Determining the Viability and Effectiveness of a Roughing Biofilter for use in Drinking Water Treatment Plants

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Dedication

I would like to dedicate this thesis to my parents, Bruce and Jenny Shoemaker, my wife, Andrea Shoemaker, and my siblings, Danielle and Megan Shoemaker. Without their love and unwavering support, I would not be where I am today.
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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ............................................................................................................................. ii

LIST OF FIGURES ........................................................................................................................................ vi

LIST OF TABLES ........................................................................................................................................ viii

1.0 Introduction ........................................................................................................................................ 1

  1.1 Introduction to Biologically Active Filtration ................................................................. 2
  1.2 Objective and Direction of Research ..................................................................................... 3

2.0 Literature Review ............................................................................................................................. 7

  2.1 The Origins of Filtration ............................................................................................................. 7
  2.2 Biofiltration through the Years ................................................................................................. 8
    2.2.1 Slow Sand Filtration ............................................................................................................. 8
    2.2.2 Rapid biofiltration .............................................................................................................. 10
    2.2.3 Riverbank Filtration .......................................................................................................... 12
    2.2.4 Trickling Filters ................................................................................................................... 14
  2.3 Disinfection Byproducts and Biofiltration ....................................................................... 16
    2.3.1 Regulated Disinfection Byproducts ............................................................................. 17
    2.3.2 Surrogate measurement for DBP precursors .......................................................... 18
  2.4 Microbial Activities in Biofiltration ..................................................................................... 20
    2.4.1 Fundamentals of Biodegradation of Waterborne Contaminants ..................... 20
    2.4.2 Biofilm Development ........................................................................................................ 22
  2.5 Current Biofilter Research ...................................................................................................... 25
    2.5.1 Influence of Media ............................................................................................................. 25
5.1 Introduction ................................................................................................................................. 55

5.2 Results ............................................................................................................................................ 56

5.2.1 Acclimation Period............................................................................................................. 56

5.2.2 Flow Reduction ................................................................................................................... 58

5.2.3 Analysis of Possible Biological Inhibiting Conditions ........................................... 60

5.2.4 Biofilm Development in Pilot-Scale Experiment .................................................... 62

5.2.5 Impacts of Roughing Biofiltration on Water Quality Parameters ................... 63

5.3 Discussion ..................................................................................................................................... 68

6.0 Summary and Suggested Future Research ........................................................................... 70

6.1 Summary ....................................................................................................................................... 70

6.2 Suggested Future Research .................................................................................................... 72

References .................................................................................................................................................... 74
LIST OF FIGURES

Figure 1-1: Conventional drinking water filter layout (USEPA 1990). ........................................ 2

Figure 1-2: Comparison of roughing (A.) and polishing filter (B.) locations and water
characteristics. ................................................................................................................................. 5

Figure 2-1: 1927 Slow Sand Filter used in the United Kingdom ................................................. 8

Figure 2-2: Typical Slow Sand Filter Layout ............................................................................. 9

Figure 2-3: Qualitative depiction of the fate of contaminants as they infiltrate through a
riverbank ........................................................................................................................................ 13

Figure 2-4: Typical trickling filter configuration ....................................................................... 15

Figure 2-5: Representation of biofilm composition and interaction with flowing water ...... 23

Figure 3-1: A.) Fluvial Biomax filter media; B.) AqWise Biomass Carrier filter media. ...... 34

Figure 3-2: Typical 12-well plate setup .................................................................................... 35

Figure 3-3: Schematic of pilot-scale roughing biofilter setup .................................................. 39

Figure 3-4: Four-tap copper manifold utilized for biofilter influent feed ............................... 39

Figure 3-5: Pilot-scale roughing biofilters installed at Marceline WTP, with media depth
indicated in red ............................................................................................................................. 40

Figure 4-1: Preliminary CV testing results between Biomax and ABC5 incubated in
Maysville Raw water ....................................................................................................................... 46
Figure 4-2: Stained Biomax media resistant to full CV solubilization. ........................................48

Figure 4-3: Plot of the biofilm development curves for the four raw water sources used to inoculate ABC5 media. Lake water sources are shown as dashed lines. .........................49

Figure 5-1: Overall influent (Raw) and effluent DOC concentrations in the filter columns during initial 35-day period. .........................................................................................................................57

Figure 5-2: DOC concentrations over Impact of flow rate on DOC concentration in filter effluents. ........................................................................................................................................59

Figure 5-3: Graph quantifying the establishment of biofilm in the ABC5 roughing biofilter over the duration of its operation. ..................................................................................................................63

Figure 5-4: Overall influent (Raw) and effluent DOC concentrations observed in the pilot study. ........................................................................................................................................64

Figure 5-5: DOC reductions attributable to the ABC5 roughing biofilter. .........................65

Figure 5-6: DOC reductions attributable to the Biomax roughing biofilter. .........................66

Figure 5-7: Overall influent (Raw) and effluent $\text{UV}_{254}$ concentrations observed in the pilot study. ........................................................................................................................................67

Figure 5-8: Overall influent (Raw) and effluent $\text{NH}_4^+$-N concentrations in the filter columns. ........................................................................................................................................68
LIST OF TABLES

Table 3-1 – Hach nutrient testing information ................................................................. 32

Table 4-1: Absorbance at 600 nm and statistical data from Maysville biofilm development experiment ......................................................................................................................... 46

Table 4-2: Results of control plates for the four water sources used. ................................. 50

Table 4-3: Raw water characteristics and peak biofilm development measured as absorbance using the CV assay. ........................................................................................................ 51

Table 5-1: EBCT and hydraulic loading rate at each flow rate interval ............................... 58

Table 5-2: Raw NH$_4^+$-N and PO$_4^{3-}$-P concentrations for each sampling interval and overall average concentrations for experiment duration. ................................................................. 61

Table 5-3: Influent DO concentrations at two sample collections. ..................................... 62
1.0 Introduction

Filters come in various designs and operate under various conditions, but their objective is similar: to separate nonsettleable solids from water by passing it through a porous medium (Viessman et al. 2009). Water either passes through the filters by gravity (gravity filters) or through applied pressure (pressure filters.) The “porous medium” can either consist of loose media or a membrane, usually consisting of hollow fibers packed in a pressure vessel.

The majority of conventional filters in the United States are gravity granular-media filters, operated as physical treatment units where contaminant removal is achieved through interception, straining, flocculation, and sedimentation. A typical filter setup is shown in Figure 1-1. The most common filters consist of a coarse anthracite coal underlain by finer sand (Viessman et al. 2009). Modern conventional treatment processes disinfect influent filter waters, which inhibits microbial growth within filters. These conventional filters rely solely on physical processes to strain out larger organic matter and their removal rate is approximately 30 percent (Simpson 2008). Filters are operated until the first of two conditions are met: 1.) solids begin to breakthrough the media and are carried into the effluent, increasing effluent turbidity to a predetermined level or 2.) the building up of solids in media pores causes the pressure required to pass water through filter media to become unmanageable and the resulting increased pore velocity decreases removal efficiency. When either of these conditions are met, clean water (possibly mixed with air) is forced through the filters in reverse to remove the particulate matter from the media in a process called
“backwashing.” This water is then disposed of or sent back through the treatment process.

Figure 1-1: Conventional drinking water filter layout (USEPA 1990).

1.1 Introduction to Biologically Active Filtration

Filters whose influent filter waters are not disinfected are considered biologically active. This biological activity can improve treatment performance and can remove contaminants not normally removed during conventional filtration, such as disinfection by-product precursors (Evans 2010). When microbial growth is allowed in filters, a biological mass or “biofilm” can begin to grow on filter media. The biofilm is capable of removing a portion of waterborne nutrients, dissolved organic matter, minerals, and microorganisms (Simpson 2008).

Although a major advantage of biological filtration is the virtually immediate reduction in unwanted waterborne contaminants, an added benefit is the positive impact on
other treatment processes and the improved stability of water. For example, the reduction of organic matter and microorganisms helps reduce overall chlorine demand – the quantity of chlorine necessary to sustain a residual chlorine level - as the chlorine has less contaminants to react with. Also, as nutrients essential for bacterial growth are reduced and microorganisms are removed, the risk of bacterial regrowth in the distribution system is also reduced (LeChevallier 1998).

Biofilms are composed of microbial cells embedded in an extracellular organic polymer matrix. As suspended microbial cells fix themselves to a surface, they begin to extend vertically into the bulk solution by enclosing themselves in an adhesive matrix of extracellular polymeric substances (EPS) secreted by the cells. Surfaces are important to microbial habitats because nutrients can adsorb to them, creating a microenvironment with much higher nutrient levels than in the bulk solution. Thus, microbial numbers and activities are much greater on media surfaces than in water (Madigan and Martinko 2006). The composition of biofilm can be evaluated by physical measurements (i.e., biofilm thickness, total dry weight) or through observing physiochemical parameters (i.e., total organic carbon, nutrient content, etc.). One of the major concerns in the operation of biologically active filtration is the clogging of filter pores due to the overproduction of EPS from bacterial stress induced by an insufficient balance of carbon, nitrogen, and phosphorus (Brown 2011).

1.2 Objective and Direction of Research

Biological treatment has conventionally been utilized for the removal of contaminants from wastewater, but recent research has suggested it may also be advantageous in the drinking water treatment process. More specifically, biologically active
filters (or “biofilters”) have been shown to be capable of oxidizing metals, removing nutrients, and the degradation of organic materials responsible for the formation of disinfection byproducts (Huck 2000). Primarily, research has focused on the conversion of polishing filters to biofilters as the last process unit in the treatment train. However, common pretreatment processes such as coagulation and sedimentation can lead to nutrient limitation in biofilter influent waters. This can cause an overproduction of EPS within the filters, resulting in headloss, underdrain clogging, and shortened filter run times (Brown 2011).

A “roughing biofilter” ahead of the entire process has been proposed as an alternative to the polishing biofilter as a way to alleviate nutrient limitations experienced later in the treatment process. Figure 1-2 shows a comparison of the conventional water treatment process with the proposed roughing biofilter process. In the figure, blue lines indicate water flow additional details are marked using red leader lines. An advantage of the roughing biofilter configuration (Figure 1-2b) over the conventional treatment process (Figure 1-2a) is that raw water with higher biodegradable carbon and nutrient concentrations would be fed to the roughing filter, avoiding the clogging associated with nutrient limitation experienced in polishing biofilter full-scale studies. The roughing biofilters would consist of more coarse media with higher porosity than those in polishing filters to avoid clogging that may be associated with sediment laden raw water. An added benefit is that the roughing biofilter configuration would not affect chlorine addition location as chlorination can still occur early in the treatment process contrary to a biologically active polishing filter whose influent waters should be void of chlorine. A major trade-off in this case is the large specific surface area associated with anthracite or granular activated carbon in polishing filters.
However, if significant reductions in biodegradable contaminants are observed, the roughing biofilter would provide a passive treatment step with minimal maintenance required. Overall, it is hoped that roughing biofilters will provide a biological pretreatment step capable of oxidizing natural organic matter (NOM) responsible for the formation of disinfection byproducts (DBPs), thereby reducing chlorine demand while increasing the biostability of the water throughout the treatment process.

Figure 1-2: Comparison of roughing (A.) and polishing filter (B.) locations and water characteristics.

Before implementation, research must be performed to evaluate the viability and effectiveness of a roughing biofilter for drinking water treatment. To begin research on the novel idea of a roughing biofilter, lab-scale experiments were carried out to observe biofilm establishment on coarse filter media suitable for use in the roughing biofilter. After adapting a suitable methodology for biofilm measurement, establishment of biofilm on filter media surfaces over time were observed. Various waters were used in order to compare biofilm development capabilities of surface and groundwater sources. An effort was also made to correlate the quickness of development and overall robustness of biofilm with measurable water characteristics.
Another research effort was to carry out a pilot-scale roughing biofilter experiment at a cooperating water treatment plant. In this way, observations were made in a setting where raw water representative of a typical surface water treatment plant could be fed through the filters while carefully observing their effect on water quality characteristics. A baseline characterization period of approximately one month was carried out where the filters were able to operate while they were biologically acclimating. After this period, adjustments were made to evaluate the optimal operation conditions for the filters. The overall objective of this research is to vet roughing biofilters as a possible treatment process, capable of degrading a portion of waterborne contaminants that might not otherwise be removed in a conventional drinking water treatment process.
2.0 Literature Review

2.1 The Origins of Filtration

Although adequate water sources have always been paramount to society, ancient civilization was much more concerned with the quantity of water over the quality. Early historical documents indicate methods such as charcoal filtration, sunlight exposure, and even chemical alum used by the Egyptians as early as 1500 B.C (USEPA 2000). Although efforts such as these were taken to improve aesthetic qualities, such as taste, odor, and smell, it was not until 1855 when Dr. John Snow linked cholera outbreaks in London to contaminated well water that serious focus on waterborne disease began to rapidly evolve modern drinking water treatment (Markel 2013).

It was in 1829 that the Chelsea Water Company became the first to provide filtered water supply to London. They were spurred in large part by a public outcry over the issue of industrial and sewage pollution of drinking water sources and increasing concern over the novel idea of waterborne diseases. Several London-based treatment plants soon followed suit, and by 1888 there were seven companies employing filtration processes. The early filtration processes, however, were only uniform in general operation: pumped river water was made to pass vertically through various media and collected by drains. Without standards, media type, media depth, and flow rates varied greatly (Hardy 1984). By 1900, the European practice of slow sand filtration had begun to emerge in the United States.
2.2 Biofiltration through the Years

2.2.1 Slow Sand Filtration

Filtration processes can be distinguished as being either slow or rapid, the former being the predominant early filtration process that gave way to the latter in preference. Slow sand filtration (Figure 2-1) was the earliest and most prominent form of filtration. It works through a combination of “cake filtration,” a form of straining, and biological consumption of organic matter that takes place in a robust layer of biological matter (called “schmutzdecke”) formed at the surface of the filter (Lahlou 2000). Straining occurs when particles are larger than the pores of the media or membrane through which they travel. The biological mechanisms within the schmutzdecke are possible due to the absence of pre-disinfectant. This absence combined with the limited need of pre-filtration treatment process units combine for cost savings.

Figure 2-1: 1927 Slow Sand Filter used in the United Kingdom (PortsmouthWater)
Figure 2-2 shows the typical arrangement of a slow sand filter. Influent water is held in a 1 – 1.5 m water reservoir above the sand bed, whose primary function is to provide the pressure that carries water through the filter. As it moves downward, water enters the intensely active schmutzdecke, where various microorganisms entrap, digest, and break down organic matter contained within. Bacteria contained in the raw water are also consumed here and nitrogen is oxidized. Other advantages of passage through the schmutzdecke include color removal and mechanical removal of suspended particles. The remainder of filtration is carried out through adsorption as water slowly percolates through the pores and open spaces in the fine sand bed (Huisman and Wood 1974).

![Figure 2-2: Typical Slow Sand Filter Layout](image)

However, due to the reliance on the straining mechanism, loading rates in slow sand filtration (from 0.015 – 0.15 gallons per minute/ft²) must be significantly lower than rapid filtration (Lahlou 2000). This contributes to a larger required filter surface area and, therefore, higher infrastructure investment costs. For example, a WTP running at a relatively slow 500 gallons per minute (gpm) would require a slow sand filter of approximately 3500 ft². For comparison, a modern rapid-rate filter, operating at a typical 2 gpm/ft² would require
a modest 250 ft\(^2\) at an equivalent flow rate. Slow sand filters are also prone to clogging when treating high turbidity water and require a fairly expensive and time consuming cleaning process (Logsdon et al. 2006). Generally during the cleaning process, slow sand filters are drained, their schmutzdecke layer dried and removed along with attached sand. This can be achieved by shovel or specialized scraper (Huisman and Wood 1974).

In certain rural and specialized settings where unskilled labor and land is plentiful, slow sand filtration is still a viable and effective form of filtration. However, increasing water demands and rising property costs have forced most water treatment plants to abandon the slow sand filtration in exchange for the more recognizable rapid filtration utilized today.

### 2.2.2 Rapid biofiltration

Increasing water demand in modern water treatment lead to the emergence of rapid-rate filtration at the beginning of the twentieth century, coinciding with the widespread use of chlorine as a primary disinfectant in the United States. Chlorine was adopted primarily for the control of cholera and typhoid fever, but it also led to the suppression of microbial activity within the filters. In this way, the biological filtration that had revolutionized water treatment in the 1800s had mostly been phased out less than a century later (Brown 2011).

Rapid-rate filtration offered several advantages over slow sand filtration. Water can be filtered at a rate of approximately 2 - 3 gpm/ft\(^2\), a significant (more than 10 times) increase over the previously used slow sand filters capable of less than 0.15 gpm/ft\(^2\). Rapid filtration also performed far better in the presence of high turbidity influent water (USEPA 1990). However, unlike slow sand filtration, rapid rate filtration generally requires pretreatment. It is much more effective when used in conjunction with coagulation and
flocculation, as these processes remove a large portion of dissolved and suspended solids and thus lengthen filter run times significantly (Logsdon et al. 2006).

The rise in popularity of ozone as a preoxidant in Europe in the late 1970s led to the emergence of biological treatment in a rapid filtration setting. In general, ozonation occurred prior to sand filtration followed by filtration through granular activated carbon (GAC) bed. This configuration fostered a symbiotic relationship, where ozone increased the biodegradable fraction of organic matter and increased dissolved oxygen (DO) levels to create an environment more amenable to biological activity in the filter beds (Rice and Overbeck 1998). In the absence of a residual disinfectant (a practice more common in United States water systems using primarily chlorine), biodegradable organic molecules encountered microorganisms within the filters. A 1982 EPA-sponsored survey of European and Canadian water utilities confirmed that water treatment utilizing properly designed and operated combined ozonation and GAC biofiltration resulted in enhanced organic chemicals removal and reduced the frequency of regeneration of activated carbon media (Rice et al. 1982). Ozonation and its impact on biofiltration will be discussed more in-depth in Section 2.5.3.

Although both have unique limitations, slow sand filtration and rapid biofiltration can be viable forms of biofiltration when used properly and under the right circumstances. However, both require significant maintenance and observation. Conversely, processes such as riverbank filtration provide a more passive biofiltration step that requires little maintenance after initial construction and implementation.
2.2.3 Riverbank Filtration

A common form of drinking water biofiltration known as riverbank filtration requires passing water to be purified through the banks (soils along the river reach) of a river using extraction wells some distance away from a river. As river water infiltrates the alluvial deposits contained within riverbanks, it undergoes passive exposure to adsorption, reduction, physiochemical filtration, and biodegradation. These alluvial deposits are typically dominated by sand and gravel, but layers of silts and clay are also deposited in floodplains, making them highly heterogeneous. However, the sediments form permeable channels that have an overall large hydraulic conductivity (Rosenshein 1988).

As shown in Figure 2-3, water passes through several regions of aerobic, anoxic, and anaerobic conditions, each having significant impacts on water quality. In aerobic conditions, free oxygen (O$_2$) is readily available as an electron acceptor, whereas anoxic conditions do not contain free oxygen but do have bound oxygen available as nitrite and nitrate (NO$_2^-$ and NO$_3^-$). Anaerobic conditions are void of oxygen in all forms. In the early stages of infiltration where oxygen levels are high, degradation of organic matter is carried out by microbial activity. Consequently, this intense microbial activity consumes more oxygen than is supplied by infiltrating river water, resulting in an anoxic zone. Here, weathering can increase Mg, Ca, and bicarbonate concentrations, while denitrifying and sulfur-reducing bacteria can further decrease the redox potential of the system. Under these extremely reduced conditions, dissolution of manganese oxides occurs (Bourg and Bertin 1993). As water infiltrates to the “mixing zone,” reaeration occurs and manganese can be removed by a series of precipitation reactions (Tufenkji et al. 2002). Because alluvial deposits can vary greatly in composition, characteristics such as those depicted in Figure 2-3
can be used to determine the aerobic, anoxic, and anaerobic zones of a riverbank alluvial system.

Figure 2-3: Qualitative depiction of the fate of contaminants as they infiltrate through a riverbank (Tufenkji et al. 2002).

Riverbank filtration has been shown capable of effectively reducing a variety of microbial contaminants, organic carbon, and DBP precursors before entering the drinking water process (Weiss et al. 2003). However, North American processes typically use shorter retention times of hours, days, and weeks that generally focus on the removal of pathogens and little else before entrance to treatment facilities. Under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), 0.5-log credit can be acquired for wells at least 25 feet from surface-water sources and 1.0-log credit for those over 50 feet away, barring certain minimum criteria are met (Regli 2003). European facilities typically use retention times on the order of months, capable of removing additional biodegradable organic carbon and trace organic pollutants (Tufenkji et al. 2002). In this fashion, bank
filtration is utilized as a major part of European water treatment rather than as limited pretreatment step, as in North America.

### 2.2.4 Trickling Filters

Trickling filtration is a form of biofiltration utilized in the wastewater treatment process. In this fixed-growth biological process, wastewater is spread over the surface of media that support microbial growth, where waterborne contaminants are removed by biological action. Unlike aforementioned forms of biofiltration, trickling filters do not involve physical filtration. This is due to configurations that commonly use filter media with greater than 90% void space (Viessman et al. 2009).

Figure 2-4 presents a schematic diagram of a trickling filter. Typically, a rotating distributor arm applies wastewater uniformly to the media, where it percolates downward. Simultaneously, oxygen required for the metabolic needs of microorganisms within the filter pores is provided by the upward flow of air through the filter bed. Effluent water then enters a final settling tank (clarifier) to remove biological growths that are washed off the filter media. Optionally, trickling filter effluent may be recirculated and mixed with the influent wastewater prior to its application to the trickling filter (Grady et al. 2011).
Trickling filtration and other forms of biofiltration come in many shapes and sizes and are operated under various conditions but their basic treatment mechanisms are similar. Biofilm established on the surface of the biofilter media remove a portion dissolved organic matter, nutrients, microorganisms, and other waterborne contaminants through biological processes. In drinking water treatment, the removal of dissolved organic matter is of special concern, as these can serve as precursors for regulated disinfection byproducts.
2.3 Disinfection Byproducts and Biofiltration

Disinfection is quite likely the single most important step in the water treatment process as it effectively combats the most serious bacterial waterborne diseases, including typhoid fever, dysentery, and cholera. However, it can result in the formation of disinfection by-products (DBPs), which are formed by the reaction of bromide and/or natural organic matter (NOM) with disinfectants (usually chlorine) and have been associated with cancer and other adverse health effects (Viessman et al. 2009). Research has shown that biologically active filters may more effectively remove compounds such as disinfection by-product precursors from water and may provide more cost-effective water quality improvements than traditional filtration (Evans 2010).

A filter is considered biologically active when there is no disinfectant in the filter influent. This lack of disinfection allows for microbial growth within the filter, which leads to a combined physical/biological treatment in a single process unit. In many traditional filtration systems, influent filter waters are chlorinated prior to filtration. This allows chemical reactions responsible for the production of disinfection by-products to take place while disinfection by-product precursors (i.e., bromide or natural organic matter) are in higher concentrations. Logically, removing biodegradable DBP precursors before disinfectant is applied would reduce DBP concentrations and lead to more effective and targeted disinfection of harmful microbial contaminants.
2.3.1 Regulated Disinfection Byproducts

Drinking water is disinfected during the treatment process to rid drinking waters of pathogenic microorganisms. Typically, disinfection consists of the addition of chlorine in the latter stages of the treatment process. However, the application of disinfection to treatment waters can result in the production of aforementioned DBPs. The EPA currently has regulations established for four DBPs including trihalomethanes (THMs), haloacetic acids (HAA5), bromate, and chlorite (USEPA 2012). These DBPs have been shown to be carcinogenic and/or to cause adverse reproductive or developmental effects in toxicological studies (USEPA 2011).

The reaction of natural organic matter (NOM) with chlorine is responsible for the production of THMs and HAA5. Decaying vegetation is a main source of NOM and will form DBPs if not fully removed before disinfectant is applied. Four THMs, chloroform, bromodichloromethane, dibromochloromethane, and bromoform, are regulated as a group. The EPA has established a maximum allowable annual average level of 80 parts per billion for total THMs. The five haloacetic acids currently regulated by the EPA include monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid. The maximum annual average for HAA5 is set at 60 parts per billion (USEPA 2012).

Bromate is produced primarily when ozone is used as a disinfectant and reacts with NOM. Bromate is regulated at a level of 10 parts per billion annually. Chlorite is a byproduct formed primarily when carbon dioxide is used as a disinfectant. It is regulated at 1 part per million monthly (USEPA 2012). Both ozone and carbon dioxide use as a
disinfectant has increased in recent years because they do not readily produce total THMs and HAA5 (Viessman et al. 2009).

2.3.2 Surrogate measurement for DBP precursors

Surrogate measurements are often used to detect DBP precursors in the drinking water industry in place of more expensive or difficult analyses. Although they may not be as precise as more intensive measurements, these surrogate measurements have been shown to be good indicators. Because DBP formation has become a significant concern in the drinking water industry, attention has been focused on using the reduction of total organic carbon (TOC) and dissolved organic carbon (DOC) as indicators of potential DBP formation. This approach has proven to be a useful although somewhat inaccurate predictor of DBP formation potential, especially THMs. Although reactivity of organic compounds with chlorine varies, TOC is widely accepted as a surrogate measure for DBP precursor concentrations and is used for precursor removal compliance in the EPA’s Stage 1 Disinfectant Byproduct Rule (USEPA 2006).

Ultraviolet absorbance at 254 nm (UV\textsubscript{254}) is another strong surrogate of DBP formation potential. This bulk chemical characterization technique generally measures the aromatic and conjugated nature of natural organic carbon, a characteristic that has been shown to participate in the formation of DBP formation reactions (Lavonen et al. 2013). Most likely due to the varied reactivity of organic compounds with chlorine, UV\textsubscript{254} has been shown to be a stronger surrogate for DBP formation potential than DOC. Furthermore, recent research has shown UV\textsubscript{254} to be the strongest indicator of DBP formation potential among a suite of other surrogates (Pifer and Fairey 2014).
TOC, DOC, and UV$_{254}$ are useful measurement parameters in drinking water treatment because they give some indication of the amount of organic materials present in water. These are of particular concern because they serve as DBP precursors. Because one of the potential advantages of biofiltration is the biological removal of waterborne organics, parameters such as TOC, DOC, and UV$_{254}$ should be monitored to observe the reduction of organics due to biofiltration in order to gain some insight into the possible reduction of DBP formation potential. The biological processes responsible for organic reduction in biofilters are detailed in the following section.
2.4 Microbial Activities in Biofiltration

Although the biological treatment of drinking water has been limited until recently, the use of microbial biomass to degrade contaminants, nutrients, and organics has been common in wastewater over the past century. This experience provides invaluable knowledge of microbial processes that can help guide the implementation of biological treatment in drinking water.

2.4.1 Fundamentals of Biodegradation of Waterborne Contaminants

Bacteria gain energy by the transport of electrons from reduced compounds to oxidized compounds. A reduced compound (sometimes referred to as an electron donor) is one that will readily donate electrons, while an oxidized compound (or electron acceptor) is one that will readily accept electrons. After the reduced compound donates electrons, they undergo a series of internal oxidations-reduction reactions while traveling through the membrane embedded electron transport chain. The electrons are ultimately donated to the terminal electron acceptor (TEA). Energy from the electron transport chain is used to pump hydrogen ions across the membrane. The resulting charge separation across the membrane creates an electrochemical gradient referred to as the proton motive force. The proton motive force then provides the driving potential for adenosine triphosphate (ATP) synthesis, as the primary energy shuttle for the cell (Maloney et al. 1974).

The exchange of electrons between donor and acceptor compound often leads to the formation of less harmful, more thermodynamically stable products. This can be illustrated by the redox reactions between acetate and dissolved oxygen (equation 2-1) and nitrogen (equation 2-2) and their associated Gibb’s free-energy values, below (Brown 2007):
\[
CH_3COO^- + 2O_2 \rightarrow 2HCO_3^- + H^+ , \Delta G^o' = -844 \frac{kJ}{mol} \text{acetate}
\]

Equation 2-1

\[
CH_3COO^- + \frac{3}{5} NO_3^- + \frac{13}{5} H^+ \rightarrow 2HCO_3^- + \frac{13}{5} H_2O + \frac{4}{5} N_2 , \Delta G^o' = -792 \frac{kJ}{mol} \text{acetate}
\]

Equation 2-2

In the microbially mediated redox reaction of acetate and oxygen, acetate is converted to relatively innocuous bicarbonate when oxygen serves as the electron acceptor, while nitrate can be converted to the similarly innocuous nitrogen gas when it is used as the electron acceptor. The Gibb’s free-energy value (\(\Delta G^o\)) indicates thermodynamic stability of each reaction. The more negative the Gibb’s free-energy value, the more thermodynamically unstable a reaction is and the greater the energy yield for the bacteria participating in the reaction (Brown 2007). Furthermore, Gibb’s free energy values serve as an indication of microbial preference of electron acceptors (Dolfing and Harrison 1992). In the example reactions above, as in most instances, oxygen is the preferred electron acceptor and results in a greater energy yield (Madigan and Martinko 2006).

Biological treatment is based on the capabilities of bacterial communities to use unwanted waterborne contaminants in redox reactions similar to those described above. The biological processes used by heterotrophic bacteria are the primary focus in biofiltration as these organisms utilize organic carbon as their electron donor, although other beneficial water and wastewater treatment processes can be carried out by other classes of bacteria (i.e. – nitrification by autotrophic bacteria).
2.4.2 Biofilm Development

There are several reasons for bacteria to form biofilms, but one important reason is the availability of nutrients at media surfaces. When in a nutrient-rich medium suspended microbial cells readily attach to surfaces and develop biofilms. These biofilms will continue developing while fresh nutrients are provided. However, when deprived of nutrients, microbial cells have been observed detaching and returning to suspension in search for more nutrient-rich conditions (O’Toole et al. 2000). Other reasons biofilms form include providing a defense against toxic materials and predation, allowing a closer association with other bacterial cells, and the ability to enter a viable but non-culturable state (Davey and O’Toole 2000).

Biofilms form highly complex structures, both physically and microbiologically, that are not fully understood. Initially, a few microbial cells attach themselves to a suitable solid surface. At this point, important cell-to-cell communication occurs that triggers EPS formation critical for biofilm development. Quorum sensing is an example of cell-cell communication used to signal the production and regulation of EPS production, triggering entry into a biofilm state (Grady et al. 2011). Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density. Quorum sensing bacteria release molecules called autoinducers at a constant rate. Thus, larger concentrations of autoinducers accumulate in the environment as the cell populations increases. When a minimum concentration of autoinducers is detected, expression of biofilm-specific genes that initiate polysaccharide (EPS) formation is induced. The ability to communicate with each other and to coordinate gene expression, and therefore behavior, of the community is very important in the formation of biofilms (Miller and Bassler 2001).
After attachment has been initiated and nearby cells have been recruited to microcolonies, further development and maturation occurs. During this time, complex biofilm architecture can form depending on the hydrodynamic characteristics of the system and the microorganisms contained within the biofilm. Interestingly, *Pseudomonas aerugiosa*, a commonly studied biofilm forming bacteria, has been shown to also require cell-to-cell communication to form mature biofilms. Davies et al. showed that a *P. aerugiosa* mutant unable to synthesize the quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone was capable of early cell-surface interactions, but unable to form complex, multicellular structures that had previously rendered it resistant to biocides and antibiotics (Davies et al. 1998). This behavior has been shown to occur in other biofilm forming organisms as well (O'Toole et al. 2000).

Biofilms can be conceptualized as having a base film zone attached directly to the support and a surface film that extends from the base film into the bulk liquid, as seen in
Figure 2-5. Often, biofilms form mushroom- and pillar-like structures that extend into the bulk liquid. These structures are composed of microbial cells embedded in an EPS matrix. The voids formed vertically and horizontally act as water channels through which fresh medium can flow. The transport of substrates and nutrients from the bulk fluid to the biofilm is dominated by advection and turbulent diffusions, while transport between bacteria within the biofilm is achieved by molecular diffusion (Grady et al. 2011).
2.5 Current Biofilter Research

Increasingly stringent finished water quality requirements and the increased use of ozone have led to the increased use of biofiltration in drinking water. A large portion of increased usage can be attributed to the capability of biofilters to effectively reduce biodegradable organic carbon (BDOC) that is responsible for disinfection byproduct formation and distribution system regrowth. Biofiltration also has the potential to remove taste-and-odor compounds, nutrients, microbial pathogens, and many other undesirable water constituents.

2.5.1 Influence of Media

A major design consideration for biofilters is the proper media selection. The major focus is the difference performance of GAC filters and those with a combination of anthracite and sand (A/S). GAC has a very high specific surface area and an adsorptive capacity, while A/S is less adsorptive. While GAC has been shown capable of greater BDOC removal, A/S has been shown capable of comparable steady state removals. One study found BDOC removals of 75% in A/S compared with 86% in GAC filters (LeChevallier et al. 1992). Trends seem to indicate that GAC are the preferable media for biofiltration, but that A/S can perform adequately. This is a positive observation, as GAC filters can be far more expensive, while current A/S non-biologically active filters could be converted to biofilters without the intensive process of filter media replacement.
2.5.2 Biofilm Development in Drinking Water Filters

In biologically active filtration, indigenous bacteria establish biofilms on the surface of filter media. These biofilms are critical to the performance of biofilters, yet minimal research has been done to characterize this biological component in drinking water. Biofilm development in drinking water filters is a complicated process that depends largely on several factors including media type, substrate and nutrient availability, and filtration rate.

GAC filters are often used in drinking water for their ability to remove DOC through adsorption, but have also become the preferred media type for rapid biofiltration (Huck 2000; LeChevallier et al. 1992). The cracked surface and porous structure of GAC media creates a large specific surface area that is very amenable for bacteria colonization and biofilm formation. Initially DOC removals in GAC filters are due primarily to physical adsorption. However, even when biofiltration isn’t desired, GAC filters that are not regularly regenerated through chemical washing evolve naturally into biofilters (Velten et al. 2011).

GAC biofilter startup was observed by Velten et al (2007) using an adenosine triphosphate (ATP) measurement method to quantify biofilm development. It was found that 50 days were required to achieve “steady-state” biofilm on fresh media, although biological activity is present much earlier. During startup, DOC reductions gradually decreased as a result of oversaturation of GAC adsorption sites. Maximum ATP concentrations, correlating to biofilm thickness, were experienced at 33 days and decreased until reaching equilibrium at 50 days. Additional research showed that DOC removals decreased as filters became biologically acclimated due to the change from physical DOC removals to removal of strictly biodegradable organic carbon (Velten et al. 2011). Although DOC removals are
typically lower, the GAC biofilter avoids the necessity of yearly GAC regeneration to maintain DOC adsorption and provides a process unit that can increase effectiveness of other processes such as membrane filtration or ozonation (Simpson 2008).

### 2.5.3 Pretreatment

Biofiltration has been shown to be an effective stand-alone treatment process, capable of removing several types of waterborne contaminants, but its utility as a combined treatment process is also well documented. Often biofiltration can have a harmonious effect on other treatment processes and can be more effective when used in conjunction with other process units or a preoxidant.

The use of ozone as a preoxidant can exemplify this relationship. Since the Safe Drinking Water Act of 1973, the use of ozone as a primary disinfectant has been increasingly encouraged in United States. This is largely due to its effectiveness at inactivating certain microorganisms and lower TTHM formation when compared to other disinfectants (Rice and Overbeck 1998). It is also well established that ozonation increases the fraction of biodegradable organic carbon in water and recent research has shown that microbially available phosphorus (MAP), an important nutrient for microbial growth, is also increased (Huck 2000; Lehtola et al. 2001).

However, the substantial increase of BDOC from ozonation can encourage bacterial regrowth in the distribution systems of water treatment plants (Van der Kooij et al. 1989). Because of this, biofiltration and ozonation can be mutually beneficial to each other. Ozonation can provide effective disinfection to water treatment facilities, while increasing the readily BDOC and MAP to biofilters, improving their overall removal abilities.
Biofilters can be used to improve the efficiency of other treatment process units or be a part of multi-stage water treatment for more efficient or selective contaminant removal. Biofilters are often membrane filtration pretreatment (Huck 2000). By improving the biostability of water, biofilters reduce the occurrence of biofouling in membrane filters. In other cases, nutrient or substrate augmentation can be used to improve overall performance of biofilters.

2.5.4 Optimal Nutrient Conditions

The proper balance of carbon, nitrogen, and phosphorus has become a major concern recently in biofilter operation, as it has been shown that an improper balance can lead to the overproduction of EPS in biofilters. The optimal growth of heterotrophic bacteria require a carbon, nitrogen, and phosphorus molar ratio (C:N:P) of 100:10:1 (LeChevallier et al. 1991). This equates to a concentration of 0.117 mg/L NH$_4^+$-N: 0.026 mg/L PO$_4^{3-}$-P for every 1 mg/L biodegradable carbon substrate. Nutrient limited conditions have been shown to cause microbial stress. Liu et al. found that a number of quorum-related genes were expressed in bacteria under phosphorus-limited conditions. This led to consistent increases in EPS formation (Liu et al. 2006).

Recent research has shown that EPS overproduction in nutrient limited conditions results in clogging of pores within biofilters, leading to shorter run times. Brown et al. displayed that minimal phosphorus dosing (0.20 mg/ L PO$_4^{3-}$-P) decreased biofilter terminal headloss by approximately 15 percent. Additional contaminant removals were also observed (Brown 2011). Phosphorus and other nutrient limitations can adversely affect biological treatment in drinking water. This research seeks to evaluate roughing biofiltration as an
alternative that may alleviate the issue of nutrient limitation in order to improve the effectiveness of biofiltration.
3.0 Materials and Methods

3.1 Water Quality Characterization

The goal of this research is to determine whether biological filtration earlier in the drinking water treatment process is an effective technology for reducing organics present in finished waters. This determination is accomplished by measuring both organic precursors for formation of disinfection by-products, total and dissolved organic carbon and UV$_{254}$. The molar ratio of carbon substrate and major nutrients also should be measured to confirm that desired conditions for biofilm development are present. Details for these various analyses and the experimental plans for biofilm development and pilot process performance follow.

3.1.1 Total and Dissolved Organic Carbon

TOC analysis was performed following EPA Method #415.3 using a Shimadzu TOC-V$_{CPN}$ analyzer using high purity air as the carrier gas at a flow rate of approximately 150 ml/minute. The TOC-V$_{CPN}$ uses a 680°C catalytically-aided combustion oxidation/non-dispersive infrared detection (NDIR). Samples were decanted into 40-mL vials and acidified automatically to a pH of 2 to 3 using a Shimadzu ASI-V autosampler, followed by sparging to remove inorganic carbon. The remaining organic carbon was measured as an NDIR signal and converted to a TOC equivalent result based on a calibration curve. DOC analysis was performed similarly as TOC, with the exception that samples were filtered with a 0.45 µm pore-diameter membrane filters to remove particulate matter with 48 hours of collection. DOC analysis was performed following EPA Method #415.1. Samples for TOC and DOC were stored refrigerated in the dark and analyzed within a week.
3.1.2 UV\textsubscript{254}

UV\textsubscript{254} provides a quantitative measure of the aromatic content within the organic carbon contained in a sample, which has been shown to correlate well with DBP formation potential (Kitis et al. 2001; Pifer and Fairey 2014). UV\textsubscript{254} samples were filtered with a 0.45 \textgreek{m} membrane filters within 48 hours of collection and were stored refrigerated in the dark and analyzed within a week. Analysis was carried out following EPA Method #415.3 on a Varian Cary 50 Conc UV-Visible Spectrophotometer using a 1-cm saprasil quartz cuvette. The instrument was zeroed using reagent grade DI water.

3.1.3 Nutrient Testing

Nutrient analysis was performed with a Hach DR 2400 spectrophotometer using 10-mL glass sample cells and appropriate Hach reagents. The suite of Hach reagent tests use colorimetric methods to evaluate various waterborne characteristics by comparing blank sample vials (those filled with DI or unreacted water samples) with those that have reacted with specified powder pillow reagents. This research was primarily concerned with ammonia-nitrogen (NH\textsubscript{4}\textsuperscript{+}-N), nitrate-nitrogen (NO\textsubscript{3}-N), and orthophosphate (PO\textsubscript{4}\textsuperscript{3-}-P) as these are important for optimal microbial growth and as indicators of microbial activities. Information about the specific Hach tests utilized is detailed in Table 3-1, below.
Table 3-1 – Hach nutrient testing information

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Method No.</th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia-Nitrogen</td>
<td>Salicylate</td>
<td>8155</td>
<td>0.01 – 0.50</td>
<td>mg/L NH$_4^+$-N</td>
</tr>
<tr>
<td>Nitrate-Nitrogen</td>
<td>Cadmium Reduction</td>
<td>8039</td>
<td>0.3 – 30.0</td>
<td>mg/L NO$_3^-$-N</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>Ascorbic Acid</td>
<td>8048</td>
<td>0.02 – 2.50</td>
<td>mg/L PO$_4^{3-}$</td>
</tr>
</tbody>
</table>
3.2 Static Biofilm Development

3.2.1 Introduction

In order to observe the ability of select filter media to support biofilm development, a bench-scale experiment was carried out utilizing the selected filter media as substrate and selected water treatment plant (WTP) source water (river, lake, and alluvial groundwater) as the growth medium in a static laboratory environment. From this experiment, observations were made about the “start-up” period of a biologically active roughing filter. This “static biofilm development” experiment should yield some insight into biofilm formation time for various source waters.

Two significantly different types of media were used as growth substrate. The substrates were AqWise Biomass Carriers (ABC5) and Fluval Biomax filter pieces shown in Figure 3-1. ABC5 is a plastic, honeycomb-style media conventionally used as a trickling filter media. Its manufacturer-estimated specific surface is approximately 7.12 ft²/L. The ABC5 surfaces are relatively smooth. Conversely, Fluval Biomax is a ceramic ring with a porous structure, leading to a significantly higher specific surface area, estimated as 1460 ft²/L. Biomax is conventionally used as an aquarium filter media. These two media types were selected in an effort to compare their effectiveness as biologically activated media and to contrast the effect of specific surface area on biofilter performance.

The two types of media were concurrently incubated in sterilized DI water (a medium essentially void of bacteria and nutrients for use as a control) and four different source waters. The biomax and ABC5 media are shown in 12-well plates in a typical experimental setup in Figure 3-2. Biofilm growth was quantitatively measured with respect
to time and overall thickness using a crystal violet staining method adapted from O'Toole et al. (1999). These results were then compared to source water characteristics, such as nutrient concentrations, and organic carbon content, in an effort to correlate optimal conditions for biofilm growth.

Figure 3-1: A.) Fluval Biomax filter media; B.) AqWise Biomass Carrier filter media.
3.2.2 Preparation of substrate and analysis solutions

**Equipment sterilization**

The 12-well plates were sterilized with UV for at least 5-10 minutes. 200 μL and 5 mL pipette tips, deionized (DI) water for the control wells, and adequate filter substrate (generally, 12 per plate is sufficient) were autoclaved at 121°C for 25 minutes and allowed to cool/dry.

**Solution preparation**

A 0.1% (w/v) crystal violet (CV) solution was prepared by dissolving crystal violet powder into ultrapure water and mixing on a stirring plate for at least 1 hour to ensure homogenous mixture. Afterward, the solution was filtered with coarse filter paper. A 30% (v/v) acetic acid solution was prepared for crystal violet solubilization by diluting glacial
acetic acid in ultrapure water. 30% acetic acid was chosen over other alternative solvents as it was recommended for more efficient solubilization of CV stains from a wider range of microbes (Merritt et al. 2005).

3.2.3 Testing Protocol

1.) A piece of substrate was inserted into each well with 5mL of growth medium. For the control plate, previously sterilized DI water was utilized as the growth medium. For all others, raw WTP water was used. When multiple sources of water were being tested simultaneously, only one control plate per type of filter substrate was monitored. The plates and media were incubated at 30°C to optimize heterotrophic bacteria growth until crystal violet readings were taken at defined timepoints.

2.) **Filter substrate base readings** – Sterilized filter substrate (media) was initially measured using the CV assay to establish a baseline. Each filter substrate was rinsed vigorously with sterile DI water to remove planktonic cells and blotted dry. They were then placed in separate microtiter plate wells with 5 mL of 0.1 % CV solution for at least 10 min. After staining, substrate was rinsed with DI water until the rinse was colorless (and blotted on a Kimwipe to check). After blotting dry, each substrate was placed in 50 mL centrifuge tubes with 5 mL of 10% acetic acid. They were then vortexed for approximately 5 seconds and allowed to incubate at room temperature to ensure CV solubilization. 200 μL of the resulting acetic acid/crystal violet solution was then transferred to a 96-well plate and the absorbance at 600 nm was measured using a Victor microreader (PerkinElmer, Massachusetts, USA).
3.) **Take base readings of growth medium** - 200μL of the control DI and WTP source water were removed from the 12-well plates and transferred into a 96-well plate to take OD₆₀₀ readings as a measure of the number of the planktonic cells.

4.) **Characterize source water** – The initial nutrient content of the source water (ammonia, orthophosphate, and nitrate) as well as total organic carbon. This was done in an effort to see if the overall amount of biofilm formed and the time to reach peak biofilm levels could be predicted by any of these easily measurable water measurements.

**Data Collection**

Starting at 3 days, readings were taken at least twice per week for at least 25 days by repeating steps 2 and 3 from *the Testing Protocol*. In this way, a curve was developed to depict the time taken for biofilm development and to quantify the amount of biofilm establishment. The results were then compared to the source water characterization data.
3.3 Pilot Scale Observations of Biofilm Development

3.3.1 Introduction

A pilot scale roughing biofilter experiment was carried out at a cooperating water treatment facility. This experiment was designed to evaluate the performance of roughing biofilters under typical surface water treatment plant conditions. For approximately one month, raw water (pumped from the reservoir source water) was fed through the filters while regular samples were taken and analyzed. During this period, the biofilters were allowed to acclimate to their environment while biofilm established on filter media surfaces. Water samples were monitored for signs of biological activity, such as organic carbon reduction, nitrification, etc. After this acclimation period, adjustments were made in an effort to determine optimal filter operation conditions.

3.3.2 Materials

The pilot-scale roughing biofilter setup included three two-inch PVC pipes plumbed to operate as down-flow filters. The general schematic of the roughing biofilter setup is depicted in Figure 3-3. Raw surface water was fed into a four-tap, one-inch copper manifold, each tap was equipped with a one-inch copper ball valve (Figure 3-4). This allowed flow adjustments to be made to the influent flow rate of each column individually. Water was fed to the biofilters through freshly purchased 5/8” vinyl tubing connected by nylon barb fittings and hose clamps at all connection points.
Figure 3-3: Schematic of pilot-scale roughing biofilter setup.

Figure 3-4: Four-tap copper manifold utilized for biofilter influent feed.
**Installation**

In order to observe biofilter startup, there was an attempt to install the biofilters in a sterile condition, so only biological activity associated with the WTP environment were observed. This was accomplished by rinsing all associated materials (excluding filter media) with a 10% bleach solution followed by flushing with hot water prior to installation in the WTP. The two types of filter media (Figure 3-1) were rinsed with clean water to remove unattached particles, dirt, etc. and were autoclaved at 121°C for 25 minutes.

The roughing biofilters were installed at the WTP plant on February 19, 2014. Flow to the biofilters was adjusted to desired rates by influent ball valve adjustments and effluent measurements using a graduated cylinder and stopwatch. The roughing biofilters, after installation, are pictured in Figure 3-5.

![Figure 3-5: Pilot-scale roughing biofilters installed at Marceline WTP, with media depth indicated in red.](image-url)
Operation and Sampling

During the first 35 days of filter operation, the biofilters were allowed to acclimate to their environment. It was expected during this time that the filters would establish a biofilm on their internal and external surfaces as they were exposed to indigenous bacteria present in the influent raw water.

The filters were operated initially at approximately 150 mL/min for each filter. Taking into consideration the inner surface area of the two-inch PVC pipes used, this flow rate corresponds with a hydraulic loading rate (flow per filter cross-sectional surface area) of 1.73 gpm/ft². This flow rate was chosen for a few reasons. Optimally, the highest feasible flow rate would provide a more efficient treatment step with regards to necessary surface area or the need to throttle overall flow rate through a hypothetical WTP. As previously discussed, slow sand filters used loading rates of 0.15 gpm/ft² or lower, which led to widespread abandonment due to surface area requirements. In recent research, polishing biofilters have been operated at 4.5 gpm/ft² with significant reduction in organic carbon (Brown 2011). However, while the increased porosity of the roughing biofilter media decreases the likelihood of hydraulic loading limitations, the decreased specific surface area of the media also provides fewer surfaces for biofilms to establish. Because of this, an effort to split the difference between typical slow and rapid filter flow rates was made with the awareness that changes could be made later to optimize flow rates.

Periodic sampling of the roughing biofilters was accomplished with the use of washed and dried 250-mL amber, plastic bottles transported to the site from the laboratory. Biofilter effluent flow rate was verified prior to sampling, with minimal adjustments made if
necessary to maintain uniformity across the three filter columns. After flow verification, the three filter columns were allowed to operate until a full bed volume had passed before sampling. This was accomplished by evaluating the empty bed contact time (EBCT) of the filters. EBCT is evaluated as the filter bed volume divided by the flow rate. For example, while the filter columns were operated at 150 mL/min with a bed depth of approximately 40 inches (approximately 2160 mL bed volume), the EBCT of the filters was 14.4 min. In this case, the filters were allowed 15 minutes to pass a full bed volume before samples were collected in order to account for any flow equalization to take effect. Samples were then placed in a cooler with reusable ice packs until storage in a laboratory refrigerator. Once in the lab, samples were analyzed for DOC, UV<sub>254</sub>, ammonia-nitrogen, phosphate, and nitrate-nitrogen following protocols detailed in Section 3.1.

Additionally, ABC5 filter media was sampled prior to filter installation and periodically throughout filter operation in order to gain some insight into biofilm establishment within the roughing biofilters. The ABC5 media was sampled using a threaded coupling that allowed access to filter media. During sampling, the filter was drained, three pieces of media were removed and stored in a 50 mL centrifuge tube with Marceline raw water as supernatant, and stored on ice until storage in the laboratory refrigerator. The following day, biofilm was measured as absorbance using the crystal violet assay described in Step 2 of Section 5.2.3.
4.0 Static Biofilm Development

4.1 Introduction

During the static biofilm development experiment, proposed roughing biofilter media was utilized as substrate for the establishment of biofilm. This was achieved through a bench-scale experiment, where pieces of roughing filter media were incubated in various WTP raw water sources over a period of 20-30 days. A crystal violet (CV) assay was utilized to quantify biofilm establishment on filter substrate. Four water sources were utilized as growth medium in an effort to observe biofilm establishment using waters from several origins and of various characteristics.

There are a few reasons why the static biofilm development experiment was carried out. Perhaps the most important reason was to establish a procedure that could be used to quantify biofilm establishment on roughing biofilter media in a pilot-scale study in order to correlate contaminant removal with biofilm establishment. By carrying out this experiment in a laboratory setting, observations could be made about the usefulness of the CV assay as a biofilm quantification method and the possible limitations of the procedure in evaluation of roughing biofilter media.

Another reason for this experiment was to establish an idea of the biofilm levels that could be expected on biofilter media in the subsequent pilot-scale study. Although the static biofilm development was carried out using a “batch” reactor setup rather than the continuous flow setup that would be used for the pilot-scale study, comparisons could be made because the raw water used as growth medium would be of similar composition to the influent water
used in the pilot-scale study. In both experiments, the indigenous bacteria within raw water samples established biofilms on filter media surfaces. Because of this, comparisons of the overall biomass can be made to determine whether a more robust biofilm could be established in a batch system where no shear forces are present, but necessary nutrients may be consumed over time, or in a continuous flow system where shear forces may limit biofilm formation, but where nutrients are continually replenished.

A final purpose for this experiment was to observe noticeable differences in overall biofilm formation between the various water sources utilized as growth medium. Water characterization was carried out prior to incubation by measuring TOC, ammonia, orthophosphate, and nitrate concentrations in the hopes that these relatively simple measurements might be linked to biofilm formation potential of water sources.
4.2 Results

4.2.1 Preliminary Crystal Violet Testing

A preliminary experiment was carried out using influent water from the Maysville, Missouri WTP. The water used was from a Northwest Missouri reservoir. Prior to carrying out experiments using other water sources, it was decided to carry out a preliminary static biofilm development experiment to analyze the usefulness of the crystal violet method for evaluating biofilm formation using roughing filter media pieces as substrate. Because the crystal violet method was typically used to observe biofilm development on relatively inert surfaces, such as microscope slides (O'Toole et al. 1999), there were questions regarding whether the method would translate well for the evaluation of filter media biofilm formation. This was especially true in the case of the Biomax media (Figure 3-1A), which have a very irregular and porous surface. Because the quantification of biofilm in this method requires the solubilization of CV, it was hypothesized that the sorption capabilities and irregularities of the Biomax media may interfere with the reliability of the experimental results.

Results of the Maysville biofilm development experiment are shown in Figure 4-1 with corresponding data shown in Table 4-1. The stark differences between the ABC5 media and Biomax absorbance plots are visibly apparent. The absorbance plot of ABC5 media starts at a value of 0.086 absorbance units (AU) and climbs fairly smoothly to a maximum value of 0.202 AU after 24 days of incubation in Maysville raw water. Conversely, Biomax media exhibits an irregular curve with intermittent peaks and valleys, displaying no real trend over the course of the experiment.
Figure 4-1: Preliminary CV testing results between Biomax and ABC5 incubated in Maysville Raw water.

Table 4-1: Absorbance at 600 nm and statistical data from Maysville biofilm development experiment.

<table>
<thead>
<tr>
<th>Days</th>
<th>ABC5 Control</th>
<th>ABC5</th>
<th>Biomax Control</th>
<th>Biomax</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.086</td>
<td>-</td>
<td>0.180</td>
<td>0.221</td>
</tr>
<tr>
<td>4</td>
<td>0.088</td>
<td>0.127</td>
<td>0.221</td>
<td>0.223</td>
</tr>
<tr>
<td>7</td>
<td>0.087</td>
<td>0.148</td>
<td>0.350</td>
<td>0.175</td>
</tr>
<tr>
<td>11</td>
<td>0.080</td>
<td>0.182</td>
<td>0.148</td>
<td>0.161</td>
</tr>
<tr>
<td>15</td>
<td>0.081</td>
<td>0.181</td>
<td>0.187</td>
<td>0.215</td>
</tr>
<tr>
<td>18</td>
<td>0.083</td>
<td>0.160</td>
<td>0.189</td>
<td>0.129</td>
</tr>
<tr>
<td>20</td>
<td>0.083</td>
<td>0.195</td>
<td>0.191</td>
<td>0.172</td>
</tr>
<tr>
<td>24</td>
<td>0.091</td>
<td>0.202</td>
<td>0.255</td>
<td>0.265</td>
</tr>
<tr>
<td>28</td>
<td>0.094</td>
<td>0.200</td>
<td>0.236</td>
<td>0.142</td>
</tr>
<tr>
<td>32</td>
<td>0.087</td>
<td>0.151</td>
<td>0.108</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Average = 0.086 -- 0.206 --

Std. Dev. = 0.004 -- 0.062 --

95% Confidence Interval = 0.003 -- 0.039 --
Table 4-1 also includes information from the control plates utilized in the Maysville biofilm development experiment under “ABC5 Control” and “Biomax Control.” These plates were included in the experiment with the intent of monitoring the background interferences associated with the CV biofilm measurement method. Possible reasons for background interference could be surface interactions with the CV solution - where a portion of absorbance totals are not due to biofilm presence - and sample contamination - where a biofilm is formed despite efforts to sterilize materials associated with the control plate. Statistics from the control data sets reveal that the ABC5 control readings had a low variability. At a confidence interval of 95%, the ABC5 control plate had an average absorbance of 0.086 ± 0.003 AU. On the other hand, the Biomax control data set has a large variability, with an average absorbance of 0.206 ± 0.039 AU.

The high variability of Biomax control absorbance readings, combined with the absence of visible trends in Biomax substrate incubated in raw water, indicated that Biomax was a poor candidate for biofilm evaluation using the proposed CV biofilm measurement. Although efforts were made to improve the removal of CV from the surface and inner pores of the Biomax substrate, including longer DI rinses and extended periods in the 30% acetic acid solution, Biomax substrate often remained stained as shown in Figure 4-2. Conversely, ABC5 performed fairly well using the CV biofilm measurement, displaying a low variability under control conditions and a visible trend when incubated with raw water as growth medium. Because of this, subsequent static biofilm development experiments only attempted to quantify biofilm development on ABC5 filter media.
4.2.2 Multiple Source Water Biofilm Results

The crystal violet biofilm quantification protocol overviewed in Section 3.2.3 was carried out using ABC5 filter media (Figure 3-1B) as substrate for biofilm establishment using WTP raw water as growth medium. Four water sources were compared in order to generate multiple biofilm development curves for waters of various characteristics. Two water sources were retrieved from surface reservoirs (Marceline and Maysville), one from a river source (Missouri River), and one from an alluvial groundwater source (Columbia). The results of the experiments are shown in Figure 4-3.

All four curves increase over the duration of the experiment, although sporadic outliers appeared at times. These are likely due to experimental error associated with each measurement. In order to reduce the variability, future measurements should be performed
in triplicate to remove this error and produce smoother curves. This will also enable better conclusions to be drawn about the development of biofilm over time.

Figure 4-3: Plot of the biofilm development curves for the four raw water sources used to inoculate ABC5 media. Lake water sources are shown as dashed lines.

Data for the control plates from the biofilm development experiment are shown in Table 4-2. As was the case with Maysville’s control plate (discussed in Section 4.2.1), the variability of four control plates was very small (from 0.002 - 0.004 AU). The average values of Marceline, Columbia, and Missouri River control statistics were very close to each other (0.075, 0.076, and 0.076 AU, respectively.) Interestingly, the Maysville control plate produced readings approximately 0.010 AU higher than the other three water sources. This could be due to a number of factors, but perhaps the most likely explanation is that the four waters were not tested concurrently. Maysville testing initiated on December 3\textsuperscript{rd}, 2013, while Marceline began on January 31\textsuperscript{st}, 2014, and Columbia and Missouri River were tested concurrently beginning February 21\textsuperscript{st}, 2014. This may have led to slight differences in the
composition of solutions used for the experiments, which could likely account for the minimal changes in background interference exhibited by the control plates.

Table 4-2: Results of control plates for the four water sources used.

<table>
<thead>
<tr>
<th></th>
<th>Maysville</th>
<th>Marceline</th>
<th>Columbia</th>
<th>MO River</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.086</td>
<td>0.068</td>
<td>0.069</td>
<td>0.070</td>
<td></td>
</tr>
<tr>
<td>0.088</td>
<td>0.073</td>
<td>0.076</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>0.087</td>
<td>0.082</td>
<td>0.076</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>0.080</td>
<td>0.082</td>
<td>0.074</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>0.081</td>
<td>0.078</td>
<td>0.082</td>
<td>0.082</td>
<td></td>
</tr>
<tr>
<td>0.083</td>
<td>0.074</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>0.083</td>
<td>0.069</td>
<td>0.074</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>0.091</td>
<td>--</td>
<td>0.077</td>
<td>0.077</td>
<td></td>
</tr>
<tr>
<td>0.094</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>0.087</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong> = 0.086</td>
<td><strong>0.075</strong></td>
<td><strong>0.076</strong></td>
<td><strong>0.076</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Std. Dev.</strong> = 0.004</td>
<td>0.005</td>
<td>0.004</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td><strong>95% Confidence Interval</strong> = 0.003</td>
<td>0.004</td>
<td><strong>0.002</strong></td>
<td><strong>0.002</strong></td>
<td></td>
</tr>
</tbody>
</table>

Source water characterization data is shown in Table 4-3. Several observations can be made about the similarities and differences between the various water sources. Perhaps the most striking relationship is the high TOC concentrations associated with the two lake sources (Maysville and Marceline) as opposed to the other water sources. Likely, the more stagnant conditions present in a reservoir contribute somewhat to these higher values. It would also be expected that an alluvial water source (Columbia) would have lower TOC concentrations as riverbank filtration (Section 2.2.3) can reduce TOC concentrations.

Nutrient concentrations are also shown in Table 4-3. This data was analyzed using the optimal nutrient molar ratio of C:N:P of 100:10:1 discussed in Section 2.5.4. The ammonia-nitrogen and phosphate concentrations were analyzed using this ratio to determine which of these would be considered the limiting nutrient. An NH₄⁺-N: PO₄³⁻-P ratio of 4.5 is necessary to ideally balance these two nutrients ((0.117 mg/L NH₄⁺-N)/(0.026 mg/L PO₄³⁻-
P) = 4.5). Ratios above this indicate phosphate limitations and below point to ammonia-nitrogen as the limiting nutrient. Columbia was the only source water found to be phosphate-limited. This information was then used to calculate the amount of biodegradable organic carbon that could be removed from the water sources based on the C:N:P ratio. All water sources were shown to have available nutrients in sufficient quantities to remove over 1 mg/L of BDOC, except for the Maysville water source.

Table 4-3: Raw water characteristics and peak biofilm development measured as absorbance using the CV assay.

<table>
<thead>
<tr>
<th>Source Type</th>
<th>Maysville</th>
<th>Marceline</th>
<th>Columbia</th>
<th>Missouri River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source Type</td>
<td>Surface (Lake)</td>
<td>Surface (Lake)</td>
<td>Groundwater (Alluvial)</td>
<td>Surface (River)</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>13.97</td>
<td>8.96</td>
<td>5.21</td>
<td>5.73</td>
</tr>
<tr>
<td>NH₄⁺-N (mg/L)</td>
<td>0.04</td>
<td>0.12</td>
<td>0.41</td>
<td>0.14</td>
</tr>
<tr>
<td>PO₄³⁻-P (mg/L)</td>
<td>0.153</td>
<td>0.078</td>
<td>0.042</td>
<td>0.199</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/L)</td>
<td>2.40</td>
<td>1.40</td>
<td>0.10</td>
<td>1.50</td>
</tr>
<tr>
<td>NH₄⁺-N: PO₄³⁻-P Ratio</td>
<td>0.26</td>
<td>1.53</td>
<td>9.67</td>
<td>0.70</td>
</tr>
<tr>
<td>Limiting Nutrient</td>
<td>Ammonia</td>
<td>Ammonia</td>
<td>Phosphate</td>
<td>Ammonia</td>
</tr>
<tr>
<td>BDOC Requirements (mg/L)</td>
<td>0.34</td>
<td>1.03</td>
<td>1.63</td>
<td>1.20</td>
</tr>
<tr>
<td>Peak CV Abs (1/cm)</td>
<td>0.199</td>
<td>0.159</td>
<td>0.172</td>
<td>0.274</td>
</tr>
</tbody>
</table>

The maximum absorbance values measured for each water source are also displayed in Table 4-3. In order to eliminate some issues with variability, the maximum absorbance value was evaluated as an average of the three maximum values. Using this method, Maysville, Marceline, and Columbia have similar maximum absorbance values, indicating similar amounts of biofilm formation. Maysville has a slightly higher value than Marceline or Columbia, although this difference may be slightly exaggerated due to experimental bias found in the Maysville data set, discussed previously in this section.
Missouri River is the only source water that produced a significantly higher amount of biofilm formation as measured by absorbance. This difference, however, cannot be explained solely by the water characteristics measured. While each water source has a unique set of measured characteristics, no one variable correlated well with the overall biofilm development. This likely indicates that the biofilm establishment is determined by an interaction of the nutrients available in the water as well as some unmeasured characteristics. Large differences in TOC concentration seem to have little to no effect on biofilm formation. Another parameter that measures the biodegradable portion of organic carbon may be a better indicator. One characteristic that most likely has a high impact on biofilm formation is the type and quantity of bacteria present in a water sample. A measure of bacteria presence in water, such as heterotrophic plate count, may shed some light on the biofilm formation potential of water sources in the future.
4.3 Discussion

A preliminary experiment utilizing Biomax and ABC5 media as biofilm growth substrate revealed limitations in the crystal violet biofilm assay. The results generated by the Biomax media were highly variable with no visible trend over time (Figure 4-1). Variable results were also generated by Biomax substrate under control conditions (using sterilized DI water as growth medium). Under control conditions, biofilm formation was not expected and absorbance values were expected to be primarily due to surface interaction with the CV solution. The highly variable results Biomax substrate generated under control conditions (Table 4-1) seemed to indicate that significant adhesion of the CV to outer and inner surfaces of the media interfered with the absorbance levels. Despite efforts to remove CV from the Biomax media by additional DI rinsing and increased acetic acid incubation, control Biomax remained visibly stained (Figure 4-2). Due to these issues, it was decided the CV biofilm measurement assay would only be used to quantify biofilm formation on ABC5 media.

The CV biofilm measure assay was used to observe biofilm development on ABC5 media incubated in four raw water sources. Overall, increasing absorbance for all four water sources indicated the development of biofilm on ABC5 surfaces (Figure 4-3). Although increasing trends were observed, outlier points decreased the smoothness of curves and inhibited the ability to make specific observations about biofilm development curves. In the future, samples should be taken in triplicate to avoid experimental error that may be the cause of the outlier data points.
Missouri River water resulted in the highest biofilm formation measured as absorbance. The waters from Maysville, Marceline, and Columbia resulted in similar biofilm formation. Unfortunately, the measured water characteristics did not provide significant insight into biofilm formation potential (Table 4-3). It is suggested that in the future, a greater effort be put into determining the type and quantity of bacteria present in a water sample as a possible indicator of biofilm formation potential.

Overall, the crystal violet biofilm assay seems to show good potential for quantification of biofilm formation on ABC5 media. The observations from the static biofilm development experiment were taken into consideration when using the method to observed biofilm formation in the pilot-scale roughing biofilters.
5.0 Pilot Scale Observations of Biofilm Development

5.1 Introduction

Pilot-scale roughing biofilters were installed at the Marceline WTP to evaluate their performance when placed in realistic drinking water treatment plant conditions. This was done in an effort to determine whether biological filtration earlier in the drinking water treatment process is an effective technology for reducing organics present in WTP’s. It was hypothesized that a biological pretreatment step may remove a portion of waterborne organics that may not be removed in conventional WTP processes, while encountering less nutrient-limited conditions that can be experienced in polishing biofilters due to coagulation-sedimentation processes.

This experiment was designed to evaluate the performance of roughing biofilters under typical surface water treatment plant conditions. For 35 days, raw water (pumped from the reservoir source water) was fed through the filters while regular samples were taken and analyzed. During this period, the biofilters were allowed to acclimate to their environment while biofilm established on filter media surfaces. Water samples were monitored for signs of biological activity, such as organic carbon reduction and nitrification. Filter media samples were also collected to monitor biofilm establishment. After this acclimation period, adjustments were made in an attempt to determine optimal filter operation conditions.
5.2 Results

5.2.1 Acclimation Period

During the first 35 days, the roughing biofilters were operated as described in Section 3.3.2. The flow rate through each filter was 150 mL/min, corresponding to a hydraulic loading rate of 1.73 gpm/ft². Over the course of this period, expectations were that biofilm establishment could be monitored in an attempt to correlate increases in contaminant removal with biological activity within the filters using the crystal violet (CV) assay described in Section 3.2.3.

It was expected that DOC removals initially would be very minimal but may increase over time as biofilm was established inside the biofilters. Biofilm establishment was verified on the surfaces of the ABC5 filter media during this period. Absorbance readings increased from 0.085 AU, initially, to 0.260 AU during the first 30 days of operation. This is significantly higher than the results generated from the Marceline source water during the static biofilm experiment. There are a number of possible reasons for this, but as the pilot scale filters were operated as a continuous flow reactor, continuous flow of raw water provided replenishment of nutrients. Conversely the static experiment operated as a batch reactor where nutrients were not refreshed. This most likely contributed to the higher biomass formation in the biofilters. The CV absorbance results seemed to indicate that biological activity was taking place within the biofilters at this point. Full biofilm quantification results will be presented in a later section.

The results of DOC analysis during the initial acclimation period are shown in Figure 5-1. Influent DOC concentrations are labeled as “Raw” in the chart, while the effluent...
results for the three filter columns are labeled in the legend. Influent DOC concentrations ranged from 9.3 - 14.3 mg/L. Baseline testing during installation showed similar results between raw water and filter effluents, although Biomax resulted in a slight increase in effluent DOC concentration. Sampling at 11 days revealed a dramatic increase in influent DOC concentration to 14.3 mg/L. Some reduction is evident in filter effluent results at 11 days, but the control filter results in similar levels of reduction, indicating that the reduction cannot be attributed to the filter media or biological activity within. Instead it may be due to hydraulic or pipe surface interactions. The remaining data points result in tighter bunching of the four samples that reveal no real reduction in DOC concentrations. It appears that over the 35-day acclimation period, the filters had no discernable effect on DOC concentrations. UV_{254} and nutrient analysis produced similarly inconclusive results during the acclimation period.

Figure 5-1: Overall influent (Raw) and effluent DOC concentrations in the filter columns during initial 35-day period.
5.2.2 Flow Reduction

During the initial 35-day acclimation period, biofilm growth within the roughing biofilters could not be correlated with contaminant removal in the form of DOC, UV$_{254}$, or nutrient reduction. Because biofilm establishment was verified using the CV quantification method, it was hypothesized that there was not sufficient contact time for the microbes in the biofilters to reduce the contaminants observably. Decreasing the flow rate of the biofilters was determined to be the most feasible way to increase the EBCT in an effort to produce observable contaminant reductions.

In an effort to determine if effluent DOC concentrations improved as a function of flow rate, a flow evaluation experiment was carried out. During this experiment, filter flow rates were adjusted over four increments with their effluent water quality monitored at each. The filters were sampled as described in Section 3.3.2. In this experiment, filter flow rates were adjusted from 10 mL/min to 200 mL/min. At each interval, filter flow rates were adjusted until all filters were in equilibrium, after which they were allowed to run until a full EBCT had passed. Table 5-1 shows each flow rate with its corresponding EBCT and hydraulic loading rate.

Table 5-1: EBCT and hydraulic loading rate at each flow rate interval.

<table>
<thead>
<tr>
<th>Flow Rate (mL/min)</th>
<th>EBCT (min)</th>
<th>Hydraulic Loading Rate (gpm/ft$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>216</td>
<td>0.12</td>
</tr>
<tr>
<td>50</td>
<td>43.2</td>
<td>0.58</td>
</tr>
<tr>
<td>100</td>
<td>21.6</td>
<td>1.15</td>
</tr>
<tr>
<td>200</td>
<td>10.8</td>
<td>2.31</td>
</tr>
</tbody>
</table>
The results of the flow evaluation experiment are shown in Figure 5-2. The results show very little discernable differences in DOC over the various flow rates. There is not a statistically significant reduction of raw DOC in either the ABC5 or Biomax filter. It seems that there is a slight increase in DOC from the Biomax effluent when flow rates are increased to 200 mL/min. High flow rates may result in breakthrough of previously removed DOC.

![Figure 5-2: DOC concentrations over Impact of flow rate on DOC concentration in filter effluents.](image)

A significant reduction in DOC was not observed at any of the evaluated flow rates. However, it was speculated that because the biofilters were not allowed time to acclimate at each flow rate, observable contaminant reductions might not occur under these experimental conditions. Flow rates have a high impact on the quantity of biofilm formed due to their effect on the replenishment of fresh nutrients and associated shear forces. In order to fully investigate the impact of a reduced flow rate, biofilter flow rates were adjusted to 20
mL/min for the remaining 35 days of operation. In this way, observations could be made about the long-term impact of a reduced flow rate.

5.2.3 Analysis of Possible Biological Inhibiting Conditions

In order for roughing biofilters to successfully remove contaminants through biological processes, conditions within the filters must be suited for bacteria to form biofilms capable of degrading waterborne contaminants. Adverse conditions could inhibit the biological activity of the filters in an array of ways. This could include the suppression of overall biomass growth or the limitation of desired biofilm formation, among a number of other results. In the event of a biological inhibiting condition, it would be expected that a decreased or undetectable reduction of contaminants would occur.

Due to the low or unquantifiable contaminant removal observed in the roughing biofilters during the acclimation period, efforts were made to address possible sources of biological inhibition.

Nutrient Conditions

Throughout pilot-scale roughing biofilter operation, nutrient levels were monitored in order to ensure sufficient nutrient conditions for microbial activity. As discussed in Section 2.5.4, the optimal growth of heterotrophic bacteria requires a carbon, nitrogen, and phosphorus molar ratio (C:N:P) of 100:10:1 (LeChevallier et al. 1991). For every 1 mg/L of BDOC, a ratio of 0.117 mg/L NH$_4^+$-N: 0.026 mg/L PO$_4^{3-}$-P is required to avoid nutrient-limited conditions.
Influent nutrient results for the seven sampling periods are shown in Table 5-2. On average, influent water contained 0.14 mg/L NH$_4^+$-N and 0.077 mg/L PO$_4^{3-}$-P. This exceeds the optimal nutrient conditions required to utilize up to 1 mg/L of BDOC. On only two sampling periods did the influent NH$_4^+$-N concentrations slip below these minimum requirements (highlighted in red). Whereas sufficient nutrients were available during filter operation, the lack of BDOC removals cannot be attributed to nutrient limitations.

Table 5-2: Raw NH$_4^+$-N and PO$_4^{3-}$-P concentrations for each sampling interval and overall average concentrations for experiment duration.

<table>
<thead>
<tr>
<th>Days of Operation</th>
<th>NH$_4^+$-N (mg/L)</th>
<th>PO$_4^{3-}$-P (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.11</td>
<td>0.059</td>
</tr>
<tr>
<td>11</td>
<td>0.09</td>
<td>0.062</td>
</tr>
<tr>
<td>19</td>
<td>0.13</td>
<td>0.111</td>
</tr>
<tr>
<td>25</td>
<td>0.17</td>
<td>0.088</td>
</tr>
<tr>
<td>35</td>
<td>0.20</td>
<td>0.075</td>
</tr>
<tr>
<td>39</td>
<td>0.14</td>
<td>0.072</td>
</tr>
<tr>
<td>70</td>
<td>0.15</td>
<td>0.072</td>
</tr>
<tr>
<td>Average</td>
<td>0.14</td>
<td>0.077</td>
</tr>
</tbody>
</table>

*Dissolved Oxygen*

Dissolved oxygen (DO) concentrations were measured pre- and post-filtration for the final two sample collections in another attempt to examine possible anaerobic conditions that might have inhibited desired biological activity within the biofilters. Measurements were made using a YSI 550A dissolved oxygen meter. Calibration and sampling were carried out according to manufacturer recommendations.

Results of the DO measurements are shown in Table 5-3. Although DO information was only collected from two sampling events, the limited data seemed to indicate that adequate DO was available to the biofilters. During both events, DO levels were above 70%
saturation, confirming aerobic conditions were present. These results suggest that biological activity and microbial growth should not have been inhibited by the absence of oxygen.

Table 5-3: Influent DO concentrations at two sample collections.

<table>
<thead>
<tr>
<th></th>
<th>Influent DO (mg/L)</th>
<th>DO Saturation (%)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-Mar</td>
<td>10.97</td>
<td>96</td>
<td>7.9</td>
</tr>
<tr>
<td>30-Apr</td>
<td>7.51</td>
<td>77</td>
<td>15.0</td>
</tr>
</tbody>
</table>

5.2.4 Biofilm Development in Pilot-Scale Experiment

The crystal violet (CV) assay discussed in Section 3.2.3 was used in an effort to quantify biofilm development in the pilot-scale roughing biofilters. As discussed in Section 4.2.1, Biomax filter media proved to be a poor candidate for the CV assay. Therefore, only ABC5 media were collected for biofilm measurements.

Absorbance results were used to develop a biofilm growth curve shown in Figure 5-3. Over the 70-day roughing biofilter operation period, absorbance climbed from 0.085 AU to 0.400 AU. This is over 40% higher than the maximum value encountered during the static biofilm development experiment (Section 4.2.2), granted it occurred over a significantly higher time frame. The biofilm development results seem to indicate a sustained biofilm formation within the roughing biofilter, although they do not confirm the microbial activity required for the desired biological removal of waterborne contaminants.
Figure 5-3: Graph quantifying the establishment of biofilm in the ABC5 roughing biofilter over the duration of its operation.

### 5.2.5 Impacts of Roughing Biofiltration on Water Quality Parameters

The pilot-scale roughing biofilter study was conducted over a 70-day period at the Marceline WTP. Over that time, samples were periodically collected and analyzed in an effort to evaluate whether roughing biofiltration is an effective technology for reducing organics responsible for the formation of disinfection byproducts. The research was heavily focused on the biofilters’ effects on DOC and UV$_{254}$, two strong surrogates of DBP precursors. Nutrients (ammonia, phosphate, and nitrate) were also monitored to confirm desired biofilm development conditions were present and as secondary biological activity indicators. Findings for these various water quality analyses follow.

**Dissolved Organic Carbon**

Results of dissolved organic carbon analyses are shown in Figure 5-4. Influent DOC concentrations are labeled as “Raw” in the chart, while the effluent results for the three filter
columns are labeled in the legend. As previously mentioned, during the initial 35-day acclimation period, no real trend in DOC reduction emerged as microbial activity was allowed to establish within the filters. Because of this, filter flow rate was reduced from 150 mL/min to 20 mL/min to determine if significant reductions could be achieved with longer EBCT. The three collection points following flow reduction did not produce observable DOC reduction, as seen in Figure 5-4.

![Figure 5-4: Overall influent (Raw) and effluent DOC concentrations observed in the pilot study.](image)

During the pilot-scale roughing biofilter study, a control filter column was included to account for water quality improvements that may not be a direct result of roughing biofilter removal mechanisms. It was decided that only if water quality improvements in biofilter effluent were significantly more than in the control effluent could they be attributed to the roughing biofilters. Using this logic, Figure 5-5 and Figure 5-6 were constructed by calculating the DOC percent reduction, as follows:
Using this method, reductions in DOC attributable to the roughing biofilters would result in positive percentages and vice versa.

Figure 5-5: DOC reductions attributable to the ABC5 roughing biofilter.
Figure 5-6: DOC reductions attributable to the Biomax roughing biofilter.

It can be seen from Figure 5-5 that positive DOC reductions attributable to the ABC5 biofilter were never measured. Most DOC concentrations were statistically equivalent to the control filter column. Results from the Biomax filter were more diverse, but similarly inconclusive. As seen in Figure 5-6, prior to flow reduction, the Biomax filters consistently resulted in DOC concentrations higher or equivalent to the control column. Flow reduction seemed to minimally improve the Biomax filter performance as DOC reduction averages trended positive. However, only one of the two samples resulted in a minimally significant improvement over the control filter column.

$UV_{254}$

Similarly to DOC, $UV_{254}$ reduction was monitored as a surrogate for DBP precursor reduction. Throughout the pilot study, little to no reduction in $UV_{254}$ was observed, as shown in Figure 5-7. This likely indicates that the roughing biofilters did little to reduce the water’s DBP formation potential.
Figure 5-7: Overall influent (Raw) and effluent UV$_{254}$ concentrations observed in the pilot study.

Ammonia-Nitrogen

Ammonia-nitrogen (NH$_4^+$-N) was monitored primarily as an indicator of biological activity within the roughing biofilters. The oxidation of ammonia to nitrate is an easily measurable process that can be used to confirm microbial activity. Figure 5-8 displays NH$_4^+$-N monitoring results from the 70-day pilot study.

Similar to the other monitored water quality parameters, the biofilters never caused a drastic reduction in NH$_4^+$-N. In the first three samples (Day 0, 11, and 19), effluent concentrations were essentially equal to raw concentrations. On Day 25, an NH$_4^+$-N reduction is observed, but control and biofilter effluent NH$_4^+$-N concentrations are essentially equal. However, after flow reduction, some NH$_4^+$-N reduction trends do appear to occur. On both Day 39 and 70, biofilter NH$_4^+$-N concentration are lower than influent and control concentrations. Although reductions are minimal, the limited data could indicate a
growing trend. This data could allude to a much longer acclimation period than initially expected.

![Graph showing NH₄⁺-N concentrations](image)

**Figure 5-8:** Overall influent (Raw) and effluent NH₄⁺-N concentrations in the filter columns.

### 5.3 Discussion

Pilot-scale roughing biofilters were installed at the Marceline WTP and monitored over a 70-day time period. During the first 35-day period, the filters were operated at a 150 mL/min flow rate and were allowed to acclimate to their environment with minimal operation adjustments. During this period, no discernable trends were observed in the water quality trends. Due to concerns that the 150 mL/min flow rate may be too high, the filters were adjusted to 20 mL/min in an attempt to prompt improved contaminant removal.

Simultaneously, an effort was made to address possible sources of biological inhibition. Nutrient-limiting conditions have become a subject of concern in drinking water biofilters (Brown 2011; Liu et al. 2004). Because of this, influent nutrient concentrations were evaluated to ensure optimal conditions. In general, sufficient amounts of NH₄⁺-N and
PO₄³⁻-P were available to remove at least 1 mg/L of BDOC (Table 5-2). Because these levels of organic carbon removal had not been observed, it was assumed that sufficient nutrients were available to filter microbes. Dissolved oxygen was also analyzed to ensure sufficient levels were present in filter effluent. During two sampling events, DO levels were measure as being above 70% (Table 5-3), indicating ample levels to provide an aerobic environment to the filter microbes.

Over the 70-day pilot study, multiple water quality parameters were monitored. Special attention was paid to DOC and UV₂₅₄ as these parameters serve as surrogates for DBP precursors. During the pilot study, the ABC5 biofilter never resulted in DOC reduction and, in fact, generally resulted in increased DOC when compared to the control filter column (Figure 5-5). The Biomax filter initially performed worse that the ABC5 filter in regard to DOC concentrations, but seemed to improve over the pilot study. However, although average DOC reductions appeared to improve, only one sampling provided a statistically significant minimal DOC reduction as compared to the control filter column (Figure 5-6). Overall, no drastic reductions in DOC or UV₂₅₄ were ever observed during the pilot study, indicating that roughing biofilters, as they were designed and operated in this study, provided no observable reduction of DBP precursors.
6.0 Summary and Suggested Future Research

6.1 Summary

Although biological treatment has traditionally been associated with wastewater treatment, it has recently emerged as a viable treatment process in drinking water as well. Recently, biologically active filtration, or biofiltration, has been successfully used to removed metals, nutrients, and organic carbon. However, polishing biofilters used at the end of the water treatment process often encounter coagulation-induced nutrient limitations that can cause significant adverse effects, including excessive headloss.

As an alternative to the polishing biofilter, a roughing biofilter was proposed as a way to alleviate nutrient limitation issues experienced later in the treatment process. The roughing biofilters explored in this paper consisted of two coarse filter media types: ABC5 and Biomax (Figure 3-1) whose high porosity would avoid clogging related to sediment laden raw water, but whose relatively high specific surface area might provide adequate area for biofilm formation.

A preliminary experiment explored a biofilm quantification method while observing biofilm growth in a static lab setting. The two filter media types were used as biofilm growth substrate and were incubated in four various raw water sources. This experiment revealed limitations in the crystal violet (CV) biofilm assay. Results generated by the Biomax media were highly variable with no development trend observed over time (Figure 4-1). However, ABC5 proved a good candidate for biofilm quantification using the CV assay. Although the curves developed were somewhat sporadic and contained outliers, obvious increases in
biofilm were observed. Modifications were made in the protocol to cut down on experimental error when using the method in the subsequent pilot-scale study.

A pilot-scale roughing biofilter study was carried out at the Marceline WTP over a 70-day time period. Initially, the biofilters were operated at 150 mL/min while they were allowed to acclimate biologically. During the first 35 days, no discernable trends were observed in the water quality trends, contrary to expectations. At this point, filters were adjusted to 20 mL/min in an attempt to promote improved contaminant removal.

Although biofilm establishment was verified in the roughing biofilters (Figure 5-3), little to no observable contaminant removal occurred within the biofilters (Figure 5-4 through Figure 5-7). This could be due to a number of reasons, but it is likely microbial activity within the filters is at a level that is undetectable by the methods used. Increasing this activity to an observable level could be accomplished by a number of actions. Increasing empty bed contact within the filters would be the most obvious. This would most easily be accomplished by increasing bed depth. Another suggestion might be to increase the interaction between biofilm and water. This might be achieved by applying water via spraying, similar to trickling filters, prompting water to travel over media surfaces in thin sheets, rather than as a bulk fluid.
6.2 Suggested Future Research

Future research should focus on improving roughing biofilter design to provide adequate waterborne contaminant removal. As previously mentioned, increasing the interaction time between water constituents, such as organic carbon, and biofilm might result in more observable water quality improvements. Suggestions to accomplish this are to increase media depth or consider applying water through a spraying mechanism, as is common in trickling filters. Increasing media depth increases the EBCT of the filters, allowing more time for redox reactions responsible for biodegradation to occur. Spraying water on biofilters allows water to percolate over the filter media in thin sheets rather than as a bulk liquid, fostering greater contact with surface biofilm.

Another suggestion would be to attempt to increase the amount of biomass within the biofilters in order to encourage biofilm to contaminant interaction. This could be partially accomplished by adjusting environmental conditions, such as nutrient loading, to encourage more robust biofilm formation. Another suggestion would be to evaluate more filter media types with increased specific surface area and lower porosity that are capable of greater biofilm development capabilities. However, it is important to maintain necessary hydraulic performance in biofilters while fostering optimal biomass development.

In future experimentation, it is suggested that biofilters be started up under more controlled conditions (such as in the lab) where more environmental factors can be manipulated and the biofilter responses can be observed. This is suggested, in part, because concerns about detrimental conditions such as low temperatures and minimal nutrient levels might inhibit desired biofilm growth and activity, especially during the startup period. In the
lab setting, biofilters could be dosed with high nutrient waters and biological activity of biofilms could be confirmed prior to pilot scale installation. This would also simplify experimental observations by eliminating the need to transport samples to the lab and allowing highly regular sampling. During this research, it was hypothesized that the biofilters in this study might require longer acclimation times than the experiment duration. Lab-scale experiments could be run as long as necessary to achieve steady-state performance prior to installation at water treatment plants.

Implementing additional parameters might provide a better understanding of biofilter processes. An organic carbon parameter that specifically measures the biodegradable organic carbon (BDOC) present in a sample would provide more insight into roughing biofilter performance and may lead to modeling abilities in the future. Additionally, pretreatment processes that may increase the BDOC, such as ozonation, may also be worth considering. Using additional methods that determine the fractionation of organics could determine the impact biofilters have on the type of organics, especially when no reduction in the amount of organics is detected. With regards to microbial measurements, more direct methods should be used to quantify microbial activity, such as heterotrophic plate and direct microscope counts. These may allow for better correlation between microbial activity and contaminant removal.


