# INVESTIGATION OF THE PERFORMANCE OF BIOLOGICALLY-ACTIVE GAC FILTERS FOR TASTE AND ODOR REMOVAL

A THESIS

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## Dedication

This thesis is dedicated to all of my friends and family who continuously provide love, support, and laughter.

## Abstract

Minneapolis Water Treatment and Distribution Services (MWTDS) is a surface water treatment plant that experiences occasional earthy/musty taste and odor problems caused by geosmin, an algal metabolite, during the spring and summer. Currently, MWTDS is using powdered activated carbon (PAC) to remove geosmin. PAC is inefficient, however, because only about 2 hours of contact time is available and the carbon is equilibrated with the low effluent concentration. Thus, large doses of PAC are needed, which can be costly. Granular activated carbon (GAC) as a filter media is an alternative process that can be used to remove geosmin. The GAC filter media becomes biologically active and can effectively remove taste and odor compounds to below their taste and odor threshold via a combination of sorption and biological degradation.

To investigate the performance of biologically-active GAC filters for geosmin removal, in addition to overall filter performance, an eight-column pilot-scale set-up at MWTDS was operated from November 2013 to May 2014. Three columns contained Calgon F300 GAC, two columns contained Norit 300 GAC, and three columns contained anthracite (non-sorptive control). Additionally, the microbial communities on these biofilters were characterized and the biological activity was measured. Interestingly, anthracite and Calgon F300 GAC had a high amount of bacteria ($10^6$-$10^8$ 16S rRNA genes copies per gram of dry weight) throughout the entire study, while Norit GAC did not reach ~$10^6$ 16S rRNA gene copies per gram of dry weight until February. All three media types developed highly diverse microbial communities (Shannon index 3.5-4.5) that were dominated by *Proteobacteria*. At the genera level, anthracite was dominated by *Hydrogenophaga*, *Sphingomonas*, and *Flavobacterium*. *Hydrogenohpaga* is a known

hydrogen-oxidizing bacteria and *Sphingomonas* is a strict aerobic that is widely distributed in nature and shown to survive in low concentrations of nutrients. *Flavobacterium* is a facultative anaerobic bacterium that is commonly found in soil and water. Both the Calgon F300 GAC and Norit 300 GAC were dominated by *Hydrogenophaga*, *Rhodobacter*, and *Rhizobacter*. *Rhodobacter* species have a variety of metabolic capabilities and are found in freshwater and marine environments. *Rhizobacter* are known to be root-colonizing bacteria that can fix nitrogen. Bacteria from the genus *Legionella* were detected on the filter media but they comprised less than 0.3% of the microbial community. This genus contains known pathogenic species of bacteria but we do not know whether any of the strains on the filter media are virulent.

The influent geosmin concentration varied from the target concentration (100 ng/L), however, the GAC effluent samples were consistently lower than the taste and odor threshold. The particle removal efficiency ranged from 52-59% for all three media types and was worse during the spring runoff when the filter effluent turbidity was above 0.1 NTU consistently. Overall, this study showed highly diverse microbial communities on filter media that can provide stability to the filtration process. Both media types had similar particle removal performances and both had difficultly during spring runoff. An advantage of the GAC media is its physical and biological ability to remove a portion of the organic carbon creating a more biologically stable water.

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# 1 Introduction

Ensuring access to safe drinking water is a complex and critical challenge for human societies. The amount of fresh water on Earth is finite and small (2.5%), and only 31% of the fresh water is accessible as the rest is trapped in glaciers (USGS 2014). The quality of the fresh water varies depending on the source. In general, in less developed countries, water is consumed directly from the source while more developed countries use treatment plants to increase the safety of the drinking water by removing or inactivating pathogens. In the United States, approximately 32% of the population receives water from treatment plants that use ground water and 68% of the population receives water from treatment plants that use surface water as their source water (USEPA 2009). For facilities using surface water, complying with the current federal regulation requirement to reduce the risk of infection to 1:10,000 (or lower) requires treatment facilities to reduce infectious viruses by 99.99% and protozoan parasites by 99.9% (Reynolds et al. 2008). The conventional treatment processes used to achieve these goals include coagulation, flocculation, sedimentation, filtration, and disinfection.

The primary goal of drinking water treatment plants is to provide water that is safe to drink; an important secondary goal is to provide water that is aesthetically pleasing in taste, odor and appearance because consumers associate the quality and safety of their water with these physical characteristics (Jardine et al. 1999). While aesthetic problems typically do not reflect water safety concerns, treatment plants still have to mitigate taste and odor issues in order to maintain public confidence and satisfaction with their product.

Although treatment plants encounter many taste and odor compounds, some of the most commonly found are algal metabolites. Two of the algal metabolites, 2-methylisoborneol (MIB) and geosmin, produce an earthy/musty odor that can be very problematic for surface water treatment plants because of the odor threshold concentrations are very low (15 to 18 ng/L for MIB and 1.6 to 3.8 ng/L for geosmin) (Young et al. 1996). Also, conventional treatment processes are not effective at removing these compounds (Chow et al. 1998, Hattori 1988, McGuire et al. 1988, Montiel 1983). Thus, advanced treatment processes are frequently required to remove geosmin and MIB below their taste and odor (T&O) threshold levels.

There are a variety of advanced processes that can either physically, chemically, or biologically decrease the concentration of geosmin and MIB. Physical processes include granular activated carbon (GAC) and powdered activated carbon (PAC) that remove the T&O compounds via sorption. Chemical processes include ozone and/or hydroxyl radicals that remove the T&O compounds via chemical oxidation. Biological processes include biologically-active filters that remove the T&O compounds via biodegradation. A facility can implement one or more of these processes depending on the cost and other water quality objectives desired.

Minneapolis Water Treatment and Distribution Services (MWTDS) is a surface water treatment plant that supplies drinking water to the city of Minneapolis, MN and surrounding suburbs. MWTDS withdraws water from the Mississippi River and treats the water with coagulation & flocculation, lime softening, recarbonation, sedimentation, granular media (anthracite-sand) or membrane filtration, and disinfection (Figure 1-1). The plant has a maximum capacity of 120 million gallons per day (MGD), but average

annual production is approximately 70 MGD. MWTDS used only conventional granular media filtration until 2005 when the utility built an ultrafiltration membrane plant at the Columbia Heights facility. Currently, about 30% of the water is treated by membrane filtration and 70% by granular media filtration.

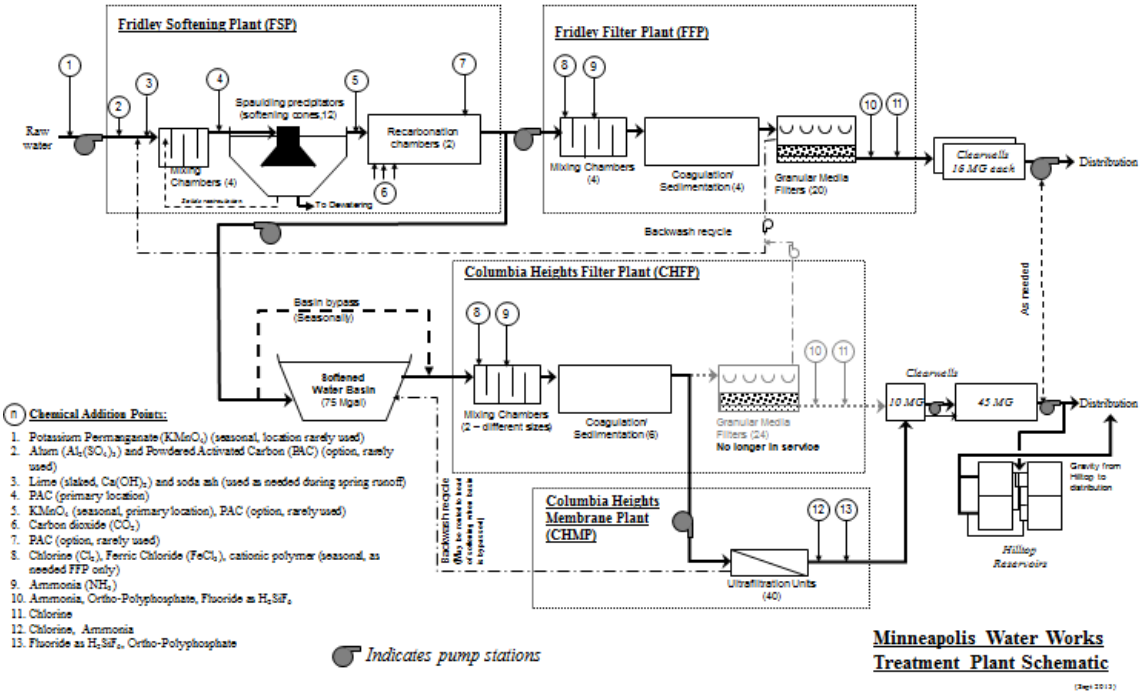


**Figure 1-1. Schematic diagram of the MWTDS treatment process.**

MWTDS occasionally experiences episodes of geosmin in their raw water above the T&O threshold. Neither membrane filtration nor granular media filtration at MWTDS is able to decrease geosmin below the taste and odor (T&O) threshold (3.8 ng/L) (Young et al 1996). From October 2012 to March 2014, 37% of the raw water samples had geosmin concentrations that exceeded the T&O threshold with the maximum observed geosmin concentration in November 2013 of 30.16 ng/L. MWTDS has received numerous complaint calls concerning taste and odor that typically increase in number

during the summer months (Figure 1-2). These complaint calls do not necessarily correspond solely to earthy/musty or geosmin-like tastes and odors. For example, a fishy odor often corresponds with the spring snowmelt. In addition to geosmin, MWTDS also measures the microbial taste and odor compounds: 2,4,6-trichloranisole (TCA), 2-isopropyl-3-methoxypyrazine (IPMP), and MIB (2-methyl-isoborneol). TCA has a moldy damp odor, while IPMP is more of a woody, stale odor. For the most part TCA has been below the detection limit (0.1 ng/L) while IPMP and MIB were often detected but remained below their T&O thresholds of 0.05 ng/L and 6.3 ng/L, respectively. Thus, the chemical or chemicals responsible for the fishy odor remains unclear. Additionally, some of these complaint calls could correspond to the taste and odor associated with chloramine residual. Regardless, MWTDS appreciates consumer feedback and strives to remediate the taste and odor problem though continuous monitoring and research into alternative technologies.

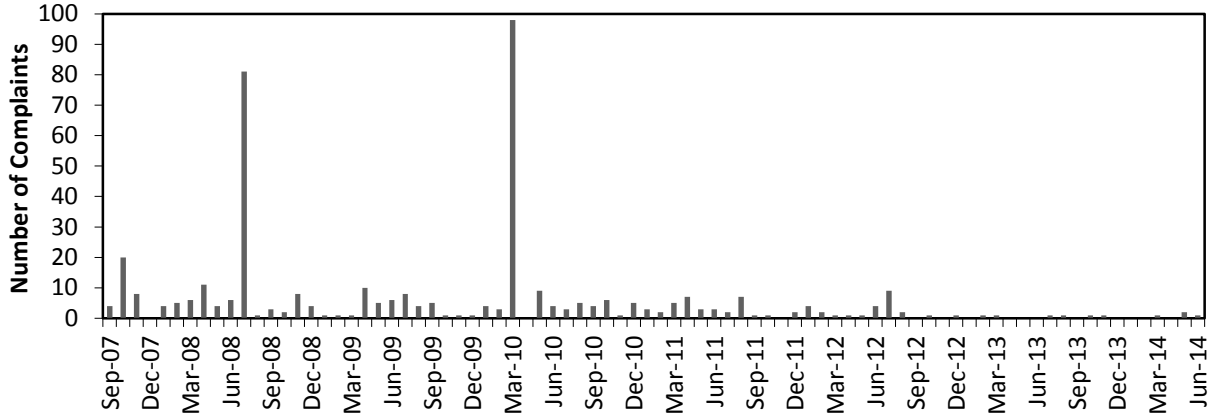


**Figure 1-2. Complaint calls MWTDS received concerning the taste and odor of the finished water.**

When MWTDS experiences geosmin episodes, powdered activated carbon (PAC) is used to lower the geosmin concentration via sorption. PAC, however, is relatively inefficient because the sorbate loading (ng geosmin/mg PAC) is dictated by the very low required target compound concentration for the finished water (< 10 ng/L). Additionally, the relatively short PAC contact time available at the treatment plant (~1-2 hrs) means that sorption equilibrium cannot be achieved. Thus, prohibitively large and costly PAC doses (>20 mg/L) (Rescorla 2012) would be needed to achieve an acceptable effluent geosmin concentration. An alternative option is to use granulated activated carbon (GAC) in a fixed bed. GAC beds can both physically and biologically remove geosmin via sorption and biodegradation, respectively.

This thesis concerns an investigation of the effects of replacing the anthracite-sand filters with GAC biofilters on geosmin removal, particle removal, and microbial community composition. A pilot-scale set-up of eight columns was run for 7 months to compare the effectiveness of anthracite (a no-sorption control) and GAC media for geosmin, particle, and organic carbon removal. The microbial community present on the granular media filters was also assayed by high-throughput Illumina sequencing of PCR-amplified 16S rRNA genes as well as by quantitative PCR (qPCR) targeting 16S rRNA genes for *Bacteria* as well as qPCR targeting *amoA* genes for ammonia-oxidizing *Bacteria* (AOB) and ammonia-oxidizing *Archaea* (AOA). Nitrifying microorganisms were studied because previous work done by Hope-Wilkinson (2012) showed *Nitrospira*, the phylum containing known nitrite-oxidizing *Bacteria*, was the most dominant on GAC biofilters representing from 17 to 24.5 % of the biofilter communities. The presence of

nitrifying organisms on these GAC biofilters suggests that nitrogen cycling is important in these systems. To better understand nitrogen cycling in these filters, ammonium, nitrite, and nitrate concentrations were also measured. Adenosine triphosphate (ATP) was measured to determine the amount of metabolically active cells on the media. This column study provided MWTDS with the data needed to assess the benefits of GAC compared to anthracite with respect to geosmin and particle removal performance. In addition, this study contributed to the general understanding of microbial community development in biofilters, as well as the function of those communities (i.e., nitrogen cycling, geosmin biodegradation).

# 2 Literature Review

## 2.1 Taste and Odor

The goal of water treatment is to provide customers with safe and clean drinking water. In addition, it is critical to produce water that is aesthetically pleasing because the organoleptic properties of water influence consumer perception of water quality (Doria et al. 2009, Jardine et al. 1999). Clarity, color, taste, and odor are the important aesthetic characteristics of drinking water (Dietrich 2006). Clarity is a function of particle concentration; color is typically due to the presence of natural organic matter (NOM) (Reuter et al. 1972, Weber et al. 1975), while taste and odor can be attributed to a wide variety of organic and inorganic compounds (Suffet et al. 1999).

### 2.1.1 Common taste and odor compounds, sources, and threshold concentrations

Taste and odor compounds originate from natural and anthropogenic sources such as sewage effluents, agriculture runoff, and algae/cyanobacteria. A taste and odor wheel was developed to better identify and understand the relationship of the most common taste and odor chemicals in drinking water and their causes (Suffet et al. 1999). The wheel consists of five different taste categories and eight different odor categories.

The odor category *earthy/musty/moldy* is a common descriptor of consumer complaint calls for treatment plants with surface water as their primary source. This category also contains the most potent taste and odor compounds that tend to be microbial metabolites such as TCA (2,4,6-trichloranisole), geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), MIB (2-methyl-isoborneol), IBMP (2-isobutyl-3-methoxypyrazine), and IPMP (2-isopropyl-3-methoxypyrazine) (Young et al. 1996). Actinomycetes and cyanobacteria (blue-green algae) are the most common producers of geosmin and MIB

(Izaguirre et al. 2004). A cyanobacterium can catalyze the cyclization of farnesyl diphosphate to geosmin (Gigilo et al. 2009). Additionally, most species of *Streptomyces* can also produce geosmin (Giglio et al. 2009). The microbial metabolites responsible for earthy musty odors have low taste and odor (T&O) threshold concentrations making them a nuisance for treatment plants (Table 2-1).

**Table 2-1. Odor and Taste theshold concentration for common microbial metabolites that produce earthy musty odors. (Source: Young et al. 1996)**

| Compound | Geometric mean odor threshold concentration (ng/L) | Lowest detectable concentration of odor (ng/L) | Geometric mean taste threshold concentration (ng/L) | Lowest detectable concentration of taste (ng/L) | Odor and taste description |
|---|---|---|---|---|---|
| **Geosmin** | 3.80 | 1.30 | 1.6 | 7.5 | Musty, earthy, grassy |
| **MIB** | 15.0 | 6.30 | 18.0 | 2.5 | Musty, earthy, peaty |
| **IBMP** | 1.0 | 0.05 | 3.0 | 0.4 | Woody, stale, coal-ash |
| **IPMP** | 0.2 | 0.03 | 20.0 | 9.9 | Sooty, dusty, cabbage |

### 2.1.2 Overview of removal mechanisms

Conventional drinking water treatment consisting of coagulation, flocculation, sedimentation and filtration does not effectively remove these earthy musty taste and odor compounds (Chow et al. 1998, Hattori 1988, McGuire et al. 1988, Montiel 1983). Thus, an advanced treatment technology such as chemical oxidation or activated carbon often is required to ensure removal to concentrations below the taste and odor threshold.

Chemical oxidation can be effective at removing a wide variety of organic pollutants. Because common oxidants such as free chlorine and chloramine are not powerful enough to effectively oxidize geosmin and MIB, so-called advanced oxidation processes are typically used. Advanced oxidation processes, in which ozone is added along with hydrogen peroxide or UV light, can effectively remove geosmin from water. Ozone can react with natural organic matter (NOM) or other constituents in the water to produce secondary oxidants such as hydroxyl radicals, but the addition of hydrogen peroxide or UV light greatly enhances free radical production. Hydroxyl radicals, because they react non-specifically and at high rates (i.e., mass transfer limited), are the key to trace contaminant removal. The second order reaction rate constants for molecular ozone and hydroxyl radicals with geosmin are $K_{O3}$= 0.10 $M^{-1}s^{-1}$ and $K_{\cdot OH}$= 7.8 x$10^9$ $M^{-1}s^{-1}$, respectively (Peter et al. 2007). The character of the NOM will influence radical formation/degradation rates, and hence, the removal efficiency of geosmin (Ho et al. 2002). Although radicals can react with a wide variety of organic compounds such as geosmin and MIB, one study suggested that ozonation altered fishy odors to a more "plastic-like" odor (Hargeshemer et al. 1996). Ozonation/advanced oxidation systems are also known to be relatively expensive to install and operate (Glaze 1987).

## 2.2 Activated carbon for water treatment

### 2.2.1 Introduction and History

Activated carbon is an advanced treatment process that can effectively remove trace contaminants such as geosmin. Activated carbon was developed in 1867 by Jon Stenhouse for use in respirators (Miles et al. 1958). It was first applied for water

treatment in the beginning of the late 19th century in Hamm, Germany. It was not until the 1970s, however, that GAC started to become more widely used. This was attributed to the need to remove organic matter and limit possible disinfection-by-product (DBP) formation (Crittenden et al. 2012).

Activated carbon is made from carbonaceous material like coal, peat, or coconut shells. The raw material goes though pyrolytic carbonization followed by activation. The primary mechanisms for removal of organic contaminants from water are adsorption and absorption. Adsorption is a process where dissolved solutes partition from the liquid phase to the surface of a solid phase. Absorption is a process where dissolved solutes are assimilated into the solid phase. It is often unclear and difficult to determine if adsorption or absorption is occurring; thus, the term sorption is used. The dissolved solutes are referred to as the sorbate and the activated carbon is referred to as the sorbent. Physical sorption of organic carbon occurs from non-specific binding mechanisms such as van der Waals forces.

The key to the effectiveness of activated carbon as a sorbent is its large surface area. Another important parameter is pore size, partly because of its effect on available surface area because there is an inverse relationship between pore size and surface area (Crittenden et al. 2012). Thus, as average pore size increases, there is less surface area available for sorption. Granular activated carbon has a range of pore sizes, including micropores (less than 20 nm), mesopores (between 20 to 500 nm), and macropores (greater than 500 nm). Calgon, US Filter, MWV and Norit are commercial manufacturers that produce activated carbons with surface areas ranging from 400 to 1500 $m^2/g$ and pore volumes ranging from 0.1 to 0.8 mL/g (Crittenden et al. 2012).

### 2.2.2 Organic compound removal

Activated carbon can be used for water treatment in either the powdered form (PAC) or the granular form (GAC), the only difference being the grain size (i.e., PAC = 20 to 50 μm and GAC = 0.5 to 3 mm). PAC is dosed directly into the water and then removed via sedimentation and/or filtration. PAC is generally added near the treatment plant influent, prior to any chemical oxidant or coagulant addition, in order to maximize the contact time between the PAC and the trace odor causing compounds. PAC is typically applied at doses ranging from a few mg/L up to about 20 mg/L. A dose of 20 mg/L PAC removed an influent concentration of 40 ng/L geosmin to < 10 ng/L (Cook et al. 2001). The dose of PAC required, however, will depend on the carbon type, the concentration of the target chemical, the concentrations of competing sorbates (e.g., NOM), and the available contact time. A competitive effect occurs between NOM and the adsorption of trace contaminants such as taste and odor compounds (Lalezary-Craig et al. 1988). The point of PAC application and flow rate through the treatment plant will determine the contact time. PAC, however, is relatively inefficient because the sorbate loading (ng geosmin/mg PAC) is dictated by the very low required target compound concentration for the finished water. Additional inefficiency results from the relatively short contact times available in treatment plants (e.g., a few hours) such that sorption equilibrium is not achieved. Hence, high and costly PAC doses may be needed to achieve an acceptable effluent geosmin concentration.

An alternative option is to use GAC in a fixed bed configuration. In this mode, the GAC provides particle removal as well as removal of dissolved organic compounds via sorption. A significant advantage of GAC over PAC is that the GAC is equilibrated

with the influent contaminant concentration, so that much higher contaminant loadings can be achieved and less carbon per unit volume of water is needed for treatment. Furthermore, the surface of GAC provides a place for bacteria to attach. The attached bacteria can remove dissolved organic compounds from the water via biodegradation. The benefits of this biological activity on GAC performance are discussed below in a section on biofiltration.

Like PAC, the effectiveness of geosmin removal by GAC is also hampered by competition for sorption sites by other sorbates such as NOM molecules. NOM is especially problematic because it occurs at concentrations approximately 3 to 4 orders of magnitude higher than geosmin. Even larger MW NOM molecules can be problematic because they can block access to smaller pores and decrease overall sorption capacity for target compounds. Thus, removal of dissolved organic carbon (DOC) prior to activated carbon treatment improves geosmin removal via sorption (Newcomb and Cook 2002). Despite issues with NOM competition, however, GAC has proven to be effective at removing geosmin (Dirkas 2009, Scharf et al. 2010).

### 2.2.3 Batch sorption experiments

Batch sorption experiments are used to determine the affinity of a sorbate to a sorbent such as GAC. The goal is to determine the sorption capacity or the amount of sorbate that will sorb to the sorbent at equilibrium and constant temperature. Batch sorption experiments are performed by dosing a range of sorbent masses into a series of batch bottles containing the same initial concentration of sorbate. After equilibrium is achieved, the aqueous concentrations of sorbate in the bottles are measured and then the

sorbed phase concentrations are determined by difference. The experimental data can then be plotted as an isotherm and fit to one or more isotherm models.

The first step, however, is to determine how long to incubate the bottles in order to achieve "equilibrium". This is done by batch kinetic experiments in which water containing the target compound is dosed with activated carbon and samples of the water are collected over time for analysis of target compound concentrations. The data can be fit to a kinetic model such as a pseudo-first order model (Equation 1), a pseudo-second order model (Equation 2), or an intraparticle diffusion model that was developed by Weber and Morris (1963). In these model equations, the amount of solute sorbed at equilibrium and time t is represented by $q_e$ (mg/g) and q(mg/g), respectively. The rate constants are represented by $k_1$ (/h), $k_2$ (mg/g/h), or $k_i$ (mg/g/$h^{1/2}$). For the intraparticle diffusion model, θ is the intercept from plotting q (mg/g) versus $t^{1/2}$ ($h^{1/2}$) (Zhang et al. 2012).

$$(1)\ \ln(q_e - q) = \ln(q_e) - k_1 t$$

$$(2)\ \frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

The kinetic model can then be used to determine the time needed to reach 95% or 99% of the true equilibrium sorbed phase concentration. For batch activated carbon experiments, the necessary incubation time is usually on the order of 1 to 3 days (Zhang et al. 2012). For GAC, the activated carbon is typically ground to a powder for the batch experiments in order to decrease the time needed to achieve equilibrium, as this decreases the length

of the pores. Because most of the surface area is internal, this conversion to PAC does not significantly alter the sorption capacity of the GAC.

Two commonly used isotherm models are the Freundlich and Langmuir models. The Langmuir isotherm (Equation 3) describes a fixed number of sites where molecules of sorbate can chemically bind in a reversible equilibrium (Langmuir 1918). It assumes a constant free-energy for each site and each site can bind one molecule making it a monolayer model. $Q_M$ (mg sorbate/ g sorbent) is the maximum sorbent-phase concentration of sorbate when the surface sites are saturated with sorbate A in solution. The term $b_A$ (L/mg) is the Langmuir sorption constant of the sorbent. The term $q_A$ (mg sorbate/ g sorbent) is the equilibrium sorbent-phase concentration of sorbate A. $C_A$ (mg/L) is the equilibrium concentration of sorbate A in solution. The linear form of the isotherm is shown in Equation 4 and can be determined by plotting $C_A/q_A$ vs $C_A$.

$$(3)\ q_A = \frac{Q_M b_A C_A}{1 + b_A C_A}$$

$$(4)\ \frac{C_A}{q_A} = \frac{1}{b_A Q_M} + \frac{C_A}{Q_M}$$

The Freundlich isotherm (Equation 5) is another two-parameter model that describes the sorption for heterogeneous sorbents (Freundlich 1906). The Freundlich parameters include the sorption capacity ($K_A$, (mg/g)(L/mg)$^{1/n}$) and the sorption intensity (1/n). The linear form of the isotherm is shown in Equation 6. The capacity and intensity parameters can be determined from a log-log plot of $q_A$ (mg sorbate/ g sorbent), the equilibrium sorbent-phase concentration of sorbate A, versus $C_A$ (mg/L), the equilibrium concentration of sorbate A in solution.

$$(5)\quad q_A = K_A C_A^{1/n}$$

$$(6)\quad log(q_A) = log(K_A) + \left(\frac{1}{n}\right) log(C_A)$$

Freundlich isotherms generally provide a better fit for adsorption to GAC because the Freundlich model accounts for more than a monolayer. The Freundlich model also assumes a distribution of sorption energies for the sorption sites, which better mimics activated carbon (Crittenden et al. 2012).

### 2.2.4 Packed-bed modeling

The sorption of a sorbate molecule to GAC involves a multistep process in which the molecules undergo external mass transfer from the bulk liquid to the surface of a GAC grain, internal mass transfer via pore diffusion and sorption to the surface of the GAC grain (Figure 2-1). Sorbed molecules can also move deeper into the pores via a parallel internal mass transfer process termed surface diffusion. These intra-particle mass transfer processes of pore and surface diffusion tend to control the rate of sorption in GAC applications (Crittenden et al. 1980).

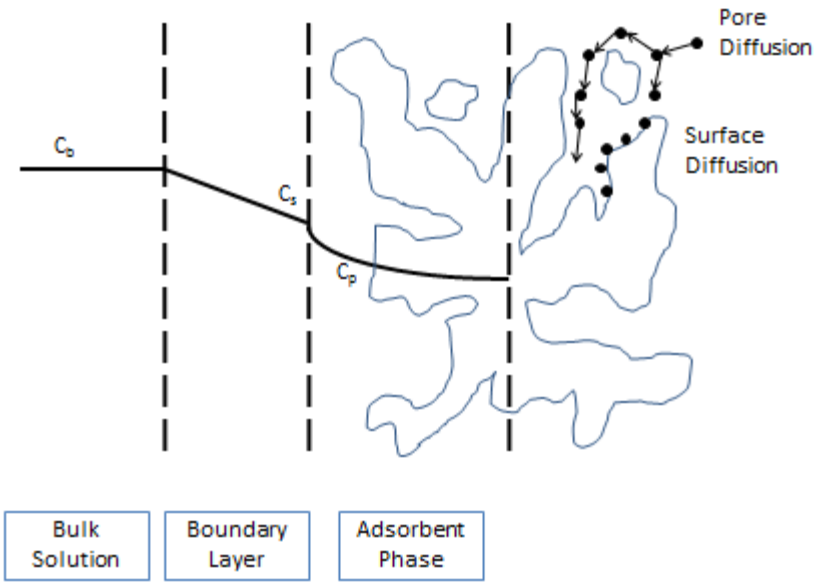


**Figure 2-1. The diffusion mechanisms involved in the adsorption process for GAC particles. (Source: Crittenden et al. 1987)**

The homogeneous surface diffusion model (HSDM), developed and finalized by Crittenden and co-workers in 1978, can be used to model the performance of packed-bed sorption columns. The HSDM model accounts for surface diffusion and assumes it is the rate-limiting step. This model has the following assumptions: plug-flow, constant hydraulic loading rate, spherical adsorbent, and Freundlich isotherms for sorption equilibrium (Hand and Crittenden 1983).

## 2.3 Biologically-active GAC filters for water treatment

### 2.3.1 Introduction

Drinking water treatment plants are increasingly using biofiltration to improve water quality and remove trace contaminants of concern. Biofiltration is an especially attractive treatment process due to the ability of the attached bacteria to biodegrade desorbed compounds and thus bioregenerate the GAC. Bioregeneration increases the life

span of the GAC by restoring sorption sites for trace compounds (Xiaojian et al. 1991), making biofiltration a very attractive and competitive with non-sorptive filter media.

When implementing biofiltration into a treatment plant, studies have shown it takes between 40-120 days for the microbial community to develop (Servais et al. 1994, Moll et al. 1998, Scharf et al. 2010). The operation of the filters, in addition to the water quality, can influence the microbial community that develops. Backwashing removes 20-40% of the original community members (Kasuga et al. 2007, Hozalski and Bouwer 1998). Nevertheless, the biofilm will build up to pre-backwashing concentration prior to the next backwash cycle and maintain similar community composition (Gilbert et al. 2013, Kim et al. 2014, Hozalski and Bouwer 1998).

An important parameter for utilities to consider when implementing biofiltration is the water temperature. Water temperature affects the organic matter removal rate, and tends to decrease with decreasing temperature (Kim et al. 2014). Increasing the empty bed contact time during colder temperatures can help to restore the organic carbon removal performance (Moll DM et al. 1999). The type of GAC used can also play a role in biofilm formation and filter performance. When chemically and thermally activated GAC types were compared, the thermally activated GAC was able to remove a higher percentage of dissolved organic carbon then the chemically activated GAC (Yapsakli et al. 2010). Although, the amount of organic carbon removal is impacted by temperature, and media type, overall, biofiltration is capable of organic carbon removal whereas anthracite media is not.

There are also some concerns with the implementation of biofilters in drinking water such as increased turbidity and possible pathogen release. Evidence shows that

some carbon fines can be released and may have attached opportunistic pathogens (Camper et al. 1986), however, disinfection is used after filtration to inactivate released microorganisms. Although biofiltration performance can vary based on the microbial community that develops, the backwashing procedure used, the water temperature and GAC type, overall the water quality is improved with this technology compared to conventional filtration.

### 2.3.2 Microbial Abundance

There are numerous reports in the literature concerning the microbial communities on GAC filter media and their activity. One approach for determining attached biomass levels on GAC involves detachment followed by conventional culture-based enumeration (e.g., plate counts). Reported bacterial populations on GAC filter media ranged from $10^5$-$10^8$ CFU/g GAC (Camper et al. 1985, Niemi et al. 2009). An alternate approach is to use so-called molecular (i.e., DNA-based) techniques for bacterial enumeration and the DNA can be extracted directly from the filter media (i.e., it is not necessary to detach the bacteria first). Gilbert et al. (2013) quantified genomic DNA throughout the 1.5 m depth of a GAC biofilter and observed a gradient from 4,263 ng/g GAC at the top to 1,576 ng/g GAC at the bottom. A bacterium typically contains about 2.5 fg of DNA per cell (Button et al., 2001), so these results equate to approximately 1.7 billion cells/g (top) and 0.63 billion cells/g (bottom). Many researchers measure adenosine triphosphate (ATP) on filter media because it provides an estimate of the amount of metabolically-active cells. ATP concentrations ranged from 50 to 10,000 ng ATP/g GAC for 30 different GAC filters throughout the Netherlands (Magic-Knevez

et al. 2004). The highest ATP concentrations from this study were from full-scale GAC filters with longer run times and ozone pretreatment. Niemi et al. (2009) obtained similar results (250 to 360 ng ATP/ g GAC). All of these studies suggest that GAC promotes the development of an active microbial community.

### 2.3.3 Community composition

Up to this point, most research has focused on determining the abundance of bacteria attached to the GAC though the measurement of ATP or quantifying the 16S rRNA *Bacteria* gene. Only a few studies have attempted to examine the composition of the microbial community on filter media. Three studies used pyrosequencing (Liao et al. 2013, Kim et al. 2014, and Pinto et al. 2012) while another study made a clone library (White et al. 2012).

White et al. (2012) made a clone library of a full-scale anthracite filter based on both 16S rRNA and *amoA* genes. The researchers reported that *Nitrosomonas*, *Nitrospira*, *Sphingomonadales*, and *Rhizobiales* dominated the clone library. Liao et al. (2013) used pyrosequencing to determine the microbial communities present in three GAC columns each fed different source water: untreated lake water, pretreated lake water, and pretreated lake water with ammonia. Pretreatment consisted of pre-ozonation, rapid mixing, flocculation, sedimentation, and post-ozonation. A sample of media from each column was collected for DNA extraction and sequencing after the columns were in operation for 5 months. The pretreated lake water column, which most closely represents the water a typical full-scale filter might receive, contained 63% *Proteobacteria*, 10.5% *Bacteriodetes*, and 6.5% *Planctomycetes* (Liao et al. 2013). The microbial community

present on the filter media is important because the filter microbial community can influence the microbial community in the distribution system (Pinto et al. 2012). Pinto et al. (2012) found that *Proteobacteria*, *Bacterioidetes*, *Actinobacteria*, and *Nitrospira* were the four dominant phyla detected in descending order of relative abundance on a full-scale BAC filter. Kim et al. (2014) collected four samples from a full-scale BAC filter, one from each season. *Proteobacteria, Bacteroidetes, Acidobacteria, Firmicutes, Actinobacteria,* and *Planctomycetes* were the six dominant phyla detected in descending order of relative abundance. Although these studies provided an in-depth analysis of the communities present, there are limitations. First, in the studies conducted by Liao et al. (2013), Pinto et al. (2012), and White et al. (2012), the filters were only sampled once, so the results represent merely a snapshot of the community composition in what is expected to be a dynamic system. Secondly, Kim et al. (2014) sampled more than once (i.e., one time point for each season), but the samples were collected from only one filter.

### 2.3.4 Function

There are many possible metabolic functions that might be carried out by the microbial community on GAC biofilters such as carbohydrate catabolism and ammonia oxidation. One metabolic process that appears to be prevalent in GAC biofilters is nitrification. Nitrification is the microbial process in which ammonia is oxidized to nitrite and then to nitrate. Typically, each step of the process is carried out by a different organism. The first step, converting ammonia to nitrite is carried out by ammonia oxidizing *Bacteria* and ammonia oxidizing *Archaea*. The second step, converting nitrite to nitrate, is carried out by nitrite oxidizing *Bacteria*. This first step is considered the

rate-limiting step as nitrite is typically not found to accumulate in the environment. Previous research on nitrifying organisms in biologically-active GAC filters is limited, and the results of the studies vary. Ammonia oxidizing *Archaea* were detected on the GAC filter media in one study (Kasuga et al. 2010) but not two others (Yapsakli et al. 2010, Feng et al. 2012). Ammonia oxidizing *Bacteria* were detected, however, in the later two studies. Similarly, in previous research conducted in our laboratory (Hope-Wilkinson 2012) concerning GAC biofilters, ammonia-oxidizing *Bacteria* were detected but not ammonia-oxidizing *Archaea.* Additionally, known nitrite-oxidizing bacteria from the genus *Nitrospira* were detected in biologically active carbon filters in which chloraminated water was used for backwashing (Pinto et al. 2012, White et al. 2012). Conversely, Kim et al. (2014) used non-disinfected water for backwashing and did not detect *Nitrospira*. Investigating the process of nitrification on these filters is important because a high concentration of nitrate (10 mg/L $NO_3$-N) in drinking water can cause methemoglobinemia, or "blue baby syndrome". Nevertheless, nitrification is preferable in the filters than in the distribution system, where ammonia oxidation can lead to the rapid loss of chlorine residual in chloraminated systems (Regan et al. 2002).

## 2.4 Implications for GAC bed life

### 2.4.1 Direct biodegradation

GAC has a finite number of sorption sites, and when those sites are filled by organic contaminants and the sorption capacity is exhausted, the carbon must be replaced. If the sorbates are also biodegradable, then the sorption capacity can be significantly extended by biological activity as the compounds are degraded before ever reaching a

sorption site (i.e., direct biodegradation) or after desorbing from the GAC (bioregeneration). Direct biodegradation is possible for all biodegradable compounds, but favorable for those that are hydrophilic and very labile, such as the organic acids and related compounds that make up what is termed assimilable organic carbon (AOC).

The microbial community that develops on GAC filters is very effective at biodegrading the AOC in the influent water. For example, a pilot-scale biological GAC filter provided 86% removal of AOC at an influent concentration of ~143 µg acetate-C/L (Chein et al. 2007). Removal of AOC from the water has multiple benefits such as reduced regrowth in the distribution system, reduced chlorine demand, and reduced disinfection by-product formation. The microbial community may also become enriched in bacteria that can aid in the removal of trace organic contaminants such as geosmin. Biofilters can also be seeded with specific bacteria, such as geosmin-degrading bacteria, to increase removal efficiencies of certain compounds of concern (McDowall et al. 2009). Overall, the biodegradation of AOC and trace organic contaminants resulting from the biological activity in GAC filters results in improved water quality compared to conventional filtration.

### 2.4.2 Bioregeneration

There are three phases of biological GAC filtration shown in Figure 2-2 (Dussert and Van Stone. 1994). The initial phase is dominated by sorption while the biofilm is becoming established. The second phase is where sorption equilibrium is reached and concurrently biodegradation starts to occur. In the third phase, biodegradation of desorbed substrates occurs which helps to maintain low aqueous concentrations. The low

aqueous concentrations provide a greater driving force for desorption, hence, the desorption rate increases. This biologically-enhanced desorption is referred to as bioregeneration. Bioregeneration is possible because of the reversibility of the sorption process (Atkas et al. 2007). Other factors that play a role in bioregeneration are physical and chemical properties of the carbon, substrate-carbon contact time, biodegradability of desorbed compounds, biomass concentration, carbon saturation and concentration gradient, and microorganism. Bioregeneration is advantageous because it extends the GAC bed life and reduces treatment costs for water utilities (Scharf et al. 2010).

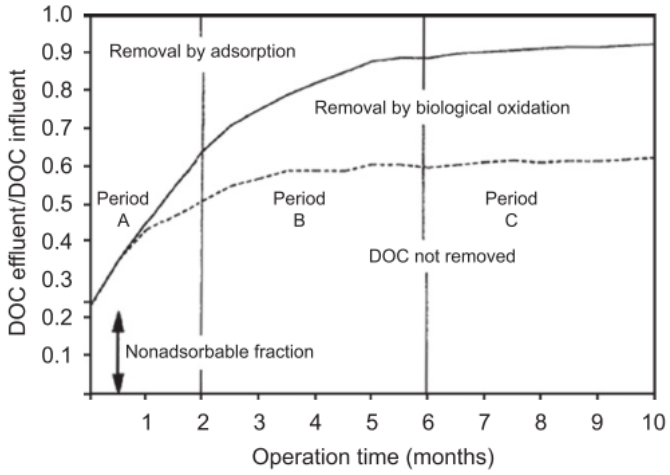


**Figure 2-2. The three phases of biological GAC filtration. The dashed line represents the amount of DOC removed via adsorption. The solid line represents the amount of DOC removed via biodegradation. (Source: Simpson et al. 2008)**

## 2.5 Summary and Research Needs

GAC has been implemented in treatment plants across the United States and the associated biological activity can improve effluent quality and reduced treatment costs by extending bed life. Although biologically-active GAC filters have been used since the 1970s to treat water, the biological processes occurring on the filter media are poorly

understood. Questions remain as to what microorganisms and specific functional genes are present, the specific biological reactions that occur, and the influence of filter operating conditions on community composition and function. An improved understanding of microbial community structure and function is expected to facilitate better control and performance of GAC biofilter.

# 3 Materials and Methods

## 3.1 Experimental Design

A pilot-scale filtration system was setup at the Minneapolis Water Works (MWW) Fridley facility to test the effects of filter media type on the removal of geosmin and particles. Ammonium, nitrite, and nitrate were also measured to determine if nitrification was occurring in the filters. Total organic carbon and dissolved organic carbon were measured as GAC biofilters can remove the organic carbon via sorption and biodegradation. There were a total of eight filter columns operated in parallel that were constructed from polycarbonate cylinders (outer diameter = 10.16 cm, inner diameter = 9.525 cm, length = 366 cm). Three columns were packed with anthracite (18 inches or 45.72 cm) over sand (10 inches or 25.4 cm) and the remaining five columns were packed with GAC (20 inches or 50.8 cm) over sand (12 inches or 30.48 cm). Four inches of pea gravel was underneath the sand in all 8 columns. The pea gravel was held up by a polycarbonate disk containing drilled 1/8” holes. Of the five GAC columns, three columns contained Calgon Filtrasorb 300 (Calgon Carbon, Pittsburgh, PA) and two columns contained Norit 300 (Cabot Norit, Marshall, TX). The anthracite media was previously collected from the full scale filters at MWTDS. All columns were first dry packed with 4 inches of pea gravel at the bottom to support the filter media. The columns were then dry packed with the filter media with periodic tapping to ensure adequate packing. The media characteristics are shown in Table 3-1. The anthracite-sand filter columns mimicked the current filter configuration at the plant while the Calgon F300 over sand mimicked the potential future media configuration. The Norit 300 columns

were included for comparison with the Calgon F300 columns to determine if GAC type plays a significant role in filter performance for this particular application.

**Table 3-1. List of properties and depth of media used in the pilot-scale column set-up at MWTDS.**

| | Sand | Anthracite | Calgon F300 GAC | Norit 300 GAC |
|---|---|---|---|---|
| Iodine Number | | | 900 mg/g (min) | 1089 mg/g |
| Effective Size | 0.41-0.46 mm | 0.66-0.9 mm | 0.8 mm | 0.8 mm |
| Uniformity Coefficient | 1.46-1.74 | 1.55-1.8 | 2 | 2 |
| Apparent Density | | | 0.56 g/cc | 0.47 g/mL |
| Abrasion Number | | | 78 (min) | 83 |

The flow of water through the pilot-scale column set-up began on November 13$^{th}$, 2013 and the columns were run until May 30$^{th}$, 2014. A detailed list of the pilot plant operational history can be found in Appendix A. Water from the full-scale recarbonation tank at MWTDS was used as the source of water for the experiments. The pH of the water was adjusted to ~8.5 using hydrochloric acid; ferric chloride was added as the primary coagulant at a dose of 3 mg/L. These chemicals were added using metering pumps (Model QG 20, FLUID Metering Inc., Syosset, NY.) and then mixed into the flow using Koflo Series 308 PVC inline static mixers (Koflo Corp., Cary, IL.). These chemical additions mimicked full-scale MWTDS operations. After chemical addition, the water flowed into a pilot-scale settling tank. From the settling tank, the water then entered a feed tank, after which geosmin (Dalton Pharma Services, Toronto, ON, Canada) was dosed into the water via a KDS Model 200 syringe pump (KD Scientific Inc., Holliston, MA.) to a target concentration of 100 ng/L. Water was then pumped using a

2.9 GPM high-head enclosed motor centrifugal pump (Cole-Parmer, Vernon Hills, IL.) to the top of each of the eight columns at a loading rate of 1 GPM/$ft^2$. Additionally, the influent and effluent of three of the columns passed through a 1720E Low Range turbidimeter (HACH, Loveland, CO.). A schematic and photo of the pilot-scale column set-up is shown in Figure 3-1 and 3-2. Detailed drawings and photos of the pilot-scale set-up are shown in Appendix C.

Backwashing of the columns was conducted every 4 days to mimic full-scale operation. The columns were backwashed using 1.3 scfm of air for 5 minutes followed by a 10-minute water wash with MWW finished water to achieve a 30% bed expansion. The MWW finished water contained chloramines at a concentration of 3.5 to 3.9 mg/L of $Cl_2$. The backwash flow rates for achieving 30% bed expansion were 9.9 gpm/$ft^2$ for the anthracite columns and 17 gpm/$ft^2$ for the GAC columns. More details on the backwashing protocol are provided in Appendix B.

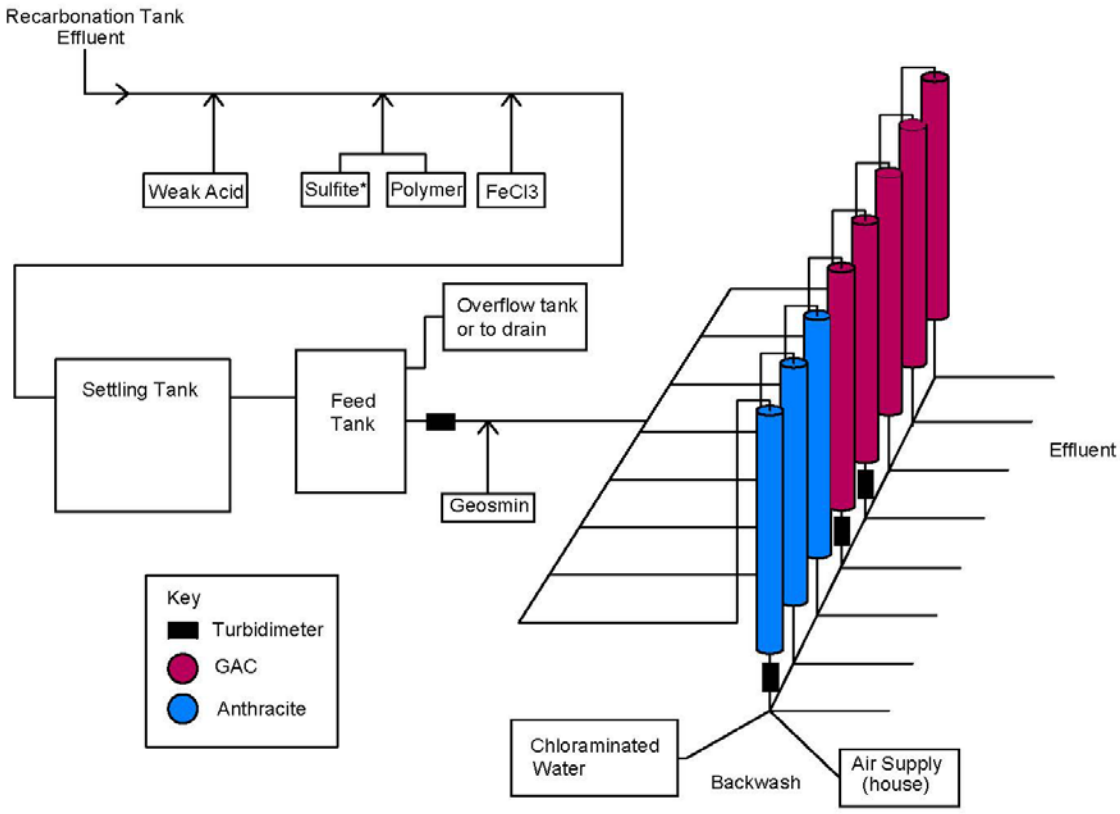


**Figure 3-1. A schematic diagram of the pilot-scale column system at Minneapolis Water Treatment and Distribution Services for studying biologically active GAC.**

**Figure 3-2. A photo of the pilot-scale column set-up at MWTDS for studying biologically active GAC.**

## 3.2 Sample Collection

Water samples were collected from the influent sample port and the effluent sample ports in 50 mL PYREX glass bottles for analysis of ammonium, nitrite, nitrate, and particle size distribution. For total organic carbon (TOC) and dissolved organic

carbon (DOC) measurements, organic carbon-free (baking at 550 °C for 5 hours) 40 mL glass sample vials were used for collection. All water samples were stored in the dark at 4 °C and analyzed within 7 days of collection. For media samples, the column was first drained and then a small amount of media was collected from the top of the filters using a scoopula that was wiped down with 70% ethanol. The media was placed in a 50 mL sterile RNase-and DNase-free falcon tubes. Media samples were collected monthly and placed in a -20°C freezer immediately after collection. The media samples were stored for up to 7 days before performing DNA extractions or 7 months before performing ATP analyses as described below.

### 3.3 Chemical Analyses

Total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations were measured via the UV-persulfate wet chemical oxidation method using a Sievers 900 Analyzer (GE Analytical Instruments, Boulder, CO). For DOC analysis, the water samples were first filtered through a nitrocellulose filter (diameter = 50 mm; nominal pore size = 0.45 μm). The organic carbon analyzer was calibrated using standards made by diluting a 1,000 mg C/L sodium hydrogen phthalate stock solution (Sigma-Aldrich, St. Louis, MO) with distilled and deionized (DI) water.

Ammonium was analyzed using the Nessler's reaction method (Thompson et al. 1951). First, a $ZnSO_4$ reagent was added to the water sample and the pH was increased to 10.5 with the addition of 6 N NaOH. This allowed for the precipitation of calcium, iron, magnesium, and sulfide from the sample. Following centrifugation, the supernatant was removed and EDTA was added to the solution to prevent further precipitation. Nessler's

reagent was then added to the solution and after mixing the sample was analyzed on a Beckman DU530 UV/Vis spectrophotometer using a 1 cm plastic cuvette (Beckman Coulter, Indianapolis, IN.) for absorbance at 420 nm. Standards were made by diluting a 7.14 mM $NH_4$-N stock solution prepared from anhydrous $NH_4Cl$ (Sigma-Aldrich, St.Louis, MO). The quantification limit was 0.5 µg NH4+-N/ mL.

Nitrite and nitrate were analyzed via ion chromatography (Metrohm, Riverview, FL.). A 3.3 mM carbonate buffer solution was used as the eluent and 100 mM $H_2SO_4$ was used as the regenerant. The anion separation was performed using a 6.1006.520 Metrosep A Supp 5 column (Metrohm, Riverview, FL.) at a flow rate of 0.7 mL/min. Nitrite and nitrate standards were made from $NaNO_2$ and $NaNO_3$ stock solutions prepared from reagent grade salts (Sigma-Aldrich, St.Louis, MO.). All water samples and standards were filtered through a 0.2 µm PTFE acrodisc prior to analysis. Method detection limits were 0.3 mg $NO_2$-N/L and 0.23 mg $NO_3$-N/L.

Geosmin was analyzed on GC/MS QP2010 SE (Shimadzu Analytical Instruments, Columbia, MD) equipped with a HPSME autosampler capable of operating in a selected ion monitoring (SIM) mode. A Shimadzu SHRXI-5MS (Shimadzu Analytical Instruments, Columbia, MD) column was used, which is a silicone-coated fused-silica capillary column. To prepare samples, 0.3 g of NaCl was added to the 20 mL screw top vial in addition to 10 ml of standard, sample, or Millipore water for blanks. Six standards using a geomsin stock of 100 µg/mL (Supleco, Cat. # 47525U) were always run each time to create a six point calibration curve. The MDL was 1 ng/L with a linear range of 1-100 ng/L. The vials were then placed in the autosampler tray for analysis. All geosmin

analysis was completed by the laboratory assistants at the MWTDS laboratory. QA/QC was carried out by MWDTS and is described fully in the SOP in Appendix E.

### 3.4 Additional Methods

The quantity and the size distribution of particles in the influent and effluents of the filters were determined using a Beckman Multisizer III Coulter Counter (Beckman Coulter, Indianapolis, IN.). For sample analysis, 9 mL of the diluent Isoton II was added to 1 mL of sample. The volume of sample analyzed was 50 µL and each sample was analyzed three times. Turbidity measurements were collected by the on-line turbidimeters at a frequency of every 15 minutes and plotted.

The adenosine triphosphate (ATP) content of the media samples was analyzed using the method of Velten et al. (2007) with BacTiter-Glo reagent (Promega Corporation, Madison, WI.). First, 100 µL of phosphate buffer (3 mg/L $KH_2PO_4$ and 7 mg/L of $K_2HPO_4$, pH 7) was added to 200 mg of media which was then incubated in a water bath at 30°C for 3 minutes. Additionally, the BacTiter-Glo reagent was placed in the water bath at 30°C for 3 minutes. After 3 minutes, 300 µL of BacTiter-Glo reagent was added to each sample. The sample was then placed back in the 30°C water bath for 1.5 minutes and mixed gently every 30 seconds. The relative light units (RLU) of the sample were measured on a Turner Biosystems Luminometer Model TD-20/20 (Promega, Madison, WI.). Standard curves were generated using an ATP standard (Sigma-Aldrich, St. Louis, MO).

The dry weight of media samples was determined to allow for comparison of media samples as the moisture content changed over time. Method 2540B in Standard

Methods was used to determine the dry weight (Std. 2540 B. Total Solids Dried at 103-105°C).

### 3.5 Microbial Community Analysis

Media samples collected from the top of each column were distributed into three sterile microcentrifuge tubes so that each tube contained ~220 mg of GAC or ~400 mg of anthracite media as wet weight. Genomic DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH) per manufacturer's instructions. Purified DNA was stored at -20°C for future use.

Real-time quantitative polymerase chain reaction (qPCR) was used to detect and quantify four genes of interest. The 16S rRNA gene for *Bacteria* was used to quantify total bacterial biomass. The ammonia monooxygenase gene for *Bacteria* (*amoA*-AOB) and the ammonia monooxygenase gene for *Archaea* (*amoA*-AOA) targeted organisms capable of carrying out the conversion of ammonia to nitrite. The primers used to target these genes are listed in Table 3-2. Positive controls were used to allow for quantification of gene copies in each sample; deionized water was used as a negative control. The quantification limit was on average $1.1\times10^4$ copies per gram of 16S rRNA genes, 620 copies per gram AOB, and 660 copies per gram AOA.

**Table 3-2. Target genes, target primers, amplicon length, melting temperatures, and reference for primers selected.**

| Gene | Sequence (5' → 3') | Amplicon length (bp) | Melting Temp (°C) | Reference |
|---|---|---|---|---|
| 16S rRNA *Bacteria* | F: GAG AGG AAG GTC CCC CAC<br>R: CGC TAC TTG GCT GGT TCA G | 116 | 60 | Layton et al. 2006 |
| *amoA*-AOB | F: TCA GTA GCY GAC TAC ACM GG<br>R: CTT TAA CAT AGT AGA AAG CGG | 204 | 56 | Harms et al. 2003 |
| *amoA*- AOA | F: STA ATG GTC TGG CTT AGA CG<br>R: GCG GCC ATC CAT CTG TAT GT | 635 | 56 | Francis et al. 2005 |

All qPCR reactions used a 25 µL volume that included 12.5 µL of BioRad iTaq SYBR Green (Life Science Research, Hercules, CA), 25 µg of bovine serum albumin, 10 µL of nuclease-free water, 12.5 pmol of the forward primer, 6.25 pmol of the reverse primer, and 0.5 µL of template DNA. This mastermix has been used in previous research and shown to provide optimal qPCR amplification to avoid primer-dimer formation (Burch et al. 2014). All qPCR reactions were performed using the Eppendorf Mastercycler *realplex* thermal cycler (Westbury, NY). The thermal profile consisted of 1 minutes at 95°C followed by 40 cycles consisted of 15 seconds at 95°C, and 60 seconds to 1.5 minutes at the primer-specific annealing temperature dependent on the target gene. After these 40 cycles, a melt curve was generated to verify that non-specific amplification did not occur.

Standard curves for qPCR were generated using 10-fold serial dilutions of a known quantity of a plasmid containing the target gene. These standards were made by amplifying a sample with the known target gene using PCR with the target gene primers. The PCR product was then purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA), ligated into the pGEM-T-Easy vector using the pGEM-T-Easy Vector

System I (Promega, Madison, WI) and then transformed into competent *E. coli* JM109 cells (Promega, Madison, WI). The cells were spread-plated onto LB ampicillin plates for blue and white screening. White colonies were picked and grown up at 37°C overnight in liquid LB ampicillin broth. The cells were then harvested and the plasmids were extracted using QIAprep Spin Miniprep Kit (QIAGEN, Valencia, CA). The plasmid DNA was then stained using Hoechst 33258 dye and quantified using calf thymus as a DNA standard on a TD-700 fluorometer (Turner Designs, Sunnyvale, CA). The positive control for the *amoA*-AOA standard was provided by Dr. Daniel Noguera from the University of Wisconsin at Madison. The nucleotide sequences of all standards were determined to confirm the validity of the standard.

### 3.6 High-throughput Illumina Sequencing

Next generation high-throughput sequencing was performed using the MiSeq Illumina platform. The sequencing targeted the V3 region of the 16S rRNA that was amplified by PCR using primer 338F and primer 518R (Muyzer et al. 1993). Targeting the 16S rRNA gene allowed for phylogenetic analysis to be performed. Bar codes (8-nucleotides of known sequence) were attached to the 5′-end of both the forward and reverse primer to allow multiplexing of up to 256 samples simultaneously (16 × 16 matrix).

Preparation of samples for sequencing required performing PCR on sample genomic DNA using the Illumina primers. The 50 µl mastermix for PCR consisted of 10 µL of buffer, 50 µg of bovine serum albumin, 4 nmol of dNTP's, 20 pmol of forward primer, 20 pmol of reverse primer, and 1.25 U of *Taq* DNA polymerase per reaction. The

thermal cycle protocol was 2 minutes at 95°C to denature the genomic DNA, followed by 25 cycles of 15 seconds at 95°C and 60 seconds at 60°C. After amplification, the PCR product was purified using a QIAquick PCR Purification kit (Qiagen Valencia, Calif.) and resolved on a 1% agarose gel for DNA quantification. The amount of DNA in each PCR product was quantified using a UVP bioimaging camera (UVP Upland, CA) by comparison to a *Hind*III digest of phage lambda. Equal amounts of DNA from each sample were pooled together and then sent to the University of Minnesota Genomics Center (UMGC) for processing and were sequenced by the Illumina MiSeq instrument (150 cycles, paired-end reads).

### 3.7 Data Analysis

Analysis of the DNA sequences from the Illumina MiSeq runs was performed using Mothur (Schloss et al. 2009). The sequences were first trimmed to 125 nucleotides and the pair-end reads were overlapped to generate contiguous sequences. The sequences were then trimmed to remove the PCR primer sequences and then screened for quality. Sequences were excluded if they had one or more ambiguous bases, more than an 8-nucleotide homopolymer, or an average quality score less than 35 over a window-size of 50 basepairs. Additionally, all sequences that appeared fewer than six times among all of the sequences were removed. Sequences were then aligned using the Silva database and chimeric sequences were identified by UCHIME (Edgar et al. 2011) and removed. The number of sequences associated with each sample were then randomly reduced to 10,000 sequences to allow comparisons among samples without bias caused by different depths of sequence. A cutoff of 0.03 was used for the distance matrix and clustering.

Phylogenetic identification was done using the Ribosomal database release 9 (Cole et al. 2009). A non-metric multidimensional scaling plot (R program language, Version 3.0.2) was generated to qualitatively compare the microbial community in each filter to each other and over time.

Microsoft Excel software was used to perform regressions and other statistical analyses including T-tests and ANOVA to determine if sample sets were significant at the 0.05 level.

# 4 Results

## 4.1 Column Operation Performance

### 4.1.1 Particle Removal

All columns exhibited similar particle removal performance but the performance varied over time. The influent particle count was elevated in fall, then lower during the winter when the river was covered by ice, and then increased substantially in spring with snowmelt and rising river discharge (Figure 4-1). Filter effluent particle concentrations tended to remain substantially below the influent concentrations, with the exception of a few samples collected immediately after startup, but followed the same temporal trend. The effectiveness of particle removal by the filters is also illustrated by the particle size distribution plots (Figures 4-2, 4-3, 4-4, and 4-5). Following the initial startup period, particle removal was relatively stable throughout the winter and spring. The mean (± standard deviation) particle removal efficiencies during this stable period (i.e., December 9$^{th}$, 2013 to May 8$^{th}$, 2014) were 59.2% ± 16.4%, 59.7% ± 19.5%, and 52.6% ± 29.1% for the anthracite, Calgon F300 GAC, and Norit 300 GAC columns, respectively. The particle removal efficiencies were not statistically significantly different (ANOVA: $P$ = 0.73) for the different media types.

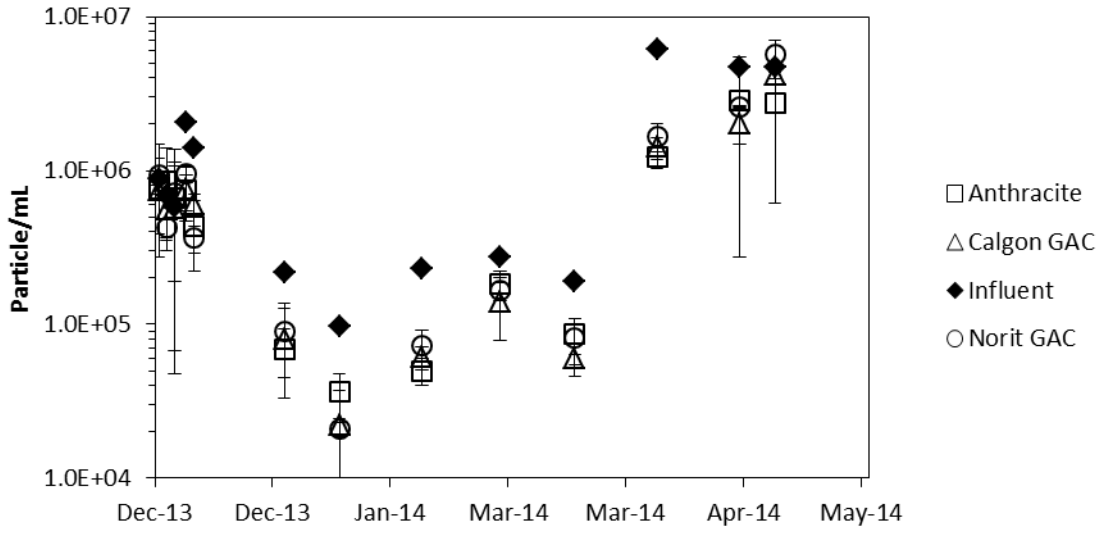


**Figure 4-1. The particle concentration in the influent and effluents of the pilot-scale filters. The effluent is the mean of the columns and the error bars represent standard deviations. The range in particle size counted is 1.3 μm to 12 μm.**

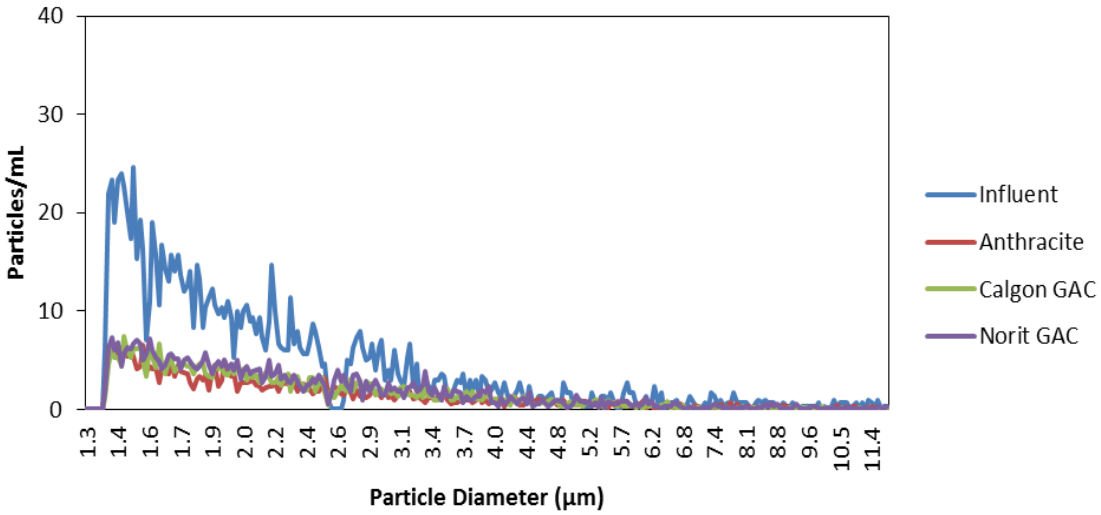


**Figure 4-2. Particle distribution of influent and effluent of the anthracite, Calgon F300 GAC, and Norit 300 GAC columns collected on 1/3/2014 at MWTDS. The particles range from 1.3 to 12μm diameter.**

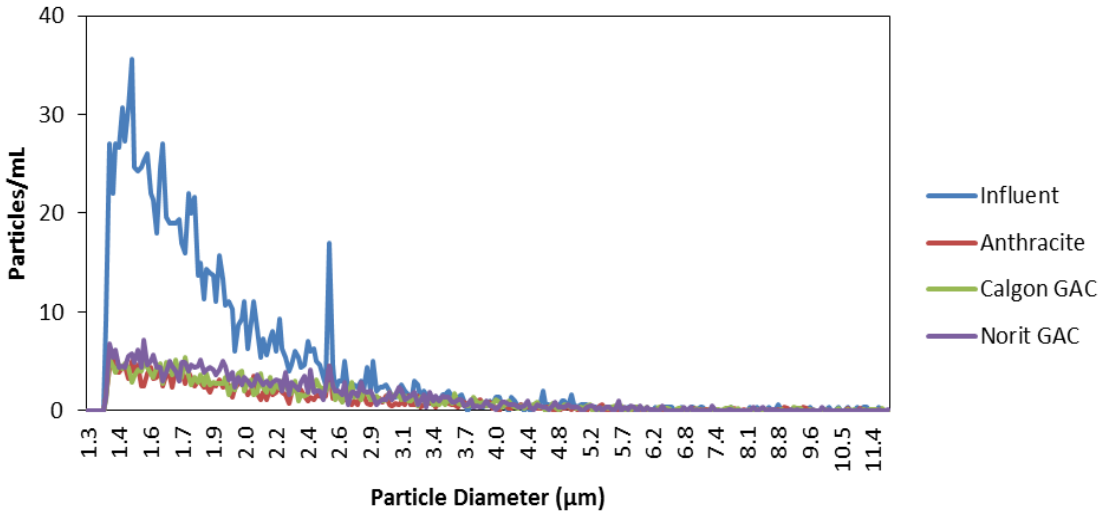


**Figure 4-3. Particle distribution of influent and effluent of the anthracite, Calgon F300 GAC, and Norit 300 GAC columns collected on 2/7/2014 at MWTDS. The particles range from 1.3 to 12µm diameter.**

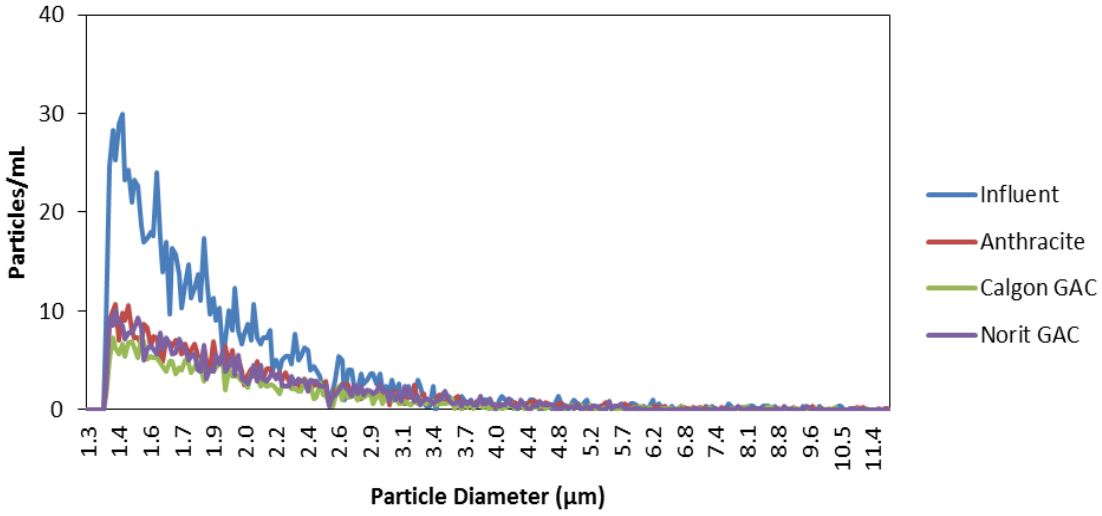


**Figure 4-4. Particle distribution of influent and effluent of the anthracite, Calgon F300 GAC, and Norit 300 GAC columns collected on 3/18/2014 at MWTDS. The particles range from 1.3 to 12µm diameter.**

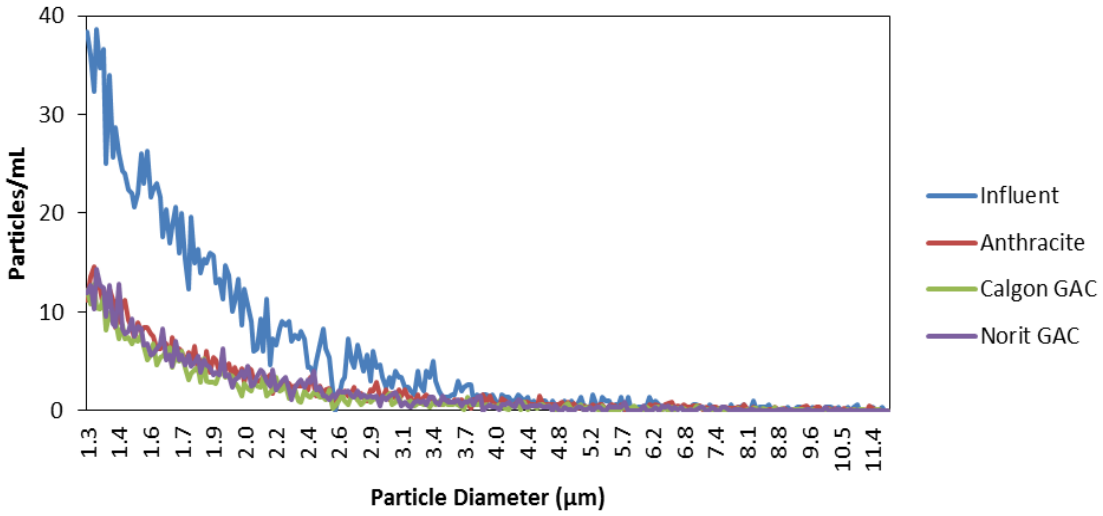


**Figure 4-5. Particle distribution of influent and effluent of the anthracite, Calgon F300 GAC, and Norit 300 GAC columns collected on 4/29/2014 at MWTDS. The particles range from 1.3 to 12µm diameter.**

The particle count data are also supported by the online turbidimeter data, which show increased turbidities in the spring for both the influent and effluents (Figures 4-6 and 4-7). In general, the filters effectively removed turbidity causing particles, but during the spring, filter effluent baseline turbidities consistently exceeded the 0.1 NTU filter effluent turbidity goal (Figure 4-7). From January 21$^{st}$ (earliest data recorded) through March 31$^{st}$, the percentage of readings below 0.1 NTU for the anthracite, Calgon F300, and Norit 300 were 58.9 %, 71.1 %, and 46.1%, respectively while after March 31$^{st}$ those values were 0.35%, 7.8%, and 12.2%, respectively (Figure 4-7). Overall, particles were consistently removed but spring runoff was a challenge for all of the filters regardless of media type.

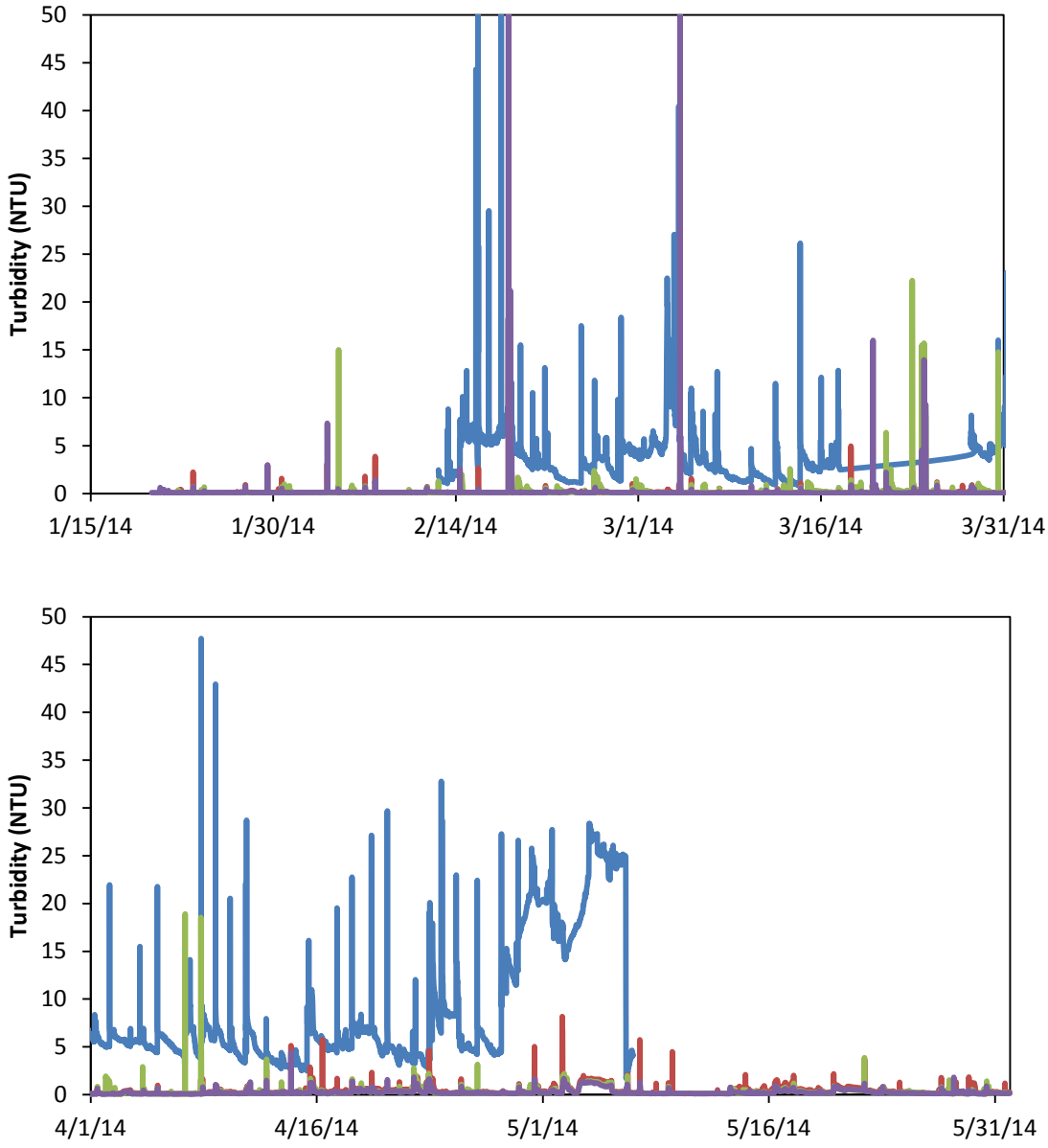


**Figure 4-6. The turbidity of the influent (blue) and effluent from column 1 (anthracite; red), column 2 (Calgon F300 GAC; green), and column 3 (Norit 300 GAC; purple) measured by the online turbidimeters at MWTDS. On 5/7/14, the influent turbidimeter became clogged and remained clogged for the remainder of May. The influent for this time period was removed from the figure.**

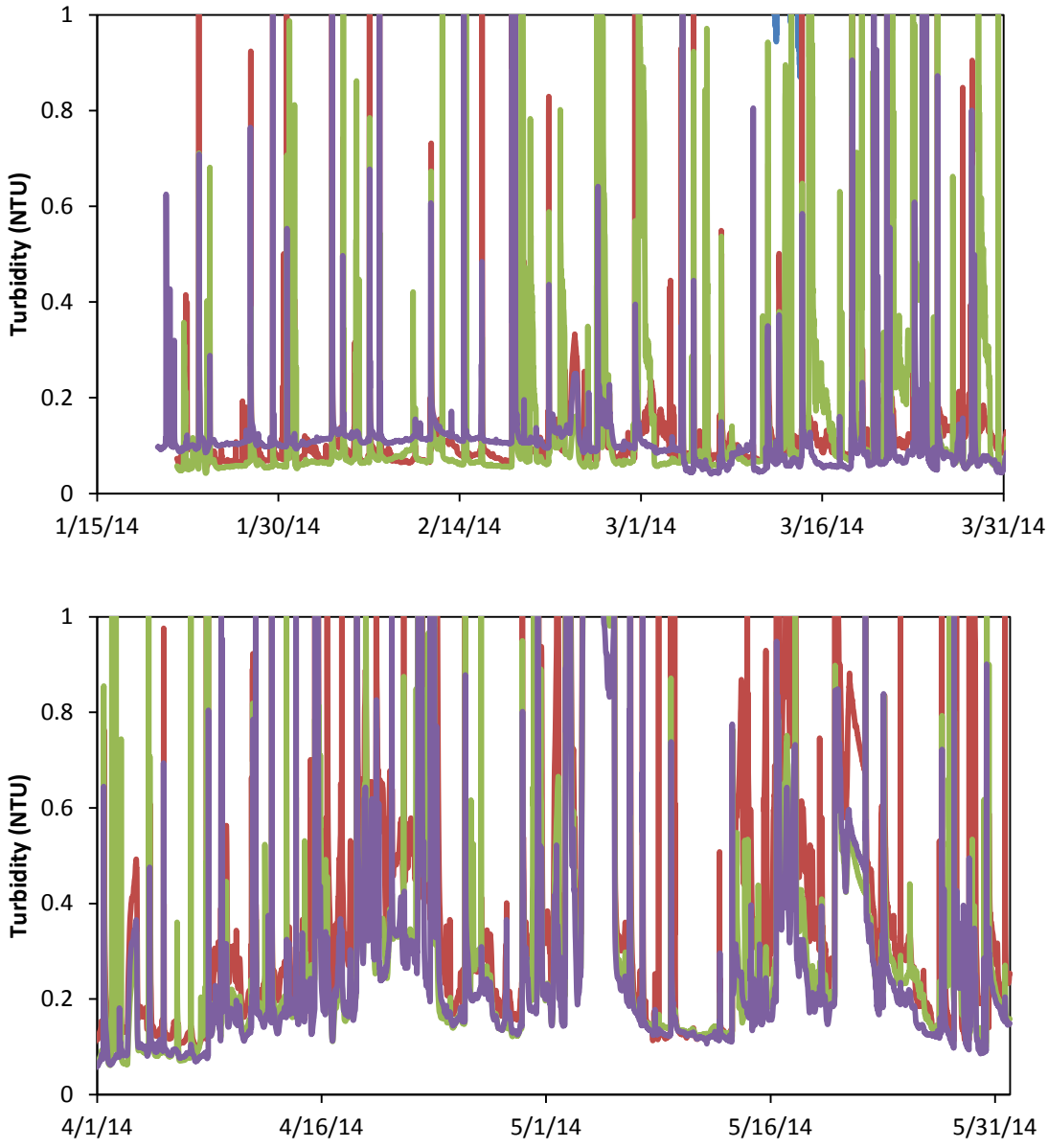


**Figure 4-7. The turbidity of the effluents from column 1 (anthracite; red), column 2 (Calgon F300 GAC; green), and column 3 (Norit 300 GAC; purple) measured by online turbidimeters at MWTDS.**

### 4.1.2 Total Organic Carbon, Dissolved Organic Carbon, and Specific UV Absorbance

Total organic carbon (TOC) and dissolved organic carbon (DOC) in the influent and effluents of all columns are compared in Figures 4-8 and 4-9, respectively. The influent TOC and DOC concentrations were low in the winter and then increased in the spring from snow melt (Brinkman and Hozalski, 2011). During the first month of operation, the Calgon F300 GAC and Norit 300 GAC columns removed 77-97% of the influent TOC and DOC. As operation continued, the organic carbon removal efficiency of the Calgon F300 and Norit 300 GAC columns declined but eventually stabilized. For example, the mean (± standard deviation) DOC removal efficiencies for the Calgon F300 and Norit 300 columns were 29.8 % ± 7.85% and 26.5% ± 6.6%, respectively over the last 3 months of operation. The Calgon F300 and Norit 300 GAC average TOC and DOC removal efficiencies from January to May 2014 were not significantly different (paired t-test: P =0.27 for TOC, P=0.22 for DOC) for TOC or DOC removal. The anthracite filters did not remove TOC or DOC effectively with mean removal efficiencies of 3.86% ± 10.54% and 3.37% ± 11.02%, respectively. The Calgon F300 and Norit 300 GAC were both statistically lower in organic carbon removal than anthracite (t-test: $P < 0.05$). Overall, the GAC exhibited significantly greater TOC and DOC removals than the anthracite filters throughout the experiment.

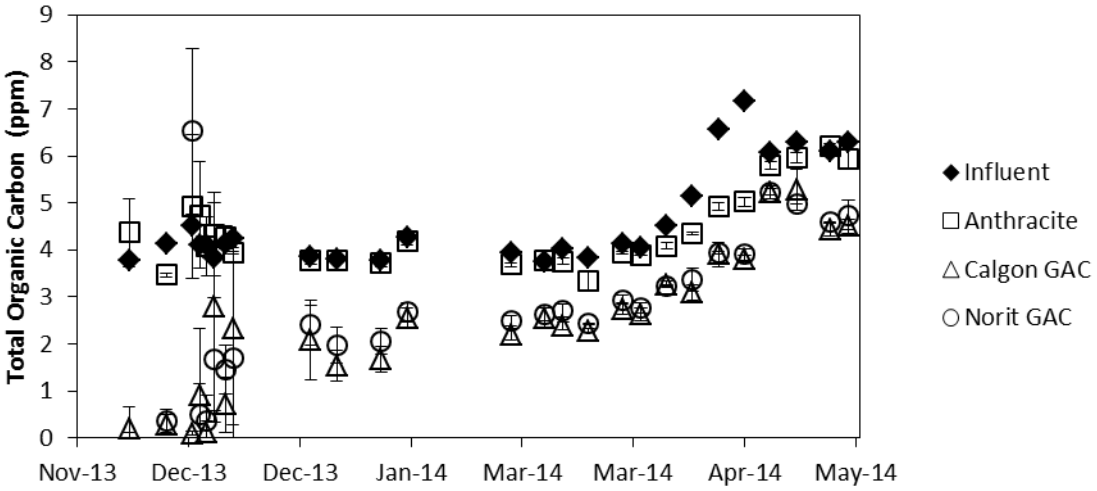


**Figure 4-2. Effect of media type on the removal of TOC through the pilot-scale filtration system. The effluent samples are means of replicate columns with standard deviations.**

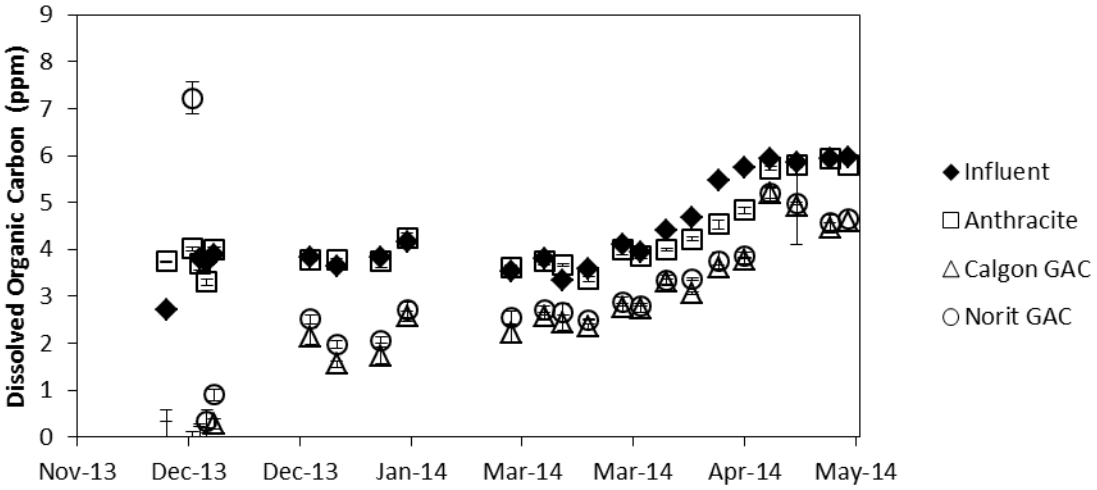


**Figure 4-3. Effect of media type on the removal of DOC through the pilot-scale filtration system. The effluent samples are means of replicate columns with standard deviations.**

Ultraviolet absorbance at 254 nm in the influent and effluent of all columns are compared in Figure 4-10. The $UV_{254}$ provides an additional measurement of the portion of aromatic organic matter in the water. Overall, the influent $UV_{254}$ followed the same trend as TOC and DOC where it was low in the winter and then increased in the spring due to snowmelt. Both the Calgon F300 and Norit 300 GAC effectively removed a portion of the influent $UV_{254}$ while the anthracite had no removal.

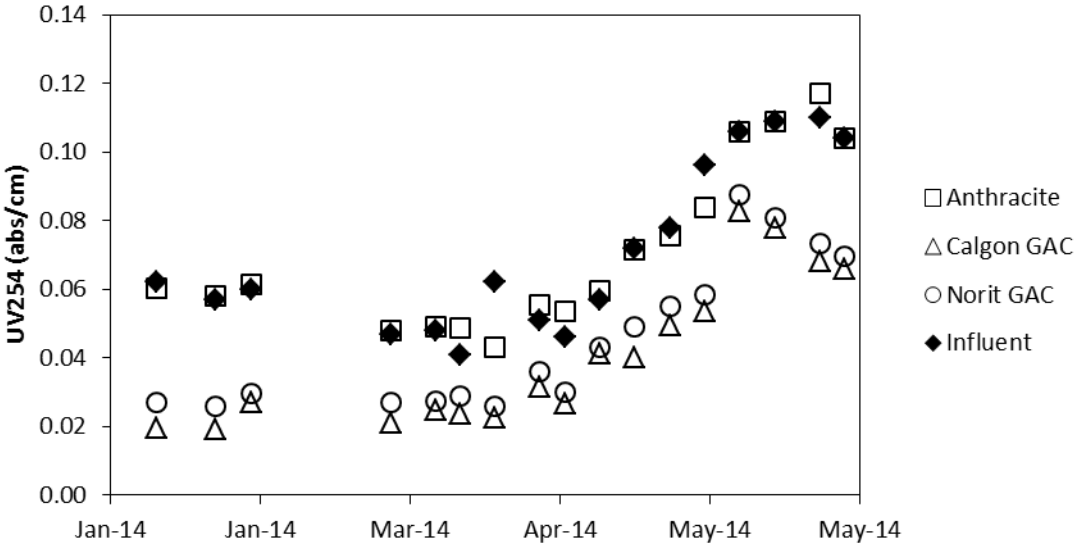


**Figure 4-4. Effect of media type on the removal of organic matter that absorbs light at 254 nm from the pilot-scale filtration system.**

Specific UV absorbance (SUVA) is the UV absorbance at 254 nm divided by the DOC concentration. SUVA values for the influent and effluents of the anthracite, Calgon F300 GAC, and Norit 300 GAC columns are shown in Figure 4-11. A higher SUVA value generally indicates a higher aromatic carbon content, which correlates with increased reactivity with chemical oxidants (Crittenden et al. 2012) but decreased biodegradability (Goel et al. 1995). The effluent anthracite SUVA values were not

statistically different from the influent values (paired t-test: P= 0.50). Overall, the effluent SUVA values for the Calgon F300 GAC and Norit 300 GAC filters were not statistically different from each other (paired t-test: P= 0.28) but were significantly lower than the influent values (paired t-test: P<0.05). Thus, the Calgon F300 GAC and Norit 300 GAC filters were more effective at removing organic matter, especially aromatic organic matter, than anthracite.

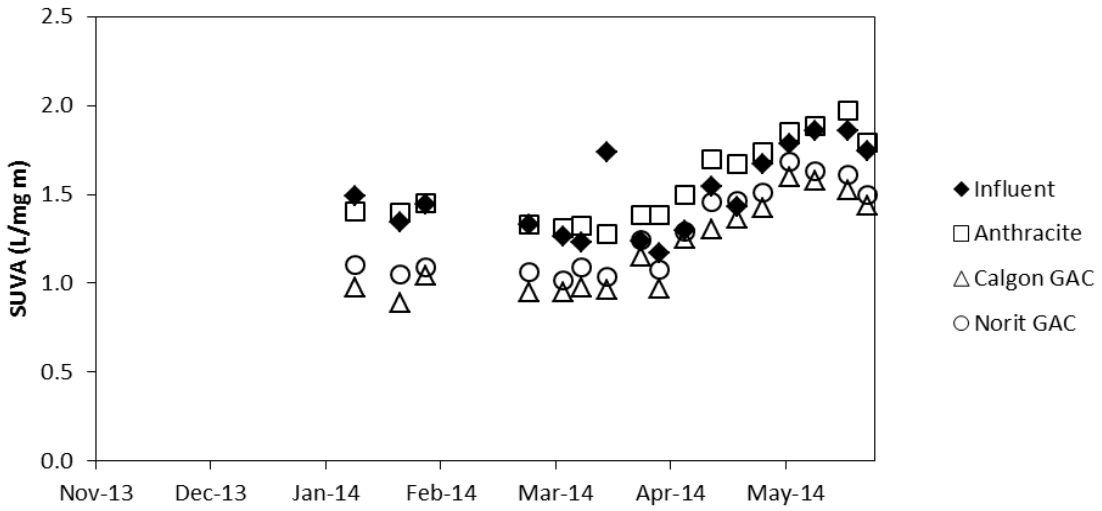


**Figure 4-5. Effect of media type on the NOM composition at the pilot-scale filtration system.**

### 4.1.3 Geosmin

Geosmin was dosed into the feed water to achieve a target influent geosmin concentration of 100 ng/L from December 31$^{st}$, 2013 to June 30$^{th}$, 2014 (with the exception of April 23rd to June 4$^{th}$, 2014 when the geosmin stock was depleted). The measured geosmin concentrations in the influent samples, however, were typically much less than the target value (Figure 4-12 and 4-13). According to the feed solution concentration and flow rate of the syringe pump, the geosmin dose rate should have

resulted in an influent concentration of 100 ng/L. Although the influent concentration at the outset was low, the anthracite column effluent concentrations for three samples from January 8$^{th}$ to the 29$^{th}$ were approximately 100 ng/L, suggesting that the target influent concentration was being achieved (assuming no removal in the anthracite columns). It was suspected that the in-line static mixer was insufficient to thoroughly mix the geosmin prior to the influent sampling port, so the geosmin dosing point was changed to immediately after the settling tank to increase the extent of mixing prior to influent sampling port. Unfortunately, this change did not improve the measured influent values and likely caused the actual feed to decrease substantially, likely due to volatilization from the open tank, based on the low effluent anthracite concentrations from March 7$^{th}$ to April 9$^{th}$, 2014. Beginning on April 11$^{th}$, 2013, the dosing point was changed back to immediately prior to the inline static mixer but at a higher pumping rate (and lower feed concentration) and the first influent sample contained 160 ng/L geosmin. The next sample collected a week later, however, had a much lower concentration of 60 ng/L geosmin. Due to the measured influent concentrations being highly variable and often much lower than expected, the geosmin removal efficiency is often unclear. The GAC geosmin concentrations, however, remained below the 4 ng/L taste and odor threshold for the majority of the study while the effluents of the anthracite columns typically exceeded 4 ng/L. The anthracite geosmin effluent concentrations were significantly higher (t-test: $P < 0.05$) than the Calgon F300 GAC and Norit 300 GAC concentrations. There was no significant difference (t-test: $P= 0.58$) between the Calgon F300 GAC and Norit 300 GAC concentrations. Although the influent geosmin concentration varied from the target concentration of 100 ng/L, in comparing the effluent geosmin concentration, both GAC

types were lower than the taste and odor threshold whereas the anthracite was statistically higher and above this threshold.

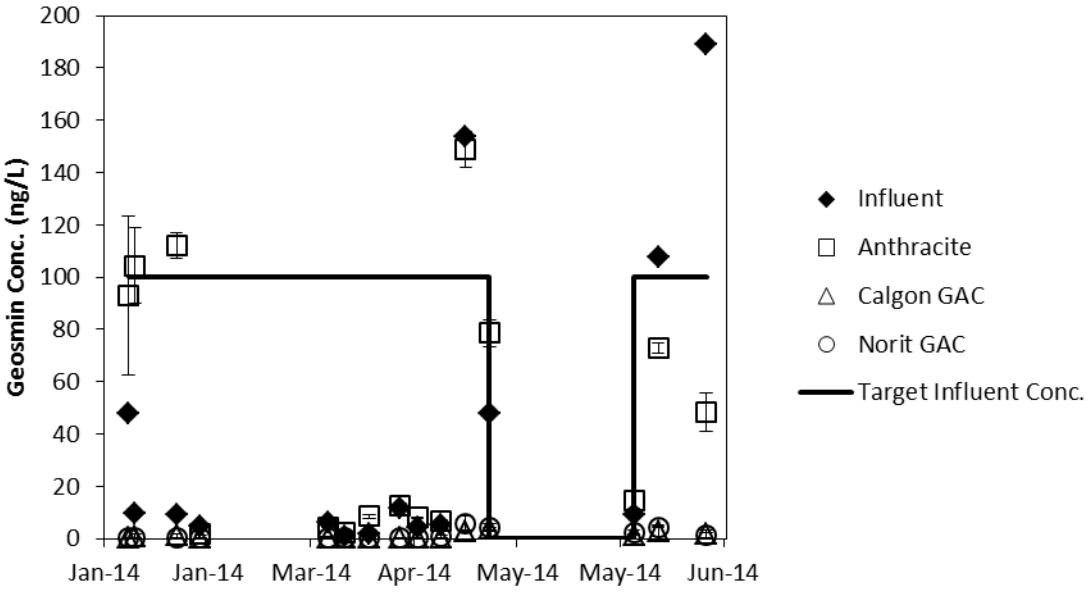


**Figure 4-6. Effect of media type on the removal of geosmin in the pilot-scale filtration system. The target geosmin influent concentration is shown by the black line. The effluent samples are means of replicate columns with error bars representing standard deviations. Geosmin was not fed from April 23rd to June 4th because the stock was temporarily depleted.**

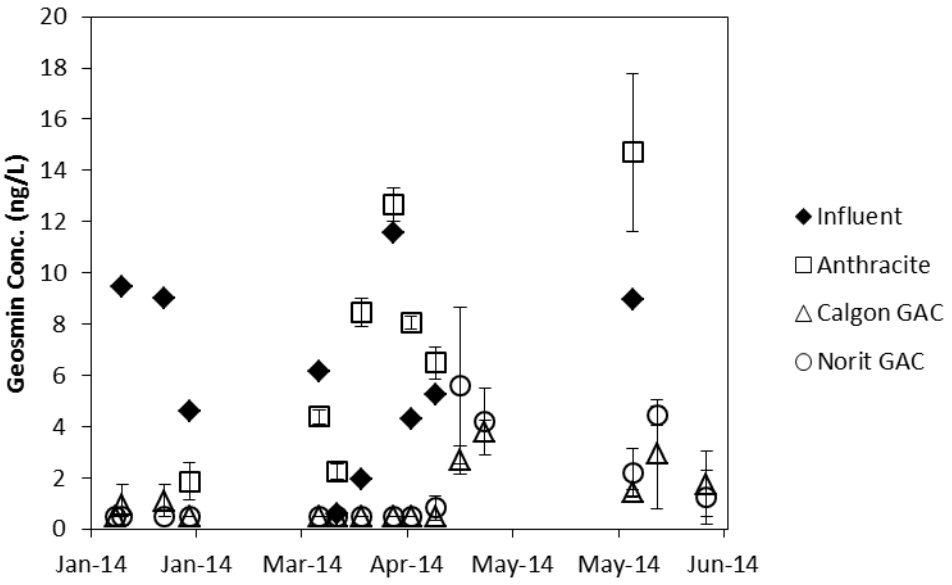


**Figure 4-7. Effect of media type on the removal of geosmin in the pilot-scale filtration system showing only data ranging from 0-20 ng/L. The effluent samples are means of replicate columns with error bars representing standard deviations.**

### 4.1.4 Ammonium, Nitrite, and Nitrate

Ammonium, nitrite, and nitrate concentrations were measured in the influent and effluent of the anthracite, Calgon F300 GAC, and Norit 300 GAC filters to determine if nitrification may be occurring in these filters. Of these compounds, only nitrate was consistently quantifiable as ammonium and nitrite remained below their respective lowest standard of 0.5 and 0.3 mg/L as N. Influent nitrate concentrations typically ranged from 0.44 to 1.0 mg/L $NO_3^{-2}$-N except for a spike to more than 2 mg/L $NO_3^{-2}$-N in April that may have been associated with spring snowmelt. The effluent nitrate concentrations of the anthracite, Calgon F300 GAC, and Norit 300 GAC columns were not statistically different (t-test: $P>0.05$ for all three media types) from the influent concentrations (Figure 4-13). The fact that the influent nitrate concentrations were not statistically different than the effluent concentrations for all the media types suggests that nitrification

was not occurring in the filters. This result is also consistent with the inability to detect ammonia in the filter influent water.

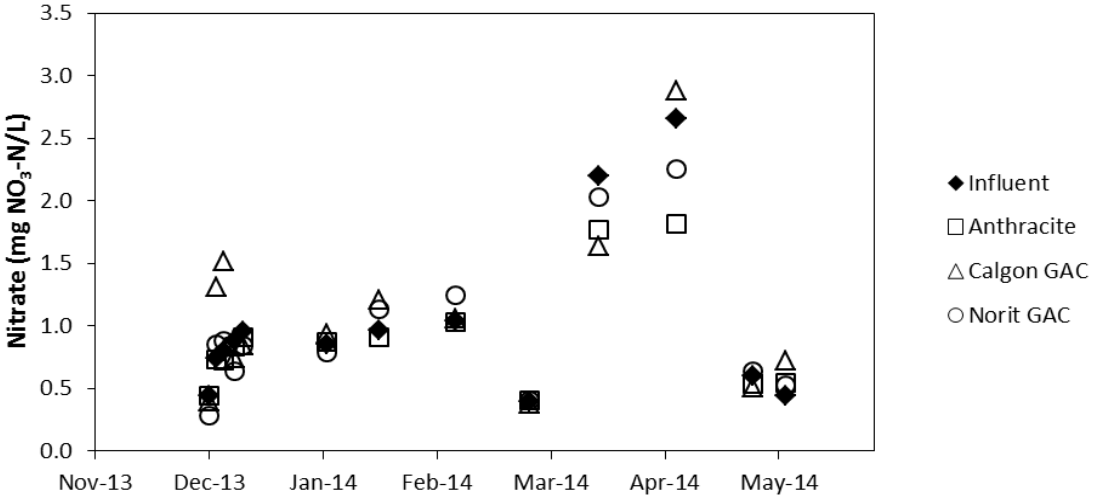


**Figure 4-8. Effect of media type on the production and removal of nitrate in the pilot-scale filtration system to determine nitrification potential.**

## 4.2 Biomass measurements and quantification of functional genes (ATP concentration, 16S rRNA gene for *Bacteria*, *amoA* gene for *Bacteria*, and *amoA* gene for *Archaea)*

### 4.2.1 Estimating Total and Active Biomass

For this study, ATP was quantified to estimate the active biomass on the anthracite, Calgon F300 GAC, and Norit 300 GAC filter media. The ATP concentrations for the anthracite, Calgon F300 GAC, and Norit 300 GAC remained low or non-detectable during the beginning of the operation and through the winter (Figure 4-14). With the onset of spring and warmer water temperatures in April (Figure 4-15), the ATP concentrations for all three media types started to increase. Thus, the filters became more metabolically active during the warmer months compared to the colder winter months. During the warmer months, the ATP concentration on the anthracite media was

significantly less than on the Calgon F300 GAC and Norit 300 GAC concentrations in April (paired t-test: P=0.007 for Calgon, P=0.022 for Norit) but greater in May (paired t-test: P= 0.006 for Calgon, P=0.002 for Norit). The ATP concentrations of two GAC types were not statistically different from each other in either April (paired t-test: P=0.60) or May (paired t-test: P=0.22). Overall, the ATP concentration correlated with the increase in water temperature but the amount of biomass also increased during this time as described below.

Real-time PCR was performed to quantify the number of copies of the 16S rRNA gene for *Bacteria* to estimate total biomass on the anthracite, Calgon F300 GAC, and Norit 300 GAC filter media (Figure 4-16). After 3 months of operation, the Norit 300 GAC had $10^4$-$10^6$ copies per gram of media, whereas the anthracite and Calgon F300 GAC initially had $10^6$-$10^8$ copies/g. The Norit 300 GAC, however, became more enriched with *Bacteria* over time and eventually reached the bacterial levels in the anthracite and Calgon F300 GAC columns. This suggests that the Norit 300 GAC was slower at accumulating bacterial biomass than the other two filter media. Additionally, the 16S rRNA gene for *Bacteria* was quantified for media from a full-scale anthracite filter at MWTDS and a full-scale Calgon F300 GAC filter at SPRWS. The full-scale anthracite filter sampled on May 14$^{th}$, 2014 had similar magnitude (~$10^8$-$10^9$ copies/g) of 16S rRNA genes for *Bacteria* as the pilot-scale filters, however, the full-scale Calgon F300 GAC filter sampled on May 1$^{st}$, 2014 had much higher biomass levels overall (~$10^9$-$10^{10}$ copies/g). SPRWS installed Calgon F300 GAC into their filter beds in 2007 and has yet to replace the media, whereas the 8-column pilot-set up at MWTDS was only operated for 7 months. The higher amount of 16S rRNA genes for *Bacteria* on the filter

media at SPRWS suggests that it may take many months of operation for filter media to fully mature in terms of biofilm development.

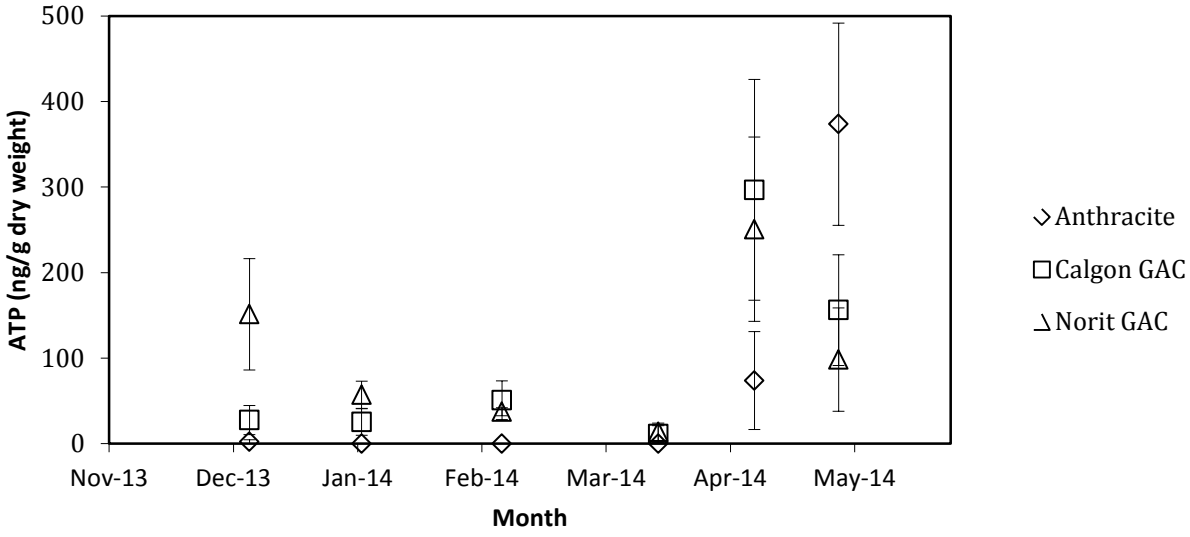


**Figure 4-9. Effect of media on the amount of biomass measured at ATP in the pilot scale filtration system. The error bars represent 95% confidence intervals.**

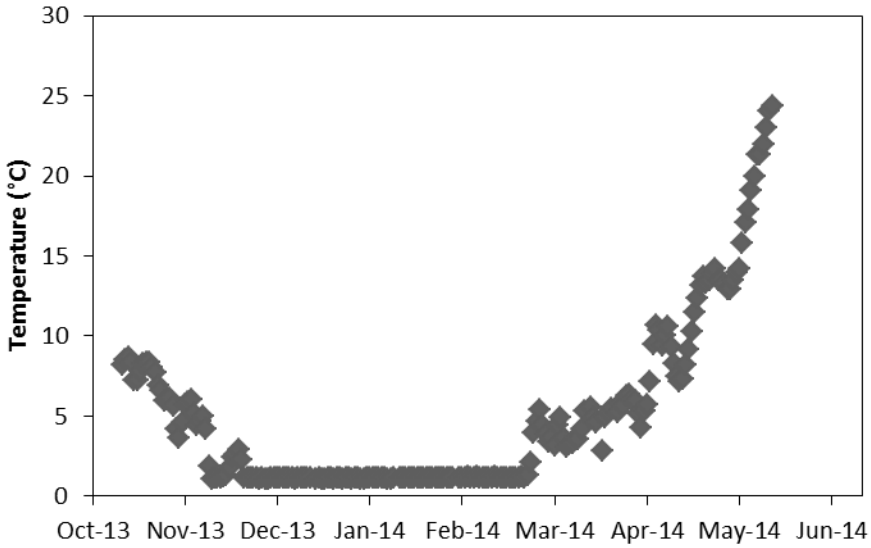


**Figure 4-10. The temperature of the raw water entering the MWTDS treatment plant over the course of the pilot-scale filtration system operation.**

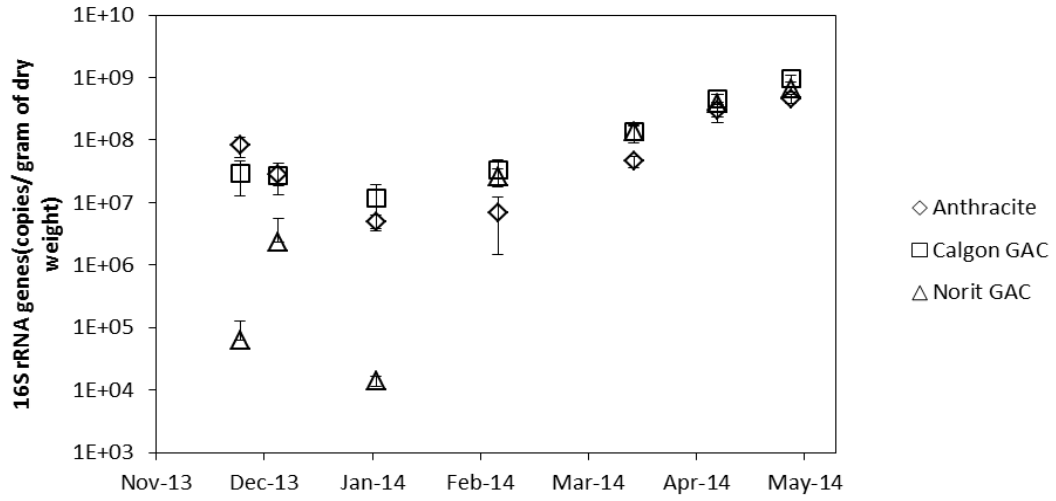


**Figure 4-11. Effect of media type on the amount of total *Bacteria* measured by the Bacterial 16S rRNA genes per gram of media in the pilot-scale filtration system. The quantification limit is on average $1.09 \times 10^4$ copies/gram of dry weight. Samples below the quantification limit were disregarded from the analysis. The error bars represent 95% confidence intervals.**

### 4.2.2 Nitrifying bacterial community

The ammonia monooxygenase gene (*amoA*) in *Bacteria* and *Archaea* was quantified in the anthracite, Calgon F300 GAC, and Norit 300 GAC to determine if the microbial community attached to the media could carry out the first step of nitrification (Figure 4-17 and 4-18). During initial operation and throughout the winter, the amount of *amoA* genes for *Bacteria* (AOB) for the anthracite, Calgon F300 GAC, and Norit 300 GAC was low ($10^2$-$10^4$ copies/g dry weight). Then, in the springtime the amount of AOB genes increased to $10^6$-$10^7$ copies/g dry weight. The *amoA* gene for *Archaea* (AOA) was initially detected only on anthracite until springtime, after which AOA was present on all three media types ($10^4$-$10^5$ copies/g dry weight). Additionally, the full-scale anthracite filter media collected from MWW had similar levels of AOB and AOA genes as the anthracite in the pilot-scale filters. The Calgon F300 GAC full-scale filter had higher

amounts of AOB genes ($10^7$ copies/ gram dry weight) and AOA genes ($10^3$ copies/ gram dry weight) than the Calgon F300 GAC in the the pilot-scale filters. This suggests the treatment system has a large impact for enriching AOB and AOA genes. Overall the amount of AOB and AOA genes was lower in the beginning of the operation and increased over time for all three media types.

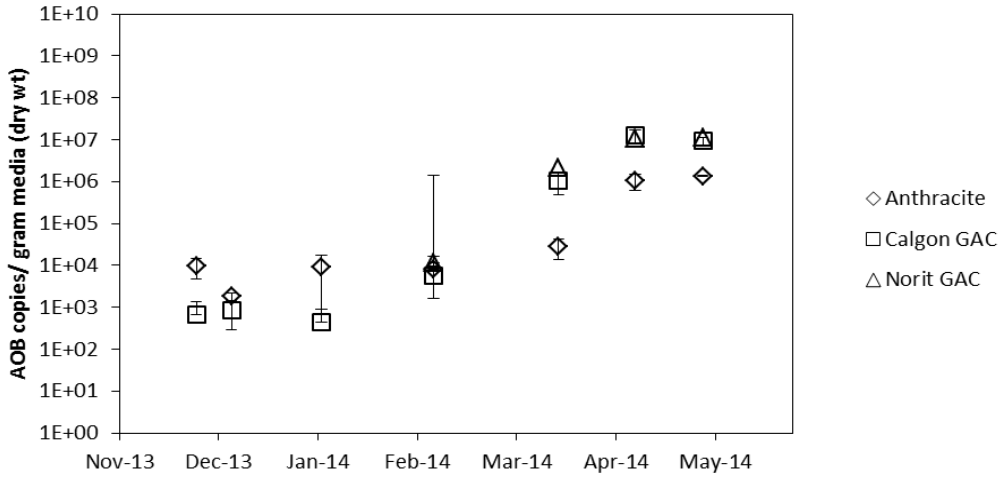


**Figure 4-12. Effect of media type on the number of Bacterial amoA genes per gram of media in the pilot-scale filtration system. The quantification limit was on average 620 copies/g dry weight. Samples below the quantification limit were disregarded from the analysis. The error bars represent 95% confidence intervals.**

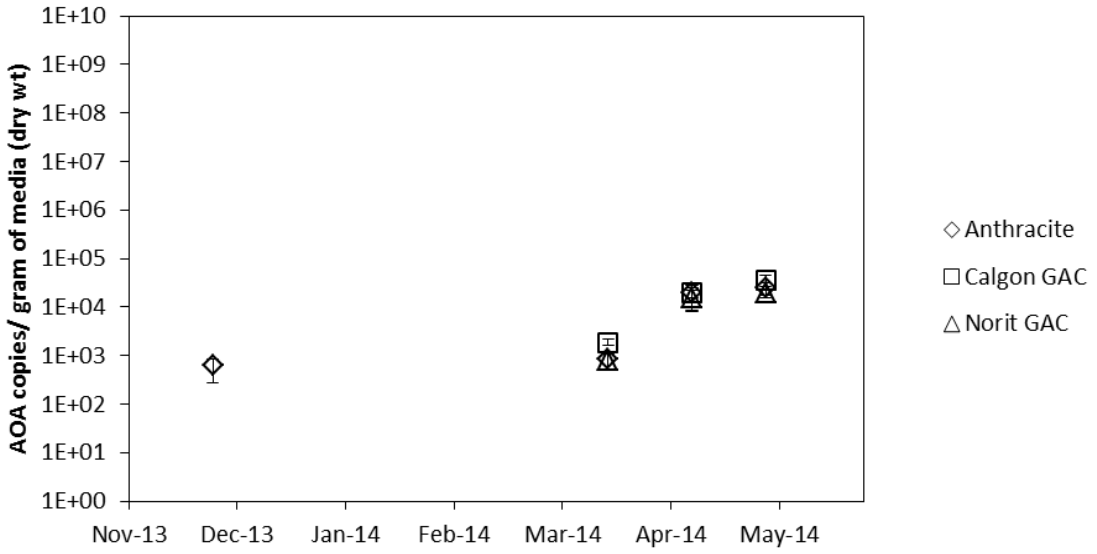


**Figure 4-13. Effect of media type on the number of Archaeal *amoA* genes per gram of media in the pilot-scale filtration system. The quantification limit on average is 659 copies/g of dry weight. Samples below the quantification limit were disregarded from the analysis. The error bars represent 95% confidence intervals.**

### 4.3 Microbial Community Composition and Diversity

The bacterial community composition was tracked in the anthracite, Calgon F300 GAC, and Norit 300 GAC filters over time. In addition to collecting media samples from the pilot-scale filtration system, full-scale media samples were also collected: one anthracite sample from a MWTDS full-scale filter and one Calgon F300 GAC sample from a SPRWS full-scale filter. Illumina MiSeq Profiles were generated for 52 samples, with each sample run in triplicate. This generated a total of 7.7 million sequences. The number of sequences for each sample ranged from 3,086 to 128,069 (Mean= 49,425, S.D.= 23,816), however, all of the samples were normalized to 10,000 sequences each. Previous research with Illumina MiSeq has shown that 10,000 sequences will provide accurate diversity index values (Hope-Wilkinson, 2012). This normalization to 10,000 sequences ended up excluding one of the replicates from 7 different samples from the

analysis. Because each sample was analyzed in triplicate, these samples are presented merely as duplicates. The normalized sequence profiles were then compared for diversity, overall community composition, and change in composition over time.

The Shannon index accounts for both richness and evenness of a community and was used to evaluate the diversity of the bacterial communities on the media from each of the pilot-scale filters (Figure 4-19). The Shannon index values ranged from 3.5 to 4.5. This is highly diverse compared to tap water ranges (1.3 to 1.7; Revetta et al. 2010) but less diverse than soils (5 to 6; Roesch et al. 2007). The anthracite columns initially had high diversity (Shannon index of 4.5), which did not change as operation continued. The diversity of both the Calgon F300 GAC and Norit 300 GAC filters was initially low (Shannon index =3.5) and then increased in both evenness and richness as operation continued. Additionally, the Shannon index of the anthracite from the MWTDS full-scale filter was 4.0 and the Calgon F300 GAC sample from the SPWRS full-scale filter was 4.3.

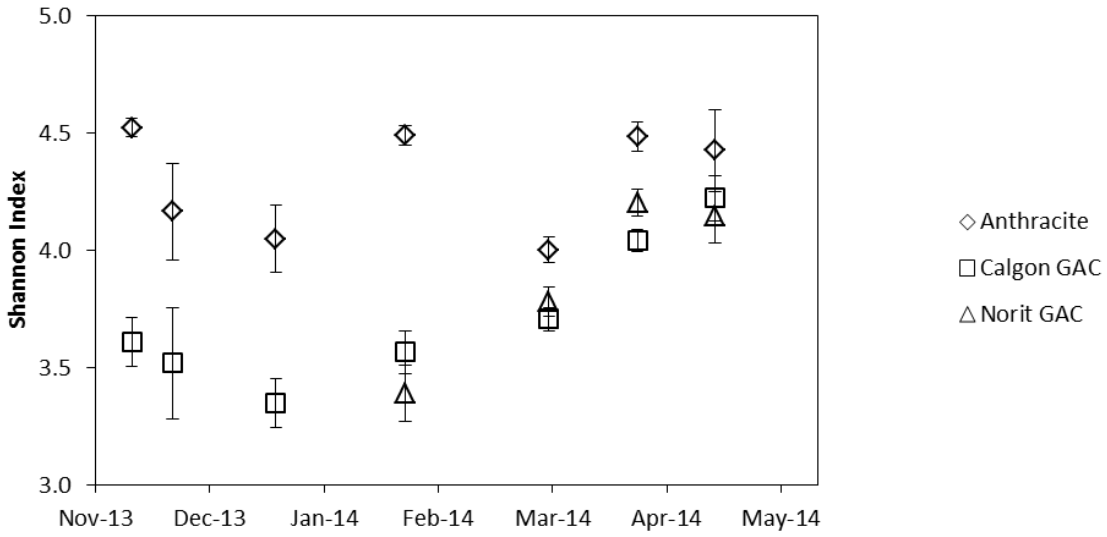


**Figure 4-14. Effect of media type on the diversity of the microbial community, measured by the Shannon index, on the media in the pilot-scale filters. The error bars represent 95% confidence intervals.**

The Illumina MiSeq Profiles were also compared for bacterial community composition at the phylum level for the anthracite, Calgon F300 GAC, and Norit 300 GAC filters (Figure 4-20, 4-21, and 4-22). The most dominant phylum was *Proteobacteria,* which made up 65.7% ± 9.9%, 89.2% ± 4.9%, and 90.4% ± 3.7% of the community for the anthracite, Calgon F300 GAC, and Norit 300 GAC filters, respectively. The second most dominant phylum was *Bacteroidetes* which made up 18.4% ± 3.9%, 6.25% ± 2.9%, and 5.83% ± 2.4% of the community for the anthracite, Calgon F300 GAC, and Norit 300 GAC columns respectively. The full-scale anthracite sample from MWW had a similar phylum profile as the anthracite samples from the pilot-scale system with *Proteobacteria* (52.9%), *Bacteroidetes* (29.1%), and *Actinobacteria* (10.1%) as the three most dominant phyla. The full-scale Calgon F300 GAC sample had *Proteobacteria* (63.9%), *Nitrospira* (14.6%), and *Bacteroidetes* (5.2%) as the three most dominant phyla, while the pilot-scale Calgon F300 GAC had *Proteobacteria* (89.2% ±

4.9%), *Bacteroidetes* (6.25% ± 2.9%), and *Actinobacteria* (3.7% ± 2.2%) as the three most dominant phyla. The microbial community composition was dominated by *Proteobacteria* for all the media types, while the remaining composition shifted over time and from sample suggesting other process factors (source water, bed life, etc.) play a role as well.

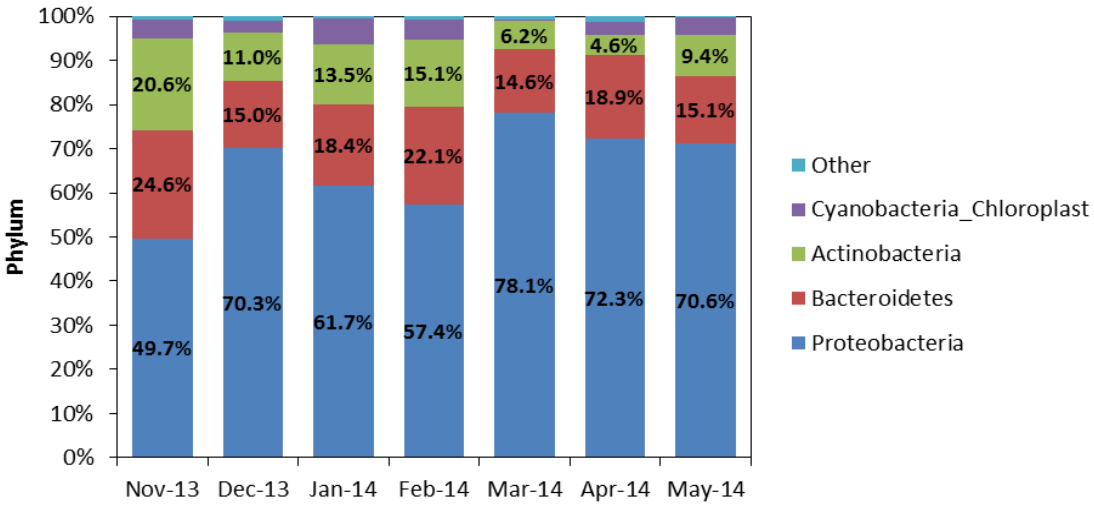


**Figure 4-15. The abundance of dominant phyla over time on the anthracite media from the pilot-scale filtration system.**

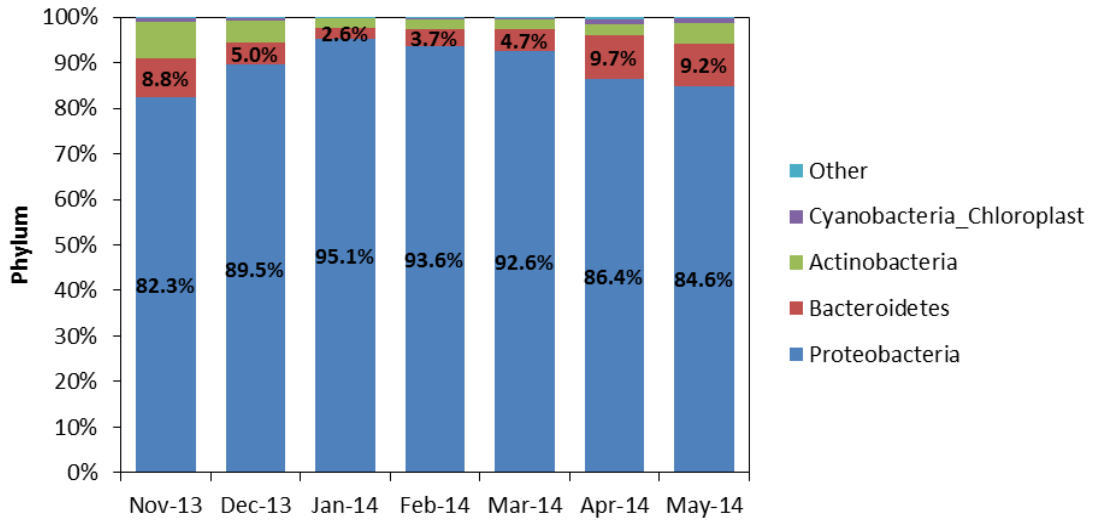


**Figure 4-16. The abundance of dominant phyla over time on the Calgon F300 GAC media from the pilot-scale filtration system.**

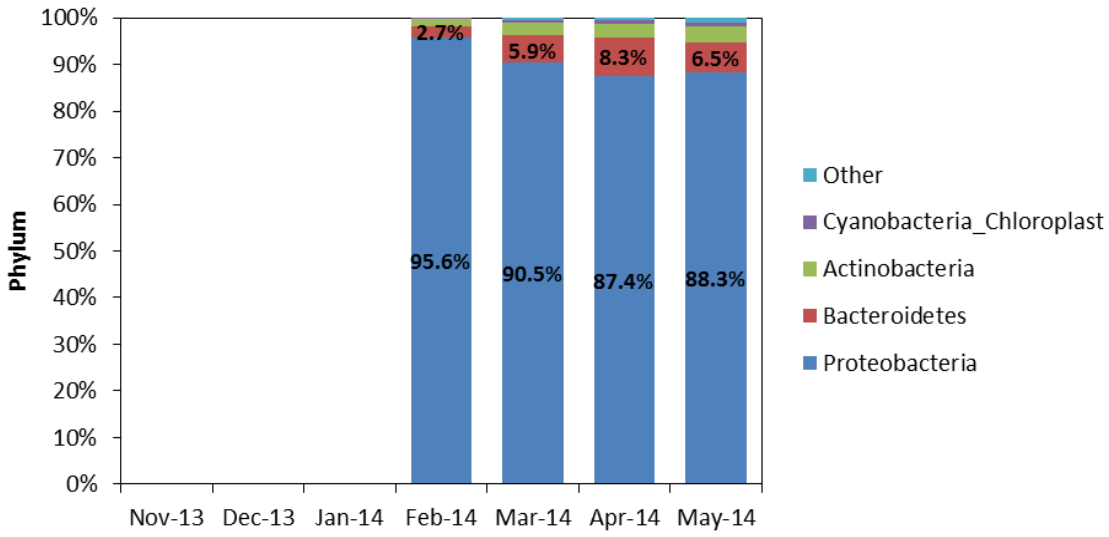


**Figure 4-17. The abundance of dominant phyla over time on the Norit 300 GAC media from the pilot-scale filtration system. November, December, and January sample did not have enough Bacteria to sequence**.

The most dominant phylum *Proteobacteria* was further broken down to the class level to compare the media types and change in community over time for the anthracite, Calgon F300 GAC, and Norit 300 GAC filters (Figure 4-23, 4-24, and 4-25).

*Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* were the three most dominant classes. During initial operation *Betaproteobacteria* was the most dominant class of *Proteobacteria* on all three media types, however, as operation continued, the amount of *Alphaproteobacteria* increased.

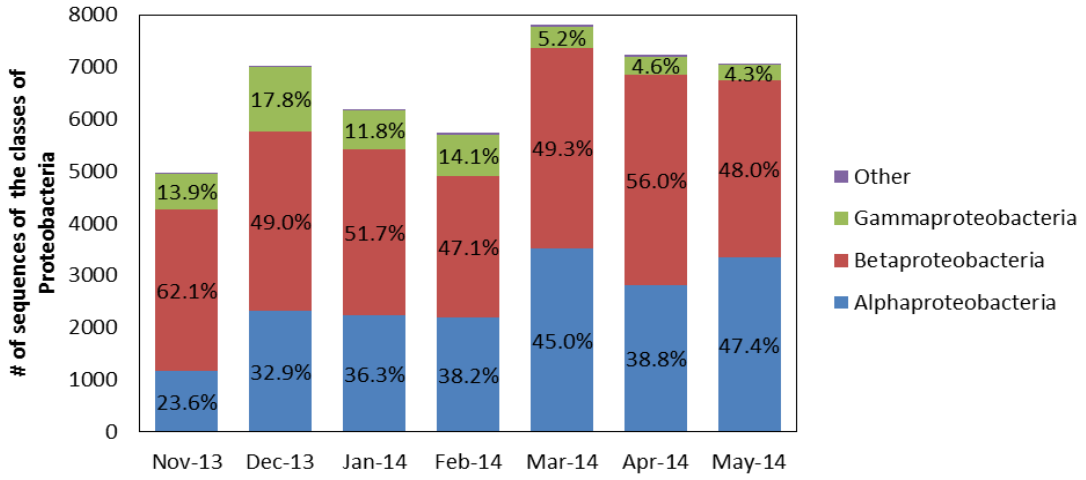


**Figure 4-18. The amount of dominant *Proteobacterial* classes over time on the anthracite media from the pilot-scale filtration system.**

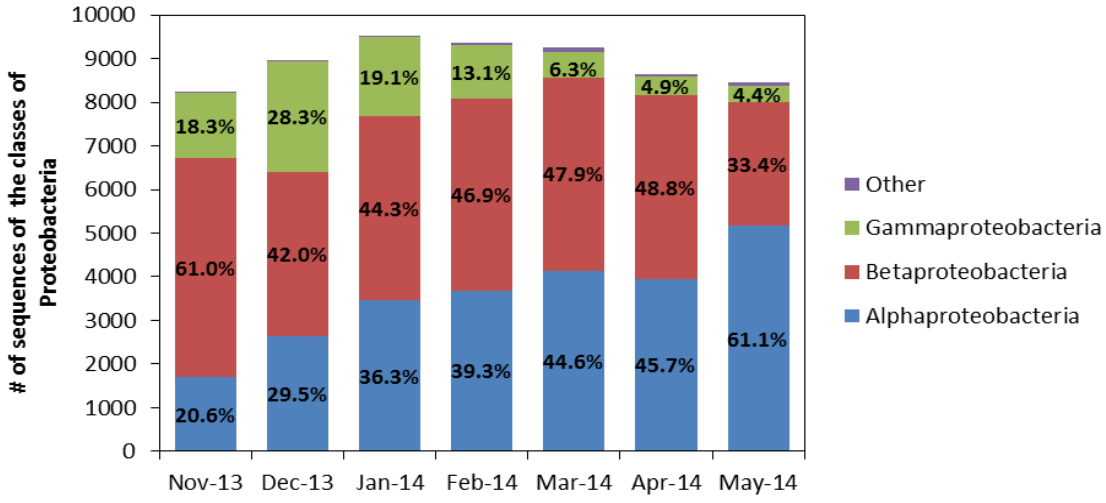


**Figure 4-19. The amount of dominant *Proteobacterial* classes over time on the Calgon F300 GAC media from the pilot-scale filtration system.**

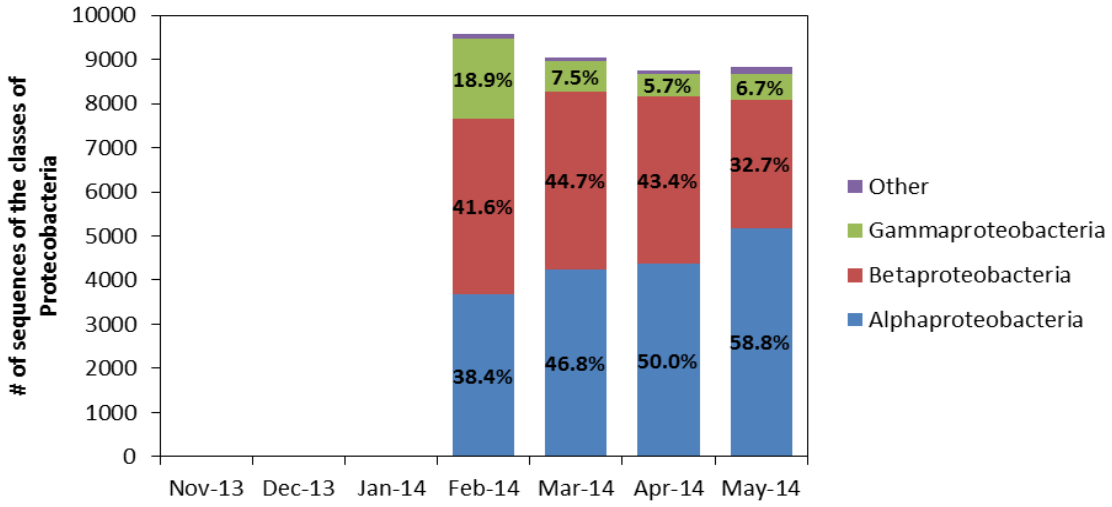


**Figure 4-20. The amount of dominant *Proteobacterial* classes over time on the Norit 300 GAC media from the pilot-scale filtration system.**

The Illumina MiSeq Profiles of the pilot-scale filtration system were further analyzed at the genus level. The three most dominant genera for the anthracite initially were *Flavobacterium*, *Rhizobacter*, and *Hydrogenophaga* (Figure 4-26). The three most dominant genera initially for the Calgon F300 and Norit 300 GAC were *Hydrogenophaga*, *Rhodobacter*, and *Rhizobacter* (Figure 4-27 and 4-28). As operation continued, all three media types saw a shift in the community over time and an increase in the abundance of *Sphingomonas*. *Legionella*, a pathogen that is regulated by the EPA in drinking water and can cause a type of pneumonia, was detected but made up less than 0.3% of the community for all media types. Investigating down to the genus level shows the microbial community is unique to each media type and the community shifts over time.

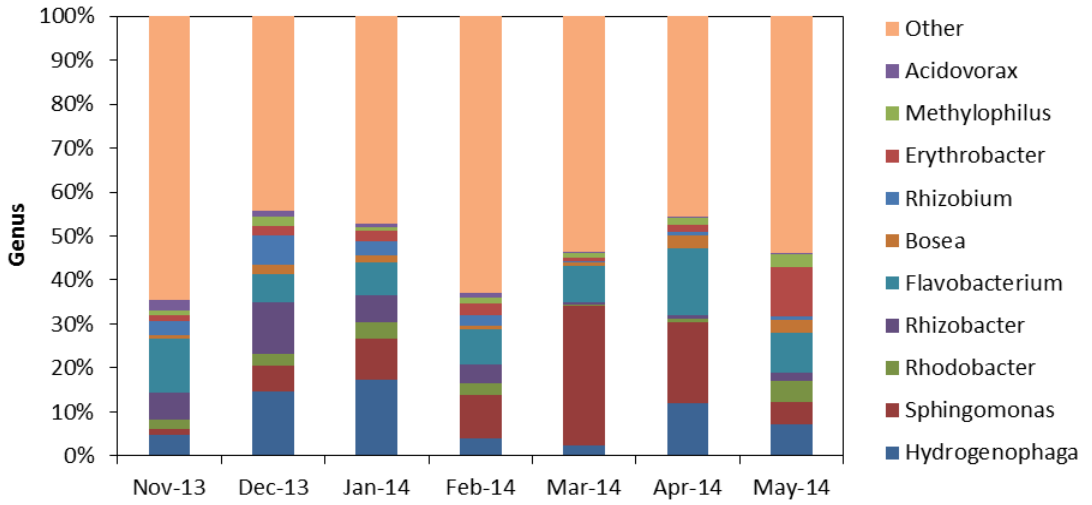


**Figure 4-21. The abundance of dominant genera over time on the anthracite media from the pilot-scale filtration system.**

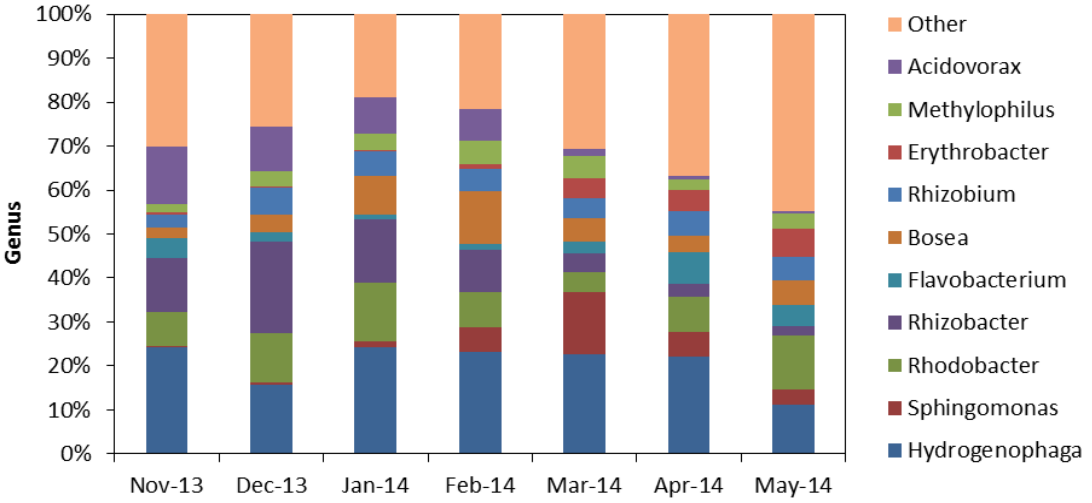


**Figure 4-22. The abundance of dominant genera over time on the Calgon F300 GAC media from the pilot-scale filtration system.**

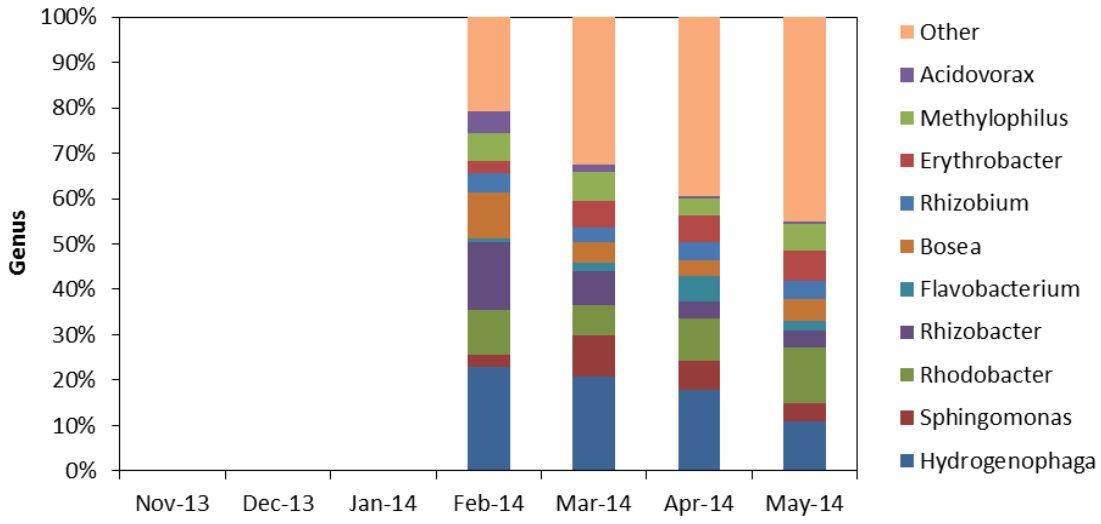


**Figure 4-23. The abundance of dominant genera over time on the Norit 300 GAC media from the pilot-scale filtration system. November, December, and January sample did not have enough Bacteria to sequence.**

A non-metric multidimensional scaling plot was generated from the Illumina MiSeq profiles to visually compare the change in community composition for the pilot-scale anthracite, Calgon F300 GAC, and Norit 300 GAC filters (Figure 4-29). The bacterial communities for each media type shifted over time but tended to cluster with each other and not with the communities from the other media. Thus, bacterial community composition was impacted by the media type and by the time since start of operation.

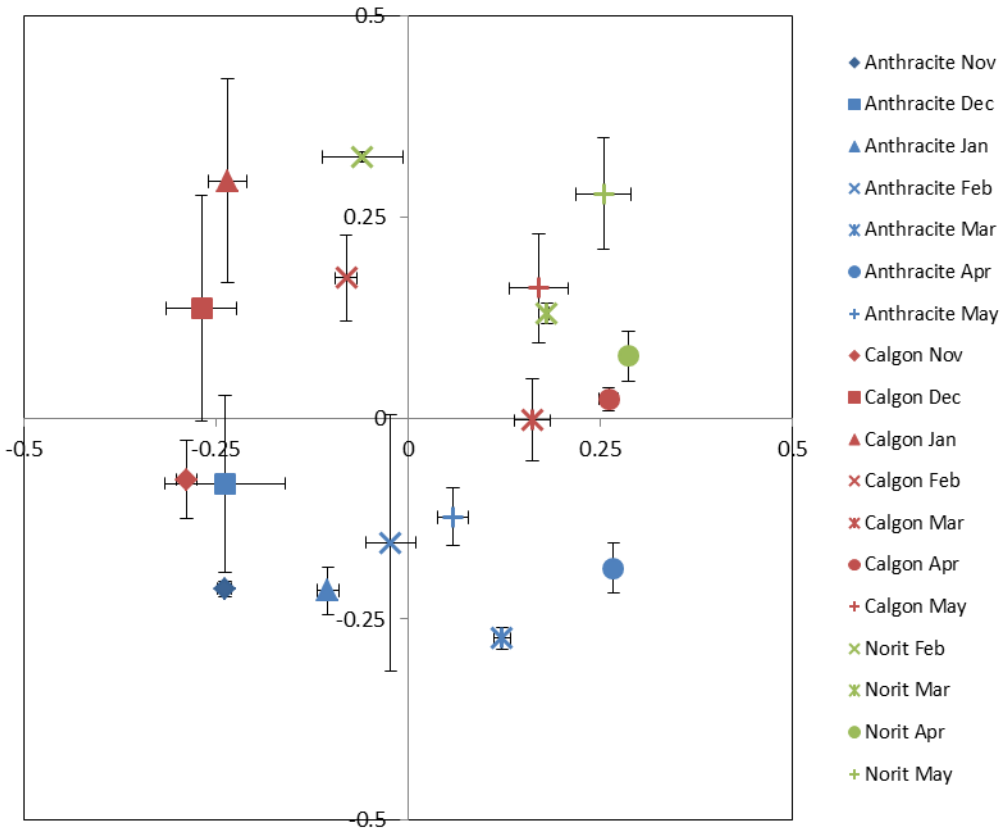


**Figure 4-24. A non-metric multidimensional scaling plot comparing the Illumina MiSeq profiles of anthracite, Calgon F300 GAC, and Norit 300 GAC media from the pilot-scale filtration system. The error bars represent one standard deviation from the mean.**

# 5 Discussion

GAC performed better as a media filter than anthracite in removal of TOC and DOC in addition to removing a higher portion of the aromatic organic matter (SUVA) known for contributing to DBP formation. In comparing the microbial community composition of the different biologically-active filter media, the anthracite initially had a higher microbial diversity than GAC, but as operation continued the diversity of both GACs increased. Furthermore, both of the GACs and the anthracite enriched for a high concentration of microbial biomass. An advantage of GAC is its ability to bioregenerate, which can effectively lower operation costs by reducing the need to replace and regenerate the carbon in order to maintain the same water quality effluent (Aktas et al. 2007, Scharf et al. 2010). Overall, GAC as a filter media is effective at both physically and biologically improving the water quality.

The geosmin concentrations in the effluent from the GAC filters was consistently lower than the taste and odor threshold, whereas the anthracite was statistically higher and above this threshold. Prior research has demonstrated that GAC can both physically and biologically remove geosmin (Hargesheimer et al. 1996, McGuire et al. 1988, Cook et al. 2001, Scharf et al. 2010), however, the low activity (i.e., ATP) during the winter suggests that if geosmin removal was occurring, it was solely via sorption. Thus, when biological activity is low during colder months from December to March, the GAC filters have to rely on sorption to remove geosmin below the taste-and-odor threshold. Furthermore, during warmer temperatures above 15°C, biological removal of geosmin may occur, providing year-round geosmin removal. Additionally, other taste and odor compounds similar to geosmin, such as MIB, would likely be removed annually.

In examining the biological ability of these biofilters, microbial community composition is significantly affected by the type of filter media and time since startup. A previous BAC filtration study (Kim et al. 2014) determined that temperature and time since startup length of operation were the two most important factors in microbial community composition; however, that study was focused solely on GAC and lacked a comparison to alternative media types. In this study, the length of operation and temperature had an influence on the microbial community composition, in addition to the type of filter media.

The microbial communities present on the anthracite and both GAC filters were diverse. Ecological theory suggests that the community stability and resilience increase with increasing diversity (McNaughton 1977, Torsvik et al. 2002, Boon et al. 2011); furthermore, a more diverse microbial community is more resistant to invasion by an outside species (Chapin III et al. 2000). Previous studies have shown BAC to have a high diversity (Hope-Wilkinson 2012, Kim et al. 2014, Liao et al. 2013); however, the diversity of the BAC filters did not change over time (Hope-Wilkinson 2012, Kim et al. 2014). In this study, GAC microbial diversity increased over time, whereas anthracite diversity did not change. Both media types had the same operating conditions, however, the anthracite media was taken from the full-scale filter and thus previously was exposed to a longer operation. In effect, anthracite diversity reached a stable level, while GAC required time to develop a more diverse bacterial community.

Although temperature did not affect the bacterial biomass in the biofilters, it did significantly influence the activity levels (measured as ATP). ATP concentrations were low during initial pilot operation, which corresponds to a similar study that discovered

low ATP concentrations during the first two weeks of operation (Magic-Knevez et al. 2004). ATP concentrations, however, did not increase until the temperature increased in the spring. Despite the fact that the biological activity remained low through the winter, the GAC filters maintained a high microbial biomass and were able to effectively remove particles TOC, DOC, and geosmin.

In this study, both GAC filters were better able to enrich for nitrifying microorganisms than the anthracite filter. Nitrifier levels were initially low during the winter, but increased in spring as the water temperature increased. This is not surprising as a previous study concluded that the optimum temperature range for potential nitrification is 20-37°C (Stark, 1996). Previous reports have detected and quantified nitrifying microorganisms in GAC filters (Feng et al. 2012, Kasuga et al. 2010). Unfortunately, the other studies lacked a comparison of GAC with other media types. The abundance of nitrifying microorganisms in the biofilters in this study was somewhat surprising given that the influent ammonia concentrations were so low. This observation suggests that an alternative nitrogen source, which we suspect is from the chloramine in the backwash water, is enriching for nitrifying microorganisms or that they have an alternative role that allows them to thrive in this environment.

# 6 Summary and Conclusions

The objective of the MWTDS pilot-scale set-up was to investigate the performance of biological GAC filters compared to anthracite filters (non-sorptive controls) for geosmin removal, particle removal, and organic carbon removal. Additionally, the microbial community composition and diversity was examined. To properly achieve these objectives, eight columns were used in this pilot-study to provide triplicate anthracite columns, triplicate Calgon F300 GAC columns, and duplicate Norit 300 GAC columns. The results from the MWTDS pilot-scale set-up showed that biological GAC filters surpass conventional anthracite filters in removing particles and organic carbon, and geosmin below the taste and odor threshold.

Throughout the majority of the operation of the MWTDS pilot-scale set-up the temperature remained very low around 1°C, which allowed for us to examine the effect of cold temperatures on the biological filters. The low temperature contributed to lower microbial community, measured as ATP, in addition to slowing the development of nitrifying biomass within the community. Despite the fact that microbial activity was low during most of the operation, the GAC filters had a sufficient sorption capacity to consistently remove TOC, DOC, and geosmin.

In addition to the effects of temperature, the media type also significantly impacted the time to enrich for bacteria and the microbial community composition. Both the Calgon F300 GAC and anthracite quickly enriched for dense bacterial communities, whereas Norit 300 GAC took almost three months before a similar bacteria concentration was measured. Additionally, the microbial community composition was evaluated with high-throughput Illumina sequencing of 16S rRNA genes, which provided a detailed

picture of the community composition at the genus level. This level of sequencing power has only been conducted once before on biological GAC filters (Hope-Wilkinson, 2012), thus the data it produced can be compared to this study in addition to providing the initial background information on the microbiome of drinking water biofilters. In this study, the microbial community compositions were more similar for the two GAC filters compared to the anthracite filters, but overall all three media types had unique microbial communities that changed over time. Thus, the media type is an important factor in shaping the enrichment and microbial community composition, however, both GAC types performed similarly in particle and organic carbon removal.

Furthermore, these biological filters exhibited moderate to high diversities and the diversity of the GAC increased overtime. Diversity is important in providing the biologically-active filters with resiliency and stability of performance, which is pertinent in lieu of changes in raw water quality from the Mississippi River that inevitably occur over time. In addition to diversity, biological GAC filters offer significant ability to biologically regenerate over time, which increases the GAC bed life and thus decreases the GAC replacement frequency– effectively lowering the operating cost for the treatment plant.

## 6.1 Recommendations

In conclusion, biological GAC filters offer significant advantages over the anthracite filters that are currently used at MWTDS. These advantages include year-round removal of organic carbon and geosmin. In addition, diverse microbial communities will develop on the GAC filters, which will help accommodate the changing

influent raw water and biologically regenerate the activated carbon capacity for sorbing geosmin. The GAC filters will offer similar particle removal performance as the anthracite filters, with difficulties during spring runoff. MWTDS should consider the use of polymer during spring runoff to improve the particle removal performance of the GAC filters during this challenging treatment period.

## 6.2 Limitations and Future research

Future research should focus on characterizing the microbial communities over long-term operation and investigate how temperature, length of operation, and influent organic matter would impact the community. In order to determine the long term impact, the pilot-scale set-up would need to be run for at least a year to determine the influence of the range of temperatures and influent organic matter that the utility annually experiences. Additionally, the microbial communities should be profiled through the depth of the media to provide a more complete picture of the influence of temperature, length of operation, and influent organic matter. Future research should also allow for the filters to reach full bed exhaustion and the biological degradation of various concentrations of taste and odor compounds and organic carbon should be studied to determine the biological regeneration limit.

A limitation of this study is the temperature remained low during the majority of the operation, which did not allow for evaluation of filter performance during warmer temperatures that would be expected throughout the summer. Additionally, it was difficult at times to accurately quantify the influent geosmin concentration, which limited the ability to quantify geosmin removal efficiency.

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# Appendix A: Pilot-scale Operation History

| | |
|---|---|
| 10/25/13 | Packed Columns 1, 2, 4, 5, 6, and 7 with media. Columns 1, 2, and 4 were backwashed with water for 10 minutes. |
| 10/30/13 | Packed Columns 3 and 8 with media. Columns 3, 5, 6, 7, and 8 were backwashed. The pilot set-up was ran for ~18 hours before the water level in the recarb chamber got too low and shut off the pumps. |
| 11/13/13 | Started up operation of pilot set-up and dosing of HCl and $FeCl_3$. Flowrate was set to high on metering pump for $FeCl_3$. Dosed in 5x concentration for ~1 hour so shut off ferric metering pump. |
| 11/17/13 | Pumps shut off due to low water level in recarb chamber. |
| 11/18/13 | Ordered new submersible pump. No pilot operation until new pump comes in. |
| 11/25/13 | Submersible pump was installed. Started up pilot set-up operation with correct dosing of $FeCl_3$ and HCl. |
| 12/2/13 | Ran out of HCl over thanksgiving break. |
| 12/17/13 | Start dosing geosmin, but was leaking out of syringe. |
| 12/18/13 | The syringe leak was fixed and started dosing geosmin. |
| 12/24/13 | Ran out of geosmin. Continued to stop dosing geosmin until MWW was ready to start analyzing for geosmin. |
| 12/30/13 | Pumps were shut off overnight due to sensor being tripped |
| 12/31/13 | Start dosing geosmin and MWW began monitoring geosmin concentrations. |
| 1/7/14 | Geosmin pump had stopped so was started up again. |
| 1/17/14 | HCl and $FeCl_3$ stocks were empty for ~6 hours |
| 2/14/14 | The recarb source water was switched from the South chamber to the North chamber. |
| 2/21/14 | Changed geosmin dosing point to overflow tank with mechanical mixer. |

| | |
|---|---|
| 4/11/14 | Changed geosmin dosing point back to above the in-line static mixer |
| 5/20/14 | Influent turbidimeter flowmeter was clogged and reading 0 NTU. The flowmeter was cleaned and the NTU increased instantly. |

## Appendix B: Backwash Protocol

**1.** Close **"Effluent"** ball valve. If column 1, 2, or 3, then also close "**Turbid**" ball valve.

**2.** Next, use a ladder to close the "**backwash drain**" ball valve.

**3.** Go behind the column set-up and close "**Influent**" ball valve. Then turn off the pump with the light switch.

**4.** From behind the columns go to the far left side and open the "**Air**" valve.

**5.** Go to the front of the columns and open the "**backwash**" valve on the bottom right hand side of the column. Let the air run for ~ 5 minutes.

**6.** After 5 minutes, close the "**backwash"** valve and open the "**backwash drain**" ball valve.

**7.** Go behind the column set-up on the far left and close the "**Air**" valve.

**8.** Open the "**Water 1**" valve located on the brick wall. Then open the "**$H_20$**" valve located above the "**Air**" valve.

**9.** Go to the front of the columns and open the "**backwash"** valve on the bottom right hand side of the column. You may have to gently pound on the column to get air bubbles out. Let the water run for ~10 minutes.

**10.** After 10 minutes, close the "**backwash**" valve. Go behind the columns and close the "**$H_2O$**" valve.

**11.** Open the "**Influent**" ball valve and turn on the pump with the light switch.

**12.** Go to the front of the column and adjust influent flow meter to between 0.075-1.0 GPM. Open slightly the "**effluent**" valve to maintain about two feet of water in the column. This "**Effluent**" valve may need to be adjusted after 5 minutes when you can see if the water in the column has maintained the same height.

**13**. Begin backwashing the next column.

# Appendix C: Drawings and Photos of Pilot-scale Column Set-up

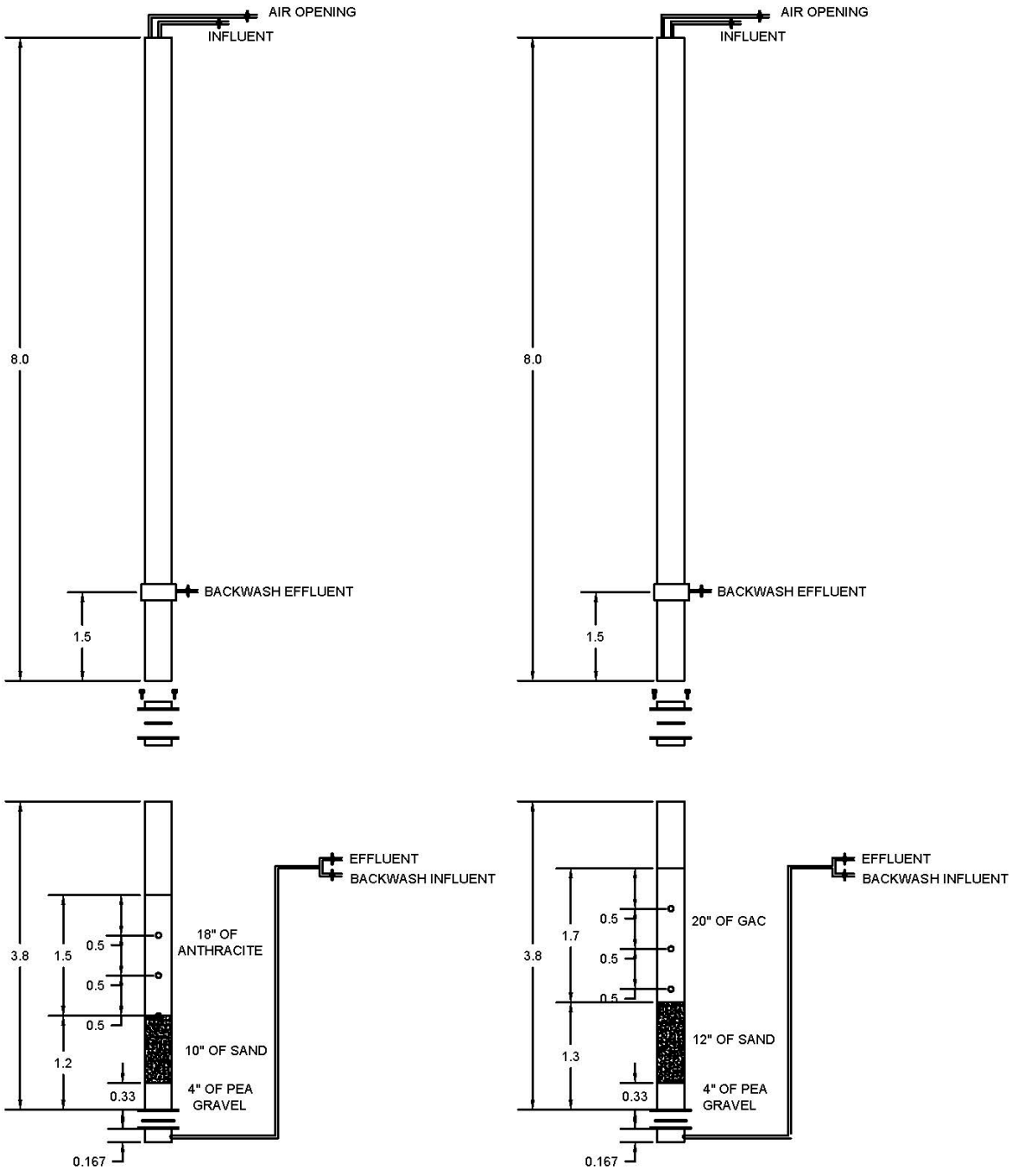



**Figure D.1. The design of an anthracite and GAC column showing the location of influent, effluent, backwash ports, and air opening.**

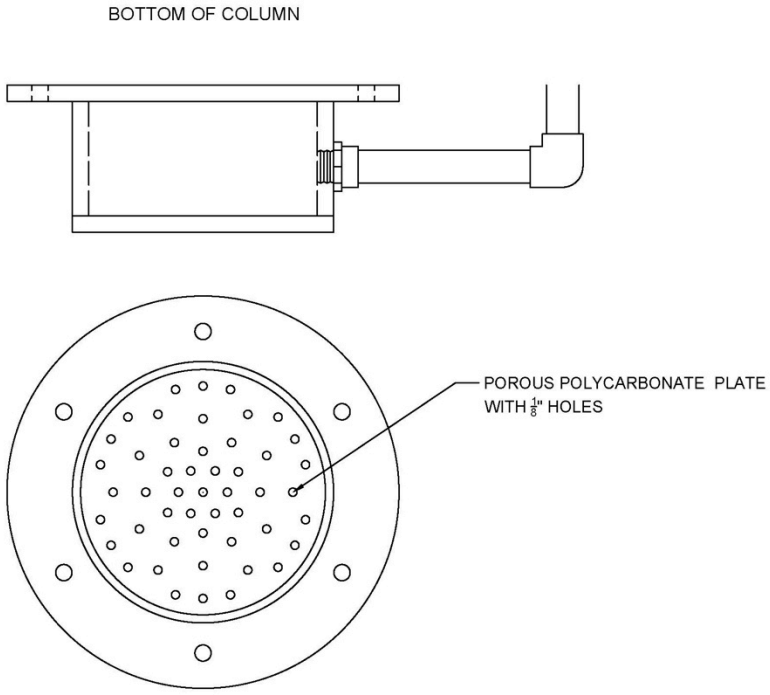



**Figure D.2. A detailed drawing of the porous plate located at the bottom of each column at MWTDS.**

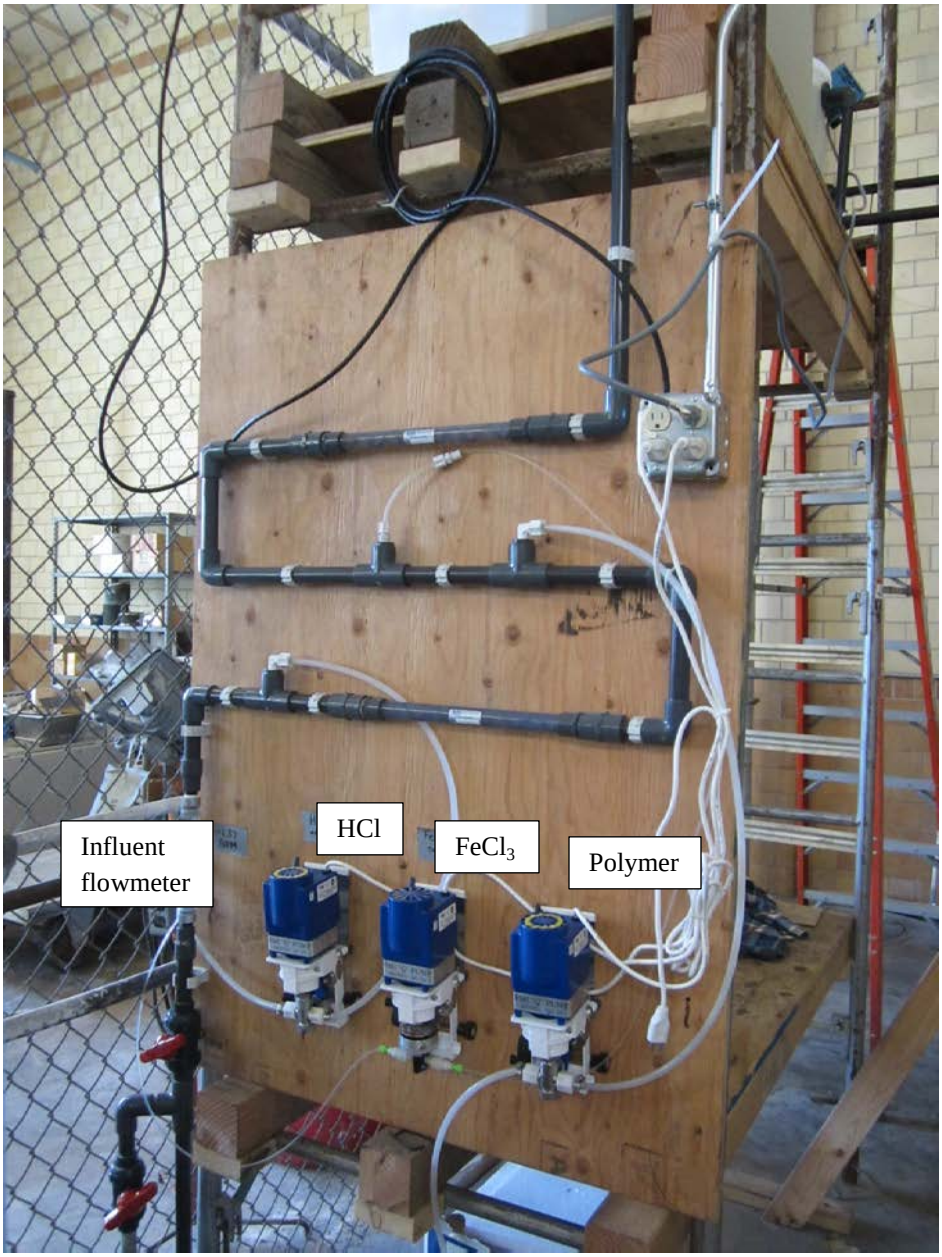



**Figure D.3. Photo of the pilot-scale set-up influent flow meter with the three metering pumps to dose in HCl, $FeCl_3$, and a polymer at MWTDS.**

**Figure D.4. Photo of the pilot-scale set-up rectangular settling tank and the circular overflow tank with mechanical mixer at MWTDS.**

**Figure D.5. Photo of the syringe pump used to dose geosmin into the pilot-scale set-up at MWTDS.**

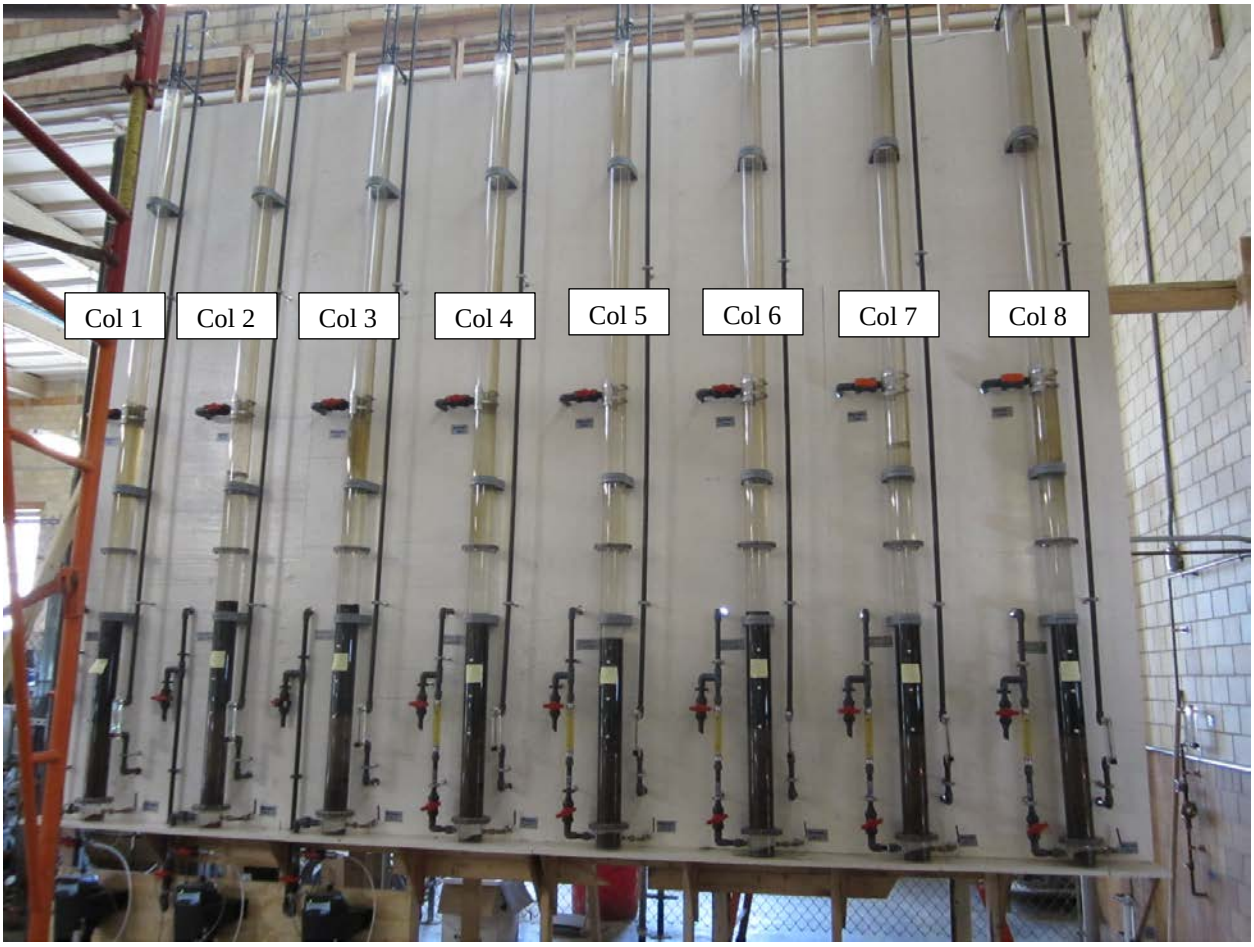



**Figure D.6. Photo of the eight columns in the pilot-scale set-up at MWTDS. Column 1, 4, and 5 contained anthracite. Column 2, 6, and 7 contained Calgon F300 GAC. Column 3 and 8 contained Norit 300 GAC.**

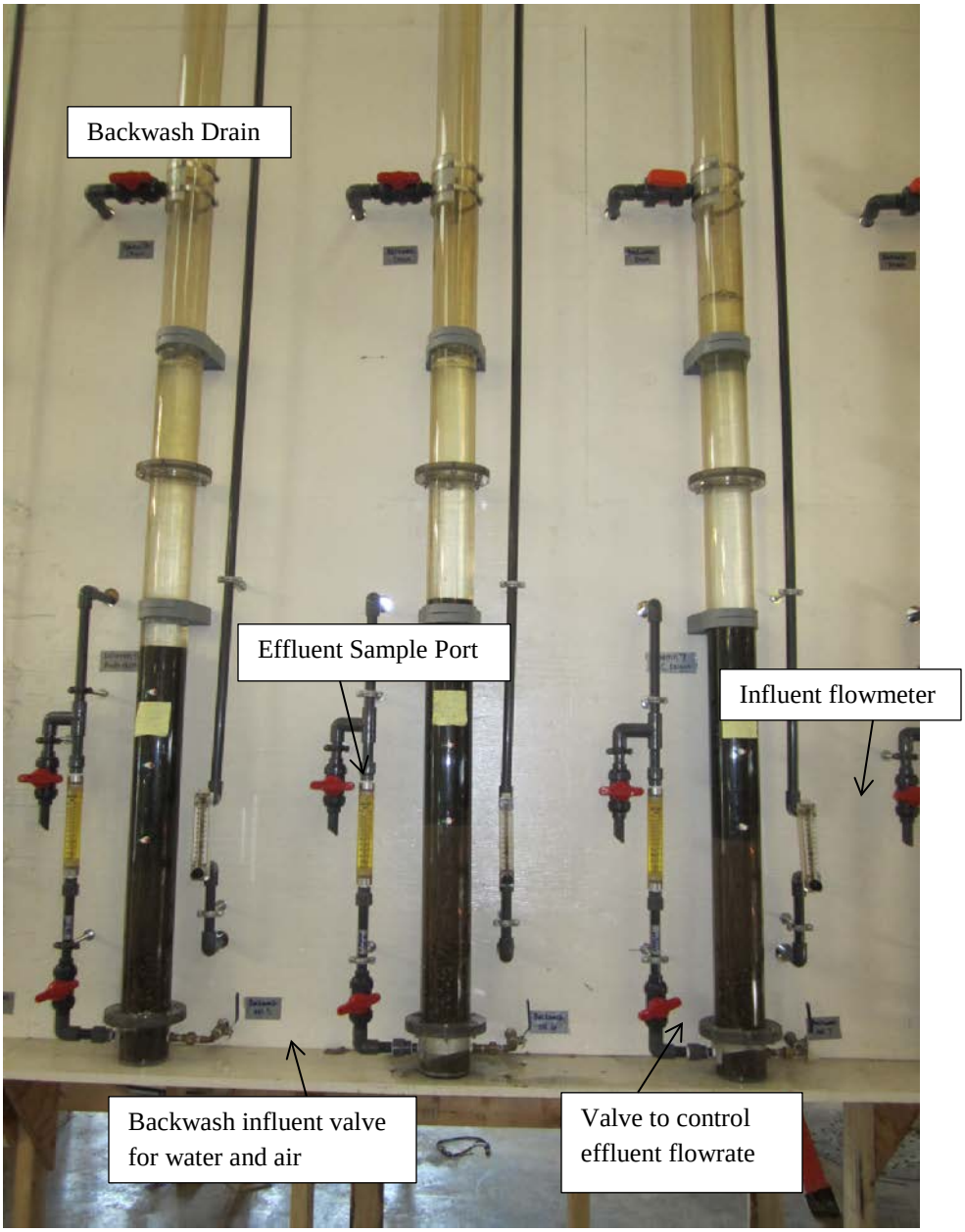



**Figure D.7. Photo of three columns pointing out the valves and flow meters for the influent, effluent, backwash, and sampling port at MWTDS.**

# Appendix E: Geosmin QA/QC from MWDTS SOP for analysis of taste and odor compounds

9.1 An initial demonstration of capability (IDC) study must be performed prior to use of the method by each analyst or after any significant changes to the method. An IDC study consists of four aliquots of reagent water spiked with target analytes and processed through the entire analytical method.

- 9.1.1 Prepare and analyze four spiked blank samples at a concentration of 20 ng/L.
- 9.1.2 Calculate the mean concentration found (X) in ng/L and the standard deviation of the concentration in ng/L for each analyte.
- 9.1.3 For each analyte X should be between 70% and 130% of the true value. The RSD should be 40% or less.
- 9.1.4 If the results from all analytes meet these criteria then the system and analyst performance are acceptable. If any analyte fails to meet the criteria then investigate and correct the source of the problem and repeat the test.

9.2 The mass spectrometer must be autotuned following the instrument manufacturer algorithms prior to calibrating.

9.3 There must be an initial calibration of the GC/MS following the procedures in Section 10.2.3 that meets the criteria listed below. The instrument conditions used for the initial calibration must also be used for sample analysis.

- 9.3.1 An initial calibration curve consisting of at least 5 points to determine instrument sensitivity and the linearity of response.
- 9.3.2 The lowest calibration point in the curve must be at or below the reporting limit. Since this point drives the reporting limit, it must always be included in the calibration curve, and may not be discarded. Calibration points may not be removed from the middle of the calibration curve. If any calibration point is removed clear documentation of this removal and the reason for removal will be written and included with the calibration curve raw data.
- 9.3.3 The use of linear and non-linear models for calibration data are allowed and are often the only usable choice for taste and odor compounds. The option for non-linear calibration may be necessary to achieve low detection limits or to address specific instrument techniques. However, one shall not allow non-linear calibration to be used to compensate for detector saturation at higher concentrations or to avoid proper instrument maintenance.
- 9.3.4 The following steps shall be adhered to when deciding on a calibration fit:
  - 9.3.4.1 Linear Least squares regression is the next possible choice in curve fit factor. This fit uses the response factor from all

data points to generate a curve; the correlation coefficient, r, must meet or exceed >0.990 to utilize this curve type. If the curve does not meet acceptance criteria and Section 9.3.3 is adhered to, then non-linear calibration models may be employed. [Line equation: Y=mx+b]

9.3.4.2 Non-linear calibration models may be used in situations where the analyst knows the instrument response does not follow a linear model over a sufficiently wide working range, or when other approaches above do not meet acceptance criteria. The coefficient of determination, $r^2$, value >0.990 must be met or exceeded to utilize this curve type. Section 9.3.3 must be adhered to in every case. [Line equation: $Y=Ax^2+Bx+C$].

9.4 Calibration verification (CV) of the initial calibration must be completed prior to the analysis of samples as well as at the end of the analytical sequence. The calibration verification is the injection and analysis of the 10 ng/L calibration standard. All compounds of interest must recover within 30% of the expected value prior to beginning the analysis of samples. If the CV fails it should be reanalyzed once to ensure that it was prepared correctly. If the CV fails criteria again recalibration may be necessary.

9.5 Blank samples must be analyzed once per day. The blank sample must be shown to be free of analytes of interest prior to beginning analysis. If analytes are detected in the blank the SPME fiber should be reconditioned or replaced.

9.6 A duplicate sample should be analyzed if sufficient sample is provided. The sample chosen for the duplicate analysis should be rotated to understand the recovery of analytes out of the various process samples analyzed. This sample should have a RPD for all analytes detected above the reporting limit of less than 40%.

9.7 A matrix spike sample may be prepared at the rate of one sample per analytical batch. The sample chosen for the matrix spike should be rotated to understand the recovery of analytes out of the various process samples analyzed. The acceptance criteria will be 50-150%.

9.8 A running log of instrument response must be kept to ensure the ongoing instrument operation maintains consistency.

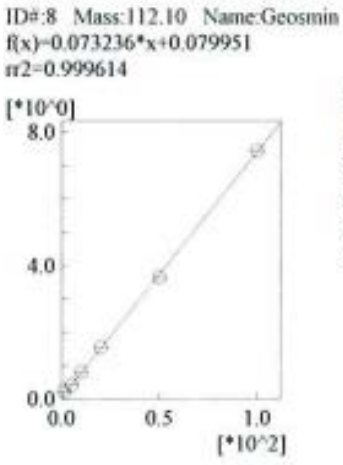
9.8.4 Record the response for the 10 ng/L calibration standard and the subsequent daily calibration verifications each day the analysis is performed.

9.9 Internal Standard – If 2-Isopropryl-3-methoxypyrazine is being utilized as an internal standard it will be added to each standard, blank, and sample at the same concentration to determine acceptability of each injection.

9.9.4 If an Internal Standard is used it will be used to quantitate the analytes of interest and the appropriate instrument method will be implemented.

9.9.5 The Internal Standard in each sample, standard, and blank will be compared to the average Internal Standard response from the most recent initial calibration. To perform this tabulate the responses from each of the calibration standards utilized in the initial calibration and determine the average value.

9.9.6 Acceptable Internal Standard response will be within 50-200% of the average internal standard response from the most recent initial calibration curve.




| # | Conc. Ratio (ppt) | Mean Area Ratio |
|---|---|---|
| 1 | 1.000 | 0.22 |
| 2 | 5.000 | 0.42 |
| 3 | 10.000 | 0.81 |
| 4 | 20.000 | 1.54 |
| 5 | 50.000 | 3.65 |
| 6 | 100.000 | 7.45 |

**Figure E-1.** Example Calibration Curve for geosmin analysis by MWTDS.