterization

Clean-bed headloss of the control biofilter was consistent through steady state testing. Table 4-1 provides the average headloss data for the control biofilter on a month-by-month basis for five months of steady state testing. Monthly clean bed headloss means remained within 9% of the calculated theoretical headloss (0.90 ft) based on Darcy's equation and information provided by the underdrain manufacturer and pilot fabricator.

Generally, the pilot control biofilter showed consistent headloss profiles for 18-hour filter runs during steady state operation. However, terminal headloss values did fluctuate. Increased terminal headloss most often occurred during periods of high biofilter feed turbidity. High turbidity episodes often coincided with operator initiated sludge pond recycle. During these events, decant from the sludge pond (a backwash wastewater and sedimentation-coagulation sludge holding tank) is recirculated to the plant influent, increasing solids loading to the JKWTP and thus, to the pilot biofilters. Settled water turbidity excursions could rise from an average 0.8 NTU to as high as 2.2 NTU (Table 4-4). Events significant enough to increase 18-hr terminal headloss by greater than 25% occurred three times during the five-month steady state evaluation. These events provided a realistic robustness characterization for turbidity removal and hydraulic performance for all pilot biofilters. Figure 4-1 illustrates the impact of a high

solids loading event on the control biofilter through consecutive filter run headloss profiles.

Table 4-1. Baseline characterization of pilot biofilter headloss

Month of steady state operation	Clean bed headloss (ft)			Terminal headloss (ft) at 18-hr		
	Mean*, †	Min.	Max.	Mean*, †	Min.	Max.
1 (Apr)	0.85 ± 0.05	0.76	0.94	5.5 ± 0.9	4.23	7.8
2 (May)	0.92 ± 0.04	0.81	0.97	7.1 ± 1.4	4.5	9.3
3 (Jun)	0.98 ± 0.04	0.85	1.05	4.7 ± 0.5	4.0	6.2
4 (Jul)	0.97 ± 0.05	0.81	1.05	4.9 ± 0.5	4.1	6.0
5 (Aug)	0.91 ± 0.03	0.83	0.98	5.9 ± 0.6	4.7	7.0
Over 5 month study	0.92 ± 0.06	0.76	1.05	5.6 ± 1.2	4.0	9.3

* Means are provided with the standard deviation of the data sets as value error.
† Hydraulic data from approximately four filter runs each month were omitted due to routine (<4 hour) process interruption for pilot maintenance or media collection.
‡ Loading rate held to 4.5 gpm/ft^2 during testing.

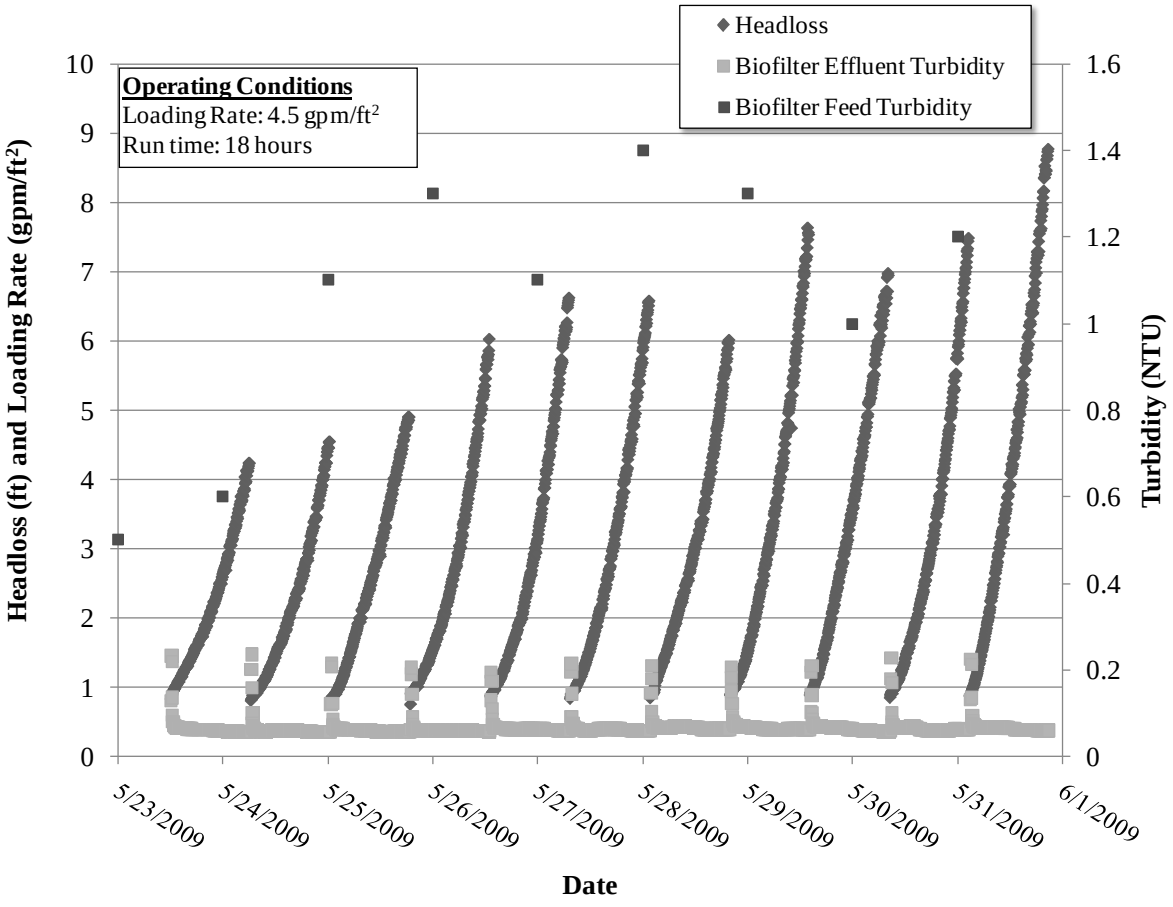

Figure 4-1. Control pilot biofilter headloss profiles impacted by sludge pond recycle to JKWTP influent

Water Quality Characterization

General

The baseline biofilter water treatment performance characterization was performed over five months of steady state operation. Full-scale and pilot biofilter effluent samples were monitored for DOC, Fe, Mn, NH_4-N, PO_4-P, MIB, geosmin, and a suite of over 150 pharmaceuticals and pesticides. These constituents were tracked to characterize biofilter feed water quality and to verify that the City's treatment objectives were met in the filter effluent. Table 4-2 summarizes the City's water quality objectives, and Table 4-3 provides the mean full-scale raw and biofilter effluent values for those criteria during pilot studies.

Table 4-2. City of Arlington biofilter water treatment objectives

Constituent	Finished Water Objective/target concentration
Turbidity	< 0.3 NTU, 95% of samples, max sample ≤ 1 NTU
DOC	Remove 10% of biofilter feed DOC concentration
Fe*	< 300 µg/L
Mn*	< 50 µg/L
MIB*	Total odor number < 3 (~10 ng/L)
Geosmin*	Total odor number < 3 (~10 ng/L)
Atrazine*	< 3 µg/L
Misc. pharmaceuticals and pesticides*	Unspecified concentration reduction

* Background metal and trace organic loads to the biofilters were low/below detection through most of the study; therefore, pilot biofilter feed contaminant spikes were performed to characterize removal performance.

Table 4-3. City of Arlington full-scale biofilter performance*

Constituent	Biofilter feed mean† water quality	Biofilter effluent mean† water quality
Turbidity (NTU)	0.8 ± 0.4 NTU	0.06 ± 0.01 NTU
DOC (mg/L)	3.50 ± 0.2	3.17 ± 0.12 (~10%)
Fe‡ (µg/L)	34 ± 75	< 8.3 µg/L
Mn‡ (µg/L)	8 ± 5	< 2.4 µg/L
MIB‡ (ng/L)	< 1.4 µg/L	< 1.4 µg/L
Geosmin‡ (ng/L)	< 1.4 µg/L	< 1.4 µg/L
Atrazine‡ (µg/L)	0.39 ± 0.07	0.47 ± 0.20
Misc. pharmaceuticals and pesticides‡	Provided in Table 1.2 (< 1 µg/L for all measured)	Provided in Table 1.2 (< 1 µg/L for all measured)

* Data collected from April 2009 through August 2009.

† Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

‡ Background metal and trace organic loads to the biofilters were low/below detection through most of the study; therefore, pilot biofilter feed contaminant spikes were performed to characterize removal performance.

Turbidity

Control biofilter effluent turbidity readings remained stable and mean monthly values were similar to full-scale values throughout steady state testing. Table 4-4 summarizes feed turbidity trends along with turbidity breakthrough from the pilot- and full-scale biofilters.

Turbidities maintained compliance with the USEPA Surface Water Treatment Rule, as greater than 95% of the effluent samples had turbidities less than 0.3 NTU. No turbidity values over 1 NTU were observed in pilot or full-scale biofilter effluents. Figure 4-1 illustrates the biofilter ripening period for the pilot control biofilter over approximately eight days of steady state operation. The ripening period was the time required for a filter to meet effluent turbidity objectives after it is put into service. Figure 4-2 provides biofilter effluent turbidity values across two filter runs.

Table 4-4. Baseline characterization of biofilter turbidity removal

Month of steady state operation	Biofilter feed[*, †] (NTU)			Pilot control biofilter effluent[†,‡] (NTU)			Full-scale biofilter effluent[*,§] (NTU)		
	Mean[**]	Min.	Max.	Mean[**]	Min.	Max.	Mean[**]	Min.	Max.[††]
1 (Apr)	1.4 ± 0.6	1	2.2	0.1 ± 0.06	0.07	0.62	0.06 ± 0.02	0.04	0.08
2 (May)	0.9 ± 0.4	0.5	1.4	0.08 ± 0.06	0.05	0.52	0.06 ± 0.02	0.04	0.08
3 (Jun)	0.7 ± 0.5	0.4	1.4	0.07 ± 0.02	0.06	0.42	0.06 ± 0.01	0.05	0.08
4 (Jul)	0.5 ± 0.3	0.3	0.8	0.05 ± 0.02	0.04	0.30	0.06 ± 0.01	0.04	0.07
5 (Aug)	0.6 ± 0.3	0.3	1.0	0.05 ± 0.02	0.05	0.30	0.05 ± 0.01	0.04	0.06
Over 5 month study	0.8 ± 0.8	0.3	2.2	0.07 ± 0.06	0.04	0.62	0.06 ± 0.02	0.04	0.08

* Biofilter feed water was JKWTP settled/ozonated water for pilot and full-scale biofilters.
† Biofilter feed and composite full-scale biofilter effluent turbidities were measured using a desktop turbidimeter. Pilot biofilter effluents were measured continuously (5 minute intervals) using inline instrumentation (Chapter 3).
‡ Pilot control biofilter effluent maximum values were observed during filter ripening.
§ Full-scale biofilter effluent turbidities were measured using a composite sample (blended from all active biofilters). These samples were collected and measured every four hours (over a 24-hour period) by plant staff.
** Means are provided with two standard deviations (to capture 95% of the data distribution) to show regulatory compliance.
†† Full-scale biofilter effluent composite samples did not show high turbidity breakthrough during ripening, as only one biofilter is backwashed at a time.

Dissolved Organic Carbon

After turbidity reduction, biodegradable organic matter (BOM) removal is typically the most important water treatment objective for biofilters. Organic carbon provides an energy and carbon source for the heterotrophic bacteria that populate the biofilter. DOC measurement is a relatively inexpensive analysis that can be performed by most water quality laboratories and provides an indication of BOM reduction. Therefore, observed improvements in DOC removal may suggest an increase in microbial activity in biofilters and a reduction of regrowth potential in the distribution system. Table 4-5 provides a summary of DOC data for the pilot control and full-scale biofilters.

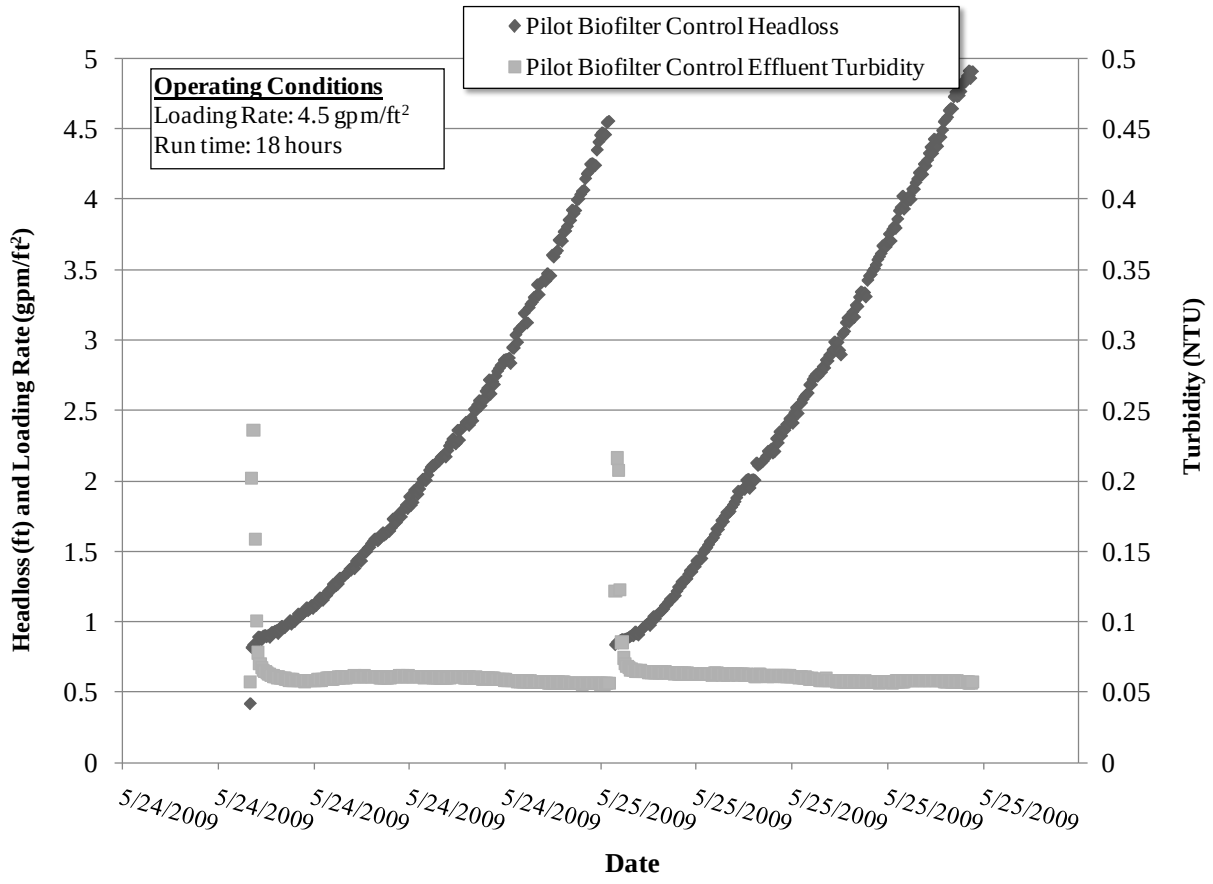

Figure 4-2. Pilot control biofilter effluent turbidity profiles across two filter runs

The data provided in Table 4-5 show low variability in the mean full-scale and pilot control biofilter effluent DOC concentrations over the five-month steady state evaluation. Figure 4-3 presents average monthly DOC removal for the pilot control and full-scale biofilters, further illustrating the pilot/full-scale similitude. Figure 4-3 also shows the average temperatures for each month of operation. The test period captured a 67% increase in average temperature. However, this appeared to have only provided a small improvement in DOC removal in the full-scale biofilter performance. No significant correlation between pilot biofilter DOC removal performance and temperature was

found, which is likely due to the limited range of temperature variation and the variability in the DOC values across the experimental period.

The control pilot- and full-scale biofilters removed an average of approximately 10% of the influent DOC, which amounted to approximately 0.4 mg/L. If 0.4 mg/L of DOC represented all BOM, 47 µg/L NH_4-N and 10 µg/L PO_4-P would be necessary in the biofilter influent to establish the target 100:10:1 C:N:P molar ratio. However, it is likely that additional nondegraded BOM is present; therefore, the nutrient requirements must be determined iteratively if additional BOM removal is observed.

Table 4-5. Baseline characterization of biofilter DOC removal

Month of steady state operation	Settled Water Temp (°C)	Biofilter feed (mg/L)			Pilot control biofilter effluent (mg/L)			Full-scale biofilter effluent (mg/L)		
	Mean*	Mean*	Min.	Max.	Mean*	Min.	Max.	Mean*	Min.	Max.
1 (Apr)	18 ± 1.7	3.43± 0.19	3.12	3.66	3.08 ± 0.11	2.89	3.21	3.19 ± 0.14	3.00	3.37
2 (May)	24 ± 1.2	3.45 ± 0.25	2.88	3.66	3.09 ± 0.10	2.98	3.25	3.20 ± 0.05	3.12	3.29
3 (Jun)	28 ± 0.5	3.71 ± 0.18	3.60	4.07	3.12 ± 0.12	3.01	3.30	3.25 ± 0.1	3.08	3.33
4 (Jul)	30 ± 0.3	3.51 ± 0.13	3.33	3.68	3.15 ± 0.25	2.80	3.55	3.13 ± 0.21	2.83	3.40
5 (Aug)	30 ± 0.3	3.52 ± 0.09	3.40	3.69	3.08 ± 0.18	2.87	3.44	3.11 ± 0.09	2.95	3.20
Over 5 month study	26 ± 4.8	3.50 ± 0.19	2.88	4.07	3.10 ± 0.15	2.80	3.55	3.17 ± 0.12	2.84	3.40

† Pilot was housed in a heated room; however, pilot effluent temperatures remained within 2 °C of settled water temperatures.

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

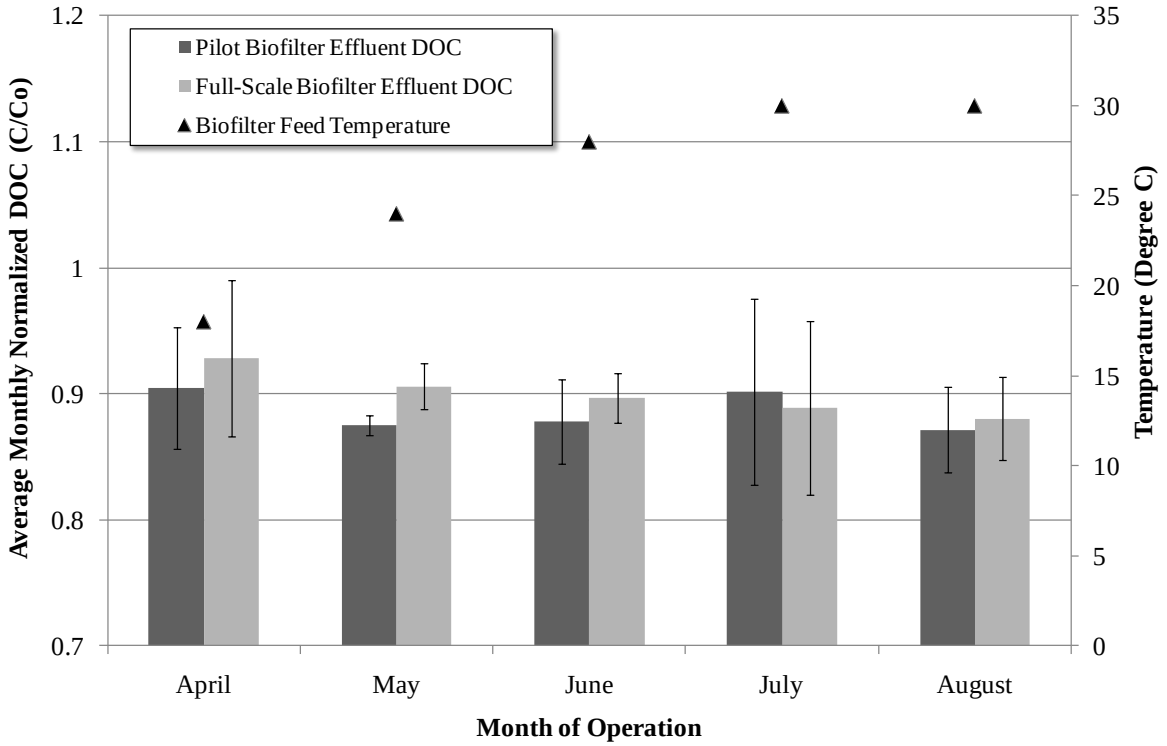

Figure 4-3. Pilot control biofilter and full-scale biofilter steady state DOC removal performance[2]

Nutrients

Nutrients (NH_4-N, PO_4-P) were monitored throughout the baseline characterization and control studies to identify potential limitations. Table 4-6 provides a summary of the collected nutrient data.

The biofilter feed PO_4-P concentrations were typically below the method detection limit of 7 µg/L, suggesting that the pilot control and full-scale biofilters were operated with a PO_4-P limitation. Furthermore, NH_4-N concentrations varied significantly during testing, indicating that occasional periods of N limitation had occurred.

[1] The error bars presented in all figures in this Chapter represent the standard deviation of the data set. This standard deviation accounts for operational variability (i.e. feed water conditions) and sampling/analytical error.

Metals

Metals characterization included monitoring Mn and Fe in pilot control and full-scale biofilter feed and effluents. As discussed in Chapter 1, Mn is a primary water quality concern for the City. During the five- month steady state analysis, background Mn concentrations in the biofilter feed were well below the 50 µg/L secondary MCL. Therefore, 50 µg/L Mn was spiked to the pilot control biofilter over the last month of testing to fully characterize baseline removal capabilities under moderate loading conditions. Subsequently, the biofilter feed Mn spike was increased to approximately 200 µg/L for a period of one week of robustness testing. Table 4-7 summarizes the baseline Fe and Mn removal performance.

The data presented in Tables 4-6 and 4-7 demonstrate similitude between the pilot- and full-scale filters for Mn and Fe removal performance at background feed concentrations. Average Mn and Fe effluent concentrations for the pilot control and full-scale biofilters were below detection. However, biofilter feed background loadings remained low throughout this phase of testing. Spiking did provide evidence of Mn removal in the pilot control biofilter, yielding average feed reductions of 76 ± 44 and 89 ± 6% (error as standard deviation) for the moderate and high spike tests, respectively. Despite the high level of treatment, Mn breakthrough near the secondary MCL was observed. It should also be noted that Mn breakthrough below the secondary MCL may also be problematic, as it may contribute to long-term accumulation of Mn oxide precipitates in the distribution system, potentially leading to periodic sloughing and colored water events. Figure 4-4 illustrates pilot baseline control mean Mn feed and effluent concentrations during the spiking tests.

Table 4-6. Baseline characterization of nutrient feed and biofilter utilization

	Biofilter feed [*, †]			Pilot control biofilter effluent [*, †]			Full-scale biofilter effluent [*, †]		
Nutrient	Mean	Min.	Max.	Mean	Min.	Max.	Mean[§]	Min.	Max.
PO_4-P[‡] (µg/L)	<MDL	<MDL	82	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
NH_4-N[§] (µg/L)	43 ± 35	13	180	15 ± 5.6	<MDL	23	20 ± 14	<MDL	55

* Data set includes five months of steady state operation (NH_4-N, N=34; PO_4-P, N=36).
† Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.
‡ PO_4-P MDL was 7 µg/L
§ NH_4-N MDL was 11 µg/L

Table 4-7. Baseline characterization of biofilter Mn and Fe removal

	Background biofilter feed[*,†]			Pilot control biofilter effluent[*,†]			Full-scale biofilter effluent[*,†]		
Metal	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Mn[‡] (µg/L)	8 ± 5	<MDL	19	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Fe[§] (µg/L)	34 ± 75	<MDL	390	<MDL	<MDL	95	<MDL	<MDL	37

	Spiked pilot biofilter feed			Pilot control biofilter effluent		
Spiked metal	Mean	Min.	Max.	Mean	Min.	Max.
Mn[**] (µg/L)	54 ± 21	26	97	11 ± 15	<MDL	48
High Mn[††] (µg/L)	220 ± 10	210	230	25± 13	18	40

* Data set includes four (Mn) and five (Fe) months of steady state operation (Mn, N=34; Fe, N=41).
† Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.
‡ MDL for Mn was 2.4 µg/L.
§ MDL for Fe was 8.3 µg/L.
** Data set includes 1.5 months of steady state operation (Mn, N=16).
†† Data set includes 1 week of steady state operation (Mn, N=3).

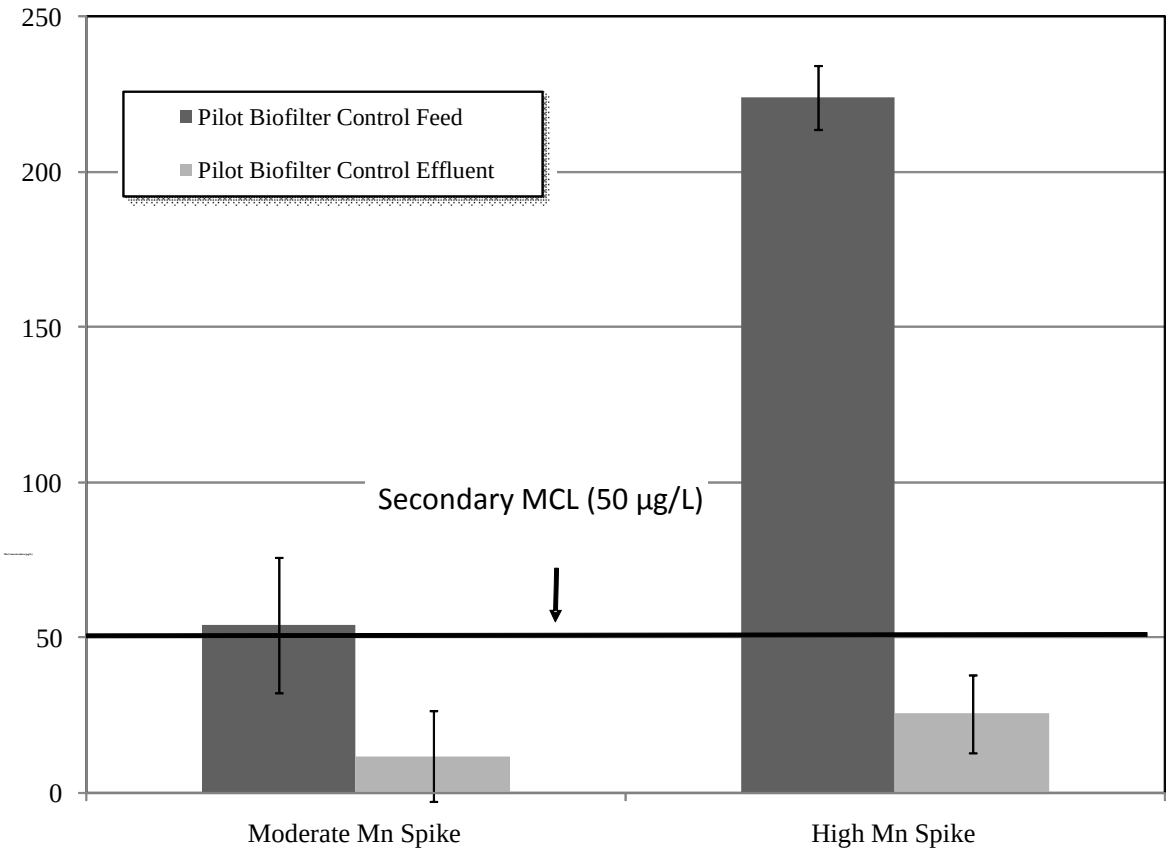

Figure 4-4. Pilot control biofilter steady state and peak load Mn removal performance

Table 4-8. Baseline characterization of biofilter taste and odor removal

	Background biofilter feed*			Pilot control biofilter effluent*			Full-scale biofilter effluent*		
Contaminant	Mean	Min.	Max.	Mean	Min.	Max.	Mean§	Min.	Max.**
MIB†,‡ (ng/L)	<MDL	<MDL	6.9	<MDL	<MDL	2.6	<MDL	<MDL	5.3
Geosmin†,§ (ng/L)	<MDL	<MDL	5.3	<MDL	<MDL	2.6	<MDL	<MDL	2.2

	Spiked pilot biofilter feed			Pilot control biofilter effluent		
Spiked contaminant	Mean	Min.	Max.	Mean	Min.	Max.
MIB** (ng/L)	31.5 ± 17.6	6.7	102	11.6 ± 11.4	0.0	37.9
High MIB†† (ng/L)	164 ± 2.3	163	167	22.3 ± 6.7	14.8	27.5

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.
† MDLs for MIB and Geosmin were 1.4-ng/L.
‡ Background MIB sample set N= 10 over approximately 6 weeks.
§ Background geosmin sample set N= 41 over approximately 5 months.
** Spiked MIB sample set N=38 over approximately 4.5 months.
†† High-spiked MIB sample set N=3 over approximately 1 week.

Tastes and Odors

The City of Arlington's water treatment objectives include the removal of MIB and geosmin. Background biofilter feed levels for these contaminants remained low, relative to historical values and their treatment objective (as described in Table 4-1). The pilot control biofilter was operated with unmodified feed for six weeks to evaluate T&O removal at the low-level background concentrations. After this initial study, 40 ng/L of MIB was dosed to the pilot feed during five months of steady state operation to better characterize removal performance. An intermittent high load (>100 ng/L) MIB spiking test was conducted at the end of steady state operation. Table 4-8 summarizes the pilot control and full-scale biofilter T&O removal performance at background concentrations and pilot control performance during the spiking tests.

Pilot control and full-scale biofilter MIB and geosmin breakthrough remained low under background loading conditions. However, the average influent concentration for both contaminants remained below their MDLs. Spiking did provide evidence of MIB removal in the pilot control biofilter, yielding average feed reductions of 66 ± 32 and 86 ± 4% (error as standard deviation) for the moderate and high spike tests, respectively. Despite the high level of treatment, the average MIB breakthrough for both high and moderate loads remained above the odor threshold concentration of 10 ng/L. Indeed, approximately 50% of effluent samples (N=38) collected from the baseline control pilot filter during the moderate spiking tests showed MIB concentrations over 10 ng/L. The observed MIB breakthrough in the pilot study illustrates the limitations of the existing biofiltration processes at the JKWTP and PBWTP. Historically, MIB and geosmin analyses on City finished water have been limited; however, the high number of

seasonal consumer complaints for earthy-musty smelling water suggests insufficient performance (Hunt, 2009).

Pharmaceuticals and Pesticides

Background biofilter feed concentrations of pharmaceuticals and pesticides remained below 1 µg/L for all tracked parameters. Table 4-9 provides a summary of the pharmaceuticals and pesticides detected in the biofilter feed and the effluent concentrations observed in the pilot control and full-scale biofilters.

Pilot biofilter feed spiking of carbamazepine, atrazine, and caffeine was conducted for one week to better characterize their removal across the pilot biofilters. Table 4-10 provides a summary of the results from this spiking test. The data in Table 4-10 indicate that the pilot control biofilter was capable of removing a portion of the spiked contaminants (22 to 40%), though end products were not identified.

Table 4-9. Baseline performance comparison of the pilot and full-scale filters*

Contaminant	Mean background biofilter feed*,†	Mean pilot control biofilter effluent*,†	Mean full-scale biofilter effluent*,†,‡
Atrazine§ (µg/L)	0.39 ± 0.07	0.42 ± 0.12	0.47 ± 0.20
Deethylatrazine§ (µg/L)	0.56 ± 0.15	0.58 ± 0.10	0.54 ± 0.12
Deisopropylatrazine§ (µg/L)	0.26 ± 0.08	0.26 ± 0.05	0.24 ± 0.05
Hydroxyatrazine§ (µg/L)	0.21 ± 0.22	0.17 ± 0.10	0.12 ± 0.03
Simazine§ (µg/L)	0.24 ± 0.44	0.17 ± 0.23	0.03 ± 0.07
Metolachlor§ (µg/L)	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Meprobamate§ (ng/L)	0.28 ± 0.04	0.23 ± 0.04	0.25 ± 0.0

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

† Samples were tested against a suite of over 150 pharmaceuticals and pesticides, those with measurable concentrations are included in this table.

‡ Samples collected from the effluent of full-scale filter No. 2.

§ Data set includes five months of steady state operation (atrazine, N = 6; meprobamate N=2, all others N = 9).

Table 4-10. Pilot biofilter treatment performance for spiked atrazine, carbamazepine, and caffeine

Contaminant	Background biofilter feed[*,†]	Pilot control biofilter effluent[*,†]
Atrazine (µg/L)	2.8 ±0.0	2.2 ±0.2
Carbamazepine (µg/L)	0.5 ±0.3	0.3 ±0.1
Caffeine (µg/L)	2.4 ±0.2	1.5 ±0.0

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

† Data set includes 1 week of steady state operation (all compounds, N=3).

Summary

The objective of this study was to characterize baseline water treatment performance, confirming similitude between pilot- and full-scale treatment and to provide control data for parallel tests occurring with other pilot filters. Similitude was characterized by operating a control biofilter under full-scale operational conditions (i.e., no enhancement strategies were used for the control biofilter) from March 5, 2009 through October 1, 2009. These tests provided approximately seven months of steady state data that confirmed and further elucidated the treatment capabilities of the existing process at the JKWTP. Monitored water quality parameters included DOC, total Mn, ammonia-nitrogen (NH_4-N), orthophosphate-phosphorus (PO_4-P), MIB, and a suite of over 150 pharmaceuticals and pesticides. These water quality data demonstrated treatment performance similitude between the pilot and full-scale filters. Measured water quality values between the control biofilter and the full-scale biofilter were within the standard deviations of their respective data sets. The baseline characterization also confirmed the process limitations of the existing full-scale system. Both Mn and MIB breakthroughs were observed under moderate and high biofilter influent load conditions.

CHAPTER 5
SUBSTRATE ENHANCEMENT STUDIES

Objectives

The objective of this study was to evaluate various primary substrate augmentation strategies for enhancing biofiltration performance. Though secondary substrates (e.g., recalcitrant DOC, MIB, geosmin, pesticides and pharmaceuticals) can be biodegraded, bacteria gain little to no energy in doing so, which means a primary substrate must be biodegraded simultaneously if any biodegradation of the secondary substrate is to be achieved. The rate of secondary substrate degradation is proportional to the concentration of active biomass present, which is, in part, a function of the concentration of primary substrate. Thus, biological treatment processes designed to biodegrade these compounds require the presence of a primary substrate. Increasing the concentration of primary substrate (e.g., by intermediate ozonation, which increases the amount of biodegradable organic matter, or primary substrate augmentation), can increase the rate of trace organic compound degradation (Lim et al., 2008). It can also enhance the removal rate of slowly degradable natural organic matter (NOM) (Hozalski and Bouwer 2001). This study evaluated the addition of four primary substrates, including acetic acid, molasses, MicroC© (a proprietary glycerin-based product produced by Environmental Operating Solutions, Inc, Bourne, MA), and ethanol. The substrates were evaluated individually during four successive two- to six-week phases, each dosed at a target of 1 mg/L as carbon (C).

Chemical Feed Observations

Dosed substrate concentration was validated twice weekly by comparing substrate-enhanced DOC concentration in the biofilter feed against the background

(pilot control) biofilter feed DOC concentration. The measured dose delivered to the pilot column often deviated from the target. Analyses of acetic acid, MicroC©, and ethanol influent samples revealed that doses of these substrates were approximately 10 to 40% higher than the target, which may have been due to inadequate mixing in the biofilter feed or siphoning from the chemical feed tanks. The mean molasses dose was approximately 130% greater than the target. Stock solution checks indicated that molasses was settling in the chemical tank, yielding higher concentrations of DOC at the tank bottom where chemical was drawn. This high dose was likely a factor in the high headloss and DOC breakthrough observations discussed later in this chapter.

Hydraulic Characterization

Substrate augmentation strategies generally impaired biofilter hydraulic performance. Clean bed headloss was unaffected by substrate addition and generally remained below 1 ft for all substrates tested. However, terminal headloss consistently exceeded that of the control biofilter. Indeed, many substrate-enhanced biofilter filter runs were terminated prematurely (prior to the typical 18-hour run) due to headloss greater than 13.5 ft, the maximum safe total dynamic head load for the pilot feed pumps. Typically, terminal headloss increased after successive filter runs for each substrate tested. Additional biodegradable carbon increases the relative nitrogen and phosphorus limitations in the biofilter feed. It is believed that the observed increase in headloss in the substrate-enhanced columns was due primarily to the increased microbial production of EPS, driven by nutrient stress. This phenomenon was described in Chapter 2 and is investigated further in Chapters 6 and 8.

Table 5-1 provides the average terminal headloss data for the substrate-enhanced biofilters compared to that of the biofilter control over the same time. Figure 5-1

provides an illustration of substrate-enhanced and biofilter control headloss profiles for a typical week of filter runs for MicroC© augmentation.

Water Quality Characterization

General

Water treatment performance characterization included routine sampling and water quality analyses (Table 4-3). pH was also closely monitored during the acetic acid substrate-enhanced biofiltration studies; however, no variation greater than ± 0.1 pH unit was observed relative to the biofilter control.

Table 5-1. Characterization of substrate-enhanced pilot biofilter headloss

Supplemental substrate tested	Substrate enhanced biofilter terminal headloss (ft)			Biofilter control terminal headloss* (ft)		
	Mean†	Min.	Max.	Mean†	Min.	Max.
Acetic acid‡	10.3 ± 2.4	5.5	>13.5	5.4 ± 1.2	3.1	8.8
Molasses§	>13.5	10.8	>13.5	7.2 ± 1.3	4.5	9.0
MicroC©**	8.8 ± 2.8	4.3	>13.5	4.7 ± 0.4	4.1	6.0
Ethanol††	>13.5	12	>13.5	5.3 ± 0.6	4.7	6.8

* Biofilter control operated without supplemental substrate.

† Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

‡ Includes 37 biofilter runs. Six acetic acid supplemented biofilter filter runs were terminated because headloss exceeded 13.5 feet.

§ Includes 38 biofilter runs. Approximately 85% of molasses supplemented biofilter filter runs were terminated prematurely because headloss exceeded 13.5 feet.

** Includes 45 biofilter runs. Clogged biofilter effluent lines artificially elevated headloss through six filter runs with MicroC© supplementation, these runs were not included in the above analysis. Three additional MicroC© supplemented biofilter runs were terminated because headloss exceeded 13.5 feet. Lines were clogged with white gelatinous material believed to be related to biological growth.

†† Includes 12 filter runs. 90% of ethanol supplemented biofilter runs were terminated prematurely because headloss exceeded 13.5 feet.

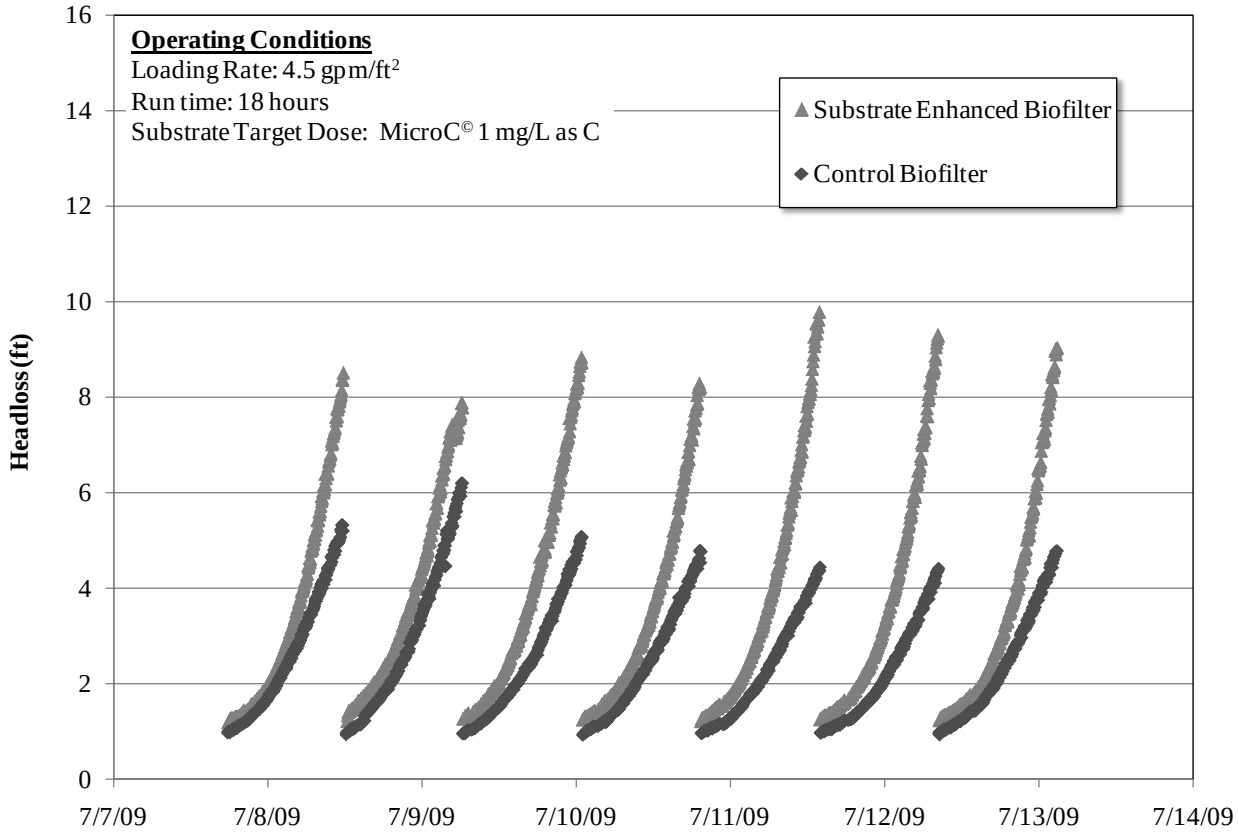

Figure 5-1. Comparison of substrate enhanced (MicroC©) and biofilter control headloss profiles

Turbidity

Substrate-enhanced biofilter effluent turbidity readings remained stable and were similar to full-scale and pilot biofilter control values throughout testing. All turbidities maintained compliance with the USEPA Surface Water Treatment Rule, as greater than 95% of the effluent turbidity samples were less than 0.3 NTU. Indeed, mean effluent turbidities remained below 0.08 NTU for all conditions tested. No turbidity values over 1 NTU were observed in substrate-enhanced biofilter effluent. Temporary headloss drops and excess turbidity was not observed during high headloss events (>13 feet), suggesting that significant short-circuiting did not occur. Figure 5-2 illustrates typical substrate-enhanced effluent turbidity profiles for a typical week of filter runs. Although

not shown in Figure 5-2, occasional turbidity breakthroughs as high as 0.6 NTU were observed in all biofilters immediately following a backwash (less than 15 minutes of production).

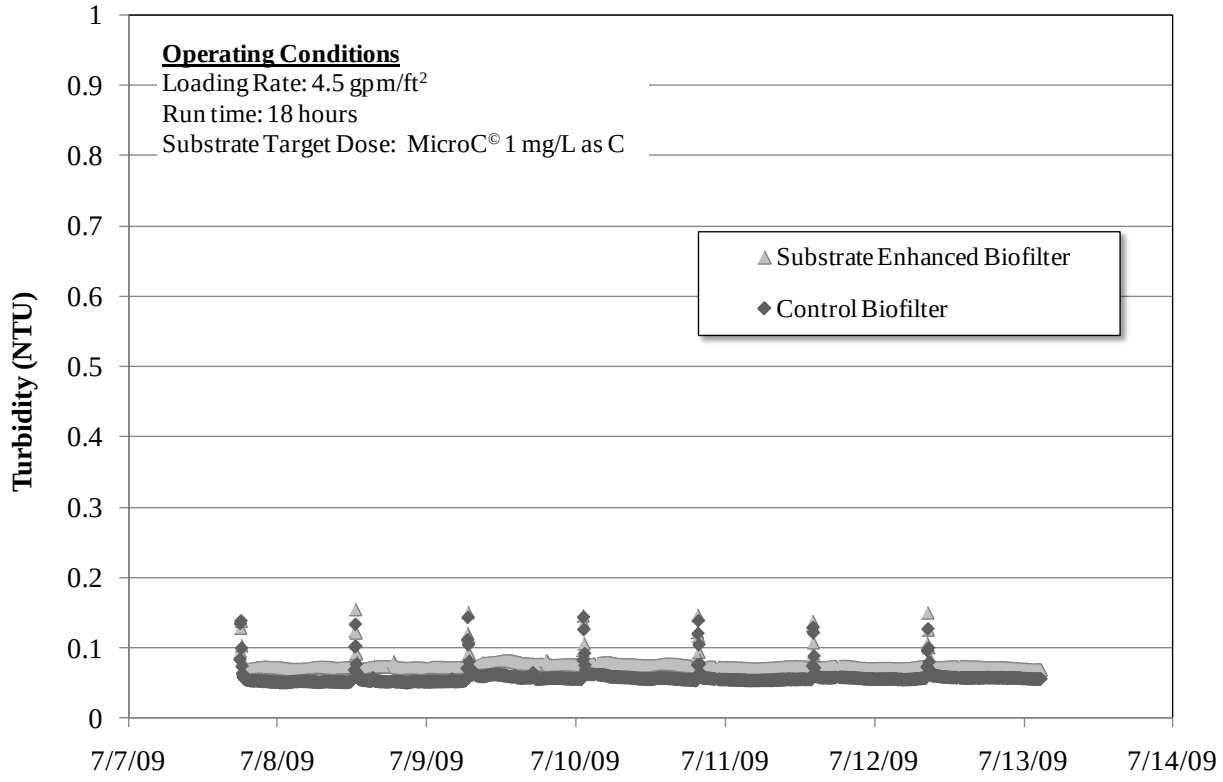

Figure 5-2. Comparison of substrate enhanced and biofilter control turbidity profiles for a typical week of filter runs

Dissolved Organic Carbon

Table 5-2 provides biofilter feed DOC (includes dosed substrate concentration) and effluent DOC for each substrate test. Table 5-3 compares substrate-enhanced biofilter effluent DOC concentrations with the control biofilter effluent DOC concentration during the same period. Effluent DOC concentrations shown are normalized to the background biofilter feed DOC (NOM) and total DOC concentrations (NOM + substrate, where added). Effluent DOC normalization to the background biofilter feed provided

simple metric to determine if substrate-enhanced biofiltration improved (or diminished) NOM removal relative to the control biofilter.

The hypothesis for the substrate-enhanced biofiltration strategy assumed that the substrates selected would be easily degradable and be removed in addition to the bioavailable carbon present in the biofilter feed.

Figure 5-3 provides a graphic representation of the data presented in Table 5-3.

Table 5-2. Substrate enhanced biofilter DOC removal characterization

Substrate tested	Background DOC (mg/L)	Dose	Biofilter feed (background + dosed DOC) (mg/L)			Substrate enhanced biofilter effluent DOC (mg/L)		
	Mean*	Mean*	Mean*	Min.	Max	Mean*	Min.	Max
Acetic acid	3.5 ± 0.2	1.4 ± 0.5	4.9 ± 0.5	4.3	5.4	3.1 ± 0.1	3.0	3.3
Molasses†	3.6 ± 0.1	2.3 ± 0.1	5.9 ± 0.1	5.9	6.0	3.6 ± 0.0	3.6	3.6
MicroC©	3.5 ± 0.1	1.4 ± 0.7	4.9 ± 0.7	4.0	6.7	3.1 ± 0.2	2.9	3.4
Ethanol	3.6 ± 0.1	1.1 ± 0.3	4.6 ± 0.3	4.3	4.8	3.0 ± 0.2	2.8	3.2

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

† Molasses partitioning in the chemical feed tank was observed. Concentrations higher than target were dosed to the substrate-enhanced biofilter.

Table 5-3. Substrate enhanced biofilter normalized DOC removal characterization

Supplemental substrate tested*	Substrate enhanced biofilter effluent normalized to feed C ($C/Co_{[Background + Dosed]}$)			Substrate enhanced biofilter effluent normalized to background C ($C_{Effluent}/C_{o,Background}$)			Biofilter control effluent normalized to background C ($C_{Effluent}/C_{o,Background}$)		
	Mean*	Min.	Max.	Mean*	Min.	Max.	Mean*	Min.	Max.
Acetic acid	0.62 ± 0.09	0.49	0.74	0.91 ± 0.04	0.88	1.00	0.89 ± 0.01	0.88	1.00
Molasses	0.61 ± 0.14	0.60	0.62	0.99 ± 0.00	0.99	0.99	0.86 ± 0.01	0.86	0.86
MicroC©	0.64 ± 0.07	0.48	0.77	0.88 ± 0.04	0.82	0.93	0.88 ± 0.02	0.81	1.03
Ethanol	0.65 ± 0.05	0.60	0.70	0.85 ± 0.02	0.82	0.87	0.90 ± 0.3	0.84	0.93

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

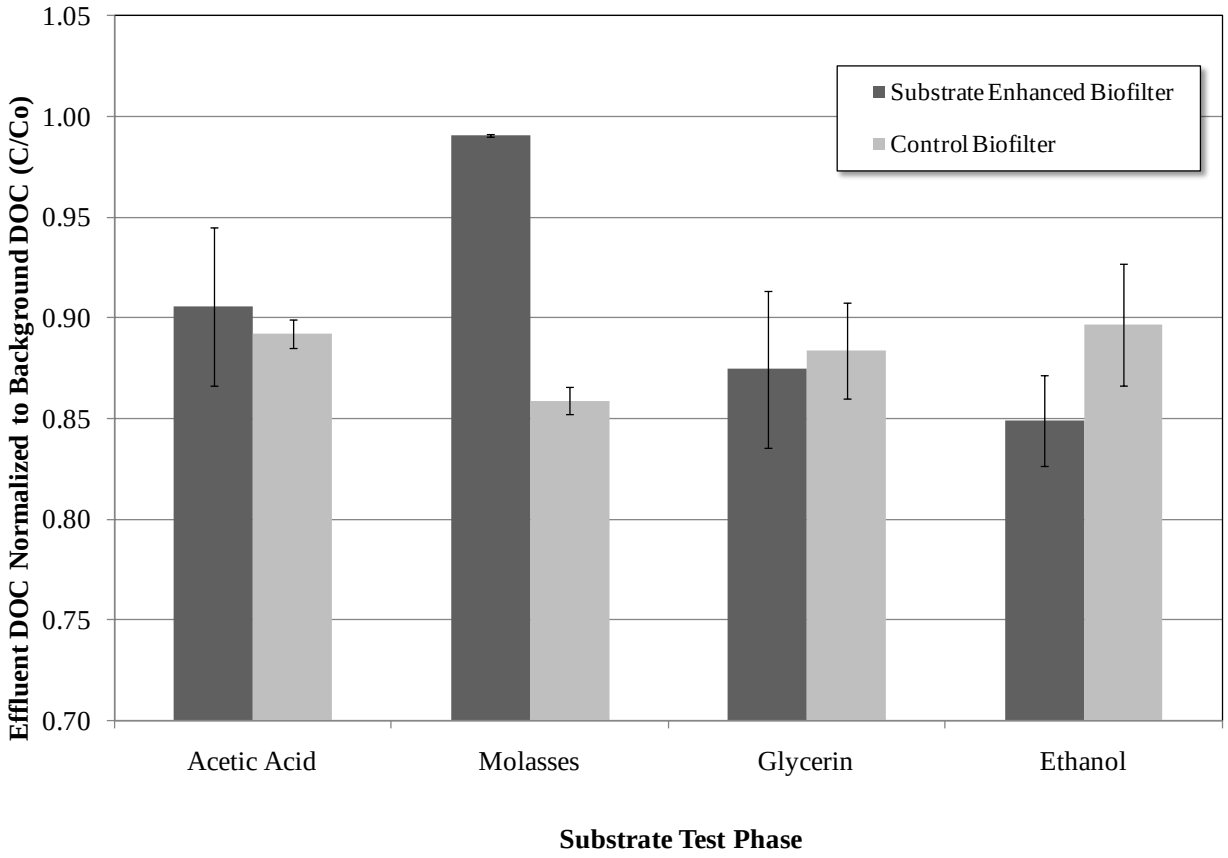

Figure 5-3. Comparison of substrate enhanced and biofilter control normalized DOC concentrations[3]

As shown in Table 5-3 and Figure 5-3 DOC removals (normalized to the background concentration) varied between the substrates tested. Acetic acid and glycerin biofilter substrate-enhancement yielded effluent DOC concentrations similar to the biofilter control. Molasses supplementation resulted in additional biofilter DOC breakthrough. This is likely due to the inconsistent dosing and the heterogeneous nature of molasses product used (i.e., molasses contains humic materials that are likely more recalcitrant than the other substrate tested).On average, ethanol appeared to perform better than the biofilter control. However, a paired t-test analyses of DOC

[3] The error bars presented in all figures in this Chapter represent the standard deviation of the data set. This standard deviation accounts for operational variability (i.e., feed water conditions) and sampling/analytical error.

removal data from the ethanol enhanced biofilter and the control indicated that the difference between the means were not statistically significant [t (3) = 3.18, two tail p = 0.17 (p>0.05)]. Thus, the substrate-enhancement studies failed to identify a sole substrate that would improve biofilter DOC removal upon supplementation. Under all substrates tested, the addition of bioavailable C exacerbated the existing nutrient limitation in the biofilter feed. Nutrient limitation may have diminished potential substrate utilitization, thus DOC removal.

Nutrients

The addition of approximately 1 mg/L biodegradable organic carbon increased the relative nitrogen and phosphorus limitations in a given biofilter feed (Table 4-3). The C:N:P ratio for the substrate-enhanced biofilter feed was approximately 100:2.6:0, significantly offset from the 100:10:1 target ratio. The effect of this offset will be discussed in Chapter 6.

Metals

Fe and Mn removal were observed for the substrate-enhanced and control biofilters under each test condition. However, metals removal performance was difficult to compare among substrates due to generally low and varied feed concentrations. Mean biofilter feed Fe concentrations were less than 100 µg/L for all substrates tested with three excursions over 300 µg/L (all during acetic acid testing). Table 5-4 summarizes the substrate-enhanced biofilter Fe removal data.

Mean biofilter feed Mn levels remained below the limit of detection (10 µg/L) during the substrate enhancement tests (Table 5-4). Therefore, moderate Mn (~50-µg/L) spiking tests were conducted to resolve removal performance with the ethanol substrate-enhanced biofilter. Mn spiking was conducted for three weeks (N=6) with a

mean dose of 63 µg/L to the ethanol substrate-enhanced biofilter and biofilter control. As shown in Table 5-5, the ethanol-enhanced and control biofilters removed Mn to below detection.

Table 5-4. Substrate enhanced biofilter Fe removal characterization

Substrate tested	Biofilter feed Fe (µg/L)*			Substrate-enhanced biofilter effluent Fe (µg/L)*			Biofilter control effluent Fe (µg/L)*		
	Mean†	Min.	Max	Mean†	Min.	Max	Mean†	Min.	Max
Acetic acid	50 ± 93	<MDL	304	41 ± 115	<MDL	365[3]	<MDL	<MDL	<MDL
Molasses	15 ± 12	<MDL	35	<MDL	<MDL	16	<MDL	<MDL	16
MicroC©	10 ± 10	<MDL	33	<MDL	<MDL	13	<MDL	<MDL	13
Ethanol	28 ± 24	<MDL	68	<MDL	<MDL	<MDL	<MDL	<MDL	11

MDL for Fe is 8.3 µg/L

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

† The acetic acid substrate-enhanced biofilter samples showed three excursions over 300 µg/L.

Table 5-5. Substrate-enhanced biofilter Mn removal characterization

Substrate tested	Biofilter feed Mn (µg/L)*, †			Substrate-enhanced biofilter effluent Mn (µg/L)*, †			Biofilter control effluent Mn (µg/L)*, †		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Ethanol	8 ± 5	<MDL	19	<MDL	<MDL	<MDL	<MDL	<MDL	15

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

† MDL for Mn was 2.4 µg/L.

Taste and Odor

One intent of biofilter substrate enhancement is to improve biological degradation of trace organic contaminants. As a contaminant of concern and potential surrogate for other trace organics, MIB was monitored closely during the substrate enhancement studies. As discussed in Chapter 4, background MIB levels remained low during pilot testing. Therefore, moderate MIB spiking (~40 ng/L) was conducted throughout the substrate enhancement studies to characterize removal performance. Table 5-6

provides the results for the MIB removal characterization during the substrate enhancement studies.

Table 5-6. Substrate enhanced biofilter MIB removal characterization

Substrate tested	Biofilter feed (ng/L)*,†			Substrate-enhanced biofilter effluent (ng/L)*,†			Biofilter control effluent (ng/L)*,†			Difference between the means for substrate-enhanced and control biofilters
	Mean‡	Min.	Max.	Mean‡	Min.	Max.	Mean‡	Min.	Max.	P Value
Acetic acid	37 ± 24	20	101	25 ± 12	4	42	23 ± 12	<MDL	38	0.05
Molasses	39 ± 8	27	47	12 ± 7	4	21	11 ± 5	4.7	17	0.72
MicroC©	26 ± 7	15	39	9 ± 5	5	21	4 ± 2	<MDL	8	0.01
Ethanol	26 ± 20	8	64	3 ± 5	<MDL	13	5 ± 4	<MDL	13	0.31

* High MIB feed concentrations observed during the acetic acid test phase were the result of intermittent background loading from algae growth in the JKWTP sedimentation basins.
† MDL for MIB was 1.4 ng/L.
‡ Means are provided with the standard deviation of the data sets as value error.

Figure 5-4 illustrates normalized MIB effluent concentrations for each substrates tested relative to the biofilter control. Figure 5-4 and Table 5-6 show marginal MIB removal improvement with ethanol supplementation and no improved MIB removal for any of the other substrates tested over the biofilter control. Possible explanations for the lack of significant MIB removal improvement include (1) MIB spiking concentrations were not sufficient to differentiate the ethanol substrate-enhanced biofilter from the biofilter control, (2) the other substrates tested did not enhance MIB secondary substrate metabolism and/or co-metabolism for any of the microbial populations present, and (3) other operational or water quality factors may play a larger role in MIB removal than substrate limitation, such as temperature, empty bed contact time, or

nutrient limitations. The effects of nutrient limitations and supplementation on MIB removal are discussed further in Chapter 6.

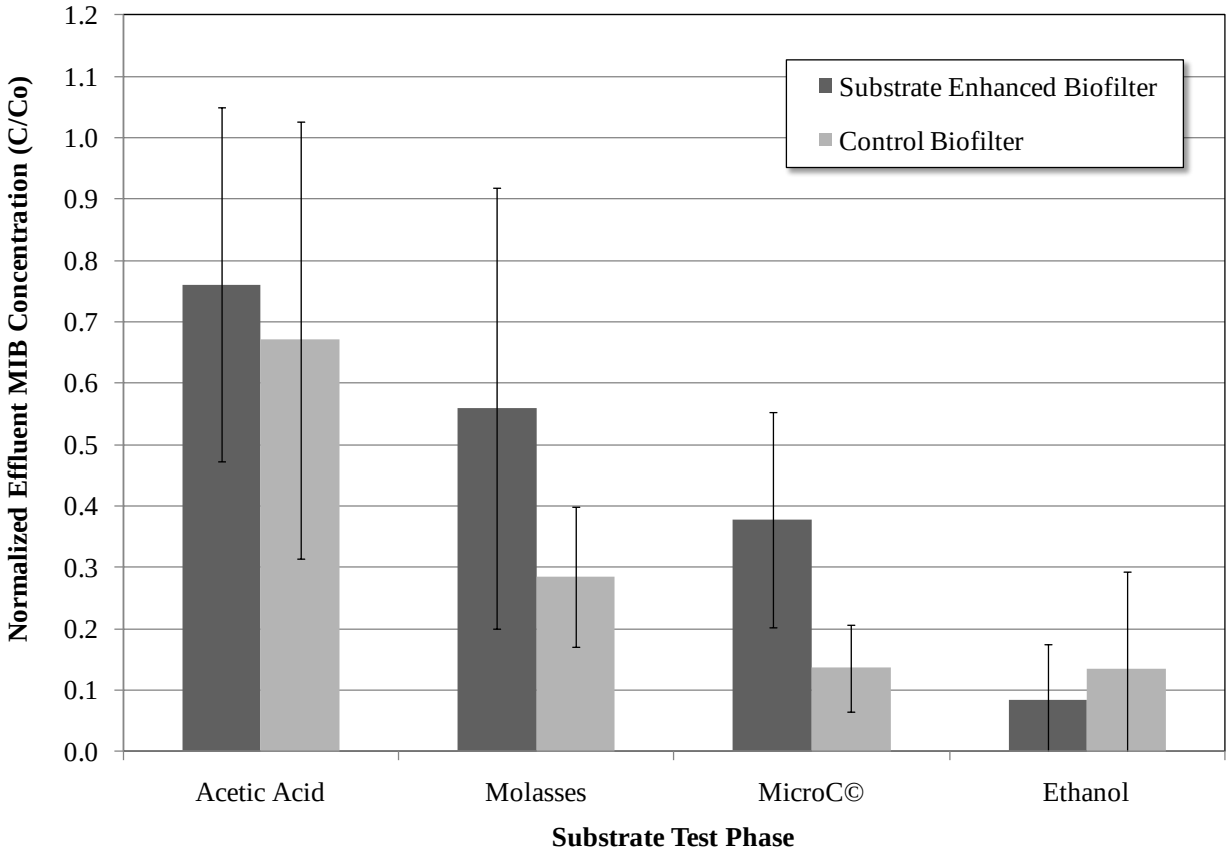

Figure 5-4. Comparison of substrate enhanced and biofilter control normalized MIB concentrations

Pharmaceuticals and Pesticides

Select biofilter effluent samples were collected during each of the substrate enhancement tests for pharmaceutical and pesticide monitoring. Atrazine, deethylatrazine deisopropylatrazine, hydroxyatrazine, simazine, metolachlor, and meprobamate were detected in the biofilter feed at ng/L concentrations. Biofiltration removals for these contaminants were not affected (favorably or adversely) by substrate-enhancement, regardless of the substrate tested.

Biological Activity

ATP concentrations in biofilter media were monitored during the substrate-enhancement studies. These tests indicated that biofilter terminal (end of filter run) biological activity (on a per media volume basis) was modestly improved (7 to 29%) relative to the biofilter control with substrate supplementation. The increased ATP concentrations measured in substrate-enhanced biofilter media samples corresponded to higher overall DOC removals (Table 5-2, Table 5-3). Table 5.4 summarizes the biofilter media ATP characterization for the substrate-enhanced biofiltration strategy.

Table 5-7. Substrate enhanced biofilter evaluation of media ATP concentrations*†

	Substrate-enhanced biofilter measured media ATP		Biofilter control measured media ATP		Effect of substrate-enhancement on ATP concentration relative to biofilter control	
Substrate tested	Start of filter run (pg/L)	End of filter run (pg/L)	Start of filter run (pg/L)	End of filter run (pg/L)	Start of filter run (% increase)	End of filter run (% increase)
Acetic acid	480,763	768,390	400,159	596,732	20	29
Molasses	532,202	713,713	488,889	667,347	9	7
MicroC©	747,156	1,209,556	503,305	1,068,987	48	13
Ethanol	567,224	880,759	471,214	742,181	21	19

* ATP test error was determined to be ~7.5% through triplicate analyses, performed monthly
† Single sample sets performed for each substrate tested

Additional data from the ATP tests (Figure 8-15) and other microbial tracking data for the substrate-enhancement studies are presented in Chapter 8 Microbial Tracking

Summary

The objective of this study was to evaluate various primary substrate augmentation strategies for enhancing biofiltration performance. Increasing the concentration of primary substrate (through intermediate ozonation or primary substrate augmentation) in a biological treatment application may increase the rate of recalcitrant

and trace organic compound degradation through secondary substrate metabolism and/or cometabolism. This study evaluated four primary substrates, including acetic acid, molasses, MicroC©, and ethanol. The substrates were evaluated individually through four successive one- to two- month phases, each dosed at target of 1 mg/L as C. The impact of filter history (i.e., the order in which the substrates were augmented to the filter) was not characterized. However, some substrates, such as molasses, appeared to have an immediate impact on biofilter headloss performance. The substrate-enhanced biofilters yielded 25 to 150% higher terminal headloss than the control biofilter during 18-hour filter runs (Table 5-1). However, sustainable (>1 week continuous operation) 18-hour filter runs were not achieved with any substrate tested due to excessive headloss.

Substrate augmentation strategies did not provide significant improvement of trace organics or metals removal relative to the control biofilter. Percent DOC removals were also comparable between the control and substrate-enhanced biofilters when acetic acid and MicroC© were used (Table 5-2 and Table 5-3). The biofilter supplemented with molasses showed twice as much DOC breakthrough as the control biofilter, while the biofilter operated with ethanol supplementation showed an average of 50% higher background DOC removal relative to the control, though the differences in the mean breakthroughs were not shown to be statistically significant. The increase in net biofilter DOC removal (background + dosed carbon) corresponded to an increased ATP concentration in the substrate-enhanced biofilter media.

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CHAPTER 6
NUTRIENT ENHANCEMENT STUDIES

Objectives

The objective of this study was to evaluate various nutrient augmentation strategies for enhancing biofiltration performance. Optimal microbial growth is dependent on a nutrient balance of carbon, NH_4-N, and PO_4-P. This balance is typically targeted at a molar ratio of 100:10:1, bioavailable C:N:P. The molar ratio translates to a concentration ratio of 1 mg/L: 0.117 mg/L: 0.026 mg/L, C:N:P. As discussed in Chapter 4, the biofilter feed at the JKWTP contained no detectable amounts of phosphorus (<0.01 mg/L), which is likely due to general source water limitation and to incidental phosphorus removal through enhanced coagulation. The background NH_4-N concentrations varied significantly during testing, indicating that occasional periods of N limitation had occurred (Table 4-6 and Table 6-7). A minimum of 0.010 mg/L PO_4-P and 0.047 mg/L of NH_4-N are necessary to prevent a biofilter nutrient limitation with background bioavailable carbon levels entering the filter process at ~0.4 mg/L C. Nutrient enhancement was performed by dosing PO_4-P (as phosphoric acid) and/or NH_4-N (as ammonium chloride) to sufficiently eliminate nutrient limitation, thereby creating a carbon, or substrate, limitation. Nutrient enhancement was evaluated through the following tests:

- Nutrient-Enhanced Biofilter Testing: The purpose of this test was to satisfy the baseline PO_4-P limitation by dosing phosphoric acid at a target of 0.020 mg/L P (200% of stoichiometric requirement) to a pilot biofilter operated with assumed 0.4 mg/L of background bioavailable C (mean C removed in the pilot control biofilter). This test was conducted in parallel to biofilter control operation. The duration of this test was 6 weeks, with approximately 2 weeks of steady state operation. This test was evaluated across all hydraulic and water treatment performance criteria, as described in Chapters 3 and 4.

- Substrate- and Nutrient- Enhanced Biofilter Testing: The purpose of this test was to satisfy the phosphorus limitation caused by substrate supplementation by dosing phosphoric acid at a target of 0.070 mg/L PO_4-P (200% of stoichiometric requirement) to a pilot biofilter operated with ~1.4 mg/L of bioavailable C (0.4 mg/L of background + 1 mg/L of supplemental C). This substrate- and nutrient-enhanced biofilter was operated in parallel with a substrate-only enhanced biofilter (same substrate) and the biofilter control. Substrate and nutrient enhancement testing was performed for each substrate tested (acetic acid, molasses, MicroC©, and ethanol). In addition, one biofilter was operated for a two-week period with ethanol, phosphoric acid, and ammonium chloride supplementation. This test was evaluated across hydraulic performance criteria and DOC removal.

- Nutrient Enhanced-Biofilter Validation Testing: During the final two weeks of pilot testing, a target 0.020 mg/L phosphoric acid as P (200% of stoichiometric requirement) was dosed to the biofilter control (thus sacrificing it as a control). The purpose of this test was to validate previous observations by evaluating nutrient enhancement on a different biofilter. This test was evaluated across hydraulic performance criteria and DOC removal.

Hydraulic Characterization

Nutrient enhancement testing

While clean-bed headloss was unaffected by nutrient addition, phosphoric acid supplementation decreased biofilter terminal headloss (following 18 hours of operation) by approximately 15% (as average decrease over 17 consecutive runs) relative to the biofilter control ($p = 0.01$, $p \leq 0.05$) In addition, phosphoric acid supplementation provided more consistent biofilter runs (50% decrease in terminal headloss standard deviation). The hydraulic improvement was sustained throughout the two weeks of testing. Table 6-1 provides a summary of the hydraulic characterization during the nutrient enhancement tests. Figure 6-1 illustrates a selection of steady state headloss profiles during the final 9 runs of this study. Terminal headloss in the nutrient-enhanced biofilter appeared to be trending down during the final filter runs, suggesting that additional hydraulic improvement may be possible with continued operation.

Substrate and nutrient enhancement testing

The substrate-enhanced biofilter operated with phosphoric acid supplementation generally showed lower headloss profiles than the biofilter operated with supplemental substrate alone (for each substrate tested). However, the mean substrate- and nutrient-enhanced biofilters terminal headloss still exceeded that of the control biofilter for each of the substrates and multiple biofilter runs were prematurely terminated due to excessive headloss (greater than 13.5 ft). Although the phosphorous nutrient requirement was satisfied, the substrate (~1.4 mg/L as C) and nutrient enhanced (~0.070 mg/L PO_4-P) biofilter was operated with a NH_4-N feed limitation (C:N:P ~100:3:2). To satisfy the NH_4-N limitation, ammonium chloride was fed (0.10 mg/L as NH_4-N) to the ethanol and nutrient enhanced biofilter during the final two weeks of testing. The addition of ammonium chloride decreased the mean terminal headloss by more than 35% as compared to ethanol and PO_4-P enhancements alone. Furthermore, the terminal headloss saw an immediate decrease of over 50% relative to the previous four filter runs. This observation confirmed that both PO_4-P and NH_4-N limitations may diminish biofilter hydraulic performance. Table 6-2 provides a summary of the hydraulic characterization during the substrate and nutrient enhancement tests. Figure 6-2 illustrates the hydraulic performance improvement realized after ammonium chloride supplementation was implemented on the substrate- and nutrient-enhanced biofilter.

Table 6-1. Nutrient-enhanced biofilter hydraulic performance

Nutrient-enhanced* biofilter terminal headloss (ft)			Biofilter control terminal headloss† (ft)			Difference between the means for nutrient-enhanced and control biofilters
Mean‡	Min.	Max.	Mean‡	Min.	Max.	p value
5.1 ± 0.7	3.0	6.6	5.9 ± 1.4	3.2	7.8	0.01

* Target PO_4-P feed in the nutrient-enhanced biofilter was 0.020 mg/L as P.
† Biofilter control operated without supplemental phosphoric acid.
‡ Includes 17 biofilter runs, means are provided with the standard deviation of the data sets as value error.

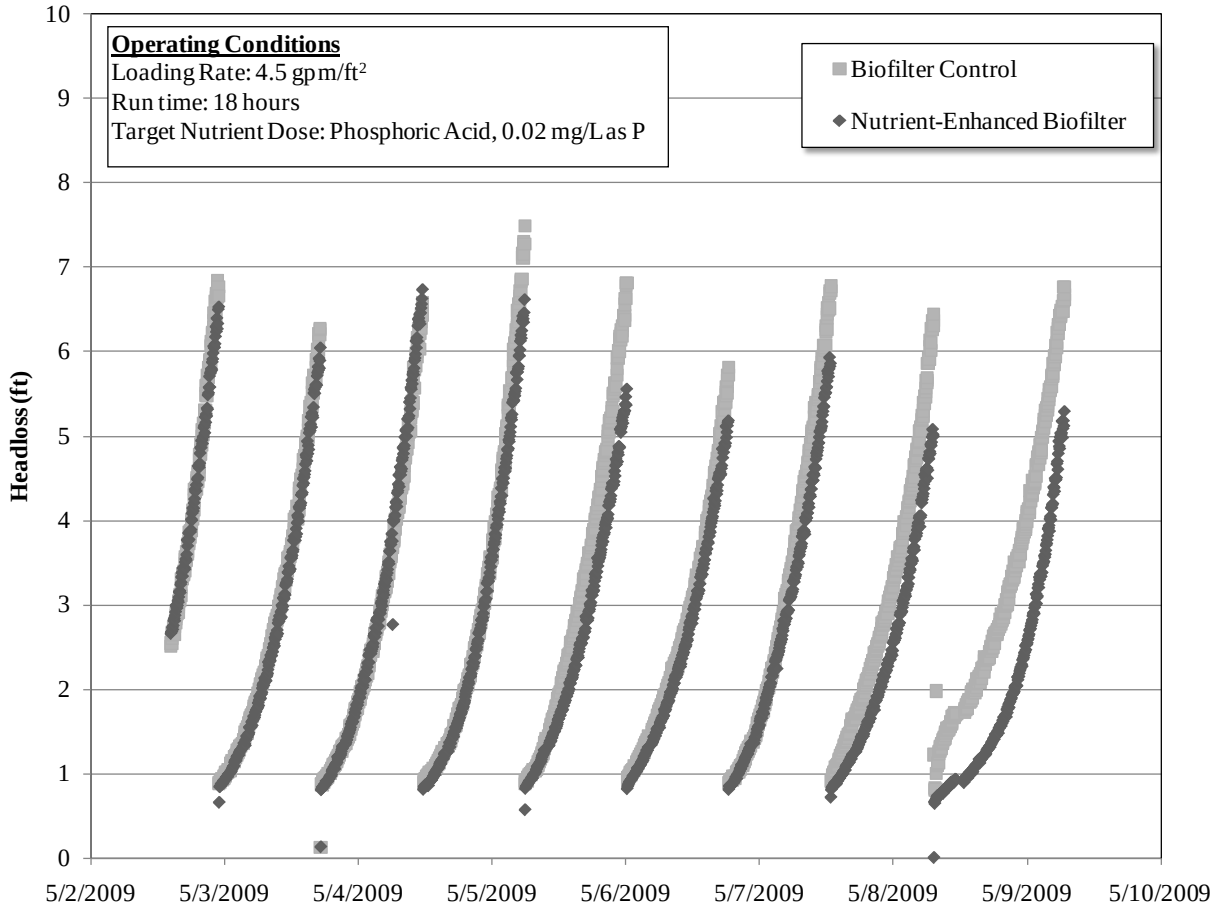

Figure 6-1. Comparison of nutrient-enhanced (PO_4-P) and biofilter control headloss profiles

Table 6-2. Substrate- and nutrient-enhanced biofilter hydraulic performance

	Substrate-enhanced biofilter terminal headloss (ft)			Substrate- and PO_4-P-enhanced biofilter terminal headloss* (ft)			Substrate-, PO_4-P-, and NH_4-N- enhanced biofilter terminal headloss* (ft)		
Condition tested	Mean†	Min.	Max.	Mean†	Min.	Max.	Mean†	Min.	Max.
Acetic acid‡	10.3 ± 2.4	5.5	>13.5	10.2 ± 2.4	5.0	>13.5	NA§§	NA§§	NA§§
Molasses§	>13.5	10.8	>13.5	>13.5	6.6	>13.5	NA§§	NA§§	NA§§
MicroC©**	8.8 ± 2.8	4.3	>13.5	8.4 ± 4.3	2.9	>13.5	NA§§	NA§§	NA§§
Ethanol††	>13.5	12	>13.5	9.9 ± 2.6	6.3	>13.5	6.3 ± 0.6	5.1	7.0

* Substrate- and nutrient-enhanced biofilters operated with a target phosphoric acid dose of 0.070 mg/L as P.

† Means are provided with the standard deviation of the data sets as value error

‡ Includes 24 (consecutive) filter runs.

§ Includes 38 biofilter runs. Approximately 85% of biofilter filter runs terminated prematurely due to headloss exceeding 13.5 feet for substrate enhanced and substrate and nutrient-enhanced biofilters

** Includes 45 biofilter runs. Clogged biofilter effluent lines artificially elevated headloss through six filter runs with MicroC© and twelve filter runs with MicroC© and phosphoric acid supplementation, these runs were not included in the above analysis. The lines were clogged with a white gelatinous material believed to be a product of biological growth.

†† Includes 12 filter runs. 90% of ethanol supplemented and 33% of ethanol and phosphoric acid biofilter runs were terminated prematurely due to headloss exceeding 13.5 feet.

‡‡ Includes 16 filter runs

§§ NA = Not applicable. No parallel operation of biofilter control.

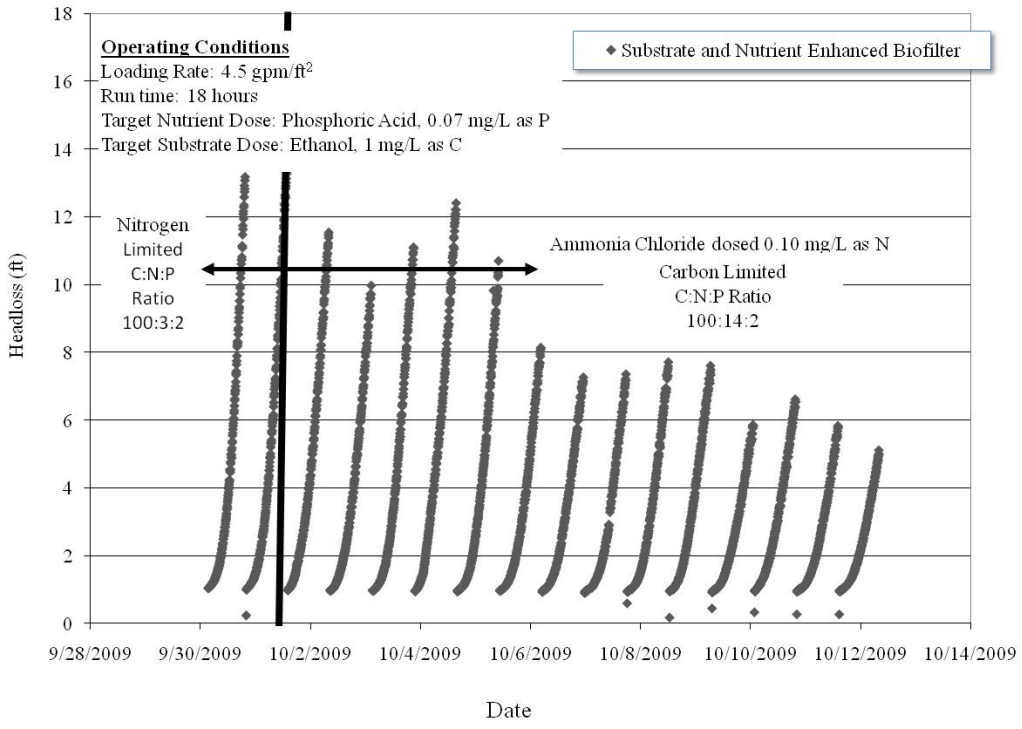

Figure 6-2. Effect of ammonium chloride supplementation on substrate and nutrient-enhanced biofilter operated with NH_4-N limitation

Nutrient enhancement validation

Validation of the nutrient-enhancement strategy was performed by dosing the biofilter control with phosphoric acid (200% of the stoichiometric requirement) to satisfy the baseline PO_4-P limitation. This required the sacrifice of the sole biofilter control. This experiment was performed at the end of the pilot testing, and use of the control filter for validation was more appropriate than using other pilot filters that had received substrate and nutrient enhancements in the recent past. Therefore, hydraulic performance was evaluated by comparing the terminal headloss of the biofilter with the previous month of steady state hydraulic data. Table 6-3 provides a summary of the hydraulic characterization during the nutrient enhancement validation test.

As shown in Table 6-3, terminal headloss data suggest that phosphoric acid supplementation may have improved the hydraulic performance of the former biofilter control (p value = 0.04, $p \leq 0.05$).

Table 6-3. Nutrient-enhancement validation on biofilter hydraulic performance

Nutrient-enhanced* biofilter terminal headloss (ft)			Biofilter control terminal headloss† (ft)			Difference between the means for nutrient-enhanced and control biofilters
Mean‡	Min.	Max.	Mean§	Min.	Max.	p value
5.4 ± 0.4	4.1	6.1	5.9 ± 0.6	4.7	7.0	0.04

* Target PO_4-P feed in the nutrient-enhanced biofilter was 0.020 mg/L as P. Table 6-7 summarizes measured PO_4-P dosages.

† Data taken from last month of steady state operation (August 2009).

‡ Includes 14 biofilter runs, means are provided with the standard deviation of the data sets as value error.

§ Includes 36 biofilter runs, means are provided with the standard deviation of the data sets as value error.

Water Quality Characterization

General

Water treatment performance characterization included routine sampling and water quality analyses (Chapter 4, Table 4-3). Selected samples were also collected for chloramine stability and DBPFP tests. Nitrite and nitrate analyses were performed on samples collected from the substrate- and nutrient-enhanced biofilter to characterize nitrification after ammonium chloride supplementation.

Turbidity

All turbidities maintained compliance with the USEPA Surface Water Treatment Rule, as greater than 95% of the effluent turbidity samples were less than 0.3 NTU. Mean turbidity breakthroughs remained below 0.08 NTU for all conditions tested. No turbidity values over 1 NTU were observed in nutrient-enhanced biofilter effluent. However, substrate- and nutrient-enhanced biofilter turbidity excursions over mean biofilter control turbidities were observed during periods of frequent backwashing due to high headloss. Figure 6-3 illustrates nutrient-enhanced effluent turbidity profiles for two typical filter runs.

Table 6-4 summarizes mean effluent turbidity breakthroughs for the nutrient-enhanced and control biofilters during parallel operation.

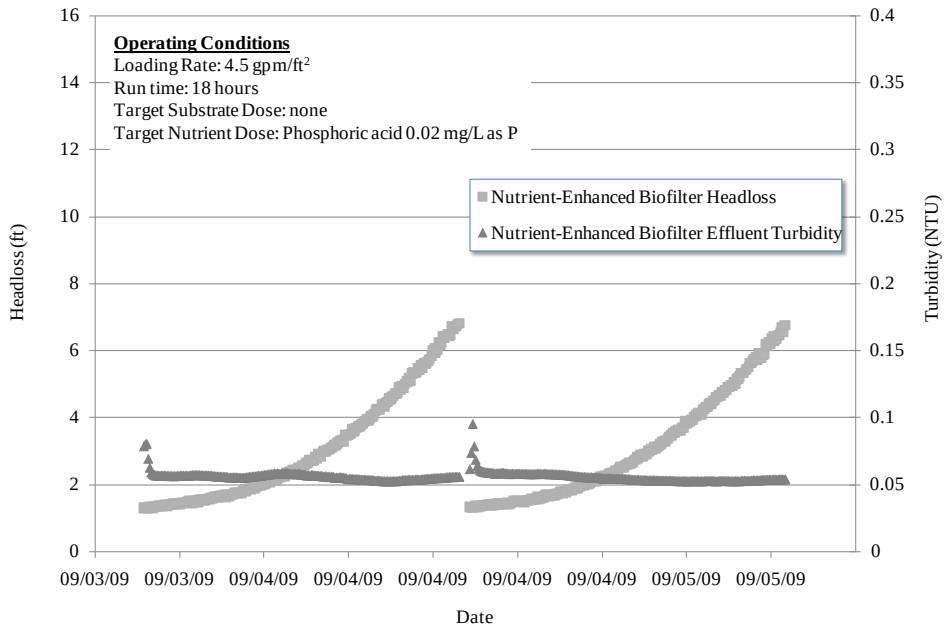

Figure 6-3. Nutrient-enhanced biofilter turbidity profiles for typical filter runs

Table 6-4. Baseline characterization of nutrient-enhanced biofilter turbidity breakthrough

Biofilter Feed*,† (NTU)			Nutrient-enhanced biofilter† effluent (NTU)			Biofilter control† effluent (NTU)		
Mean‡	Min.	Max. §	Mean‡	Min.	Max. §	Mean‡	Min.	Max. §
0.6± 0.2	0.3	0.9	0.06± 0.02	0.05	0.14	0.07± 0.02	0.06	0.17

* Biofilter feed water was JKWTP settled/ozonated water for pilot and full-scale biofilters.
† Biofilter feed turbidities were measured using a desktop turbidimeter. Pilot biofilter effluents were measured continuously (5 minute intervals) using inline instrumentation (Chapter 3).
‡ Means are provided with two standard deviations (to capture 95% of the data distribution) to show regulatory compliance.
§ Pilot biofilter effluent maximum values were observed during filter ripening.

DOC

Low-level phosphoric acid supplementation improved biofilter DOC removal performance during the nutrient-enhanced biofilter tests. The nutrient-enhanced biofilter removed a mean of 75% more DOC than the biofilter control (0.7 mg/L with nutrient enhancement as compared to 0.4 mg/L in the control). A paired t-test analyses of DOC removal data collected from the nutrient-enhanced biofilter and the control indicated that the difference in the means was statistically significant [$t(6) = 6.14$, $p = 0.0009$]. This

performance was sustained over 2 weeks of routine operation. A summary of the nutrient-enhanced biofilter DOC removal performance is provided in Table 6-5. All DOC removal data was also normalized ($C_{effluent}/C_{o,backgound}$) to illustrate changes in background BOM removal across the biofilters. Normalized DOC results for the nutrient-enhancement study are provided in Figure 6-4.

Table 6-5. Substrate- and nutrient-enhanced biofilter DOC removal performance

Biofilter feed (background C, mg/L)			Nutrient-enhanced biofilter* effluent (mg/L)			Biofilter control effluent (mg/L)		
Mean*	Min.	Max	Mean*	Min.	Max	Mean*	Min.	Max
3.6 ± 0.1	3.5	3.7	2.9 ± 0.1	2.8	3.1	3.2 ± 0.2	3.0	3.4

* Target phosphoric acid dose for the nutrient-enhanced biofilter was 0.020 mg/L as P. Table 6.7 summarizes measured PO_4-P dosages.

† Includes two weeks of steady state data (N=7). Means are provided with the standard deviation of the data sets. =

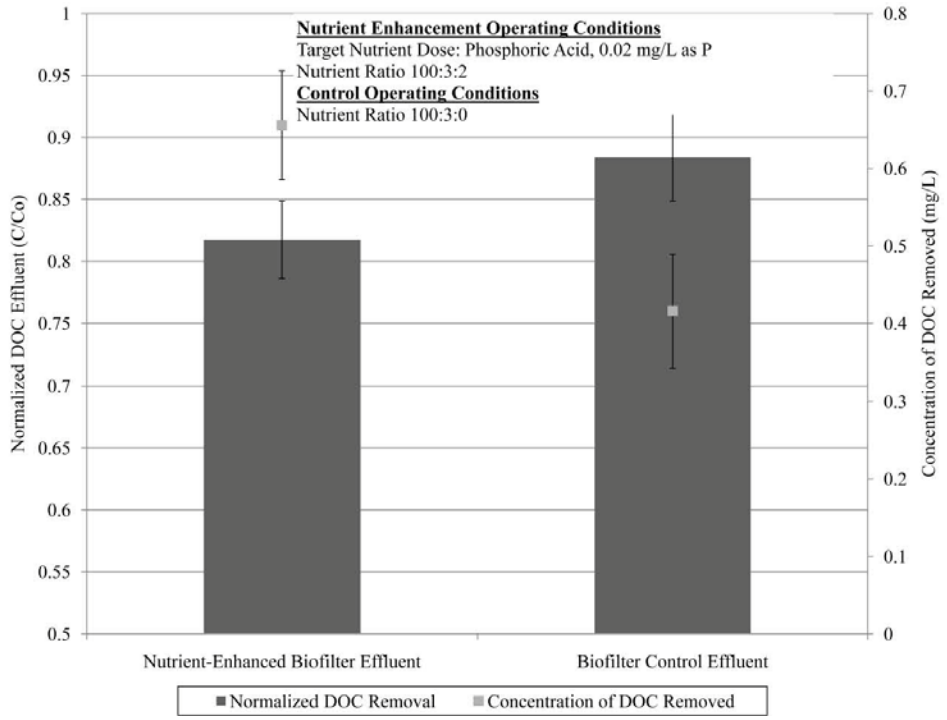

Figure 6-4. Comparison of nutrient-enhanced and biofilter control normalized DOC removals [4]

[4] The error bars presented in all figures in this Chapter represent the standard deviation of the data set. This standard deviation accounts for operational variability (i.e., feed water conditions) and sampling/analytical error.

The results provided in Table 6-5 were validated when phosphoric acid was fed to the biofilter control during the last two weeks of pilot testing (Figure 6-5). DOC removal (as % of influent) increased by 35%, when comparing the two weeks of nutrient enhancement against the preceding two weeks of biofilter control operation ($p = 0.0001$, $p \leq 0.05$).

Data collected during the substrate- and nutrient-enhanced biofilter tests suggest that nutrient-enhancement also improves DOC removal for the substrate-enhanced biofilters. Table 6-6 compares normalized effluent DOC concentrations for the nutrient- and substrate-enhanced biofilter, the substrate-enhanced biofilter, and biofilter control for samples collected during parallel operation. Normalized biofilter effluent DOC data are also provided for the substrate- and nutrient-enhanced biofilter operated with phosphoric acid and ammonium chloride supplementation. Figure 6-6 illustrates the data collected during the parallel studies described in Table 6-6. The data presented in Table 6-6 and Figure 6-6 show no DOC removal improvement with nutrient- and substrate-enhancement relative to nutrient enhancement alone. A hypothesis for this observation is that most labile BOM was effectively removed with nutrient supplementation alone (to achieve substrate limitation). In addition, the data suggest that the supplemental substrates tested did not promote significant secondary substrate metabolism or cometabolism of recalcitrant BOM.

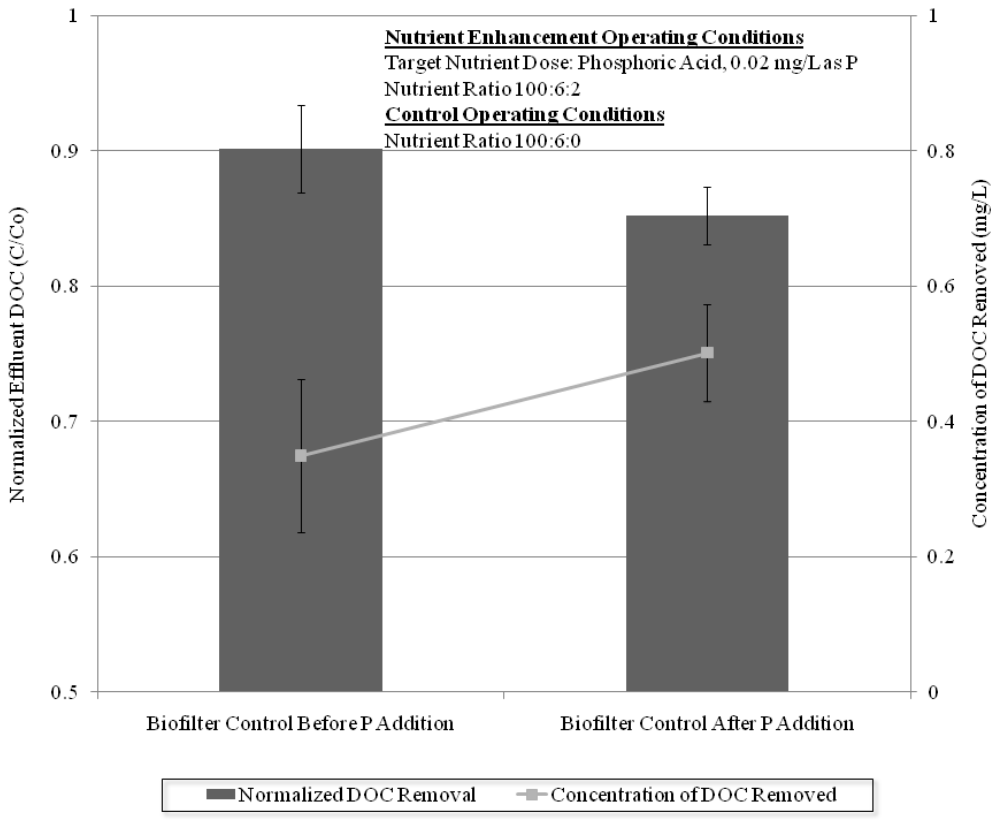

Figure 6-5. DOC removal performance improvement with nutrient-enhancement of (previous) biofilter control

Parallel studies between nutrient- and substrate-enhanced and nutrient-enhanced biofiltration were limited; therefore, additional testing must be performed to better characterize relative performance.

As shown in Table 6-5 and Figure 6-6, relative DOC removals between the substrate-enhanced biofilter and substrate and nutrient-enhanced biofilter were similar for all conditions tested excluding molasses. Steady-state water treatment performance (DOC removal) with supplemental molasses was only achieved with supplemental phosphorus.

The biofilter operated with supplemental ethanol and phosphoric acid removed an additional 0.2 mg/L of background DOC relative to the biofilter control (Figure 6-6). A

paired t-test analyses of this DOC removal data indicated that the difference between the means were statistically significant [t (7) = 3.3, two tail p = 0.013]. However, paired t-test analyses of all other substrates tested showed no statistically significant difference (p>0.05) from the substrate-enhanced biofilter, the substrate- and nutrient-enhanced biofilter, and the biofilter control.

The molasses and ethanol substrate- and nutrient-enhanced biofilter data support the nutrient enhancement tests, suggesting that PO_4-P limitations during supplemental substrate addition may inhibit optimal DOC removal in biofilters. Furthermore, the PO_4-P limitations may be resolved with phosphoric acid supplementation for improved DOC removal performance. The data in Table 6-6 also suggest that the other substrates may be less labile than ethanol or limit background DOC utilization.

Table 6-6. Substrate- and nutrient-enhanced biofilter normalized DOC removal characterization

Condition tested[*,†,‡]	Substrate-enhanced biofilter effluent[§] ($C_{effluent}/C_{o,backgound}$)			Substrate- and nutrient-enhanced biofilter effluent[§] ($C_{effluent}/C_{o,backgound}$)			Biofilter control effluent[§] ($C_{effluent}/C_{o,backgound}$)		
	Mean[**]	Min.	Max.	Mean[**]	Min.	Max.	Mean[**]	Min.	Max
Acetic acid	0.91 ± 0.04	0.88	1.00	0.89 ± 0.03	0.86	0.95	0.89 ± 0.01	0.88	1.00
Molasses[††]	0.99 ± 0.00	0.99	0.99	0.85 ± 0.03	0.80	0.90	0.86 ± 0.01	0.86	0.86
MicroC©	0.88 ± 0.04	0.82	0.93	0.85 ± 0.03	0.80	0.90	0.88 ± 0.02	0.81	1.03
Ethanol	0.85 ± 0.02	0.82	0.87	0.83 ± 0.04	0.74	0.88	0.89 ± 0.03	0.84	0.93
Substrate (Ethanol), NH_4-N and PO_4-P	NA[‡‡]	NA[‡‡]	NA[‡‡]	0.84 ± 0.04	0.80	0.88	NA[‡‡]	NA[‡‡]	NA[‡‡]

* Target substrate dosage was 1 mg/L as C for all substrate-enhanced and substrate-and nutrient-enhanced biofilter conditions tested.
† Target phosphoric acid dose was 0.070 mg/L as PO_4-P for all substrate- and nutrient-enhanced biofilter conditions tested. Mean biofilter feed PO_4-P concentrations are provided in Table 6-8.
‡ Target ammonium chloride dose was 0.10 mg/L as NH_4-N, mean dosage provided in Table 6-9.
§ Effluents normalized to background feed DOC concentrations.
** Means are provided with the standard deviation of the data sets as value error.
†† Molasses partitioning in the chemical feed tank was observed. Concentrations higher than target were dosed to the substrate-enhanced biofilter.
‡‡ NA = Not applicable. No parallel operation of substrate-enhanced biofilter or biofilter control.

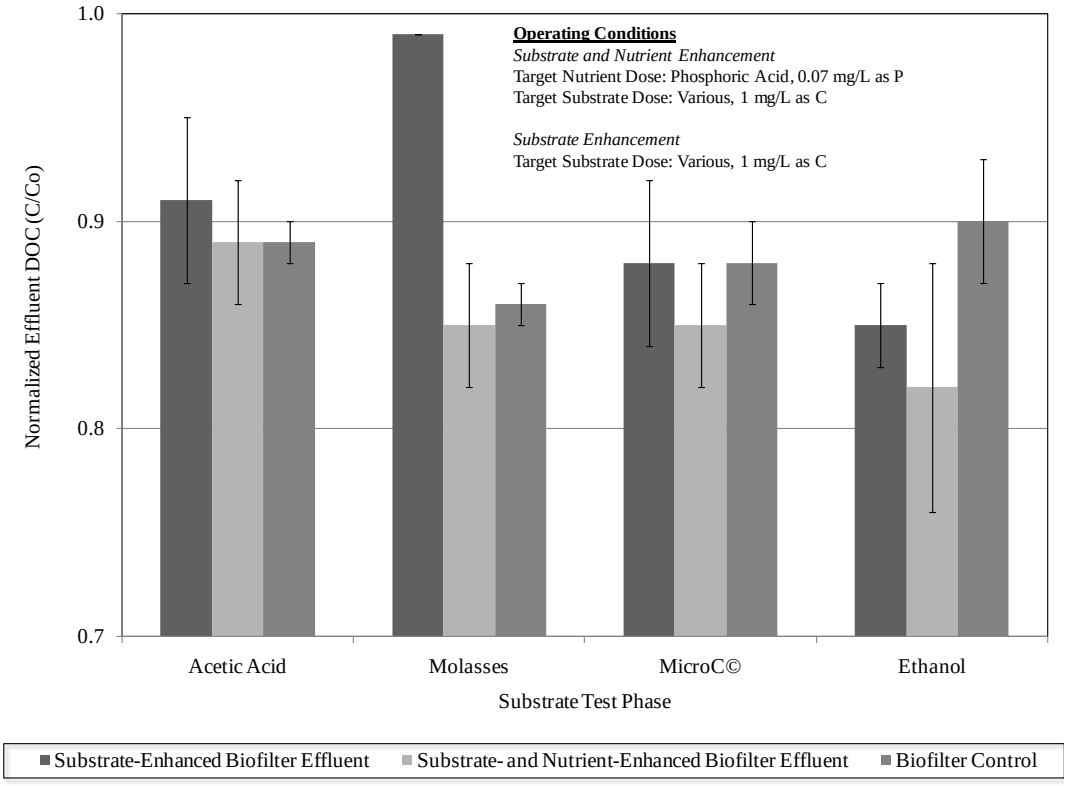

Figure 6-6. Characterization of normalized DOC removal for substrate- and nutrient-enhanced biofilters

Nutrients

Nutrient balance evaluation

Biofilter effluent PO_4-P and NH_4-N concentrations were monitored during the nutrient-enhancement studies to validate dosage, characterize utilization, and monitor breakthrough. The primary objective of these analyses was to verify whether sufficient PO_4-P and NH_4-N concentrations were established in the biofilter feed to shift the C:N:P ratio from a nutrient-limited condition to a carbon-limited condition. Table 6-7 presents the nutrient ratio for each nutrient enhancement test by providing the mean bioavailable C, PO_4-P, and NH_4-N for each biofilter feed. The mean DOC removed was used as a

conservative estimate of bioavailable C to identify the minimum nutrient concentrations required to satisfy the 100:10:1 bioavailable C:N:P ratio.

The data in Table 6-7 indicate that under all conditions tested, phosphoric acid supplementation was sufficient to meet minimum PO_4-P requirements. The 5-month mean background NH_4-N feed concentration (0.043 mg/L, see Chapter 4, Table 4-6) met nutrient requirements under control conditions. However, periodic fluctuations created nutrient limitations during the nutrient-enhanced biofilter validation tests. Substrate enhancement further exacerbated the observed NH_4-N limitations by increasing the concentration of bioavailable C.

Table 6-7. Nutrient enhancement studies: nutrient balances

Condition tested	Feed bioavailable C* (mg/L)	Feed NH_4-N * (µg/L)	Feed PO_4-P* (µg/L)	C:N:P stoichio-metric ratio	Limitation	Hydraulic Performance**
Nutrient-Enhanced Biofilter†						
Initial phosphoric acid test	0.7	77	19	100:10:1	None	Highly Improved
Validation w/phosphoric acid (performed at end of study, control biofilter + PO_4-P)	0.5	32	23	100:6:1	NH_4-N	Improved
Substrate- and Nutrient-Enhanced Biofilter‡						
Acetic acid w/phosphoric acid	1.3	61	170	100:4:4	NH_4-N	Highly Decreased
Molasses w/phosphoric acid	1.0	25	211	100:2:8	NH_4-N	Highly Decreased
MicroC© w/phosphoric acid	1.1	31	91	100:2:3	NH_4-N	Highly Decreased
Ethanol w/phosphoric acid	1.7	59	82	100:3:2	NH_4-N	Highly Decreased
Ethanol w/phosphoric acid and ammonium chloride	0.8	130	51	100:14:2	Carbon	Similar to Control

Table6-7. Continued

* Means for data collected during test duration. Value error is not provided in table for clarity, it can be found in Tables 6-4, 6-5, 6-7, and 6-8 as standard deviation.

† Target phosphoric acid dose was 0.020 mg/L as PO_4-P for all nutrient-enhanced biofilter conditions tested.

‡ Target phosphoric acid dose was 0.070 mg/L as PO_4-P for all substrate- and nutrient-enhanced biofilter conditions tested. Poor chemical flow control observed during acetic acid and molasses testing. However, mean PO_4-P dosages well exceed minimum requirement to satisfy nutrient limitation.

** Relative to control biofilter

Nutrient breakthrough evaluation

Biofilter effluent NH_4-N and PO_4-P concentrations were monitored to characterize breakthrough during nutrient enhancement studies. Under some conditions, excessive nutrient breakthrough during biofilter nutrient enhancement may contribute to biological regrowth in the distribution system. The PO_4-P data collected indicate that nutrient-enhanced biofilters show 55 to 65% breakthrough of feed PO_4-P (mean effluents of ~14 to 16 µg/L). The observed breakthrough was likely due to multiple factors including excess feed (200% of nutrient requirement fed under most conditions), fluctuations in chemical delivery, and fluctuations with alum flocculant carryover (thus precipitation of PO4-P in filter media).These PO_4-P breakthrough levels were considered low, as they were below the minimum reporting limits (MRLs) for many utilities, including the City. However, the long-term impact of low-level PO_4-P breakthrough on the City's distribution system is unknown and, therefore, must be studied further. Mean PO_4-P breakthroughs of 55 to 94% (mean effluents of ~38 to 160 µg/L) were observed in the substrate- and nutrient-enhanced biofilter, although biofilter influent overfeeding (>200%) was observed during some tests. It is important to note that PO_4-P breakthrough does not necessarily suggest excess PO_4-P biofilter feed or non-utilization. PO_4-P does not biologically transform and may appear in effluent samples as

sloughed biomass or extracellular materials. Table 6-8 summarizes the PO_4-P characterization for the nutrient enhancement studies.

Table 6-8. Nutrient enhancement studies: PO_4-P characterization

	Feed PO_4-P (µg/L)			Biofilter effluent PO_4-P concentration (µg/L)			Normalized biofilter effluent PO_4-P concentration (C/C_o)		
Condition tested	Mean*	Min.	Max.	Mean*	Min.	Max.	Mean*	Min.	Max
Nutrient-enhanced biofilters†									
Initial phosphoric acid test	20 ± 5	12	25	14 ± 4	11	20	0.55 ± 0.36	0.95	0.08
Validation w/phosphoric acid	23 ± 1	14	36	16 ± 5	10	25	0.65 ± 0.19	0.81	0.28
Substrate- and nutrient- enhanced biofilters‡									
Acetic acid w/phosphoric acid	170 ± 80	186	147	160 ± 23	132	197	0.94 ± 0.08	0.84	1.0
Molasses w/phosphoric acid	211 ± 14	86	360	98 ± 73	33	178	0.67 ± 0.50	0.09	0.97
MicroC© w/phosphoric acid	91 ± 79	255	0	57 ± 62	0	194	0.55 ± 0.22	0.27	0.95
Ethanol w/phosphoric acid	82 ± 33	104	25	52 ± 22	22	77	0.59 ± 0.19	0.28	0.88
Ethanol w/phosphoric acid and ammonium chloride	51 ± 15	40	72	38 ± 21	16	66	0.72 ± 0.22	0.40	0.91

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.

† Target phosphoric acid dose was 0.020 mg/L as PO_4-P for all nutrient-enhanced biofilter conditions tested.

‡ Target phosphoric acid dose was 0.070 mg/L as PO_4-P for all substrate- and nutrient-enhanced biofilter conditions tested. Poor chemical flow control was observed during acetic acid and molasses testing. However, mean PO_4-P dosages well exceeded minimum requirement to satisfy nutrient limitation.

Table 6-9 provides NH_4-N characterization through the nutrient enhancement studies. Under NH_4-N limiting conditions, NH_4-N mean utilizations (assimilation and oxidation) ranged from 28 to 52% across the conditions tested. The NH_4-N breakthrough levels were higher than expected; however, the limitation may shift

biofilter microbial communities to those requiring less NH_4-N than assumed in the nutrient balance. (Sekar et al., 2002; Davidson et al., 2007). Interestingly, NH_4-N supplementation increased utilization to a mean of 85%. This increase is possibly due to a biofilter population shift to nitrifying bacteria.

Table 6-9. Nutrient enhancement studies: NH_4-N characterization

	Feed NH_4-N (µg/L)			Biofilter effluent NH_4-N concentration (µg/L)			Normalized biofilter effluent NH_4-N concentration (C/C_o)		
Condition tested	Mean*	Min.	Max.	Mean*	Min.	Max.	Mean*	Min.	Max
Nutrient enhancement									
Initial phosphoric acid test†	77 ± 41	46	161	39 ± 22	16	65	0.65 ± 0.41	0.1	1.0
Validation w/phosphoric acid†	32 ± 10	18	42	15 ± 4	12	29	0.48 ± 0.15	0.36	0.70
Substrate and nutrient enhancement									
Acetic acid w/phosphoric acid†	61 ± 56	16	180	24 ± 11	11	42	0.72 ± 0.15	0.56	0.88
Molasses w/phosphoric acid†	25 ± 10	13	39	17 ± 10	10	34	0.38 ± 0.16	0.16	0.59
MicroC© w/phosphoric acid†	31 ± 13	14	50	16 ± 6	10	27	0.31 ± 0.07	0.22	0.40
Ethanol w/phosphoric acid†	59 ± 17	42	88	21 ± 5	17	30	0.49 ± 0.22	0.31	0.66
Ethanol w/phosphoric acid and ammonium chloride‡	130 ± 72	44	210	16 ± 5	9	21	0.15 ± 0.06	0.08	0.20

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.
† Feed NH_4-N levels include background only.
‡ Feed NH_4-N levels include background and dosed ammonium chloride.

Table 6-10 summarizes biofilter effluent nitrogen speciation during the ammonium chloride supplementation. At non-chloraminating utilities, NH_4-N breakthrough may decrease effluent stability and increase chlorine demand. However, the data show near

complete nitrification of all NH_4-N to NO_3-N in the test biofilter. Nitrification in the biofilter is likely driven by the oxidation processes of autotrophic bacteria that utilize ammonia and nitrite as electron donors. It is important to note, NH4-N is not oxidized during assimilation. However, assimilated NH4-N (e.g., proteins, amino acids, nucleotides) may be cycled within the biofilter and ultimately nitrified. Effluent nitrate remained well below current drinking water limits (10 mg/L as N). Figure 6-7 illustrates nitrogen speciation before and after treatment through substrate- and nutrient-enhanced biofiltration. The, a portion of the influent NO_3^--N would be retained in the filter as part of the biomass.

Table 6-10. Biofilter nitrification characterization after ammonium chloride supplementation

Nitrogen species	Mean* feed NH_4-N (µg/L)	Mean biofilter effluent NH_4-N (µg/L)	Δ (µg/L)
NH_4-N†	130 ± 70	16 ± 5	- 111 ± 65
NO_2-N‡	4 ± 5	2 ± 1	- 2 ± 5
NO_3-N†	442 ± 37	585 ± 75	+ 143 ± 80

* Means are provided with the standard deviation of the data sets as value error. Statistical analyses included assigning one-half the limit of detection/quantification values to constituents with non-detected concentrations.
† Feed NH_4-N levels include background and dosed ammonium chloride.
‡ Feed NO_2-N and NO_3-N levels included background only.

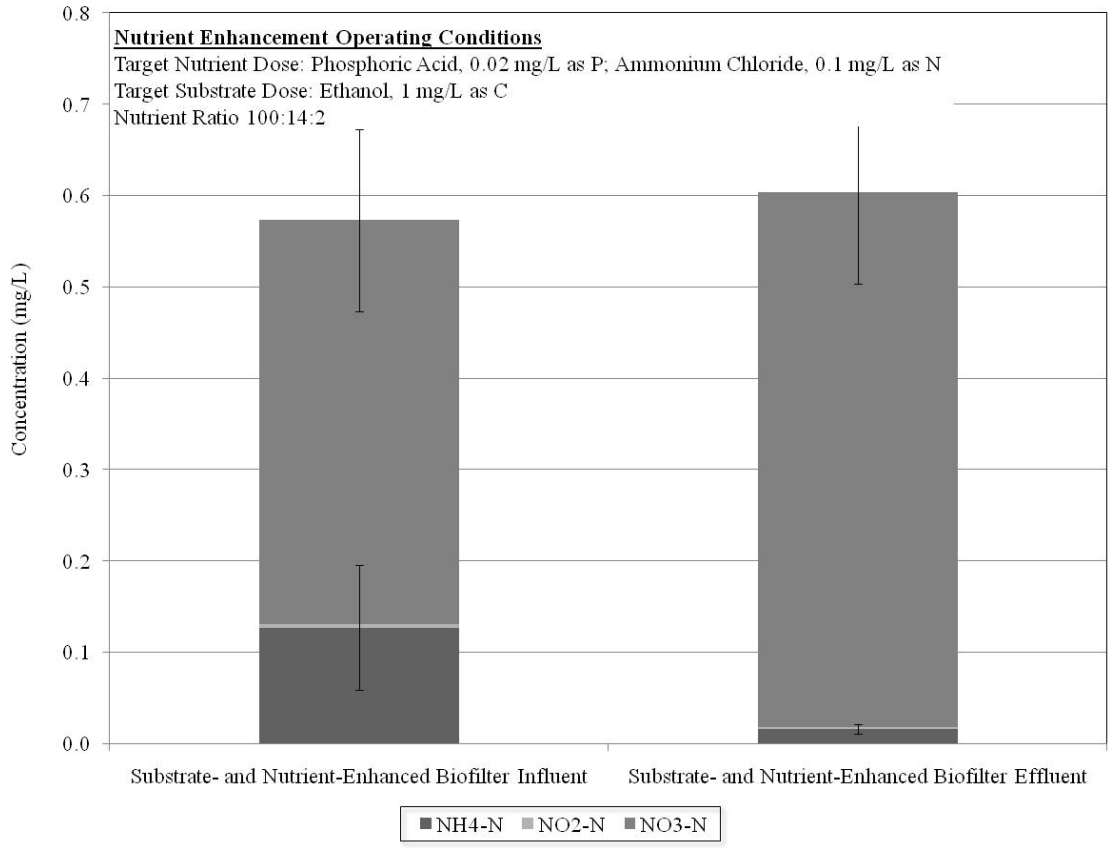

Figure 6-7. Biofilter nitrification characterization after ammonium chloride supplementation to the substrate (ethanol)- and nutrient-enhanced biofilter

Metals

Fe and Mn removal were observed for the nutrient-enhanced, nutrient- and substrate-enhanced, and control biofilters under each test condition. However, relative metals removal performance was difficult to establish because of generally low and varied feed concentrations. Mean effluent concentrations remained below the Mn and Fe MDLs for all conditions tested including the control performance (Chapter 5, Table 5-4). Therefore, selected nutrient enhancement test conditions were further characterized for metals removal performance. The nutrient-enhanced biofilter and the substrate- and nutrient-enhanced biofilter (ethanol and phosphoric acid) were evaluated in parallel with the biofilter control over the following conditions:

- Simulated long-term, moderate Mn load event - 1 month of steady state operation with a Mn load of ~50 ng/L Mn.
- Simulated short-term, high Mn load event - 1 week of steady state operation with a Mn load of ~220 ng/L.

Figure 6-8 shows the results of long-term, moderate load test. The average removals in the enhanced biofilters were slightly lower than that observed in the control biofilter. The control biofilter also demonstrated greater fluctuations in Mn removal. Figure 6-9 shows the results of the short-term, high load test. The nutrient-enhanced and the substrate- and nutrient-enhanced biofilter showed no Mn breakthrough while the control biofilter showed an average Mn breakthrough of 25 µg/L. Maximum breakthrough for the biofilter control was near the secondary MCL for both loading conditions tested (40 to 48 µg/L), supporting the observation of occasional colored water complaints during high load events at the JKWTP and PBSWTP. These data suggest that nutrient limitations may create unfavorable conditions for the microorganisms responsible for Mn oxidation, thus inhibiting its overall removal. Figures 6-8 and 6-9 summarize the results from Mn removal characterization study. Chapter 8 discusses the identified microbial communities in the pilot biofilters and their possible roles in contaminant cycling.

Taste and Odor

MIB and geosmin removal were observed for the nutrient-enhanced, nutrient- and substrate-enhanced, and control biofilters under each test condition. However, relative taste and odor removal performance was difficult to establish due to generally low and varied feed concentrations. Mean background geosmin and MIB feed concentrations remained below their MDLs, as described in Chapter 4, Table 4-5. Therefore, MIB spiking tests were conducted for additional characterization.

MIB spiking was performed on the substrate- and nutrient-enhanced biofilters and substrate-enhanced biofilters while in parallel operation. These tests were performed to identify whether PO_4-P limitations may inhibit MIB degradation in the presence of supplemental organic substrates. Phosphoric acid supplementation marginally improved MIB removal performance when acetic acid, MicroC©, and ethanol were the dosed substrates. The nutrient-enhanced biofilter (PO_4-P) was also evaluated for MIB removal performance across both long- and short-term MIB loading events. This biofilter was operated in parallel with the substrate- and nutrient-enhanced biofilter and the biofilter control over the following conditions:

- Long-term, moderate load - 1 month of steady state operation with an MIB load of ~30 ng/L.
- Short-term high load - 1 week of steady state operation with an MIB load of ~160 ng/L.

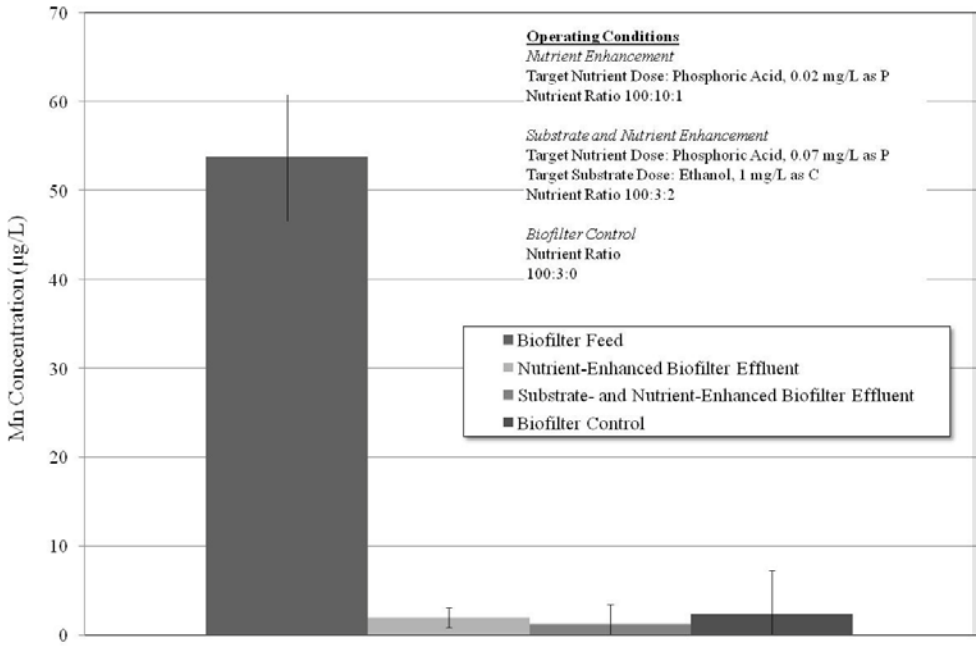

Figure 6-8. Mn removal performance for the nutrient-enhancement strategies during simulated moderate long-term loading event

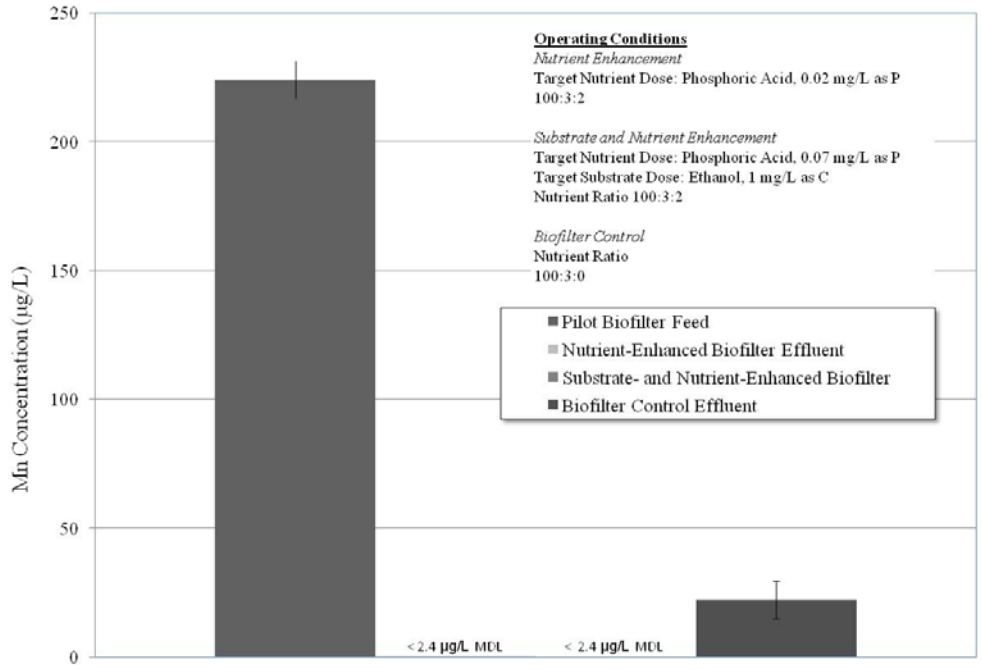

Figure 6-9. Mn removal performance for the nutrient-enhancement strategies during simulated high short-term loading event

Figures 6-10 and 6-11 show the results of the long-term/moderate MIB load and short-term/high MIB load tests, respectively. During the simulated long-term, moderate MIB loading event, mean biofilter effluent MIB concentrations remained below the OTC for all strategies tested. Paired t-tests showed the difference between biofilter mean effluent values were not statistically significant between any test conditions. However, the biofilter control showed significant breakthrough (~200% of OTC) during the short-term, high MIB loading event, while minimal breakthrough (~ 60% of OTC) was observed in the nutrient-enhanced biofilter. These data suggest that nutrient limitations may create unfavorable conditions for the microorganisms responsible MIB degradation, thus inhibiting its overall removal. Figures 6-10 and 6-11 summarize the results from MIB removal characterization study. Chapter 8-Microbial Tracking discusses the

identified microbial communities in the pilot biofilters and their possible roles in contaminant cycling in further detail.

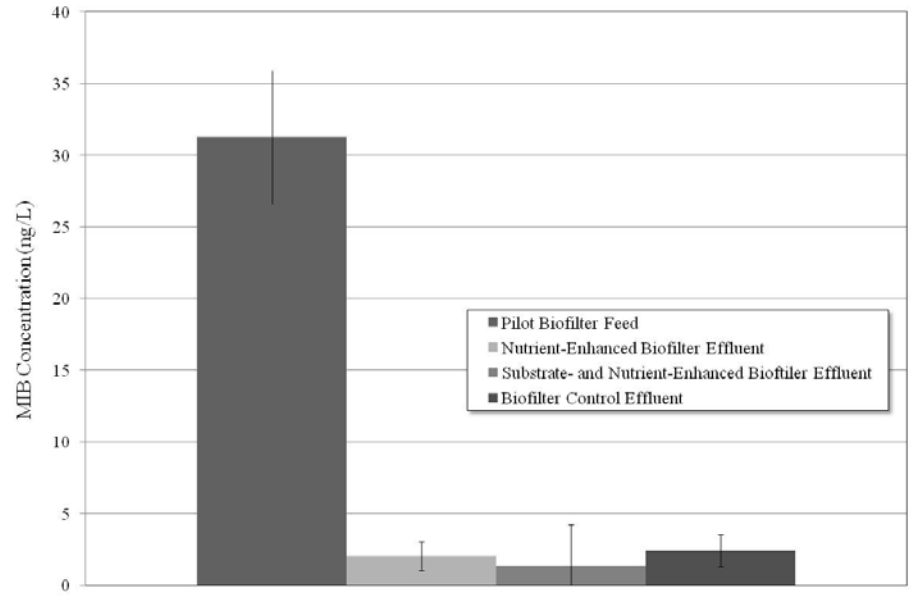

Figure 6-10. MIB removal performance for the nutrient-enhancement strategies during simulated moderate long-term loading event

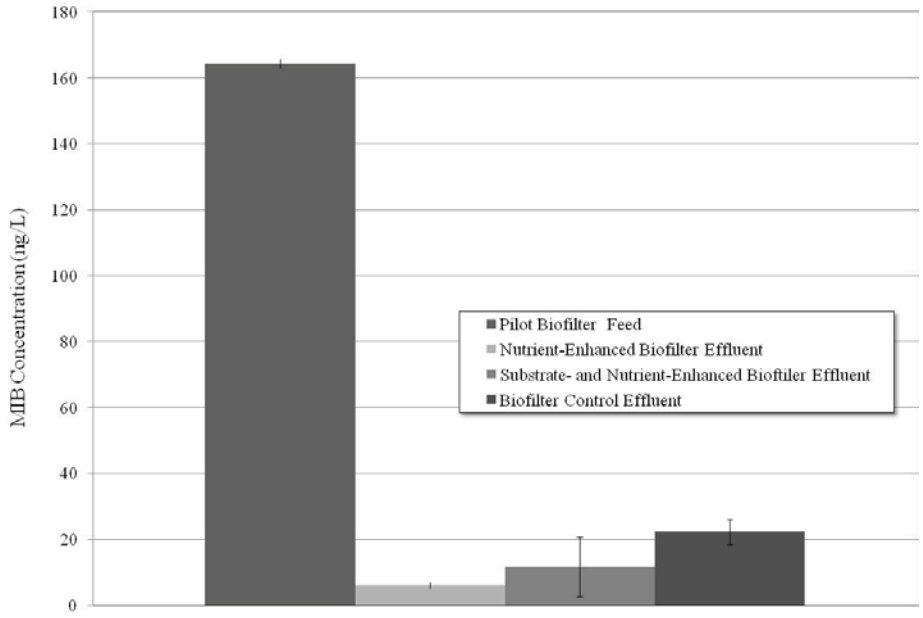

Figure 6-11. MIB removal performance for the nutrient-enhancement strategies during simulated high short-term loading event

Chloramine Decay and DBPFP Testing

Aqueous samples were collected from batch chloramine study bottles at the end of the 200 hr test and analyzed for DBPs. Nutrient-enhancement provided no stability performance was not improved over the 200 hour maximum detention time. As shown in Figure 6-13, the results from these analyses indicated that the nutrient-enhanced and control biofilter effluent samples had DBPFP values that were not significantly different, and both were well below the HAA5 and TTHM MCLs. NDMA was also non detect (<2 ng/L) in both samples.

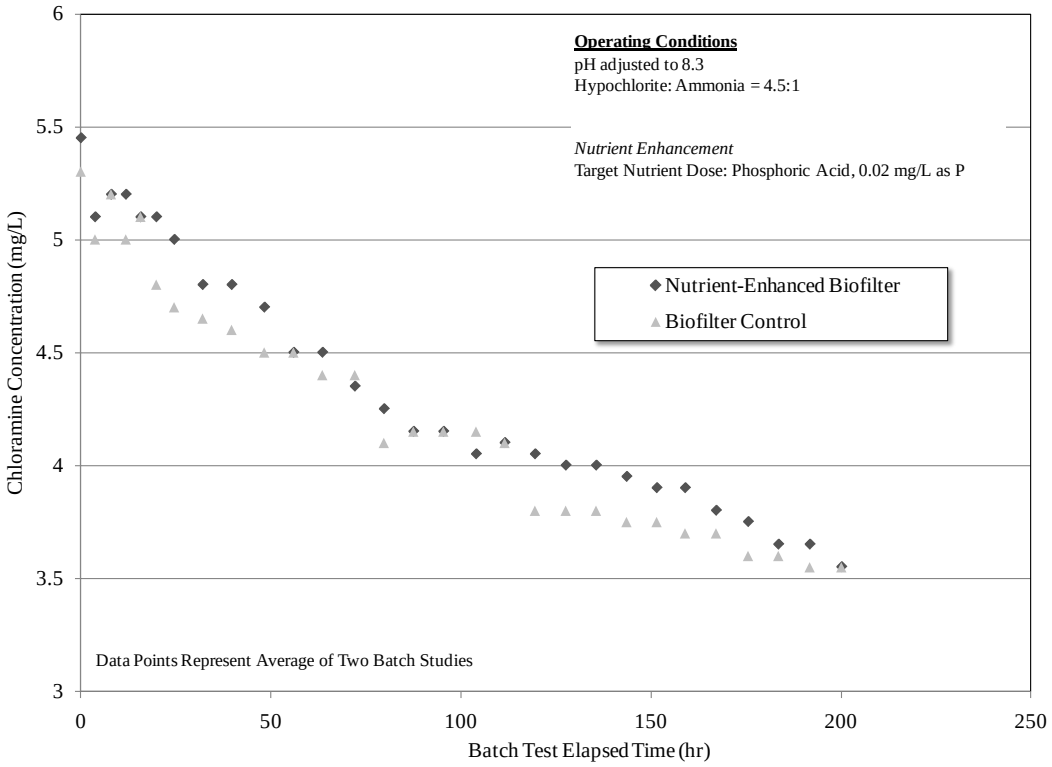

Figure 6-12. Chloramine decay results for the nutrient-enhanced biofilter and biofilter control

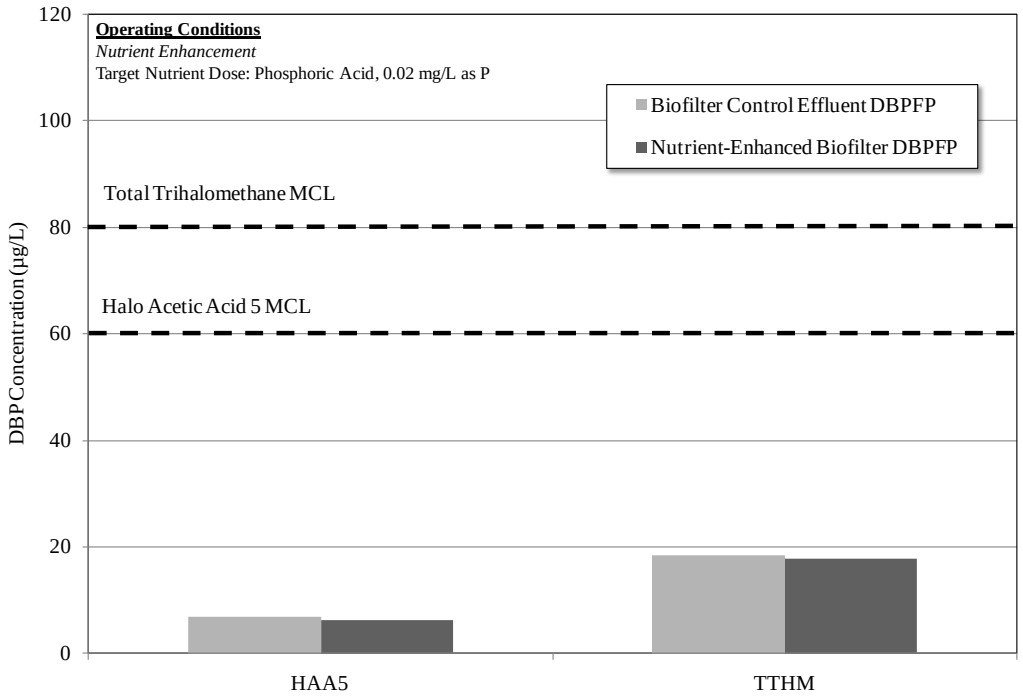

Figure 6-13. DBPFP results for the nutrient-enhanced biofilter and biofilter control

Pharmaceuticals and Pesticides

Biofilter feed and effluent samples were collected during the nutrient-enhancement tests and monitored for pharmaceuticals and pesticides. Atrazine, deethylatrazine deisopropylatrazine, hydroxyatrazine, simazine, metolachlor, and meprobamate were detected in the biofilter feed at concentrations below 1 µg/L. Due to the low concentrations; the nutrient-enhancement strategies could not provide a discernable improvement to pharmaceutical and pesticide removal relative to the control under background loading conditions (Chapter 4, Table 4-5). Therefore, pharmaceutical and pesticide spiking was performed to differentiate the removal performance of the nutrient-enhanced biofilter, the substrate- and nutrient-enhanced biofilter (ethanol and phosphoric acid), and biofilter control. Atrazine, carbamazepine, and caffeine were spiked for one week at low µg/L levels to the pilot biofilter feed. These contaminants were monitored along with the degradative atrazine byproducts, deisopropylatrazine

and deethylatrazine, and background levels of meprobamate. The results of the spiking studies (Figure 6-14) showed that nutrient-enhancement provided negligible benefit for removal of the contaminants tested. However, each biofilter provided at least 20% removal for all tested contaminants. In addition, concentrations of deisopropylatrazine and deethylatrazine increased through all of the biofilters, possibly reflecting the biotransformation of atrazine.

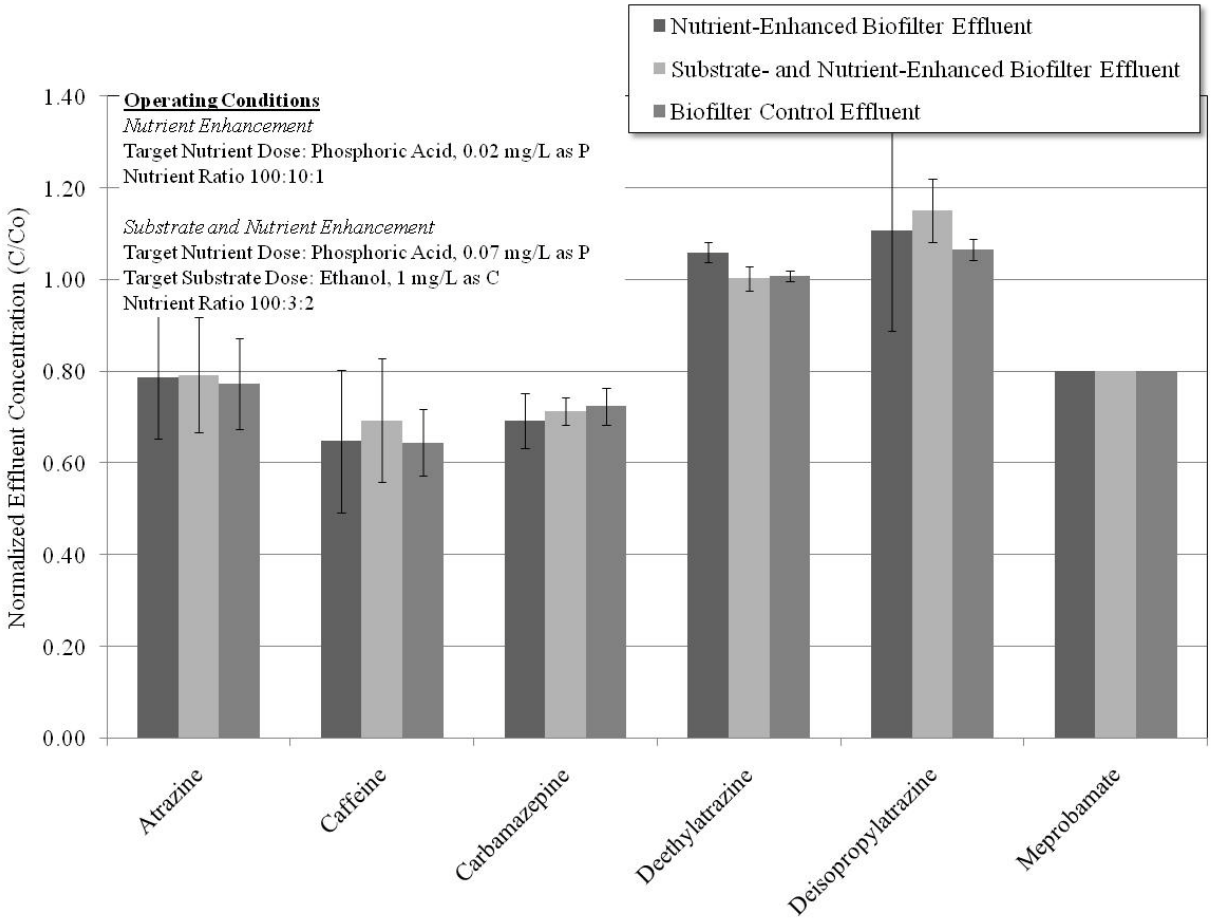

Figure 6-14. Normalized pharmaceutical and pesticide removal performance during the nutrient-enhancement studies

Biological Activity

ATP concentrations in biofilter media were monitored during all nutrient enhancement tests. Biofilter ATP concentrations were consistently higher in biofilters with nutrient

supplementation. The nutrient-enhanced biofilter showed 30% higher terminal (end of filter run) ATP concentrations relative to the biofilter control. The substrate- and nutrient-enhanced biofilter showed 70 to 250% (varied by substrate) higher terminal ATP concentrations relative to the substrate-enhanced biofilter (Figure 8-15). The increase in ATP correlated with higher DOC removals, suggesting that PO_4-P supplementation may enhance cell synthesis or cellular metabolic activity in PO_4-P limited conditions. Additional data from the ATP tests and other microbial tracking data for the substrate-enhancement studies are presented in Chapter 8 - Microbial Tracking.

Robustness Testing

Limited robustness testing was performed on the nutrient-enhanced biofilter with PO_4-P supplementation. The purpose of these tests was to determine if nutrient-enhancement affected biofiltration process robustness. Robustness tests included a 48-hour complete pilot shutdown and a 48-hour phosphoric acid feed shutdown. Neither test created an observable hydraulic nor water treatment upset across any of the monitored parameters. Additional investigation must be conducted to further stress the nutrient-enhanced biofiltration system and characterize improvements and/or degradation of process robustness.

Summary

The objective of this study was to evaluate various nutrient augmentation strategies for enhancing biofiltration performance. Optimal microbial growth is dependent on a nutrient balance of carbon, ammonia- (or nitrate-) nitrogen, and orthophosphate-phosphorus. This balance is typically targeted at a molar ratio of 100:10:1, bioavailable C:N:P. This study evaluated nutrient enhancement by dosing

0.020 mg/L phosphoric acid as P to satisfy the biofilter feed nutrient requirement for 0.8 mg/L of bioavailable DOC.

Phosphorus supplementation decreased biofilter terminal headloss by approximately 15% relative to the control (Table 6-3). This improvement could translate to longer filter run times and decreased backwash frequencies. Furthermore, this strategy may extend underdrain life by decreasing the formation of EPS, which could be responsible for underdrain cap clogging, as experienced by Arlington and other utilities (Zhu, 2010). Phosphorus supplementation also enhanced biofilter water treatment performance. The phosphorus-enhanced biofilter showed higher removals of multiple constituents relative to the control biofilter, including spiked MIB, spiked Mn, and DOC (Table 6-5. Figures 6-8 through 6-11). The results of the nutrient enhancement biofilter tests were validated at the end of the pilot study by dosing phosphoric acid to the biofilter control. Improved filter operation through decreased headloss development was observed during the validation study.

Another component of this study was to evaluate the significance of NH4-N as a limiting nutrient for biofilter operation. Testing was performed by dosing ethanol and phosphoric acid to a biofilter at concentrations that created an artificial NH_4-N limitation (C:N:P equaled 100:3:2). This condition was maintained for 7 weeks and resulted in decreased hydraulic performance as measured by gradual increase in 18-hr terminal headloss during the pilot filter runs. The average terminal headloss for this biofilter was 9.9 feet (60% greater than that of the control), with many runs ending before 18-hours due to a headloss trigger. Ammonium chloride was then dosed to eliminate the NH_4-N limitation. Consequently, average terminal headloss in the enhanced biofilter column decreased to

6 feet (56% improvement), indicating that ammonia limitations may be as deleterious to hydraulic performance as phosphorus limitations during biofilter operation (Figure 6-2). These results suggest that both N and P limitations may increase microbial EPS production in drinking water biofilters.

ATP measurements were performed on the nutrient-enhanced biofilter media to characterize biological activity relative to the control. The data show that nutrient supplementation to achieve a nutrient balance increased biofilter media ATP concentrations by up to 30%. These data suggest that the additional DOC, MIB, and Mn removal observed in the nutrient-enhanced biofilter is the result of increased biological activity. This leads to the important conclusion that appropriate nutrient supplementation produces more biological activity but at the same time improves filter performance because the bacterial production of EPS is reduced with the removal of the nutrient limitation stress.

CHAPTER 7
OXIDANT-ENHANCEMENT STUDIES

Objectives

The objective of this task was to screen hydrogen peroxide supplementation as a potential strategy for improved biofilter hydraulic and water treatment performance. The intention is to augment the oxidative action and response of the biofiltration. Many microorganisms express a class of enzymes, known as oxidoreductases, when exposed to hydrogen peroxide. The resulting reaction neutralizes the hydrogen peroxide, and some (e.g., peroxidase) release free radicals. These free radicals may then oxidize natural organic matter, including recalcitrant forms, thereby further reducing the concentration of DOC and trace organics. In addition, inactive microorganisms and/or their extracellular materials may also be oxidized by the hydrogen peroxide and free radicals, thereby improving biofilter hydraulics.

Testing Conditions

Preliminary testing of this strategy was conducted by dosing 1 mg/L of hydrogen peroxide to a pilot biofilter for approximately ten days. The test biofilter had previously been operated under the nutrient-enhancement strategy with phosphoric acid supplementation (0.02 mg/L as PO_4-P). The phosphoric acid feed was suspended one biofilter run prior to oxidant-enhancement testing. No other nutrients or substrates were dosed to the oxidant-enhanced biofilter during testing. The oxidant-enhanced biofilter was operated in parallel with the nutrient-enhanced (validation testing) biofilter and substrate- and nutrient-enhanced biofilter.

Hydraulic Characterization

Clean-bed headloss generally remained below 1 ft for all conditions tested. However, headloss profiles over a given run decreased dramatically immediately after hydrogen peroxide supplementation was initiated. Figure 7-1 presents serial headloss profiles before and after implementation of the oxidant-enhancement strategy. Oxidant-enhanced biofilter terminal headloss remained below three ft throughout the 10-day testing period, yielding the lowest mean terminal headloss observed for all enhancement strategies tested during the entire pilot study. The oxidant-enhanced biofilter hydraulic profiles appear more consistent than the biofilter control and other strategies tested, with the lowest observed deviations between biofilter terminal headloss. Table 7-1 summarizes the terminal headloss data collected during the oxidant-enhancement strategy testing. The data in Table 7-1 is a parallel comparison of the oxidant-enhancement study to the nutrient-enhancement validation study and historical control biofilter operation.

As shown in Table 7-1 and Figure 7-1, headloss data suggest that hydrogen peroxide supplementation may significantly improve biofilter hydraulic performance. Indeed, the oxidant-enhanced biofilter mean terminal headloss remained 2.4 ft below the lowest monthly mean terminal headloss observed for the biofilter control (Chapter 4, Table 4-1).

Table 7-1. Oxidant-enhancement validation on biofilter hydraulic performance

Oxidant-enhanced biofilter terminal headloss* (ft)			Nutrient-enhanced biofilter terminal headloss† (ft)			Biofilter control terminal headloss‡ (ft)		
Mean§	Min.	Max.	Mean§	Min.	Max.	Mean**	Min.	Max.
2.3 ± 0.2	2.0	2.5	5.4 ± 0.4	4.1	6.1	5.9 ± 0.6	4.7	7.0

* Target hydrogen peroxide feed in the oxidant-enhanced biofilter was 1 mg/L.
† Target PO_4-P feed in the nutrient-enhanced biofilter (validation study) was 0.020 mg/L as P.
‡ Biofilter control terminal headloss data from last month of steady state operation (August 2009).
§ Includes 14 biofilter runs, means are provided with the standard deviation of the data sets as value error.
** Includes 36 biofilter runs, means are provided with the standard deviation of the data sets as value error.

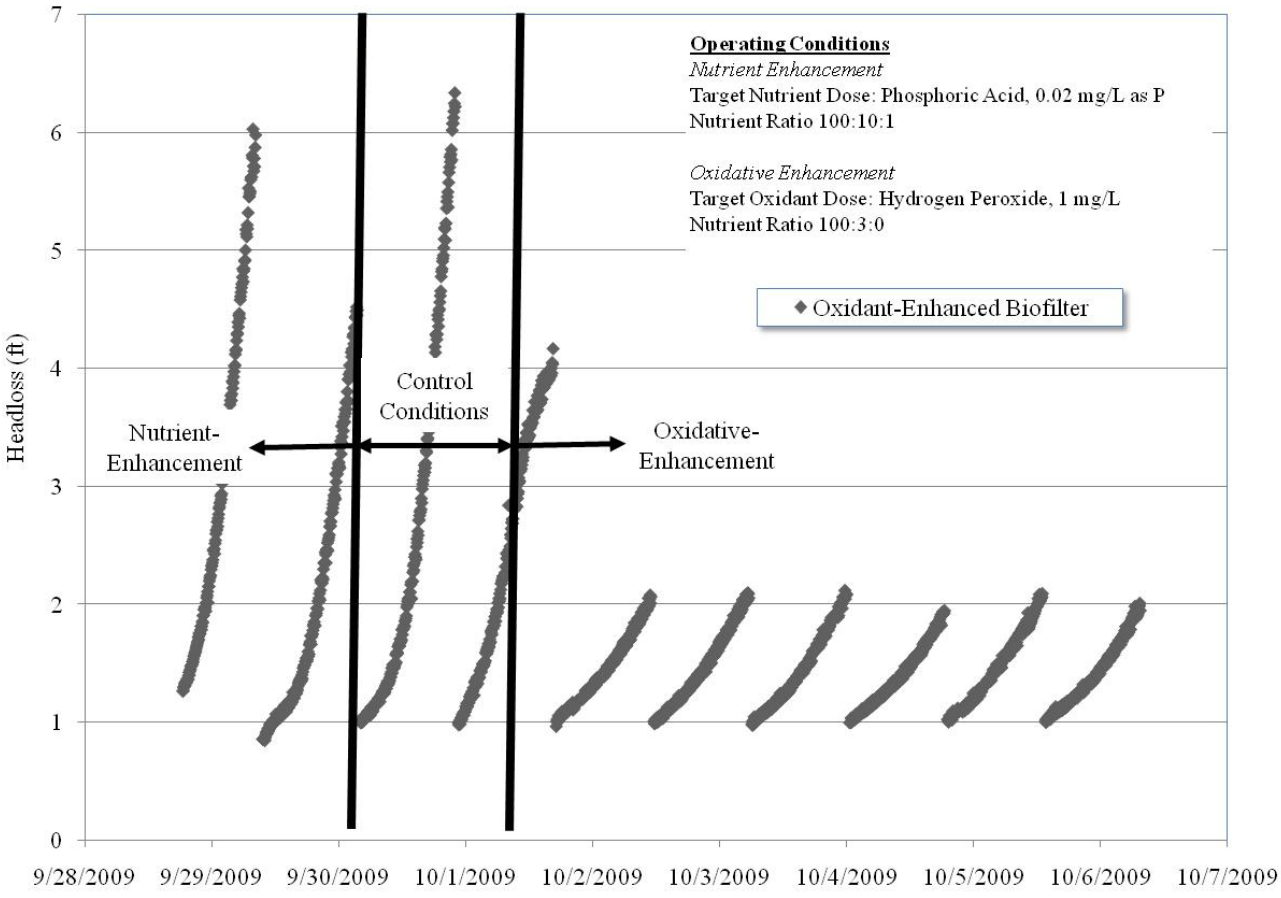

Figure 7-1. Effect of oxidant enhancement on biofilter headloss profiles

Water Quality Characterization

General

The oxidant-enhanced biofilter water treatment performance characterization included routine sampling and water quality analyses (Chapter 4, Table 4-3). The study period was ten days. Therefore, the data provided below should be considered preliminary. Further evaluation is required to fully characterize performance of the oxidant-enhancement strategy.

Hydrogen Peroxide

Dosed hydrogen peroxide concentrations were verified by sampling oxidant enhanced biofilter feed daily. Measured concentrations remained on the target dose of 1 mg/L throughout testing (MDL of 0.1 mg/L). Oxidant-enhanced biofilter effluent was also tested daily for hydrogen peroxide residual; however, it was never detected.

Turbidity

All turbidities maintained compliance with the USEPA Surface Water Treatment Rule, as greater than 95% of the effluent turbidity samples were less than 0.3 NTU. No turbidity values over 1 NTU were observed in oxidant-enhanced biofilter effluent. Figure 7-2 illustrates oxidant-enhanced biofilter effluent turbidity profiles for two typical filter runs.

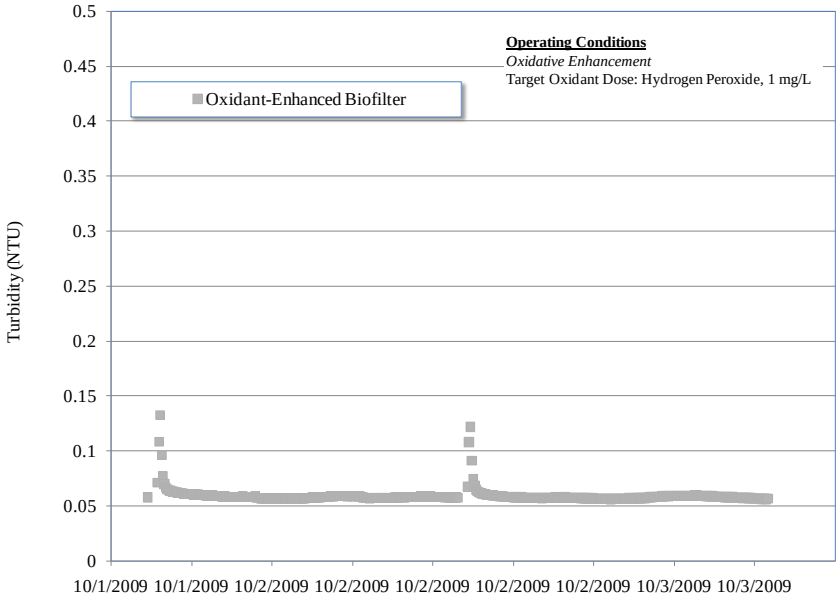

Figure 7-2. Oxidant-enhanced biofilter turbidity profiles for typical filter runs

DOC

Influent and effluent oxidant-enhanced biofilter DOC data were collected during the ten-day oxidant-enhancement study. These preliminary data showed that DOC removals during hydrogen peroxide supplementation tests were similar to those observed during nutrient-enhancement conditions. Furthermore, mean oxidant-enhanced biofilter DOC removals also remained below the historical means for the biofilter control. Table 7-2 summarizes influent and effluent DOC data for the oxidant-enhance biofilter and the parallel nutrient-enhanced biofilter (validation study, see Chapter 6).

DOC removal performance was also characterized by comparing the oxidant-enhanced biofilter effluent against that of the preceding 2 weeks of operation under nutrient enhancement conditions. During this study, hydrogen peroxide supplementation appeared to have little observed impact on DOC removal performance as compared to the nutrient-enhanced study data (Figure 7-3).

Nutrients

The oxidant-enhanced biofilter was operated without nutrient supplementation. The mean biofilter feed nutrient concentrations during the study were 0.0325 NH_4-N and less than 0.010 PO_4-P, yielding an approximate C:N:P ratio of 100:3:0. Thus, the oxidant-enhanced biofilter was operated with both NH_4-N and PO_4-P limitations relative to the available organic carbon.

Table 7-2. Oxidant-enhanced biofilter DOC removal performance

Biofilter feed* (background C) (mg/L)			Oxidant-enhanced biofilter† effluent (mg/L)			Nutrient-enhanced biofilter‡ (mg/L)			Historic biofilter control (mg/L)		
Mean§	Min.	Max	Mean§	Min.	Max	Mean§	Min.	Max	Mean§	Min.	Max
3.4 ± 0.1	3.3	3.5	2.9 ± 0.1	2.8	3.0	2.9 ± 0.1	2.8	3.0	3.2 ± 0.1	2.8	3.4

* Samples were collected of biofilter feed before and after hydrogen peroxide addition to evaluate direct mineralization of background DOC. None was observed.
† Target hydrogen peroxide dose for the oxidant-enhanced biofilter was 1 mg/L.
‡ Target phosphoric acid dose for the nutrient-enhanced biofilter was 0.020 mg/L as P.
§ Includes ten days of steady state data (N=4). Means are provided with the standard deviation of the data sets as value error.

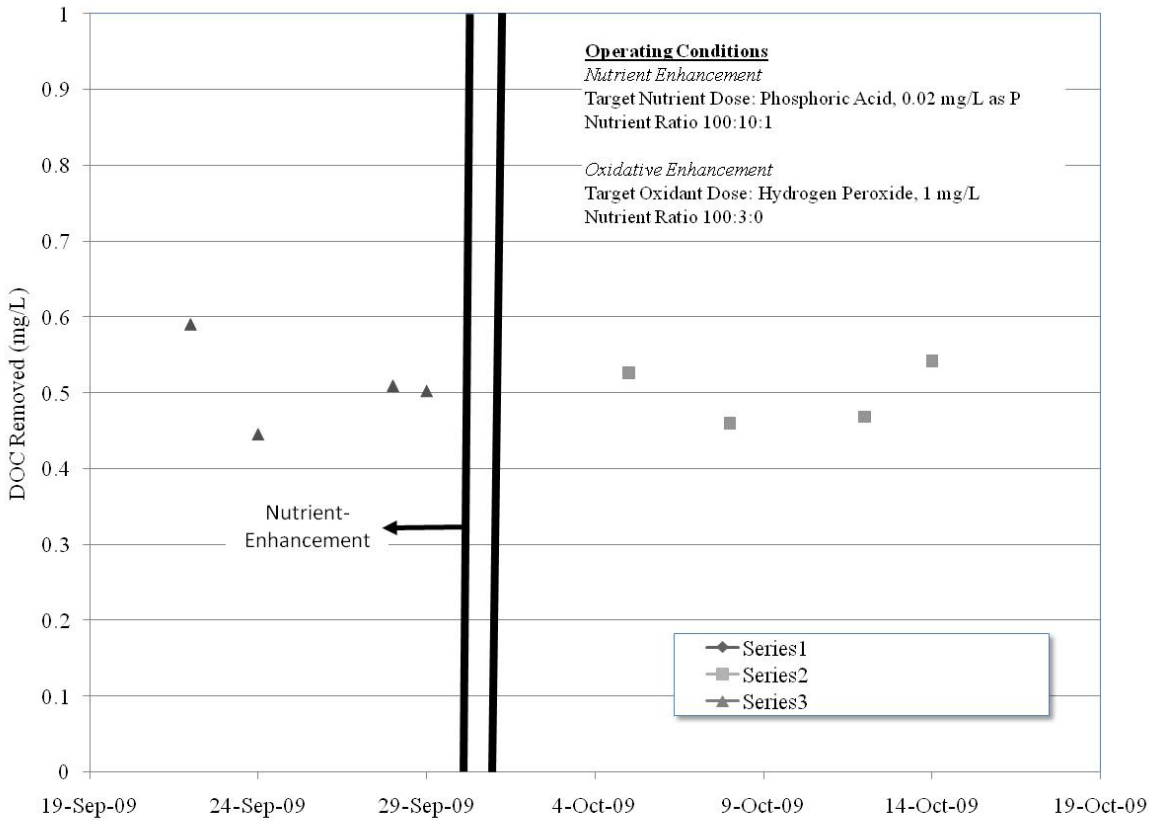

Figure 7-3. The effect of oxidant enhancement on DOC removal performance

Metals

Background biofilter feed Fe and Mn levels remained below their respective MDLs (10 µg/L) during the oxidant-enhancement studies. Therefore, high biofilter feed Mn loading (~180 µg/L) was performed to characterize metals removal performance. The oxidant-enhanced biofilter successful removed all loaded Mn to non-detect levels (less than 10 µg/L). Figure 7-4 illustrates the extent of Mn removal through the oxidant-enhanced biofilter. Mn speciation of oxidant-enhanced biofilter influent was not performed; therefore, it is unknown whether direct oxidation of Mn by peroxide had occurred. The parallel operating nutrient enhanced biofilter performed similarly. The ten-day test duration and limited sample set (N=4) provide only a preliminary characterization of Mn removal performance. Therefore, additional testing and

characterization are necessary. Furthermore, the mechanisms for Mn oxidation were uncharacterized. Therefore, it is unknown whether hydrogen peroxide supplementation improved removal via direct oxidation or improved conditions for microbial oxidation.

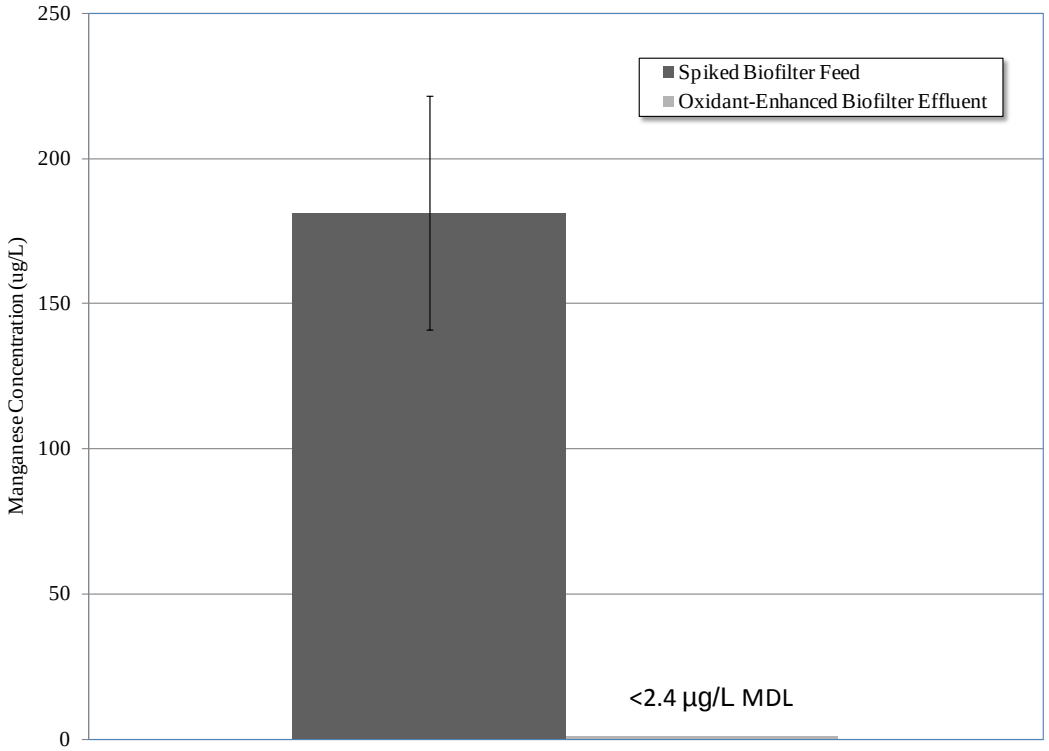

Figure 7-4. Oxidant-enhanced biofilter Mn removal performance[5]

Taste and Odor

Background biofilter feed geosmin and MIB levels remained below their respective MDLs (1.4 ng/L) during the oxidant-enhancement studies. Therefore, moderate biofilter feed MIB loading (~40 µg/L) was performed throughout the oxidation-enhancement studies. Samples were collected of biofilter feed before and after hydrogen peroxide addition to evaluate direct transformation of MIB. No MIB was observed in the effluent. The oxidant-enhanced biofilter successfully removed all loaded MIB to non-detect levels

[5] The error bars presented in all figures in this Chapter represent the standard deviation of the data set. This standard deviation accounts for operational variability (i.e. feed water conditions) and sampling/analytical error.

(less than 1.4 ng/L). Figure 7-5 illustrates the extent of MIB removal across the oxidant-enhanced biofilter. The parallel operating nutrient-enhanced biofilter performed similarly. Analyses of oxidant-enhanced biofilter influent samples suggested that direct peroxide oxidation of MIB did not occur (no degradation observed). The ten-day test duration and limited sample set (N=4) provide only a preliminary characterization of MIB removal performance Therefore; further testing and characterization are necessary.

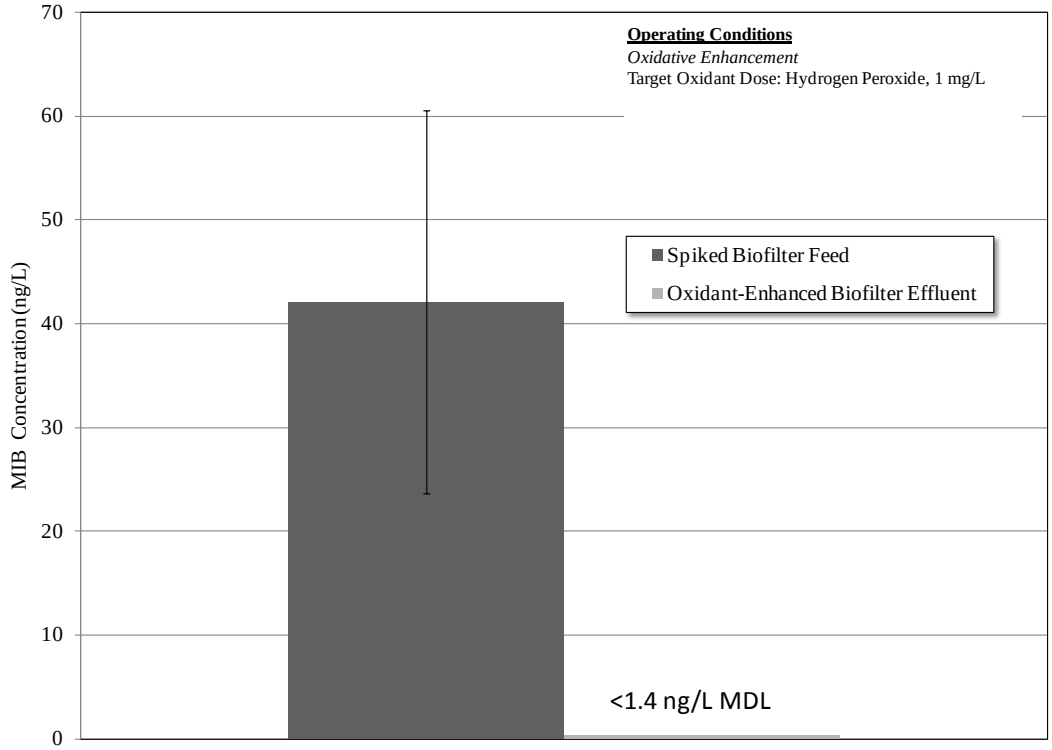

Figure 7-5. Oxidant-enhanced biofilter MIB removal performance

Biological Activity

ATP measurements were performed on the oxidant-enhanced biofilter media to characterize biological activity relative to the (historical) control. Terminal filter media ATP concentrations for the oxidant-enhanced and historical biofilter control were 1,007,000 and 1,009,000 pg/L, respectively. The data suggest that hydrogen peroxide supplementation did not decrease biofilter media ATP concentrations to levels below

those of the biofilter control (Figure 8-16). These data suggest that biological activity is not negatively impacted by 1 mg/L hydrogen peroxide supplementation. The drastic decrease in headloss might be due to peroxide oxidation of inactive microorganisms and EPS (direct or via activated peroxide/free radicals). Additional microbial tracking data for the oxidant-enhancement studies are presented Chapter 8 - Microbial Tracking.

Summary

The objective of this task was to evaluate peroxide supplementation for augmenting the oxidative action and response of the biofiltration process. Preliminary testing of this strategy was conducted by dosing 1 mg/L of hydrogen peroxide to a pilot filter for two weeks. The test filter demonstrated 15% removal of filter feed DOC (50% greater than that observed with the control and full-scale filters), and complete removal of Mn and MIB (Figure 7-2). The oxidant-enhancement strategy also decreased terminal headloss to 2.2 feet, or 66% less than the control biofilter (Figure 7-1).

ATP measurements were performed on the oxidant-enhanced biofilter media to characterize biological activity relative to the control. The data showed that hydrogen peroxide supplementation did not decrease ATP concentrations in the biofilter media. These data suggested that biological activity is not negatively affected by 1 mg/L hydrogen peroxide supplementation. Additional microbial tracking data for the oxidant-enhancement studies were performed as part of microbial tracking study (Chapter 8).

CHAPTER 8
MICROBIAL TRACKING

Objectives

The objective of this task was identify preliminary correlations between microbial ecology/activity in the biofilters and hydraulic and water treatment performance. The microbial tracking task included the following analyses: SEM, biofilter media ATP quantification, HPCs, biofilm formation potential, EPS quantification, T-RFLP, and clone libraries.

Scanning Electron Microscopy

Multiple SEM micrographs, at varying magnifications, were taken of biofilter media samples collected during each test condition in the pilot-scale experiments. SEM micrographs also were taken from full-scale biofilter and biofilter control media samples. Morphological changes were observed on pilot-scale biofilter biofilms as they matured through the study and were exposed to various treatments. These observations were used only as a qualitative reference for potential biological responses to the conditions of the biofilter feed (i.e., nutrient limitation/supplementation, hydrogen peroxide supplementation, substrate supplementation, etc.). The sections below provide a summary of the observations made for the full-scale and pilot-scale biofilters.

General Observations

Bacteria were observed in all biofilter media samples, colonizing large regions of the surface. In addition, a “biofilm matrix,” was observed in all samples at varying levels. While the biofilm matrix was not directly characterized, the images suggest that it is a combination of plant materials, sequestered minerals, and products of microbial origin (EPS). There appeared to be diverse morphologies present in the biofilm microbial

community and biofilm. Filaments of varying diameters and shapes (round and flat cross sections) were observed in the biofilm, the larger of which where likely plant materials (Raskin, 2009; Bennett-Stamper, 2009). Similarly, biofilm matrices had varying textures and surface areas. Biofilm qualitative appearance was the primary tracked criteria during the SEM analyses and was characterized by the observed unique characteristics and surface area between samples.

Full-scale Biofilter

The full-scale biofilter media appeared to be encapsulated by the biofilm (Figure 8-1) throughout the study. Bacteria in the full-scale biofilter were abundant with diverse cell morphologies. Bacilli (rod-shaped), cocci (spherical), and coccibacilli (oval), and helical bacteria were observed in a variety of sizes. The highest levels of filamentous materials were observed in the full-scale biofilter samples. The size and shape of the filaments likely indicate microbial stalks, (Kirisits, 2010[*]), flagella (Xi, 2009), or filamentous bacteria (Raskin, 2009). Overall, the Full-scale filter (FSF) samples appeared consistent with each other and the biofilter control. All full-scale filter SEMs exhibited an extensive (relative to surface area) heterogeneous biofilm matrix on the GAC with observable bacterial concentrations.

[*] Personal communication with Mary Jo Kirisits, Assistant Professor at University of Texas-Austin, various dates 2010.

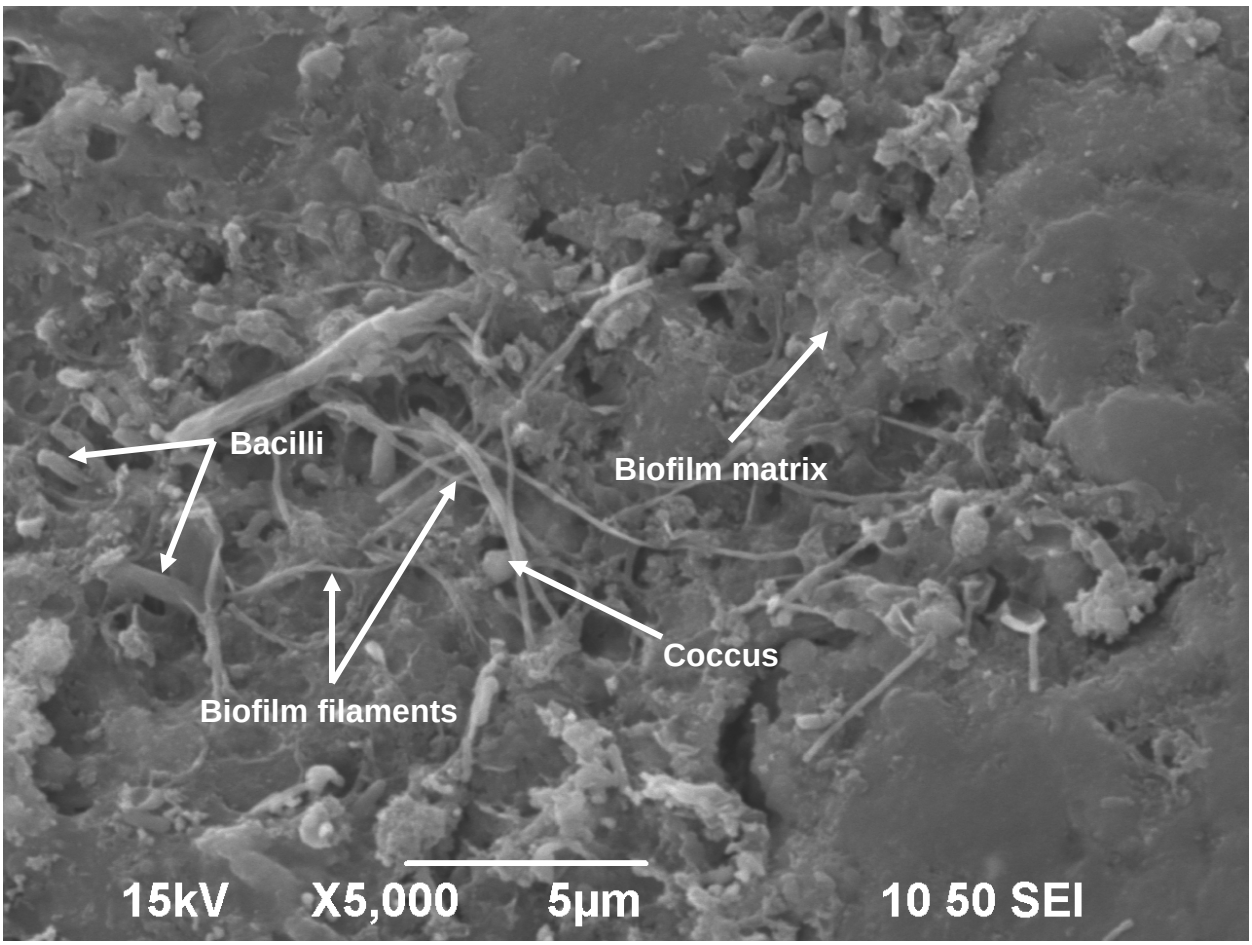

Figure 8-1. Full-scale biofilter GAC media SEM micrograph

Biofilter Control

SEM micrographs of the pilot baseline control biofilter appeared consistent throughout the study (Figures 8-2 and 8-3). The baseline control biofilter also had a large number of filaments connected to microbial cells. The size and shape of these filaments suggest stalked bacteria (Kirisits, 2010). Bacterial stalks may be an evolutionary adaptation to life in nutrient limited conditions (Madigan et al., 2009). The elongations of the stalks are potentially a response to nutrient limiting conditions (Wagner et al., 2006). The stalk is an extension of the cell that maintains the diffusion (nutrient uptake) capabilities of the cell proper. Thus, by extending stalk length, a

microorganism may increase its surface area with little net increase in cell volume (and the accompanying metabolic requirements). Bacteria with increased surface-to-volume ratios maintain an increased ability to take up nutrients and expel wastes (Madigan et al., 2009). During instances of severe nutrient limitation, terminal stalk lengths may exceed 30 µm (Gonin et al., 2000).The prevalence of stalked bacteria and their corresponding stalk lengths on biofilter media SEMs appeared to coincide with influent nutrient conditions.

The control biofilter also displayed an increased abundance of biofilm matrix, relative to the nutrient-enhanced biofilter. Indeed, many bacteria observed on the media were difficult to differentiate due to their embedment in the biofilm

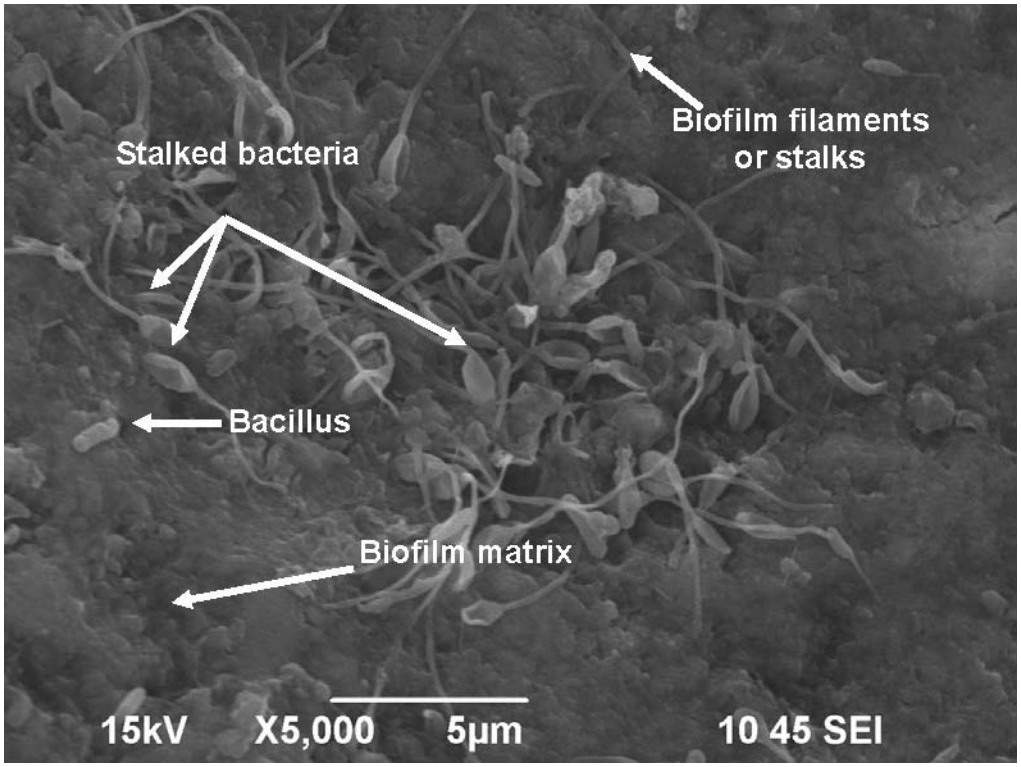

Figure 8-2. Biofilter control media SEM micrograph: 2 weeks of pilot operation

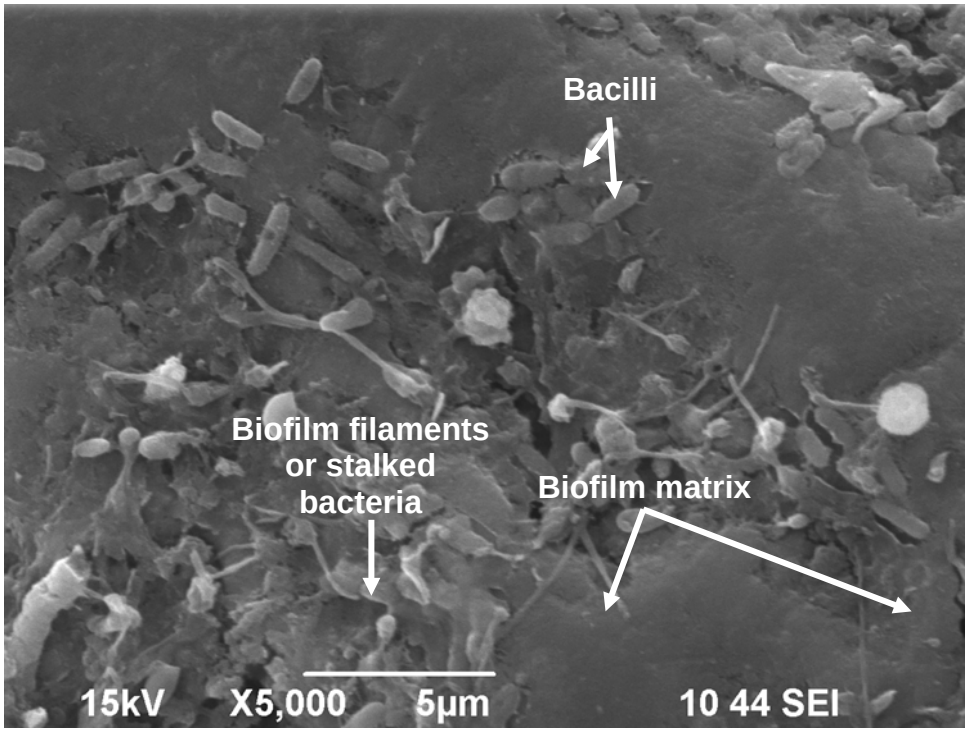

Figure 8-3. Biofilter control media SEM micrograph: 38 weeks of pilot operation

Several clusters of small white granules were observed on the control biofilter media (Figure 8-4). The white appearance suggests that the clusters are mineral in nature rather than biological. These granules are seen in several of the samples but could not be fully characterized. However, a trial with energy dispersive X-ray spectroscopy indicated potentially high amounts of sequestered phosphorous. Some research has shown phosphorus precipitation may be mediated by bacterial extracellular enzymes (Kerdachi and Roberts 1980). These precipitates may then chemically bind to the EPS. The excessive biofilm material and potential sequestration of phosphorus suggests that the control biofilter biofilm responded to the PO_4-P limitation by producing higher levels of EPS. This EPS may then serve to assist with capture and storage of the limiting nutrient. Further investigation is necessary to fully characterize the biofilm and observed clusters.

Substrate-Enhancement Studies

SEM micrographs of media collected from the substrate-enhanced biofilters generally showed extensive biofilm, regardless of the substrate tested. The biofilm matrices appeared more pervasive than that observed on the media samples collected from the control biofilter. Furthermore, micrographs taken from successive substrate-enhanced biofilter media samples showed increased biofilm prevalence and decreased morphological diversity. Indeed, the matrix coated most of the bacteria within the biofilm, making cell differentiation challenging. Figures 8-5 and 8-6 illustrate the extent of the biofilm in the substrate-enhanced filters.

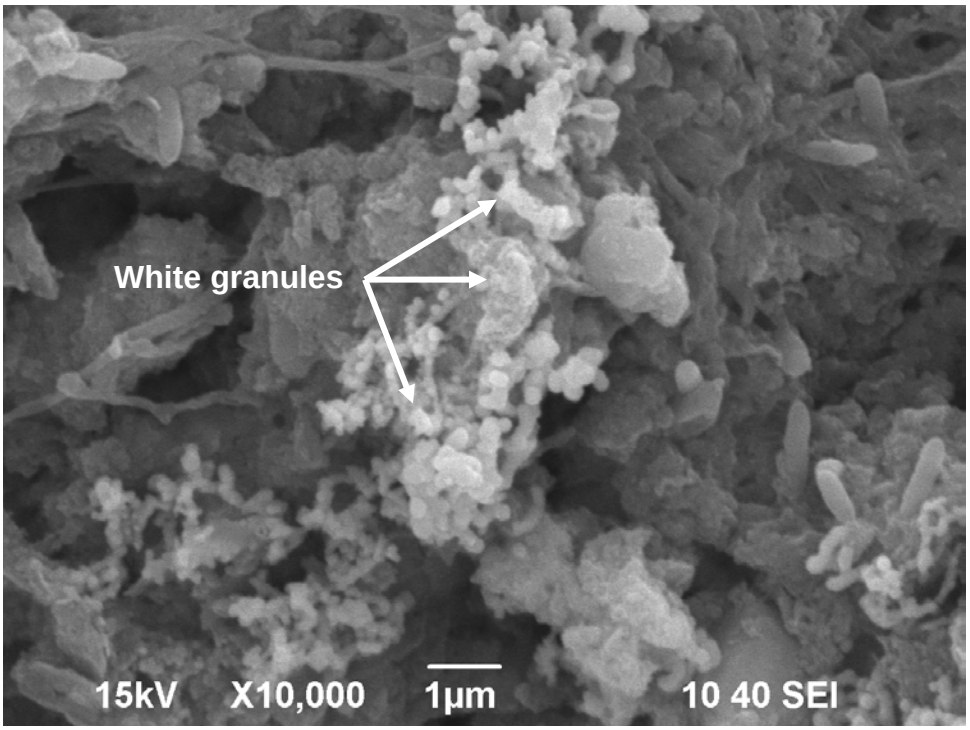

Figure 8-4. Biofilter control media SEM micrograph: white granules

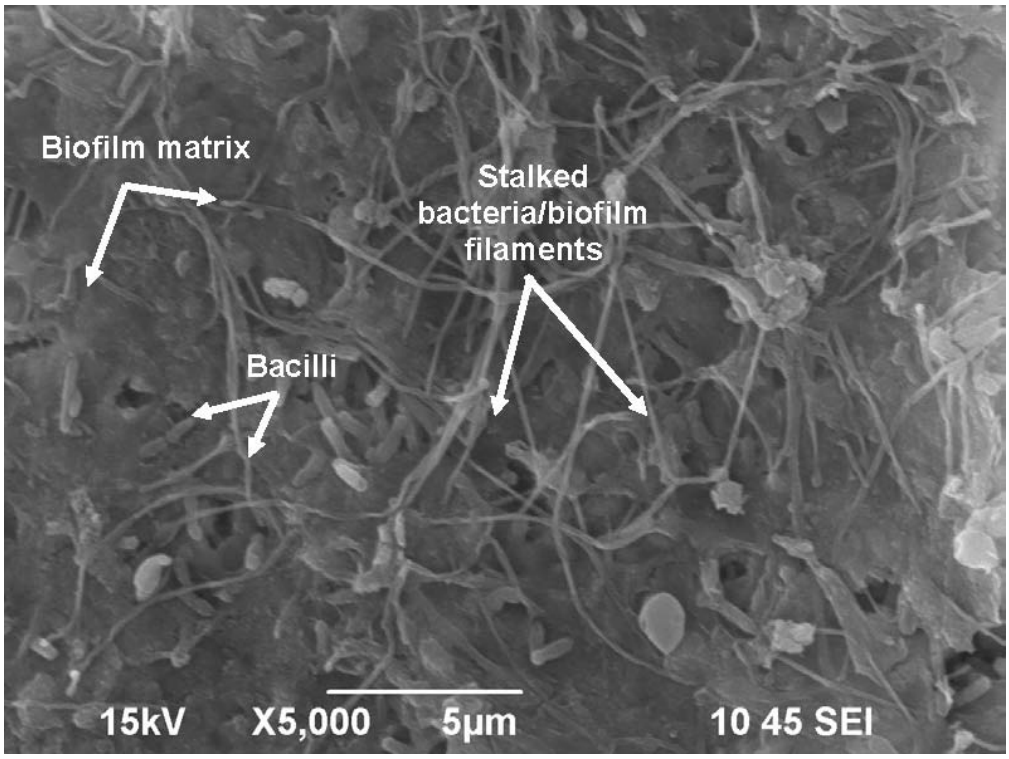

Figure 8-5. Substrate-enhanced, nutrient-limited biofilter: MicroC© substrate

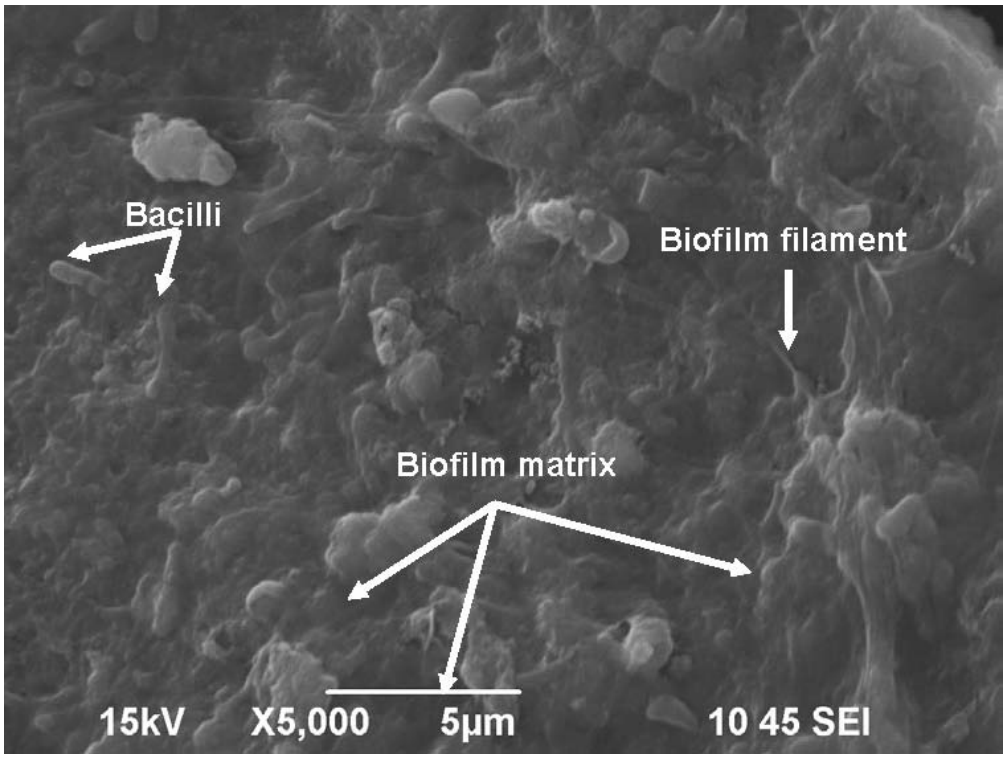

Figure 8-6. Substrate-enhanced, nutrient-limited biofilter media SEM micrograph: ethanol substrate

Nutrient Enhancement Studies

The nutrient-enhanced biofilter media samples showed the lowest prevalence of biofilm matrices and filaments relative to all micrographs analyzed (Figure 8-7). Polymeric strands are visible between individual cells. However, the pervasive biofilm matrix observed on other samples was not seen. Furthermore, these samples showed the highest levels of cell morphological diversity and abundance. Substrate- and nutrient-enhanced biofilter media samples showed increased biofilm prevalence when operated with a NH_4-N limitation than with ammonium chloride supplementation (Figures 8-8 and 8-9). Indeed, biofilter media from the NH_4-N limited substrate- and nutrient-enhanced biofilter exhibited thick, ribbon-like filaments in a majority of the micrographs.

These images suggest that both NH_4-N and PO_4-P may play an important role in the formation and morphology of biofilm materials.

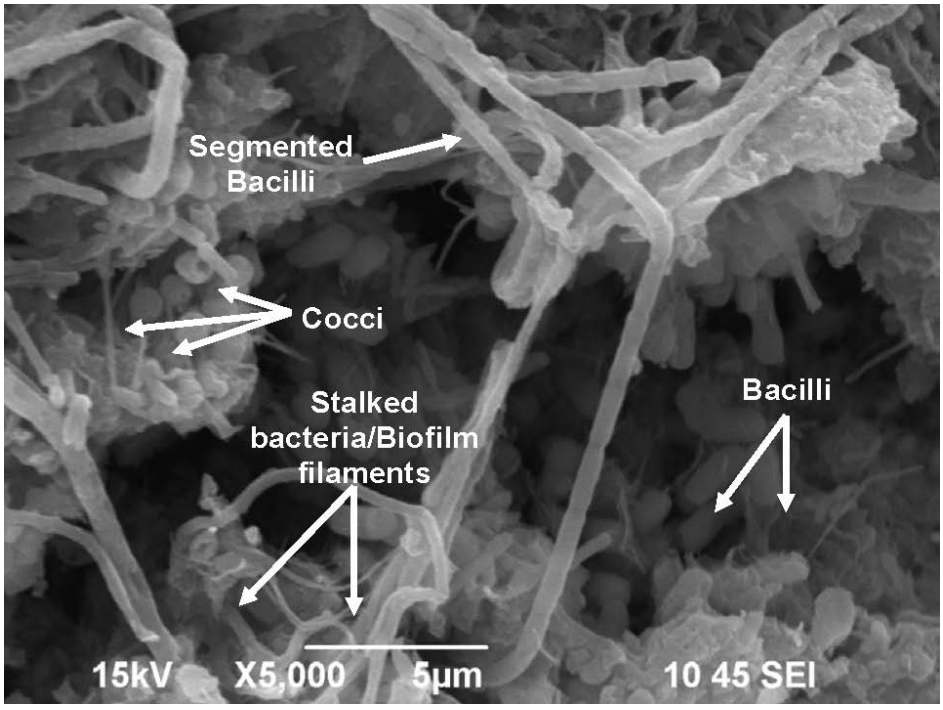

Figure 8-7. Nutrient-enhanced biofilter media SEM micrograph: phosphoric acid

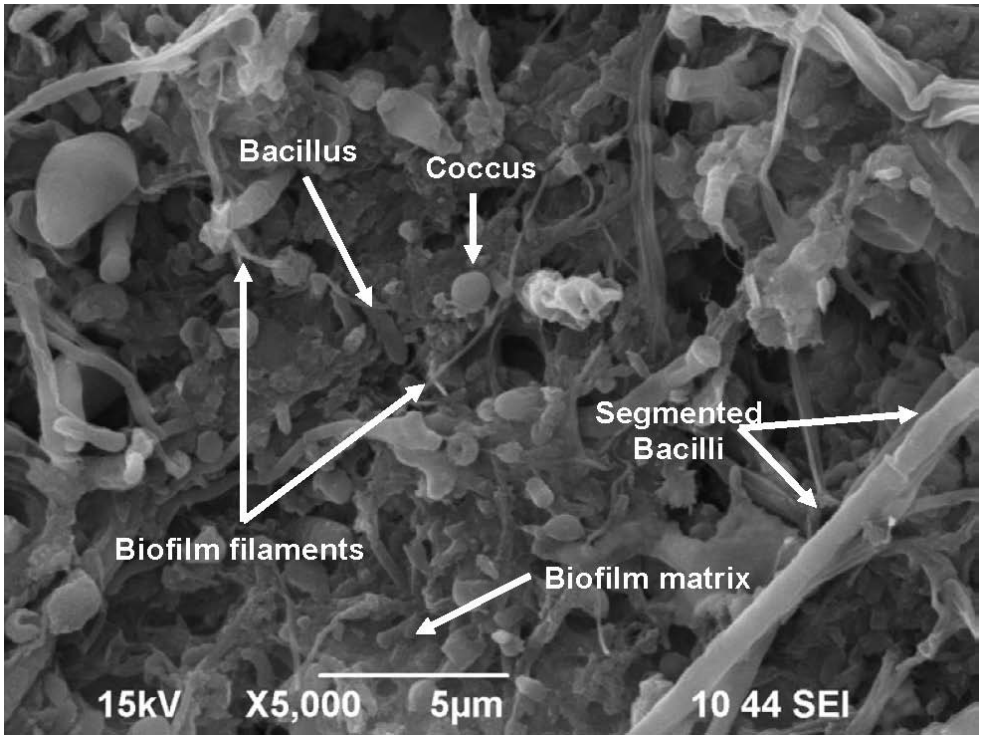

Figure 8-8. Substrate- and nutrient-enhanced biofilter media SEM micrograph: ethanol substrate, phosphoric acid, ammonium chloride

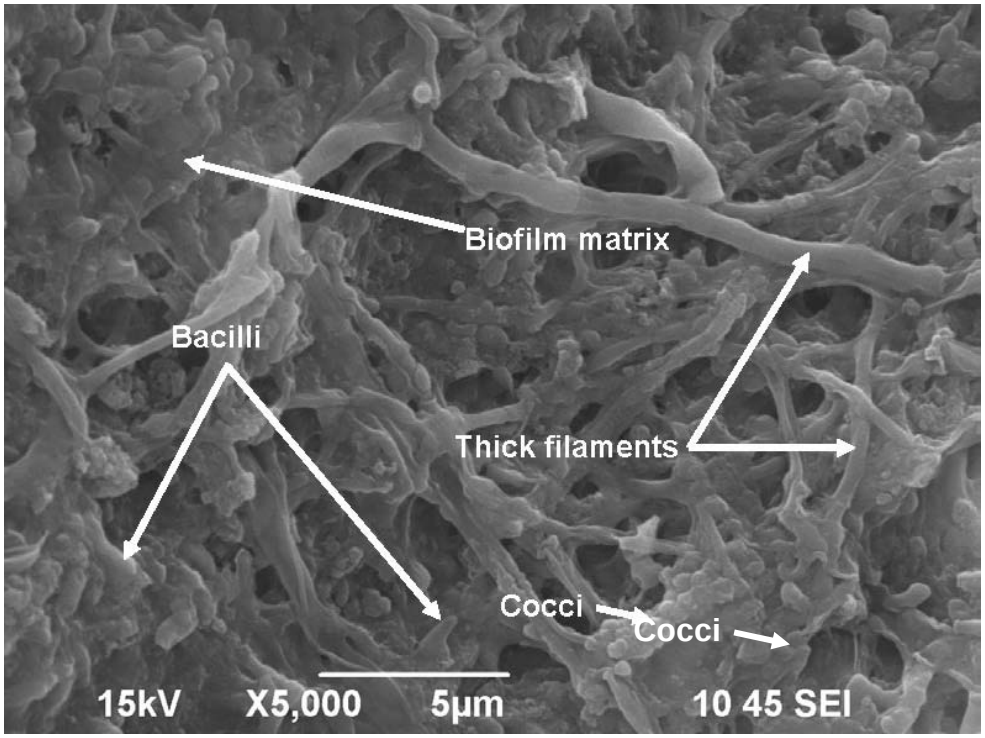

Figure 8-9. Substrate-enhanced biofilter media SEM micrograph: ethanol substrate, phosphoric acid

Oxidation Enhancement Studies

The oxidant-enhanced biofilter media samples showed microbial abundance and morphological diversity, including multiple sizes of bacilli, cocci, and filamentous bacterial morphologies. Stalked bacteria also had a high prevalence in these samples. However, the biofilm matrix was not as significant as in the substrate-enhanced biofilter media samples (Figure 8-10). These images suggest the hydrogen peroxide supplementation may change biofilter communities over a short period. Additional studies are necessary to fully characterize the effects of oxidation enhancement on biofilm formation.

Summary of SEM Micrographs

The studied enhancement strategies produced observable changes in biofilter media biofilm appearance when analyzed through SEM. Images taken suggest that biofilm and cellular morphologies may be influenced by nutrient limitations and subsequent supplementation. Table 8-1 summarizes the observations made through the SEM analyses during the study.

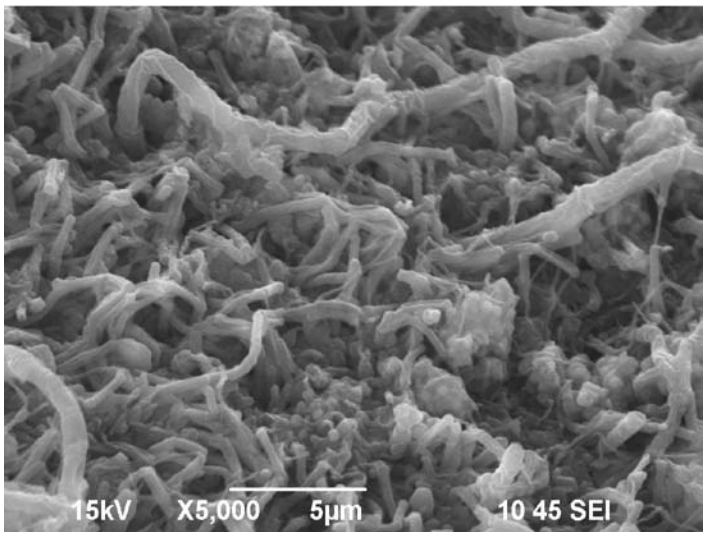

Figure 8-10. Oxidant-enhanced biofilter media SEM micrograph: hydrogen peroxide

Table 8-1. Summary of observations made during SEM analysis of biofilter media

Test Condition		*Observed* qualitative element frequency relative to the biofilter control		
Condition	Nutrient Ratio (added chemicals)	Cellular abundance	Biofilm matrix prevalence	Biofilm filaments
Biofilter control	100:6:0 (none)	-	-	
Nutrient-enhanced biofilter	100:10:1 (0. 02 mg/L PO_4-P)	High	Very low	Low
Substrate-enhanced biofilter*	100:2:3 (1 mg/L C [MicroC©])	Low to high	Very high	Low to high
Substrate- and nutrient-enhanced biofilter	100:3:2 (1 mg/L C [ethanol], 0.04 mg/L PO_4-P)	High	Same	High
Substrate- and nutrient-enhanced biofilter†	100:14:2 (1 mg/L C [ethanol], 0.1 NH_4-N, 0,04 mg/L PO_4-P)	High	Low	Same
Oxidant-enhanced biofilter	100:6:0 (1 mg/L H_2O_2)	Very high	Low	High

* Biofilm and cellular morphologies varied with substrate tested. However, all substrate-enhanced biofilter samples exhibited high levels of Biofilm matrices

† Substrate- and Nutrient-Enhanced Biofilter operated with supplemental ammonium chloride feed (non-nutrient limited)

Plate Count

Select biofilter media samples were surveyed for HPC. Overall, these results suggest that phosphorus is responsible for increasing the number of viable cells in the filter. Figure 8-11 illustrates relative HPC between the nutrient-enhanced biofilter and the biofilter control media. The data included in Figure 8-11 represent a single sample set that was analyzed in triplicate. These data suggest that nutrient supplementation may increase the prevalence of viable heterotrophic bacteria on biofilter media ($p = 0.005$, $p \leq 0.05$). The HPC data support the observed increases in biofilter activity and DOC removal during the nutrient enhancement study (Table 6-2 and Figure 8-16).

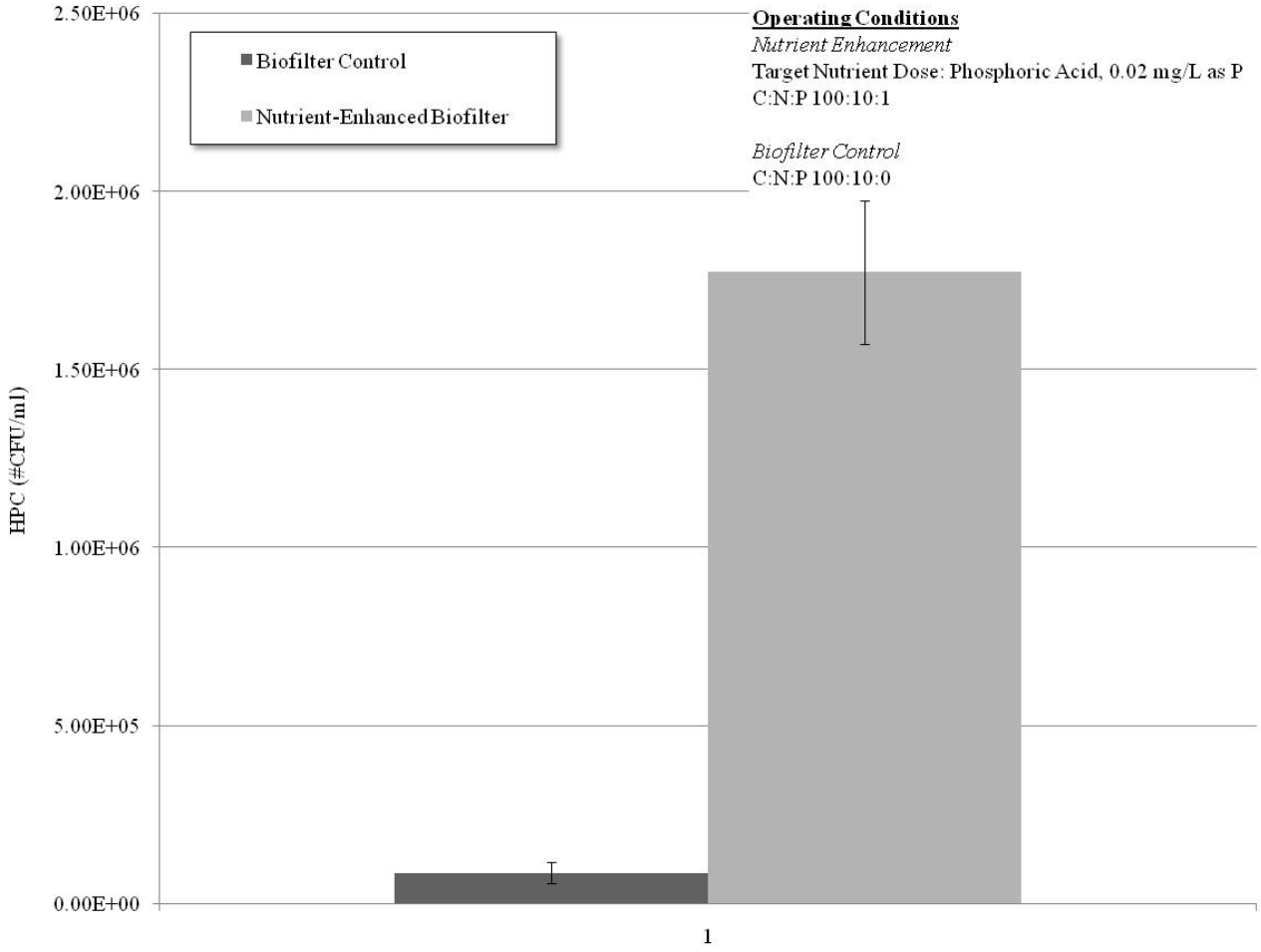

Figure 8-11. Biofilter media HPC per mL of phosphate buffered saline media samples: biofilter control and nutrient-enhanced biofilter

Biofilm Formation Characterization

The biofilm formation capacity was assessed using crystal violet (CV) assay as described by O'Toole and Kolter (1998). The CV assay is best used to identify populations or communities of microorganisms that have significantly increased biofilm formation. Since the CV assay results are heavily dependent on the initial cell concentration of the inoculum, the biofilm formation capacity of the cells from the filter samples was compared among inocula with similar cell concentrations (as determined by HPC). As shown in Figure 8-12, biofilter media biofilm formation potential was lower

for the nutrient-enhanced biofilter relative to the control for the one sample tested. All substrate-enhanced media showed higher biofilm formation potentials relative to the control biofilter ($p = 0.01$). Conversely, the substrate- and nutrient-enhanced biofilter media showed similar biofilm formation potential to the control biofilter (Figure 8-13) ($p = 0.14$). These results suggest that nutrient limitations may drive biofilm formation potential in a biofilter with or without substrate supplementation. The biofilm formation potential results generally corresponded to relative filter hydraulic performance between the control, nutrient-enhanced, substrate-enhanced, and substrate- and nutrient-enhanced biofilters (Figures 5-1, 6-1, and 6-2).

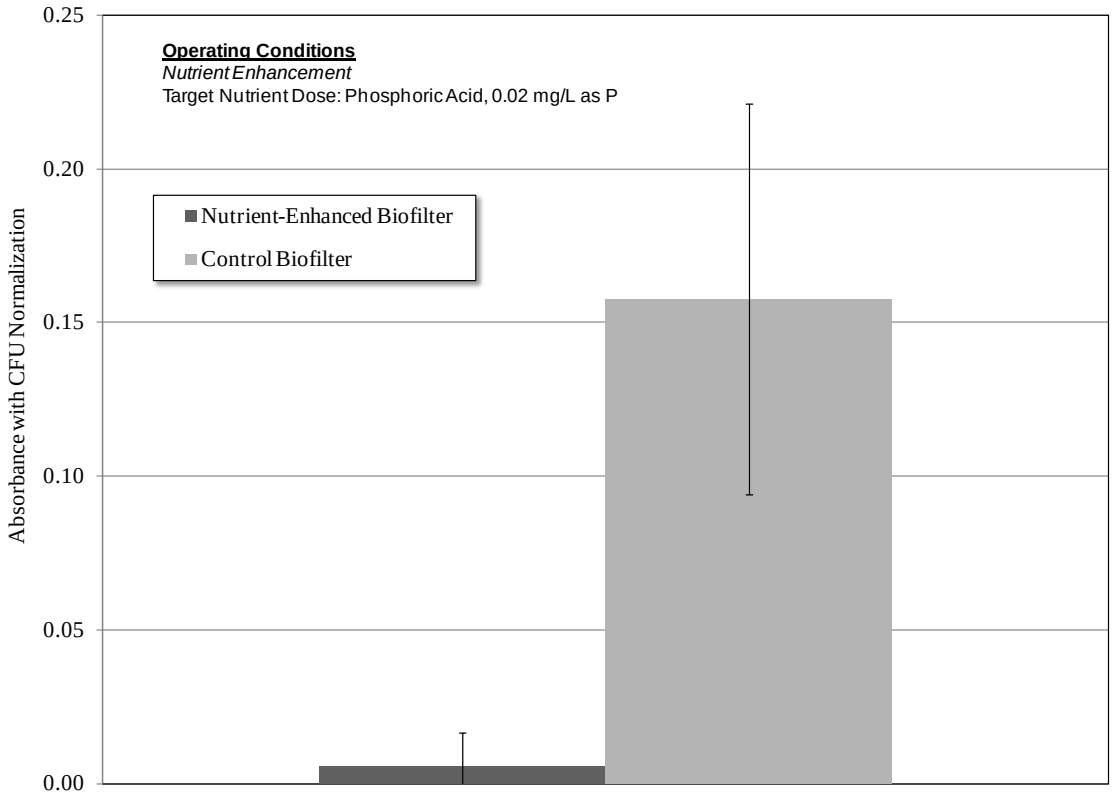

Figure 8-12. Relative biofilm formation potential between biofilter control and nutrient-enhanced biofilter[6]

[6] The error bars presented in all figures in this Chapter represent the standard deviation of the data set. This standard deviation accounts for operational variability (i.e. feed water conditions) and sampling/analytical error.

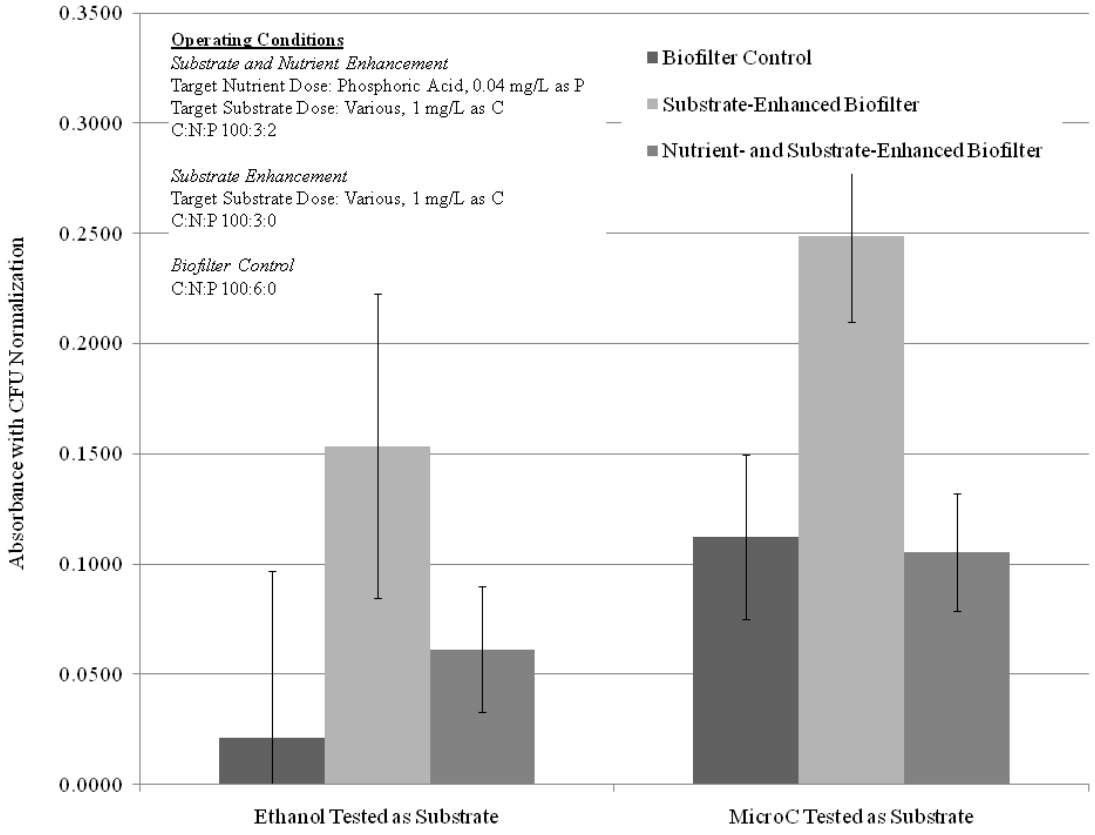

Figure 8-13. Relative biofilm formation potential between biofilter control, substrate-enhanced biofilter, and substrate- and nutrient-enhanced biofilter

EPS Quantification

EPS was quantified in glucose equivalents. EPS concentrations were found to vary significantly during the study, even in control biofilter samples. Therefore, conclusive characterization of enhancement strategies was not possible. The variation in EPS is likely to multiple factors including – lack of spatial homogeneity in the media samples (Kirisits, 2010), limited sampling frequency, and variability in sample preparation and hold time due to operator error and shipping methods. In general, it was found that the EPS was lowest for the nutrient enhanced biofilter, relative to the control biofilter (Figure 8-13). Substrate-enhancement appeared to increase free and bound EPS concentrations, while substrate- and nutrient-enhancement had little effect on EPS

production as compared to the control biofilter (Figure 8-15). The decreased presence of EPS in the nutrient-enhanced and substrate-and nutrient-enhanced biofilter samples corresponded with decreased headloss (Figures 5-1, 6-1, and 6-2) relative to the control and substrate-enhanced biofilters, respectively. The results suggest that supplementation of substrates increased the normalized (to HPC) production and quantity of biofilter EPS in nutrient limited conditions. However, both EPS production potential and the EPS concentration were reduced to levels found on the biofilter control media when the nutrient requirements were satisfied (Figures 8-13, 8-15).

This data supports the work of Mauclaire et al. (2004) that identified EPS as a significant source of fouling and decreased hydraulic conductivity in biological filters.

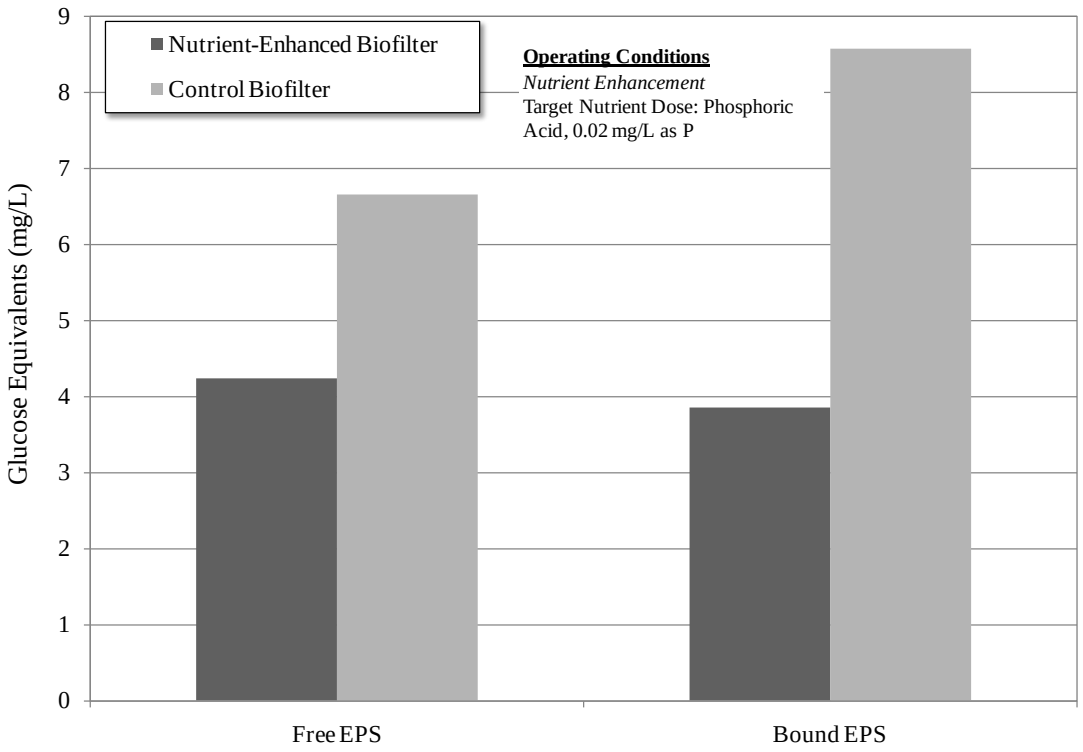

Figure 8-14. Nutrient enhancement influences on biofilter media EPS relative to the control biofilter

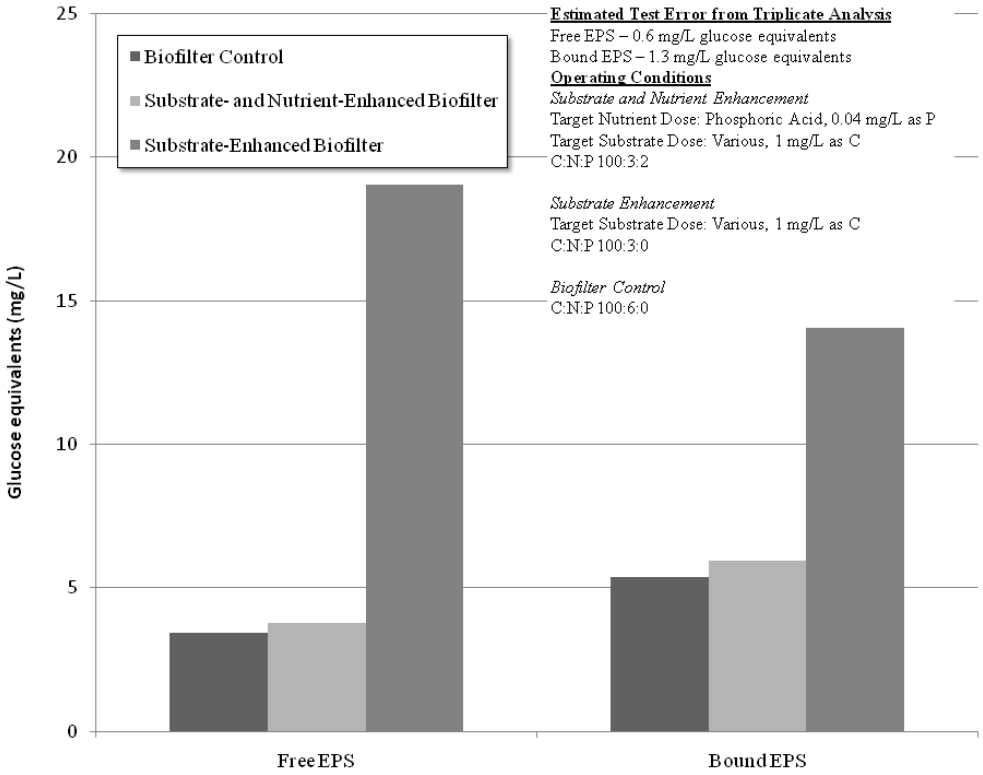

Figure 8-15. Substrate-enhancement greatly increased EPS concentrations under nutrient limited conditions

ATP Characterization

Baseline Control

ATP concentrations were measured on the biofilter media at the beginning and end of selected biofilter runs. All ATP data presented in the tables below represent the mean data of the enhanced biofilters and the biofilter control during parallel operation. Generally, ATP increased in the control biofilter media by approximately 50% from the start of a filter run to the end of a filter run. However, this increase varied from 0 to 115% through the study. These variations were likely due to uncharacterized fluctuations in feed water quality.

Substrate Enhancement Studies

The substrate-enhanced biofilter yielded higher media ATP concentrations than the control biofilter (Table 5-7). However, the substrate-enhanced biofilter media yielded

lower ATP concentrations relative to the substrate-and nutrient-enhanced biofilter media (Figure 8-14) ($p = 0.02$, $p \leq 0.05$). This observation was consistent for all substrates tested. One possible explanation for this observation is that biofilter microorganisms utilized the excess available carbon in nutrient-limited conditions (Table 5-6) to produce additional EPS relative to the control (Figure 8-15). This transformation does not produce new microbial cells, and thus it has a lower impact on ATP concentrations.

Nutrient Enhancement Studies

ATP concentrations in biofilter media were monitored during all nutrient enhancement tests. Biofilter ATP concentrations were consistently higher in biofilters with nutrient supplementation. The nutrient-enhanced biofilter showed 30% higher terminal (end of filter run) ATP concentrations relative to the biofilter control. The substrate- and nutrient-enhanced biofilter showed 70 to 250% (varied by substrate) higher terminal ATP concentrations relative to the substrate-enhanced biofilter. The increase in ATP correlated with higher DOC removals, suggesting that PO_4-P supplementation may enhance cell synthesis in PO_4-P limited conditions. Increased HPC counts on the nutrient-enhanced biofilter media (Figure 8-11) support this hypothesis. Figure 8-14 illustrates effect of nutrient limitation on biofilter media ATP observed during parallel operation of (ethanol) substrate-enhanced and (ethanol) substrate- and nutrient-enhanced biofiltration (single sample set). A 3-week characterization (N=4) of the effects nutrient-enhancement on biofilter media ATP concentrations is provided in Figure 8-15.

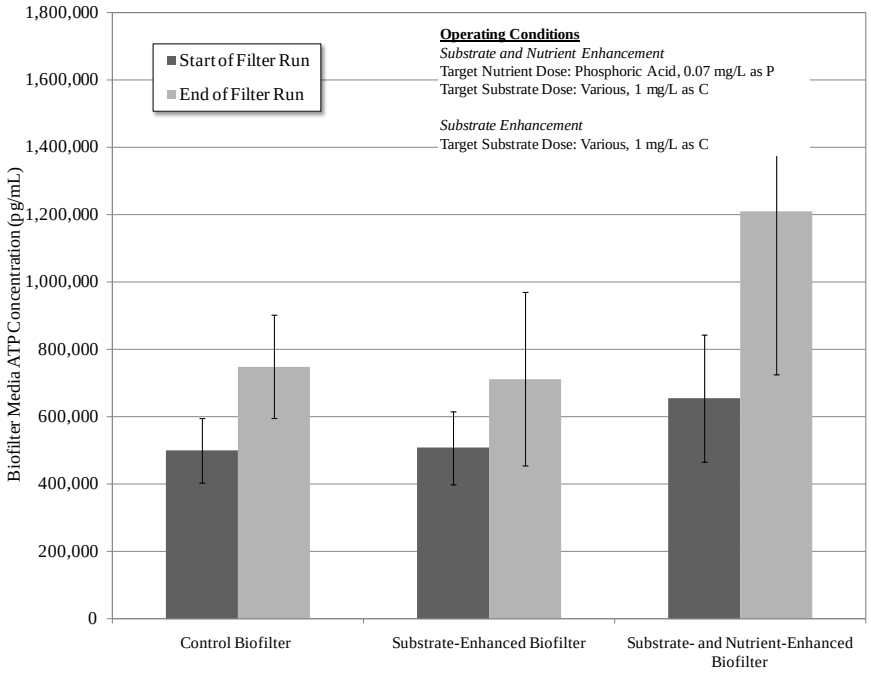

Figure 8-16. Effects of nutrient supplementation on substrate-enhanced biofilter media ATP concentrations

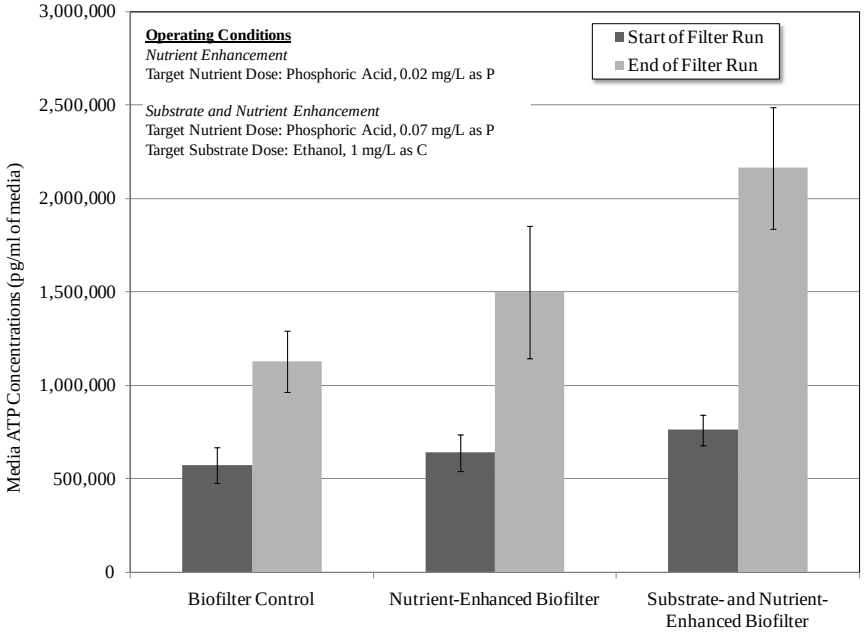

Figure 8-17. Nutrient-enhancement and nutrient- and substrate-enhancement ATP characterization

Oxidant-Enhancement Studies

The pilot biofilter control was not operated in parallel to the oxidant-enhanced biofilter. However, hydrogen peroxide supplementation did not decrease ATP

concentrations to levels below the historical levels observed in the biofilter control. These results support the DOC removal data presented in Chapter 7, suggesting that 2 weeks of steady-state hydrogen peroxide supplementation may not negatively impact biological activity in a biofilter. As stated in Chapter 2, some microorganisms are capable of expressing catalase and other oxidoreductase enzymes to reduce peroxides to innocuous water and oxygen. Figure 8-16 illustrates ATP concentrations in the oxidant-enhanced biofilter media relative to those collected during the previous month of biofilter control operation.

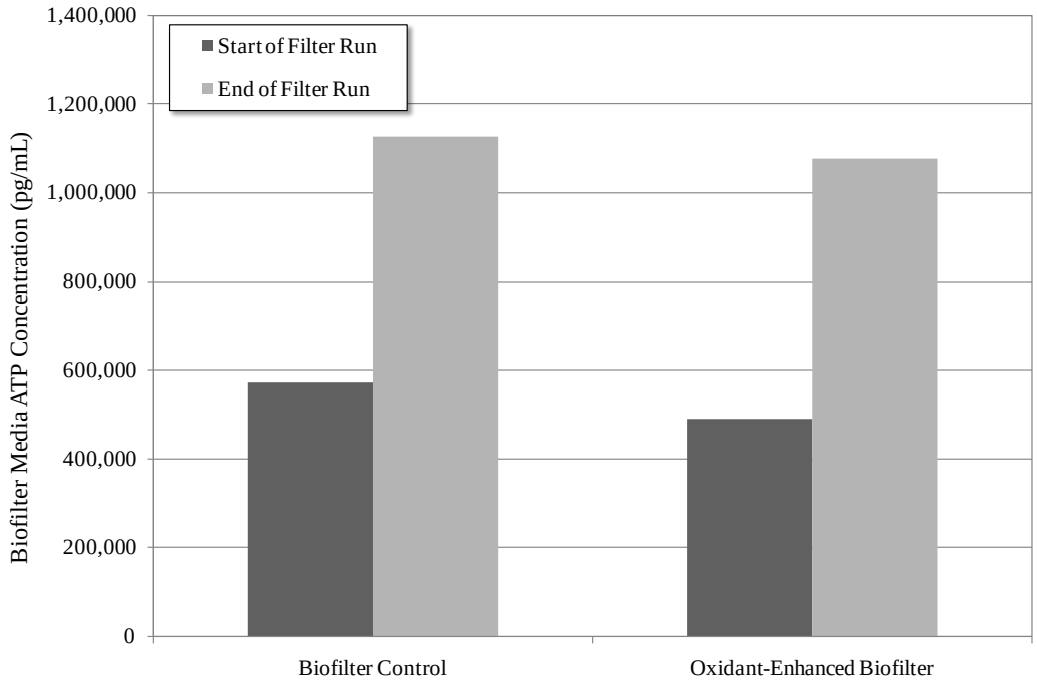

Figure 8-18. Nutrient-enhancement and nutrient- and substrate-enhancement ATP characterization

T-RFLP

T-RFLP was performed on select biofilter media samples to characterize relative shifts in community diversity/similarity. The Shannon-Weaver index (SWI) and Sørenson index (SI) were used to characterize sample diversity and sample set

similarity, respectively. The SWI accounts for both species richness and evenness, with higher values suggesting higher community diversity. A value of 0 for the SI indicates that there are no OTUs in common between two samples (no similarity), while a value of 1 for the SI indicates that two samples have identical OTUs.

The following bullets provide a summary on the test conditions evaluated:

- Test 1: Media from the nutrient enhancement validation study was compared against media collected from the same biofilter two weeks prior when it was operated under control conditions.
- Test 2: Substrate- and nutrient-enhanced biofilter (ethanol and phosphoric acid) before and after ammonium chloride supplementation.
- Test 3: Biofilter control and the oxidant-enhanced biofilter (hydrogen peroxide).
- Test 4: Biofilter control 36 weeks of operation and 38 weeks of operation.
- Test 5: Biofilter control and the full-scale biofilter.
- Test 6: Substrate-enhanced biofilter (ethanol) and the substrate- and nutrient-enhanced biofilter (ethanol and phosphoric acid).

Table 8-2 summarizes the results from this analysis.

Diversity did vary across each test and during long-term operation of the control (SWI = 3.30 to 4.32 for all samples, 3.30 to 3.75 for biofilter control). According to the SWI, the highest community diversity was present in the full-scale filters. This increase in diversity might due to the introduction and proliferation of microbial populations in the open-air filters that would not occur in the pilot system (e.g., blue green algae). Higher diversity was observed for the nutrient- enhanced biofilter and the biofilter control. The biofilter control media sample collected after 36 weeks of operation still showed 68% similarity in diversity relative to the full-scale biofilters. Decreased similarities were observed when comparing the biofilter control against substrate-enhanced and nutrient-

enhanced biofilters. Furthermore, nutrient-enhancement appeared to have the greatest effect on shifting microbial communities (i.e., lowest similarity with biofilter control).

Table 8-2. Diversity and similarity indices for various biofilter media samples

Test number	Sample set A	A diversity index (SWI)*	Sample set B	B diversity index (SWI)	A and B similarity index (SI)†
1	Nutrient-enhancement validation	3.40	Biofilter control (week 38)	3.30	0.46
2	Substrate- and nutrient-enhanced biofilter w/ammonium	4.08	Substrate- and nutrient-enhanced biofilter w/o ammonium	4.11	0.83
3	Biofilter control (week 38)	3.30	Oxidant-enhanced biofilter	3.66	0.67
4	Biofilter control (week 3)	3.74	Biofilter control (week 36)	3.85	0.74
5	Biofilter control (week 36)	3.74	FSF	4.32	0.68
6	Substrate-enhanced biofilter	3.37	Substrate- and nutrient-enhanced biofilter	3.97	0.61

* Diversity index of each sample was determined by the SWI.
† Similarity between samples was determined by the SI using ±0.5bp comparisons.

Microbial community similarities also varied over test duration. The biofilter control showed 74% similarity in microbial community in weeks 3 and 36.

Clone Libraries

Clone libraries were performed on media collected from the control, nutrient-enhanced validation, oxidant-enhanced, and the C:N:P balanced substrate- and nutrient-enhanced biofilters. Specific hydraulic and water treatment observations from each biofilter during the time of sample collection include

- Biofilter control: Biofilter was operated under full-scale/control conditions. Media sample was collected immediately before nutrient enhancement validation testing was performed. Water treatment and hydraulic performance was representative to data collected throughout the study. The observed C:N:P ratio at the time of sample collection was 100:7:0.

- Nutrient-enhancement biofilter: Biofilter was operated with phosphoric acid supplementation. Media was collected after 1 month of steady state operation. Water treatment performance was representative data collected throughout the study. However, hydraulic performance was less than

recorded means (terminal headloss of ~6 ft). The observed C:N:P ratio at the time of sample collection was 100:7:1.

- Substrate- and nutrient-enhanced biofilter: Biofilter was operated with ethanol, phosphoric acid, and ammonium chloride supplementation. Media was collected after 10 days of steady state operation. Water treatment and hydraulic performance was representative to data collected throughout the study. The observed C:N:P ratio at the time of sample collection was 100:14:2.

- Oxidant-enhanced biofilter: Biofilter was operated with hydrogen peroxide supplementation. Media was collected after 10 days of steady state operation. Water treatment and hydraulic performance was representative to data collected throughout the study. The observed C:N:P ratio at the time of sample collection was 100:6:0.

The clone libraries are provided in Tables 8-2, 8-3, 8-4, and 8-5. Identified genera were compared against the literature to identify whether they contained potential MIB, atrazine, or Mn-cycling bacteria. In addition, the clone libraries were examined for organisms known to produce EPS under nutrient-limited conditions. The clone library data presented in Tables 8-3 through 8-6 identifies communities that may support the potential functionalities described. However, testing did not seek to identify the genes required to express these functions. Therefore, this discussion is speculative and is presented to solely to support the hydraulic and water treatment performance observed in the biofilters.

The clone libraries from each biofilter included genera that contained MIB, atrazine, and Mn cycling bacteria (Ishida and Miyaji, 1992; Egashira et al., 1992; Stucki et al., 1995; Lauderdale, 2004; Kohl et al., 2006). However, all genera contain multiple strains that may or may not express reported functionality. The substrate- and nutrient-enhanced biofilter held the highest number *Burkholderia* clones. This genus contains known Mn oxidizing bacteria, supporting the water quality treatment observed in this biofilter.

Table 8-3. Biofilter control clone library

Phylum	Class	Order	Family	Genus	No. of clones	Potential functionality		
						Atrazine degradation	MIB and geosmin degradation	Mn oxidation
Proteobacteria	Alpha-Proteobacteria	Rhodospirillales	Rhodospirillaceae	*Azospirillum*	2			
		Rhizobiales	Rhizobiaceae	*Agrobacterium*	3	O		
				Rhizobium	2	O		
			Bradyrhizobiaceae	*Bradyrhizobium*	11			
				Afipia	1			
			Mesorhizobium	*Mesorhizobium*	1			
			Ochrobactrum	*Ochrobactrum*	1			
		Sphingomonadales	Sphingomonadaceae	*Novosphingobium*	1			
		Rhodocyclales	Rhodocyclaceae	*Sterolibacterium*	1			
	Beta-Proteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	1			O
				Ideonella	1			
		Nitrosomonadales	Nitrosomonadaceae	*Nitrospira*	5			
		Thiotrichales	Thiotrichaceae	*Beggiatoa*	2			
		Legionellales	Legionellaceae	*Legionella longbeachae*	5			
	Gamma-Proteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	3	O	O	
			Moraxellaceae	*Acinetobacter*	3			
		Xanthomonadales	Xanthomonadaceae	*Dokdonella*	1			
		Chromatiales	Ectothiorhodospiraceae	*Halorhodospira halophila*	1			
		Methylococcales	Methylococcaceae	*Methylocaldum*	1			
	Delta-Proteobacteria	Desulfobacterales	Desulfobacteraceae	*Desulfobacter*	3			
		Desulfovibrionales	Desulfovibrionaceae	*Desulfovibrio*	4			
		Desulfuromonadales	Geobacteraceae	*Geobacter*	1			
	Epsilon-Proteobacteria	Nautiliales	Nautiliaceae	*Nautilia*	5			
Acidobacteria	Actinobacteridae	Acidobacteriales	Acidobacteriaceae	*Acidobacteria*	6			
		Actinomycetales	Micrococcacea	*Arthrobacter*	1	O	O	
			Streptomycetaceae	*Streptomyces*	1	O		
			Nocardiaceae	*Rhodococcus*	1	O	O	
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	1		O	
	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	*Lacibacter*	1			
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	1			

Table 8-4. Substrate-and nutrient-enhanced biofilter clone library (carbon limited)

						Potential functionality		
Phylum	Class	Order	Family	Genus	No. of clones	Atrazine degradation	MIB and geosmin degradation	Mn oxidation
Proteobacteria	Alpha-Proteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	1	O		
			Bradyrhizobiaceae	*Bradyrhizobium*	1			
		Rhodospirillales	Acetobacteraceae	*Acetobacter*	3			
		Rhodobacterales	Rhodobacteraceae	*Rhodovulum*	1			
	Beta-Proteobacteria	Burkholderiales	Comamonadaceae	*Simplicispira*	1			
				Variovorax	1			
			unclassified	*Sulfurovum*	1			
			Burkholderiaceae	*Burkholderia*	17			O
				Cupriavidus	2			
			Derxia gummosa	*Derxia*	2			
		Rhodocyclales	Rhodocyclacease	*Zoogloea*	3			
		Neisseriales	Neisseriaceae	*Aquaspirillum*	2			
		unclassified Betaproteobacteria	Ferrovum	*Ferrovum myxofaciens*	3			
		Rhizobiales	unclassified	*Methylocystis*	1			
		Nitrosomonadaceae	Nitrospiraceae	*Nitrosospira*	1			
	Gamma-Proteobacteria	Chromatiales	Ectothiorhodospiraceae	*Halorhodospira*	2			
		Legionellales	Legionellaceae	*Legionella*	2			
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	5	O	O	
		Acidithiobacillales	Acidithiobacillaceae	*Acidithiobacillus*	3			
		Thiotrichales	Thiotrichaceae	*Beggiatoa*	1			
		Xanthomonadales	Xanthomonadaceae	*Rhodanobacter*	1			
	Delta-proteobacteria	Desulfovibrionales	Desulfovibrionales	*Desulfovibrio*	1			
		Desulfuromonadales	Geobacteraceae	*Geobacter*	3			
		Desulfovibrionales	Desulfovibrionales	*Desulfonatronum*	2			
Actinobacteria	Actinobacteria	Rhizobiales	Bifidobacteriaceae	*Bifidobacterium*	1			
	Actinobacteridae	Actinomycetales	Mycobacteriaceae	*Mycobacterium*	1		O	
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	1			
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	1		O	
	Clostridia	Clostridiales	Clostridia	*Clostridium*	4			

Table 8-5. Oxidant-enhanced biofilter clone library

						Potential functionality		
Phylum	Class	Order	Family	Genus	No. of clones	Atrazine degradation	MIB and geosmin degradation	Mn oxidation
Proteobacteria	Alpha-Proteobacteria	Rhodospirillales	Acetobacteraceae	*Acetobacter*	3			
			Rhodospirillaceae	*Azospirillum*	1			
			Hyphomicrobiaceae	*Hyphomicrobium*	1			O
			Methylobacteriaceae	*Methylobacerium*	2			O
		Rhizobiales	Beijerinckiaceae	*Methylocapsa*	1			
				Methylocella	1			
			Rhizobiaceae	*Rhizobium*	1	O		
			Bradyrhizobiaceae	*Rhodopseudomonas*	1			O
				Bradyrhizobium	18			
		Caulobacterales	Caulobacteraceae	*Brevundimonas*	1			
		Sphingomonadales	Sphingomonadaceae	*Sphingobium*	3			
		Rhodocyclales	Rhodocyclaceae	*Zoogloea*	1			
				Sterolibacterium	2			
	Beta-Proteobacteria	unclassified Betaproteobacteria		*Thiobacter*	2			
		unclassified Betaproteobacteria		*Ferrovum*	2			
	Gamma-Proteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	2			
		Aeromonadales	Aeromonadaceae	*Aeromonas caviae*	1			
		Thiotrichales	Thiotrichaceae	*Beggiatoa*	1			
		Enterobacteriales	Enterbacteriaceae	*Enterobacter*	1		O	
		Chromatiales	Halothiobacillaceae	*Halothiobacillus*	1			
			Pseudomonadaceae	*Pseudomonas*	1	O	O	
		Enterobacteriaceae	Enterobacteriaceae	*Serratia*	2			
		Pseudomonadales	Thiotrichaceae	*Thiothrix*	1			
Acidobacteria	Actinobacteria	Acidobacteriales	Acidobacteriaceae	*Acidobacteria*	1			
			Streptosporangiaceae	*Streptosporangineae*	1			
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Flavisolibacter*	2			
	Sphingobacteria	Sphingobacteriales		*Sphingobacterium*	3			
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	1			

Table 8-6. Ammonia-limited, nutrient-enhanced biofilter clone library

Phylum	Class	Order	Family	Genus	No. of clones	Potential functionality		
						Atrazine degradation	MIB and geosmin degradation	Mn oxidation
Proteobacteria	Alpha-Proteobacteria	Rhodospirillales	Acetobacteraceae	*Acidiphilium*	1			
		Sphingobacteriales	Sphingomonadaceae	*Sphingomonas*	4			
				Afipia	2			
		Rhizobiales	Bradyrhizobiaceae	*Agromonas*	1			
				Bradyrhizobium	19			
			Methylobacteriaceae	*Microbacterium*	2	O		
			Nordella	*Nordella*	2			
			unclassified	*Rhizobiales*	2			
		Rhodobacterales	Rhodobacteraceae	*Rhodobacter*	3			
	Beta-Proteobacteria	Burkholderiales	Alcaligenaceae	*Achromobacter*	1			
			Burkholderiaceae	*Burkholderia*	2			O
		Rhodocyclales	Rhodocyclaceae	*Azoarcus*	1			
				Sterolibacterium	1			
		Neisseriales	Neisseriaceae	*Chitiniphilus shinanonensis*	1			
		Nitrosomonadales	Nitrosomonadaceae	*Nitrospira*	2			
		unclassified Betaproteobacteria		*Thiobacter*	2			
	Gamma-Proteobacteria	Aeromonadales	Aeromonadaceae	*Aeromonas*	2			
		Pseudomonadales	Moraxellaceae	*Acinetobacter*	1			
			Pseudomonadaceae	*Pseudomonas*	7	O	O	
		Enterobacteriaceae	Enterobacteriaceae	*Serratia*	1			
		Xanthomonadales	Xanthomonadaceae	*Xanthomonas*	1			
	Delta-Proteobacteria	Desulfovibrionales	Desulfovibrionaceae	*Desulfovibrio*	3			
		Desulfobacterales	Desulfobacteraceae	*Desulfobacter*	1			
			Geobacteraceae	*Geobacter*	1			
				Lacibacter	1			
		Bdellovibrionales	Bacteriovoracaceae	*Bacteriovorax*	1			
Actinobacteria	Actinobacteridae	Actinomycetales	Pseudonocardiaceae›	*Actinobispora*	1			
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	3		O	
	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	*Chitinophaga*	1			
		Sphingobacteriales	Chitinophagaceae	*Niastella*	1			
		Sphingobacteriales		*Sinorhizobium*	2			
	Sphingobacteria	Sphingobacteriales	Cyclobacteriaceae	*Aquiflexum*	1			
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	1		O	
	Clostridia	Clostridiales	Clostridia	*Clostridium*	1			

Perhaps most significant observation in the clone libraries for these samples was the drastic reduction in the *Bradyrhizobium* population when the stoichiometric C:N:P ratio was implemented. *Bradyrhizobium* constituted only 1.5% of the clones in the stoichiometric C:N:P sample, but constituted 31% in the oxidant-enhanced biofilter, 27% in the nutrient-enhanced biofilter (with NH_4-N limitation), and 15% in the biofilter control. *Bradyrhizobium* has been shown to increase EPS production under N-limitation (Quelas et al., 2006) and related rhizobia have been shown to increase EPS production under PO_4-P limitation (Skorupska et al., 2006). Figure 8-17 shows relative population sizes of *Bradyrhizobium* (blue) and *Burkholderia* (yellow) in clone libraries obtained from the substrate- and nutrient-enhanced biofilter (100:14:2 C:N:P) and the biofilter control (100:6:0). In addition, using the calculated length of the OTU corresponding to *Bradyrhizobium*, T-RFLP showed that the *Bradyrhizobium*-OTU maintained between 10 and 15% abundance (Figure 8-18) in the biofilter control media throughout the study; however, during the nutrient enhancement validation study, *Bradyrhizobium* abundance decreased to approximately 1% in this biofilter. Figure 8-18 illustrates the observed shift in *Bradyrhizobium* in the biofilter with phosphoric acid supplementation. This shift correlated with improved filter hydraulic performance (The prevalence of *Bradyrhizobium* in the oxidant-enhanced biofilter is potentially due to a completive advantage provided by EPS production. Studies have shown EPS provides microbial resistance to oxidative stress (Király, 1998). Nutrient-enhanced biofilter media samples with the lowest *Bradyrhizobium* abundance also maintained the lowest filter run terminal headloss.

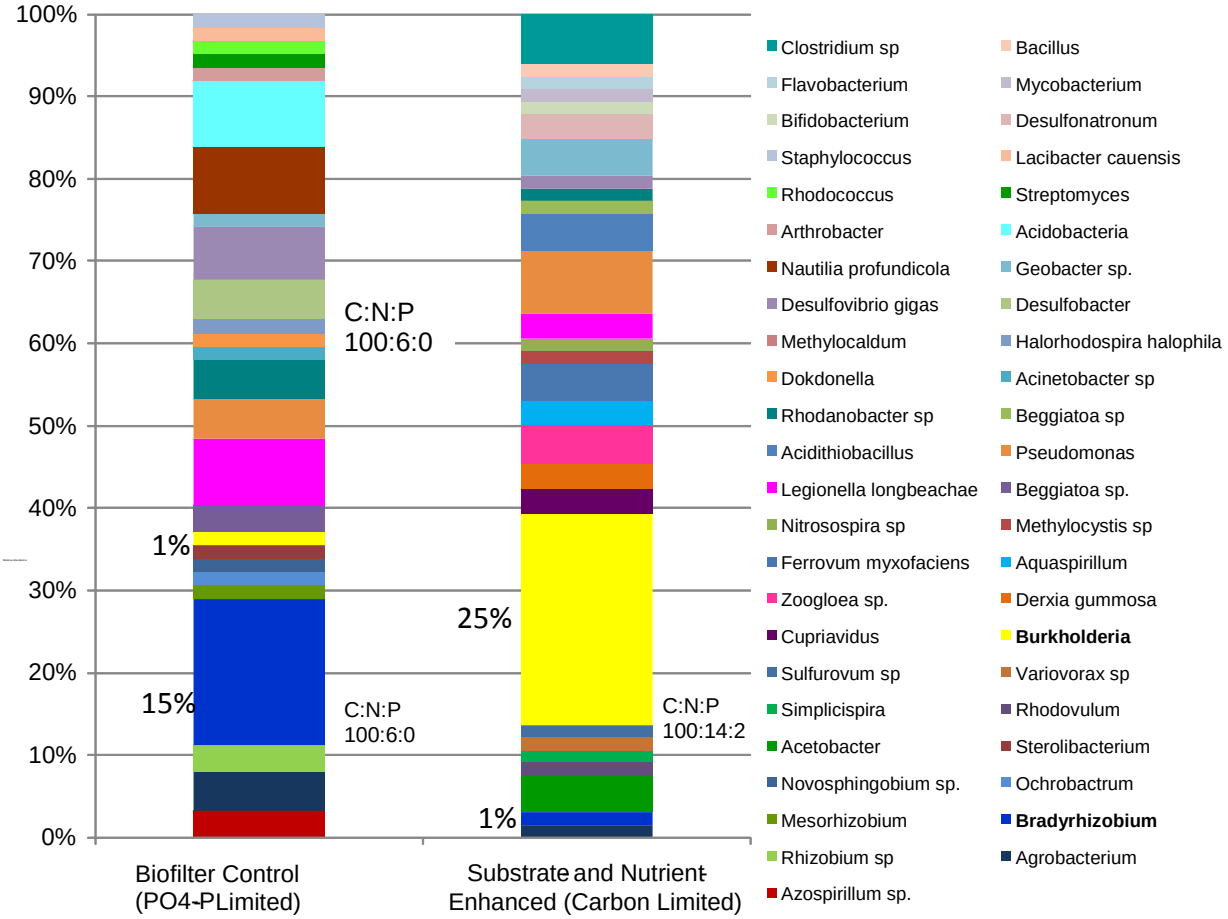

Figure 8-19. Comparison of biofilter media clone libraries under phosphorus-limited and carbon-limited conditions

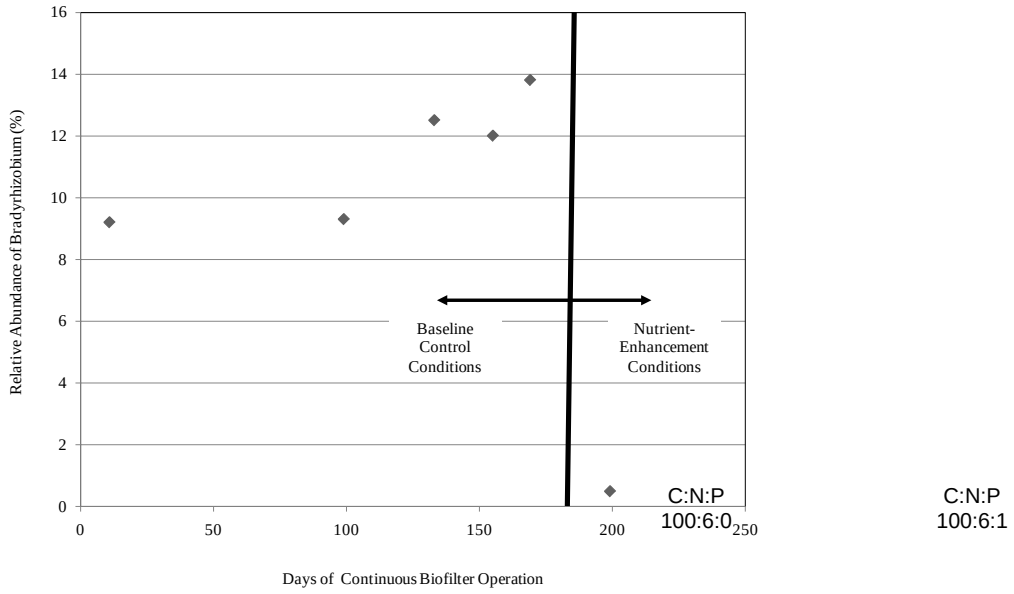

Figure 8-20. Relative abundance (via T-RFLP) of *Bradyrhizobium* before and after phosphoric acid supplementation (0.02 mg/L as P)

CHAPTER 9
FULL-SCALE PROCESS INTEGRATION ASSESSMENT AND ECONOMIC EVALUATION

Objectives

During pilot testing, the substrate enhancement, nutrient enhancement, and enzyme enhancement strategies all demonstrated some water treatment benefits. The enzyme and nutrient enhancement strategies also provided significant hydraulic benefits, making them particularly promising for full-scale implementation. The oxidant enhancement strategy was evaluated only briefly, and additional testing is required to better understand process mechanisms and characterize long-term performance. In addition, dosage sensitivity and optimization must still be performed because a 1 mg/L continuous dose of hydrogen peroxide may be cost prohibitive for many utilities (~$12/MG treated at a bulk cost of $1.5/lb). The chemical cost of oxidant-enhancement strategy implementation would exceed the total cost for backwashing (Table 9-5). Therefore, the full-scale process integration assessment and cost evaluation was conducted solely on the nutrient-enhanced biofilter strategy. Design requirements and cost estimates were developed around the JKWTP and PBSWTP process configurations, capacities, and requirements (phosphorus limitation only). All costs are presented as $/MG produced.

Process Integration

Conceptual Design and Implementation

Nutrient-enhanced biofiltration can be implemented at the Arlington facilities with the installation of a phosphoric acid feed system that would satisfy the PO_4-P limitation through continuous feed at 20 µg/L as P. The phosphoric feed system would include a peristaltic pump skid housing two pumps (1 duty, 1 standby) with variable frequency

drives (VFDs) and supervisory control and data acquisition (SCADA) integration. The pumps would have flow pacing capabilities with automatic control from the raw water flow meters. The system would be located in each facility's chemical room. The assumed phosphoric acid feed rate would be 0.044 gal/MG treated (assuming 85% acid purity). At peak capacity (PBSWTP - 72 mgd, JKWTP - 97.5 mgd,), the feed rate would be 3.2 to 4.3 gallons per day of 85% phosphoric acid. The acid would be pumped through flexible tubing and injected into a carrier water line containing finished water. The carrier water system would convey approximately 10 gpm of carrier water (finished water + phosphoric acid) to a nozzled manifold located within a common filter influent channel. The manifold would be arraigned perpendicular to flow with chemical injected countercurrent to flow to improve chemical dispersement. Chemical storage would include two standard, self-contained, 250-gallon totes (1 duty, 1 standby) provided by the phosphoric acid supplier. Each tote would be capable of providing over 50 days of storage at peak flow. Empty totes would be replaced upon each chemical delivery. Approximately 75 to 150 ft of carrier piping would be required to convey phosphoric acid from the chemical room to the biofilter feed channel. Figure 9-1 illustrates a conceptual layout for the phosphoric acid feed system.

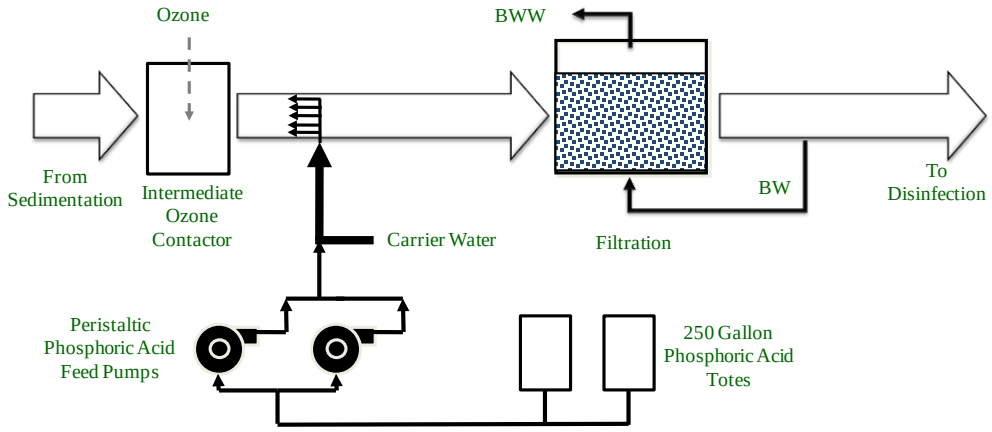

Figure 9-1. Conceptual integration schematic for nutrient enhancement

Process Monitoring

The implementation of biofilter nutrient-enhancement or any engineered biofiltration strategy requires not only the intentional design on operation of the biofilters as a biological system, but also intentional monitoring of the biological activity and health. Nutrient-enhancement shows significant promise as a biofiltration operational strategy; however, not all water treatment plants should be treated equally. Monitoring is critical, as process optimization is an iterative approach that is unique to each water source and treatment train. Many analytical tools (including those discussed in this dissertation) are currently available that provide utilities with cost efficient and easily accessible onsite monitoring. Headloss monitoring and ATP analyses are two examples of real-time tools that may indicate successful implementation of biofiltration enhancement or provide an early warning for system upset. It is recommended that utilities consider these and other methods for process monitoring to promote optimal biofiltration performance.

Additional Considerations

Biofilter nutrient-enhancement may affect not only biofilter hydraulics and filtered water quality, but also distribution system stability. The results presented in Chapter 6 suggest that nutrient enhancement will not increase chloramine decay or DBP formation. Biofilter nutrient-enhancement also provided significant reduction in DOC relative to the biofilter control, likely limiting regrowth potential. However, long-term distribution system impacts of nutrient-enhanced biofiltration remain unknown. Previous work evaluating nutrient levels in finished waters supports both positive and negative impacts on distribution system regrowth and disinfectant stability (Chapter 2). Biofilter nutrient breakthrough may increase biological activity in distribution systems.

Conversely, a substrate-limited condition in the distribution system may reduce EPS production and biofilm formation thus increasing microbial susceptibility to residual disinfectants and improving long-term stability. Regardless, it is recommended that concerned utilities carefully monitor nutrient addition and breakthrough to prevent undesired concentrations in the distribution system. Although dosed concentrations are likely to be less than 0.1 mg/L as N or P, low level nutrient breakthrough may be of particular concern for areas held to strict numeric nutrient criteria limits on wastewater discharge permits.

Cost Assessment

Assumptions

The assumptions used for the cost assessment are included in Table 9-1.

Capital Cost

The estimated capital cost for a phosphoric acid dosing system is $25,000, including $18,000 for the peristaltic pump skid and $7,000 for installation and the associated piping. The amortized production cost for the equipment is $0.07/MG.

Table 9-1. Cost assessment assumptions

Criteria	Units	Value
Average annual facility production	mgd	60
Discount Rate	%	3
Estimated Contingency on Power and Chemical Costs	%	25
Power cost	$/kWh	0.1214
Equipment life	Yr	20
Phosphoric acid feed requirement*	mg/L as P	0.02
Phosphoric acid cost†	$/lb	0.72

* 85% bulk solution

† Nutrient limitation is assumed for PBSWTP based on similar water quality to JKWTP – P/C mass ratio of 0.026

Operation and Chemical Cost

The peristaltic pumps are operated at a maximum of 220 watts. Assuming a continuous maximum power draw, the normalized production cost for power is approximately $0.01/MG treated. The estimated chemical cost is $0.44/MG treated. The total operation and chemical production cost is $0.45/MG treated, or $0.56 with a 25% contingency.

Total Estimated Cost for Implementation

The estimated production cost for nutrient enhanced biofiltration at the JKWTP and PBSWTP is $0.63/MG treated.

Potential Net Costs and Cost Savings

The increased hydraulic performance observed with the nutrient-enhanced biofilters may lead to real cost savings during full-scale implementation. The basis for this estimate is an assumed increase in biofilter run time. This increase in filter run time translates to a corresponding decrease in backwash frequency and all costs associated with backwashing (e.g., power and backwash wastewater retreatment). All backwash wastewater must be repumped to the head of the plant and retreated. Treatment costs vary between the JKWTP and PBSWTP. Therefore, the potential savings for nutrient enhancement are different for each plant. Tables 9-2, 9-3, and 9-4 shown below provide an estimate of the cost of filter backwashing developed for the JKWTP and PBSWTP. These estimates include the chemical and pumping costs for the recycled backwash water. Net cost and saving estimates were developed for biofilter runtime improvements of 5, 10, 15, and 20%.

Table 9-2. Backwash water production estimates*

Parameter	Treatment plant	
	PBSWTP	JKWTP†
Total flow (MG)	23,470	12,858
Total # of filters washed	3,627	3,310
Filter backwash water per filter (MG)	0.363	0.233
Total backwash water produced and returned (MG)	1335	771

* The flows (in MG) and filter numbers are based on information obtained from the annual summary report from October 2008 to September 2009

† Low production rates at the JKWTP were attributed to plant shutdowns for an expansion project that occurred during the study.

Table 9-3. Chemical costs ($/MG) to retreat backwash wastewater*

Parameter	Treatment plant	
	PBSWTP	JKWTP
Liquid oxygen (LOX)	$15.92	$11.20
Alum	$40.08	$23.08
Polymer	$5.46	$5.05
Ozone generation	$13.92	$22.12
Total cost	$75.38	$61.45

* The chemical cost numbers are based on information obtained from the annual summary report from October 2008 to September 2009

Table 9-4. Pumping costs to recycle backwash wastewater*,†

Parameter	Treatment plant	
	PBSWTP	JKWTP[3]
Backwash pump ($/MG)	43.68	-
Recycle pump ($/MG)	36.00	36.00
Total cost ($/MG)	79.68	36.00

* The pumping costs are based on information obtained from the annual summary report from October 2008 to September 2009

† Power rates are based on Arlington's 2010 rate of $0.1214/kWh

As shown in Table 9-5, cost savings may be realized by implementing a nutrient-enhancement strategy for the biofiltration process if biofilter runs are extended by greater than 10% (~11% reduction in backwash frequency required for savings at JKWTP). Arlington may achieve a net cost savings of approximately over $17,100 per year if the PBSWTP and JKWTP full-scale biofilters respond to phosphoric acid

supplementation similar to the pilot system (15% backwash frequency reduction). These saving would provide an implementation payback of less than two years. In addition, nutrient-enhancement may also provide additional cost savings through extending the life of biofilter media (via decreased attrition) and mechanical equipment (underdrains/caps, pumps, etc).

Table 9-5. Nutrient-enhancement implementation net costs or savings

	Treatment plant	
Parameter	PBSWTP	JKWTP
Backwash Cost Summary		
Chemical cost ($/MG)	75.38	61.45
Pumping cost ($/MG)	79.68	36.00
Total cost ($/MG)	155.06	97.45
Filter backwash flow (MG)	0.37	0.23
Total cost/filter backwash ($/BW)	57.37	22.41
Total backwash cost/year ($/yr)	208,088	74,189
Total backwash cost/MG treated ($/MG)	8.87	5.77
Projected savings from 5% extended filter runs*		
Backwash savings ($/MG)	0.44	0.29
Net savings from nutrient enhancement ($/MG)	-0.19 (Cost)	-0.34 (Cost)
Potential annual savings at previous annual production rates ($)	-3468 (Cost)	-6205 (Cost)
Projected savings from 10% extended filter runs*		
Backwash savings ($/MG)	0.89	0.56
Net savings from nutrient enhancement ($/MG)	0.26	-0.07 (Cost)
Potential annual savings at previous annual production rates ($)	4,745	-1,278(Cost)
Projected savings from 15% extended filter runs*		
Backwash savings ($/MG)	1.33	0.87
Net savings from nutrient enhancement ($/MG)	0.70	0.24
Potential annual savings at previous annual production rates ($)	12,775	4,380
Projected savings from 20% extended filter runs*		
Backwash savings ($/MG)	1.62	1.12
Net savings from nutrient enhancement ($/MG)	1.00	0.50
Potential annual savings at previous annual production rates ($)	18,068	8,943

* Net savings incorporate estimated capital and operating costs for nutrient enhancement. All nutrient-enhancement costs include 25% contingency on power and chemical costs.

CHAPTER 10
SUMMARY AND CONCLUSIONS

New regulatory and technological developments are driving more utilities to consider the use of biofiltration to treat their drinking water. These developments include (1) the concern over the formation of disinfection by products (DBPs); (2) the emergence of ozone for taste, odor, and color control; (3) the increased awareness of how biological activity in distribution systems contributes to disinfectant demand, aesthetic upsets, and corrosion; and (4) the push for green technologies. Currently, drinking water biofiltration is largely operated as a passive process. Particle/turbidity removal and headloss drive the design and operation of biofiltration as they would conventional filtration. Thus, biofilter design parameters are typically limited to media configuration, backwash strategy, and loading rate. The biological removal of dissolved organic and inorganic contaminants is an anticipated benefit of biofiltration. However, common practice does not seek to enhance the bioactivity responsible for those mechanisms. Indeed, in an effort to improve filter productivity and minimize headloss, many utilities employ chlorinated or chloraminated water to the filter backwash or feed. However, this practice is to the detriment of biological activity and may be ineffective at removing a primary foulant of biofilters – extracellular polymeric substances (EPS) produced by bacteria resident in the filter biofilm. EPS can occupy as much as 1,000 times the filter media void space as bacteria, playing a more significant role in both fouling and headloss (Mauclaire, 2004).

An alternative approach is to move the practice of biofiltration from a passive process designed and operated around conventional filtration objectives to an intentionally operated biological system, i.e. “engineered biofiltration.” Engineered

biofiltration targets multiple water quality objectives while maintaining hydraulic performance. These benefits can be achieved simultaneously by providing specific conditions that promote the improved biological activity. Engineered biofiltration shifts the industry-accepted paradigm so that the design and operation of biofilters should be driven not only by filtration but also by biological treatment objectives.

Problem Statement and Hypothesis

Drinking water biofilters at surface water treatment facilities commonly experience shortened run times; underdrain clogging, T&O breakthrough, and Mn breakthrough (Hunt, 2009; Zhu, 2010[*]). A pilot study was conducted at the City to evaluate methods for restoring and enhancing the performance of the biofiltration process. This evaluation entailed both a characterization and evaluation of biological activity in the filters and an examination of potential enhancement strategies. The hypothesis of this work is both water treatment and hydraulic performance of a biofilter can be improved by modifying influent conditions for enhanced biological activity. The purpose of this research was to identify strategies to enhance the biological activity in a biofilter without compromising productivity or particulate removal performance. Specific objectives included:

- Evaluate potential biofilter enhancement strategies comprised of dosing low levels of common drinking water treatment chemicals at a feed point just upstream of a biofiltration process. These chemicals were added to provide substrate, nutrient, and/or oxidant optimization of the biofilter process influent.
- Investigate of biological drinking water treatment process fundamentals (e.g., microbial ecology, bacterial metabolism, and contaminant removal mechanisms) to understand how

DOC, MIB, geosmin, and Mn can be removed effectively in a single treatment step

[*] Zhu, I. Personal communication with Ivan Zhu, Senior Engineer at F.B. Leopold, ITT, on May 15, 2010.

Biological clogging (filter headloss) can be minimized

The ultimate goal of this work is to shift an industry-accepted paradigm so that the design and operation of biofilters are driven not only by filtration but also by biological treatment objectives.

Objectives

The research included 10 months of biofiltration enhancement pilot-scale testing at the JKWTP to evaluate methods for restoring and enhancing the performance of the City's biofiltration process. This evaluation entailed both a characterization and evaluation of biological activity in the biofilters and an examination of potential enhancement strategies. The strategies tested were selected based on previously published literature and industry experience. The following studies were performed to meet research objectives:

- Characterize the baseline performance of the JKWTP operating under existing conditions. This included an assessment of the system's ability to meet the City's current treatment objectives;
- Evaluate primary substrate augmentation enhancement strategies;
- Evaluate nutrient augmentation enhancement strategies;
- Evaluate peroxide supplementation for augmenting the oxidative action and response of the biofiltration process;
- Identify and track the microbial communities within the biofilters;
- Develop conceptual full-scale design and operating parameters for the recommended modified biofiltration process and estimate the associated capital and production costs.

Results

Baseline Biofiltration Characterization

The objective of this task was to characterize baseline water treatment performance, confirming similitude between pilot- and full-scale treatment and to provide control data for parallel tests occurring with other pilot filters. Similitude was characterized by operating a control biofilter under full-scale operational conditions (i.e., no enhancement strategies were used for the control biofilter) from March 5, 2009 through October 1, 2009. These tests provided approximately seven months of data that confirmed and further elucidated the treatment capabilities of the existing process at the JKWTP. Monitored water quality parameters included DOC, total Mn, ammonia-nitrogen (NH_4-N), orthophosphate-phosphorus (PO_4-P), MIB, and a suite of over 150 pharmaceuticals and pesticides. These water quality data demonstrated treatment performance similitude between the pilot and full-scale filters (Tables 4-5, 4-6, 4-7, 4-8, 4-9). Measured water quality values between the control biofilter and the full-scale biofilter were within the standard deviations of their respective data sets. The study confirmed potential treatment deficiencies under full-scale biofilter operating conditions. MIB breakthroughs of 11 ng/L and 25 ng/L were observed during simulated moderate (~30 ng/L) and high load (~160 ng/L) conditions. In addition, Mn breakthrough of approximately 25 µg/L was observed during simulated peak load testing (220 µg/L). These results support the high frequency of T&O and (black/brown) colored water complaints received by the City (Hunt, 2009). The baseline biofiltration characterization also identified nutrient limitations in the full-scale (and pilot) biofilter feed. During the course of 5-months of steady-state testing the bioavailable C:N:P ratio varied from 100:10:0 to 100:6:0. The absence of PO_4-P in the biofilter feed was consistent

throughout the study, likely the result of the enhanced coagulation process. The observed PO_4-P limitation in the biofilter control was identified as a limiting factor for both hydraulic (15% higher terminal headloss) and water treatment performance (DOC, Mn, and MIB removal) relative to the nutrient-enhanced biofilter (C:N:P of 100:10:1). The biofilter control filter media also contained approximately 30% less ATP. In addition, the microbial characterization of the biofilter control suggests increased EPS (free and bound), biofilm formation potential, and prevalence of biofilm matrices relative to the nutrient-enhanced biofilter. 16S rRNA clone libraries were also developed for media samples collected from the biofilter control. These analyses found higher prevalence of microbial populations belonging to the genus *Bradyrhizobium* than observed in media collected from the nutrient-enhanced biofilter operated under carbon-limiting conditions (C:N:P of 100:14:2) (Figure 8-19). Select species within the genus *Bradyrhizobium* have been characterized to increase EPS production under nutrient-limiting conditions (Quelas et al., 2006). These results suggest that nutrient limitation may not only increase biofilter EPS production (and thus headloss), but also select for populations responsible for producing excess amounts of EPS under limited conditions. The ability to produce additional EPS under nutrient-limiting conditions might provides *Bradyrhizobium* a competitive advantage under normal biofilter operations (e.g., where additional EPS provides additional resistance to sloughing from abrasion and scouring during backwash).

The findings of this study all suggest that the nutrient-limited conditions may inhibit optimal water treatment performance while increasing filter headloss (and potentially

underdrain fouling). These findings are supported by the work of Mauclaire et al. (2004), Nishijima et al. (1997), and Sang et al., (2003).

Substrate-Enhancement Studies

The objective of this study was to evaluate various primary substrate augmentation strategies for enhancing biofiltration performance. Increasing the concentration of primary substrate (through intermediate ozonation or primary substrate augmentation) in a biological treatment application may increase the rate of recalcitrant and trace organic compound degradation through secondary substrate metabolism and/or cometabolism. This study evaluated four primary substrates, including acetic acid, molasses, MicroC© (a proprietary glycerin-based product produced by Environmental Operating Solutions, Inc, Bourne, Mass.), and ethanol. The substrates were evaluated individually through four successive one- to two- month phases, each dosed at target of 1 mg/L as C. The limited test durations and rapid succession of the substrates tests biased the results of these studies. The substrate-enhanced biofilter likely retained biofilm matrix/community/population history after each substrate tested, influencing the effectiveness of successive tests. However, specific substrates, such as molasses, yielded immediate negative impacts on biofilter performance (Table 5-1),

The substrate-enhanced biofilters yielded 25 to 150% higher terminal headloss than the control biofilter during 18-hour filter runs (Table 5-1). However, sustainable (>1 week continuous operation) 18-hour filter runs were not achieved with any substrate tested due to excessive headloss. Microbial analyses identified that substrate-enhancement increased biofilter media ATP (7 to 30%, Table 5-7), EPS (>180%, Table 8-15), and biofilm formation potential (>150%, Table 8-13) relative to the control, indicating a higher degree of microbial activity and biofilm formation. The C:N:P ratio

varied during these tests (Table 6-7); however, P and N limitations were persistent for all substrates tested. Interestingly, the substrate- and nutrient-enhanced biofilter showed higher ATP levels (>70%, Table 8-16) than the substrate-enhanced biofilter, yet terminal headloss, EPS, and biofilm formation potential measurements remained near biofilter control levels. These microbial analyses suggest that headloss may be attributed to a higher production of EPS produced by bacteria and not to increased overall biological activity (as measured by ATP or HPC).

Substrate augmentation strategies did not provide significant improvement of trace organics or metals removal relative to the control biofilter. Background DOC removal percentages were also comparable between the control and substrate-enhanced biofilters when acetic acid and MicroC© were used. The biofilter supplemented with molasses showed twice as much DOC breakthrough as the control biofilter, while the biofilter operated with ethanol supplementation showed an average of 50% higher background DOC removal relative to the control, though the differences in the mean breakthroughs were not shown to be statistically significant. Substrate enhancement provided no significant benefit to the pilot biofilter under the conditions and substrates tested. Therefore, the strategies tested for substrate-enhancement are not recommended. Possible strategy limitations that may have inhibited substrate-enhanced biofilter performance include:

- Substrates tested do not support necessary enzymatic activity for secondary substrate or cometabolic process for trace organic degradation

- The supplemental bioavailable C increases biofilter nutrient deficiencies resulting in EPS production and headloss; therefore, limiting the steady-state operational performance necessary for optimal substrate utilization.

Nutrient-Enhancement Studies

The objective of this task was to evaluate various nutrient augmentation strategies for enhancing biofiltration performance. The primary focus of this study was to evaluate nutrient enhancement by dosing 0.020 mg/L phosphoric acid as PO_4-P to satisfy the biofilter feed nutrient requirement for 0.8 mg/L of bioavailable DOC, achieving a 100:10:1 bioavailable C:N:P ratio.

Phosphorus supplementation decreased biofilter terminal headloss by approximately 15% relative to the control (Table 6-1). This improvement could translate to longer filter run times and decreased backwash frequencies. Microbial characterizations suggest that biofilter nutrient enhancement (to achieve a 100:10:1 C:N:P ratio) can simultaneously decrease biofilm formation potential (>1400%) and EPS media concentrations (>30%), while increasing media ATP concentrations (>30%) (Figures 8-11, 8-12, 8-17). The observed decreases in EPS and biofilm formation potential are likely the cause for the decrease in biofilter headloss. In addition, clone libraries indicated that pilot biofilter media operated under nutrient enhancement conditions had lower prevalence of organisms belonging to *Bradyrhizobium* (<2%) than did biofilter media operated under nutrient-limited conditions (where *Bradyrhizobium* communities exceeded 10%). Eliminating nutrient limitations appears to reduce the ability of biofilter populations to produce excess amounts of EPS. Communities relying on biofilm formation to persist in drinking water biofilters may wash out under substrate-limited conditions, allowing for a different community of organisms to establish on filter

media. These results suggest that the nutrient-enhancement strategy may extend underdrain life by decreasing the formation of EPS, which could be responsible for underdrain cap clogging, as experienced by Arlington and other utilities (Zhu, 2010).

Phosphorus supplementation also enhanced biofilter water treatment performance. The increased media biological activity observed with nutrient-enhancement corresponded with improved water treatment performance .The nutrient-enhanced biofilter provided higher removals of DOC and MIB relative to the biofilter control. Biofilter nutrient-enhancement improved DOC removal by over 75%, relative to the biofilter control (Table 6-5). This result confirms previous work suggesting the nutrient limitation may inhibit optimal microbial substrate utilization and organics removal (Nishijima et al., 1997; Sang et al., 2003). MIB degradation follows secondary substrate metabolic pathways (Rittman, 1995); therefore, it is possible that a portion of the additional DOC removed served as primary substrate for the populations responsible for MIB transformation. Improved biofilter Mn removal was also observed under nutrient-enhanced conditions. Although soluble Mn oxidation and removal may occur through physical/chemical processes in a biological filter (Kohl et al., 2006; Sahabi et al., 2009), it may also be oxidized by autotrophic bacteria (Diem and Stumm, 1984; Nealson, 1992; Sahabi et al., 2009). Phosphoric acid suppresses pH; therefore, its application should not contribute to the oxidation of Mn. However, complete Mn removal was observed in the nutrient enhanced biofilter under both high- and moderate-load conditions. Interestingly, clone library analyses on nutrient-enhanced biofilter media indicated a relatively high prevalence of populations belonging to the genus *Burkholderia* relative to the biofilter control (> 25% to ~1%) (Figure 8-19). The genus

Burkholderia contains some species previously identified as Mn-oxidizers (Kohl et al., 2006). These results suggest that nutrient limitation select against biofilter communities responsible for contaminant removal and improved water treatment.

An additional nutrient-enhancement evaluation sought to examine the effects of NH_4-N supplementation under N-limited conditions. Ethanol and phosphoric acid were dosed to a biofilter at concentrations that created an artificial ammonia limitation (C:N:P equaled 100:3:2). This condition was maintained for 7 weeks and resulted in decreased hydraulic performance as measured by gradual increase in 18-hr terminal headloss during the pilot filter runs (Table 6-2, Figure 6-2). The average terminal headloss for this biofilter was 9.9 feet (60% greater than that of the control), with many runs ending before 18-hours due to a headloss trigger. Ammonium chloride was then dosed to eliminate the NH_4-N limitation. Consequently, average terminal headloss in the enhanced biofilter column decreased to 6 feet (56% improvement), indicating that ammonia limitations may be as deleterious to hydraulic performance as phosphorus limitations during biofilter operation (Figure 6-2). These results suggest that both N and P limitations may result in a loss in hydraulic performance in drinking water biofilters.

The results of the nutrient-enhancement study leads to the important conclusion that appropriate nutrient supplementation produces more biological activity but at the same time improves filter performance because the bacterial production of EPS is reduced with the removal of the nutrient limitation stress.

Oxidant-Enhancement Studies

The objective of this task was to evaluate peroxide supplementation for augmenting the oxidative action and response of the biofiltration process. Many microorganisms express a class of enzymes, known as peroxidase family

oxidoreductases, when exposed to hydrogen peroxide. The resulting reaction neutralizes the hydrogen peroxide, oxidizing organic (or inorganic) substrates. Peroxidases, a specific group of enzymes within the peroxidase family, release free radicals when reducing hydrogen peroxide (Schumb et al., 1955). These free radicals may then oxidize recalcitrant organic carbon, further reducing the concentration of DOC and trace organics (Klibanov et al., 1980; Huang et al., 2004).In addition, inactive microorganisms and/or their extracellular materials may also be oxidized by the hydrogen peroxide and free radicals (Neyens, 2003), thereby improving biofilter hydraulics. Preliminary testing of this strategy was conducted by dosing 1 mg/L of hydrogen peroxide to a pilot filter for two weeks. The test filter demonstrated 15% removal of filter feed DOC (50% greater than that observed with the control and full-scale filters), and complete removal of Mn and MIB. The oxidant-enhancement strategy also decreased terminal headloss to 2.2 feet, or 66% less than the control biofilter (Figure 7-1).

ATP measurements were performed on the oxidant-enhanced biofilter media to characterize biological activity relative to the control. The data showed that hydrogen peroxide supplementation did not decrease ATP concentrations in the biofilter media relative to the biofilter control (Figure 8-18) and these data suggest biofilter populations are resistant to the levels of hydrogen peroxide dosed in this study. However, the peroxide concentration was sufficient to drive significant improvements in filter hydraulic performance. These improvements are possibly due to microbially-mediated peroxide oxidation process that destabilized EPS and other organic biofilter foulants sufficiently to be removed through mineralization or through filter backwash.

Microbial Tracking

The objective of this task was to correlate the microbial ecology and activity in the biofilters to hydraulic and treatment performance. Media samples were collected from the pilot and full-scale biofilters after each process change and analyzed for microbial activity, community structure, and morphology. The microbial tracking task included the following analyses: SEM, HPC, biofilm formation potential, EPS quantification, T-RFLP, and clone libraries.

Scanning electron microscopy

SEM provides a qualitative means to track general changes in biofilm appearance and cellular morphology. Biofilter media samples were sent to the USEPA ORD for SEM characterization. SEM analyses showed considerable differences in biofilm morphology among biofilters that employed different operational strategies. In general, biofilms developed under nutrient limited conditions appeared to have a higher prevalence of a biofilm matrix relative to biofilter with nutrient- enhancement (Figures 8-2, 8-7). The observed presence of the biofilm matrix significantly increased in samples collected from biofilter operated with substrate supplementation. In addition, the prevalence of stalked bacteria and their corresponding stalk lengths on biofilter media SEMs appeared to coincide with influent nutrient-limiting conditions. The bacterial stalk is a morphological adaptation that allows these organisms to increase their surface area-to-volume ratio, improving nutrient diffusion through the cell wall, thus allowing the organism to be more competitive under nutrient-limited conditions (Madigan et al., 2009). Conversely, SEMs of the nutrient-enhanced biofilter media showed a decrease in biofilm matrix, while exhibiting a higher degree of cellular morphological diversity and larger cells.

Biofilter media HPC

Select biofilter media samples were surveyed for HPC. Overall, these results suggest that phosphorus is responsible for increasing the number of viable cells in the filter (Figure 8-11). These data suggest that nutrient supplementation may increase the prevalence of viable heterotrophic bacteria on biofilter media. The HPC data support the observed improvements in biofilter activity and DOC removal during the nutrient enhancement study.

Biofilm formation potential

The biofilter media biofilm formation potential was lower for the nutrient-enhanced biofilter relative to the control for the one sample tested (Figure 8-12). All substrate-enhanced biofilters showed higher biofilm formation potentials relative to the control conversely, the substrate- and nutrient-enhance biofilter media showed similar biofilm formation potential to the control biofilter (Figure 8-13). These results suggest that nutrient limitations may drive biofilm formation potential in a biofilter with or without substrate supplementation. The biofilm formation potential results generally corresponded to relative filter hydraulic performance between the control, nutrient-enhanced, substrate-enhanced, and substrate- and nutrient-enhanced biofilters

EPS quantification

Measured biofilter media EPS was lowest for the nutrient enhanced biofilter, relative to the control biofilter (Figure 8-13). Substrate- enhancement appeared to increase free and bound EPS concentrations, while substrate- and nutrient-enhancement had little effect. The decreased presence of EPS in the nutrient-enhanced and substrate-and nutrient-enhanced biofilter samples corresponded with decreased headloss relative to the control and substrate-enhanced biofilters, respectively. The

results suggest that supplementation of substrates increased the normalized (to HPC) production and quantity of biofilter EPS in nutrient limited conditions. However, both EPS production potential and the EPS concentration were reduced to biofilter control levels when the nutrient requirements were satisfied (Figures 8-13, 8-15).

Adenosine triphosphate measurements

ATP is a high-energy molecule considered the energy currency of life. Higher concentrations of ATP are associated with increased metabolic activity. The ATP analyses provided a real-time assessment of biofilter activity that correlated directly with DOC removal. Nutrient-limited biofilters consistently showed lower concentrations of ATP in media samples than those that those operated under the same carbon load conditions and a bioavailable C:N:P balance of 100:10:1. When nutrients were dosed to previously nutrient-limited biofilters, biofilter ATP concentrations increased, as did DOC removal. Thus, nutrient-enhanced biofilters simultaneously demonstrated (1) increased ATP concentrations, (2) improved effluent water quality, and (3) lower headloss trends. Interestingly, biofilters operated with 1 mg/L (as C) ethanol supplementation exhibited minor improvements to ATP concentrations relative to the improvements observed during phosphorus supplementation tests.

16S rRNA and clone libraries

The most significant observation in the clone libraries for these samples was the drastic reduction in the *Bradyrhizobium* population when the stoichiometric C:N:P ratio was met on the enhanced biofilter. *Bradyrhizobium* constituted only 1.5% of the clones in the stoichiometric C:N:P sample, but constituted 15% under the baseline control conditions. *Bradyrhizobium* has been shown to increase EPS production under N-

limitation and related rhizobia have been shown to increase EPS production under P-limitation.

Full-scale Process Integration Assessment and Economic Evaluation

During pilot testing, the substrate enhancement, nutrient enhancement, and oxidant enhancement strategies all demonstrated some water treatment benefits. The oxidant and nutrient enhancement strategies also provided significant hydraulic benefits, making them particularly promising for full-scale implementation. The oxidant-enhancement strategy was evaluated only briefly, and additional testing is required to better understand process mechanisms and characterize long-term performance. A full-scale process integration assessment was performed for the nutrient enhancement strategy, as it was better characterized and validated than the oxidant-enhancement strategy, and it was shown to be operationally and economically effective when used without other supplements. Process integration includes the installation of a chemical feed system capable of dosing orthophosphate and/or ammonia to the top of the biofilter feed channel, Chemical feed system sizing is a function of biofilter production capacity and the degree of nutrient limitation.

At the JKWTP, the filter feed has a typical orthophosphate limitation of 20 µg/L as P. Less than 5 gallons per day of 85% phosphoric acid would mitigate this limitation, which means that a standard 250-gallon tote would be capable of providing in excess of 50 days of storage for this facility. The estimated installed capital cost for a nutrient dosing system is $25,000, and the associated chemical cost estimate is approximately $0.44 per MG treated. This chemical cost may be offset by increased biofilter production efficiency. Indeed, factoring in consumable nutrient costs (and contingency), Arlington Water Utilities could save over $17,100/yr in operating costs if nutrient supplementation

increased biofilter runs by 15% at both the JKWTP and PBSWTP. Implementing nutrient-enhancement strategies may provide additional cost savings through extending the life of media and biofilter underdrains/caps. All savings realized with the hydraulic improvements of biofilter nutrient-enhancement compliment the improved water treatment performance: enhanced DOC, Mn, and MIB removal.

Utilities implementing biofilter nutrient-enhancement should perform regular assessment of biofilter fed nutrient and BOM levels. The costs associated with implementation will be dependant on the presence, concentration, and requirement for both PO_4-P and NH_4-N supplementation. Nutrient limitations (and corresponding requirements) may likely vary during source water seasonal changes.

Summary

Currently, biofiltration is largely operated as a passive process in the water treatment industry. Particle/turbidity removal and headloss drive the design and operation of conventional filtration as well as biofiltration. Thus, biofilter design parameters are typically limited to media configuration, backwash strategy, and loading rate. The biological removal of dissolved organic and inorganic contaminants is an anticipated benefit of biofiltration. However, common design and operational practice does not seek to enhance the biological activity responsible for those mechanisms. Indeed, in an effort to improve filter productivity and minimize headloss, many utilities employ chlorinated backwashes and other biomass control strategies.

The purpose of this research was to identify strategies to enhance the biological activity in a biofilter without compromising productivity or particulate removal performance. The ultimate goal of this work is to shift an industry-accepted paradigm so that the design and operation of biofilters are driven not only by filtration but also by

biological treatment objectives. Strategies comprised dosing low levels of common drinking water treatment chemicals at a feed point just upstream of a biofiltration process. Of the strategies tested, nutrient enhancement and oxidant-enhancement showed the most promise for drinking water biofilter applications. Substrate-enhancement proved to be ineffective at providing either improved water treatment performance or reliable biofilter hydraulic operation. The oxidant-enhancement strategy significantly improved filter hydraulic performance without compromising biological activity. However, the chemical cost for the dosage tested (1 mg/L) is impractical for full-scale implementation ($12/MG). Therefore, additional optimization and validation are necessary to define operational parameters for full-scale implementation.

The nutrient enhancement strategy is elegant in its simplicity: operate a given biofiltration process so that an approximate bioavailable C:N:P molar ratio of 100:10:1 is maintained. Associated water quality benefits may include improved biological treatment of organic carbon, Mn, and MIB. Associated hydraulic benefits may include lower terminal filter headloss and decreased media and underdrain clogging. Therefore, nutrient enhancement strategies may be applicable to any utility with existing or planned biofiltration facilities.

The primary goal of this work was to move the practice of biofiltration from a passive process designed and operated around conventional filtration objectives to an intentionally operated biological system, i.e. “engineered biofiltration.” Engineered biofiltration targets multiple water quality objectives while maintaining hydraulic performance.

CHAPTER 11
FUTURE WORK

This research identified unique and promising biofilter enhancement strategies that may provide both hydraulic and water treatment performance improvements to surface water utilities upon implementation. However, additional work further evaluating both full-scale performance and enhanced-biofilter microbial community functionality is warranted. Currently, the City of Arlington is developing a work plan to perform full-scale nutrient-enhancement on select filters. The next phase of pilot research will be performed under Water Research Foundation Tailored Collaboration No. 4346. This research will validate the Engineered Biofiltration approach and include additional operational refinement of the hydrogen peroxide enhancement and nutrient enhancement strategies. Source water and seasonal variation, long-term steady state performance, and distribution system impacts will also be evaluated in this study. Specific research objectives should include further evaluation of the following: (1) refine operational parameter at other facilities, (2) elucidate mechanisms and mitigation strategies for underdrain clogging, (3) identify biofilter microbial communities and understand their role in EPS production and/or contaminant cycling, and (4) characterize how enhanced biofiltration affects distribution system health and water quality.

This research evaluated enhanced biofiltration strategies at one facility with a relatively stable feed water quality. Therefore, further characterization would be beneficial to examine strategy effectiveness at facilities treating different source waters. The impacts of feed water temperature, nutrient loads, bioavailable organic carbon in different water matrices may have profound effects on enhanced biofilter performance.

In addition, the effects of pretreatment process selection and optimization on the enhanced biofilter strategies should be studied (e.g., ozonation, coagulation-sedimentation, softening, etc.). The implementation of enhanced biofiltration in a pretreatment mode (i.e., direct filtration of raw water) also merits evaluation. Direct biofiltration would take advantage of the relatively higher concentrations of nutrients available in the raw water, reducing or eliminated the need for supplementation. In addition, the DOC removal and other water quality enhancements achieved through direct biofiltration may decrease operating costs for downstream processes, e.g., coagulant and polymer.

Further study is necessary to characterize the mechanisms for underdrain clogging and evaluate the ability of the enhanced biofilter strategies to prevent and/or mitigate clogged underdrains. This evaluation will require long-term biofilter operation (greater than 12 months) to adequately characterize and verify the performance of the underdrain caps under optimized steady state conditions. This task would evaluate (1) underdrain cap performance after steady state operation with biofilter enhancement strategies, and (2) clogged underdrain cap mitigation with biofilter enhancement strategies. These evaluations would include autopsies to reveal the extent and mode of underdrain cap clogging. Autopsy evaluations would include surface analysis and biological assessment techniques such as identification and abundance of microbial, bulk characterization and quantification of biofilm EPS, and in-situ characterization and imaging of biofilms. In addition, the underdrain caps would be analyzed for potential organic and inorganic accumulation by methods such as Fourier transform infrared

(FTIR) spectroscopy and SEM imagery with energy dispersive spectroscopy (EDS). Permeability tests would also be conducted on all clogged underdrain caps.

Additional biofilter microbial activity characterization is necessary to define the functionality of the present microbial communities and identify their roles in EPS production and contaminant cycling. This research constructed clone libraries targeting the bacterial 16S rRNA gene for only a few pilot biofilters. It is likely that biofilters at different utilities have different microbial community structures. In addition, change in community structure over time (e.g., seasonal variation) within each system is anticipated. The microbial community structure depends on source water quality, filter characteristics (e.g., filter media) and operational characteristics (e.g., backwash frequency and intensity, temperature). Therefore, it is unknown if the populations identified during this research are commonly present in other biofilters used for drinking water treatment. Thus, is it important to perform microbial community characterization on the biofilters for facilities interested in enhancement strategy implementation. Subsequent identification of protein coding genes through pyrosequencing and combined with reverse transcription quantitative PCR (RT-qPCR) assays combined with reverse transcriptase (RT-PCR) would then allow researchers to collected data on metabolism, function, and removal mechanisms for organisms of interest (Raskin, 2010). Additional microscopy studies may also present additional information on biofilter media microbial communities and biofilm characteristics. TEM would be used on future samples to accurately quantify biofilm thickness – a potential measure to determine relative microbial stress between biofilter operating conditions. Fluorescence in situ hybridization analyses would be used to support genetic analyses to obtain information

on possible niche differentiation of the present microbial populations (Amann and Fuchs, 2008).

Further microbial community analyses could lead researchers towards the identification of enzymes responsible for organic and inorganic contaminant cycling in biofilters. Once identified, and understood, methods to further enhance specific enzyme production and activation may be developed. The effectiveness of drinking water biofiltration on trace organic removal, e.g. tastes and odors, may be greatly enhanced if microorganisms could be stimulated to increase the production of specific enzymes, such as peroxidase. Further evaluation of biofilter hydrogen peroxide enhancement may elucidate the potential for this approach.

The effects of enhanced biofiltration on distributions systems warrant additional investigation. Studies using annular reactors and pipe loops would be beneficial in characterizing improvement or degradation of effluent stability with respect regrowth, disinfectant decay, corrosion and disinfectant byproduct formation (for both regulated and non-regulated (DBPs). Furthermore, microbiological methods could be implemented to investigate the effect of disinfection on biofilter effluent microbial community structure. Effluent samples from enhanced and control biofilters could be surveyed for microbial species that are resistant to disinfection. This information will be collected and compared to the existing body of knowledge to evaluate how the disinfected biofilter effluent may affect the distribution system.

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BIOGRAPHICAL SKETCH

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