INVESTIGATION OF BIOLOGICALLY ACTIVE GRANULAR ACTIVATED CARBON FILTERS

A THESIS SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

SEPTEMBER 2012

Acknowledgements:

I would like to acknowledge Saint Paul Regional Water Sources for funding this research and providing technical support. I would specifically like to thank Jim Bode and Che Fei Chen for their support in organizing the project and resources. The project would not have been possible without the help of Andrew Magdziarz and Daniel Finnegan who helped me put together and maintain the pilot system at Saint Paul Regional Water Services. Special thanks to Jack Storm who built the manifold and geosmin dosing tank, to Mark Cullen for building the shelves for the columns, and Steve Brown for doing all the electrical work involved in running 16 pumps, two data loggers, and a heater above a large pool of water. Additional thanks to the many operators who troubleshot those midnight emergencies during the pilot study period. And thanks to Matt Madson who babysat for the 6 weeks when I was traveling this summer.

I would like to thank my co-advisers, Dr. Raymond Hozalski and Dr. Timothy LaPara, for entrusting me with this project and giving me the opportunity to learn all that I did. Their coaching and direction led this project into new and exciting areas. Thanks to Dr. Simcik for being a member of my committee and taking the time to review my thesis. My appreciation to Dr. Matt Hamilton, Dr. Brian Badgley and Dr. Mike Sadowsky, who taught me how to do Illumina sequencing. Especially Dr. Hamilton's help processing the sequence data. Finally many thanks to my officemate and friend Andrew Rescorla who's help with the GC-MS and autosampler was invaluable.

Abstract

Geosmin is naturally produced by numerous cyanobacteria and actinomycetes in surface waters. Although it is non-toxic, it causes an unpleasant taste and odor even at very low concentrations. Water utilities, therefore, often must expend great effort and funds to remove geosmin to avoid customer complaints when the compound is present in the source water. Saint Paul Regional Water Services in St. Paul, MN successfully uses granular activated carbon (GAC) filters to remove geosmin from its drinking water, but, curiously, the useful life of their full-scale GAC filters has exceeded estimates based on batch sorption isotherms and AdDesignS modeling. It has been hypothesized that geosmin-degrading microorganisms on the GAC filters degrade geosmin, thereby extending the GAC filter bed life.

In this study, the microbial communities growing in full-scale, biologically active GAC filters at Saint Paul Regional Water Services were characterized using automated ribosomal spacer analysis (ARISA) and high throughput DNA sequencing (Illumina sequencing) of 16S rRNA gene fragments. This study showed that Saint Paul Regional Water Services has highly diverse bacterial filter communities that are functionally stable throughout year. Illumina sequencing revealed that a dominatnt bacterial phylum on the GAC filters was *Nitrospira* and that pathogen levels (e.g., *Enterobacteria)* were negligible.

Additionally, the effects of mediatype and inoculation on the development of a geosmin-degrading bacterial community on GAC filters was investigated using a pilot-scale column system that was fed geosmin. A geosmin-degrading biofilm developed after 40 days of being enriched with 100 ng/L of geosmin. Additionally, the geosmin-degrading organisms proved to be robust in that they were able to resume geosmin degradation after 6 weeks when geosmin was absent. The effects of GAC type and inoculation did not impact the biomass levels or geosmin removal.

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1.0 Introduction

Most communities are not endowed with a water source that is directly potable so they must treat their water before consuming it. Water is treated to remove pathogens and chemicals and to improve aesthetic qualities. Unless pathogens and harmful chemicals are removed, waterborne disease can occur resulting in severe illness and sometimes even death of the very young and immunocompromised (WHO, 2010). Unclean water can also cause uncomfortable gastrointestinal distress, lung irritation, vomiting, and dizziness. On average, surface waters require more treatment than ground waters; typically ground waters are of a higher quality due to their pre-treatment by long infiltration in the ground (Water Treatment, 2005, p. 258), whereas surface waters are more easily contaminated by industry, agriculture, and nature.

Surface waters are conventionally treated by screening, softening, coagulation and flocculation, sedimentation, filtration, and disinfection (Figure 0-1). Screening removes large debris, such as leaves. Softening is the removal of polyvalent cations, typically by chemical precipitation by the addition of lime ($Ca(OH)_2$) and soda ash (Na_2CO_2). Coagulation involves the addition of chemicals, such as aluminum sulfate ($Al_2(SO_4)_3$ or ferric chloride ($FeCl_3$), to decrease the repulsive interaction energies between particles thus encouraging particles to aggregate to form larger particles with higher settling velocities. Flocculation, carefully controlled mixing, is an important step in getting particles to come together by increasing particle collisions and promoting the formation of larger particles masses. Water then travels through a sedimentation basin where particles with high settling velocities are removed. After these processes the water is filtered, typically through granular media (sand, anthracite, granular activated carbon) to remove the particles that remain after sedimentation. Finally, disinfection is required to inactivate any remaining pathogens and provide protection against regrowth in the water distribution system (Water Treatment, 2005, p. 1862).

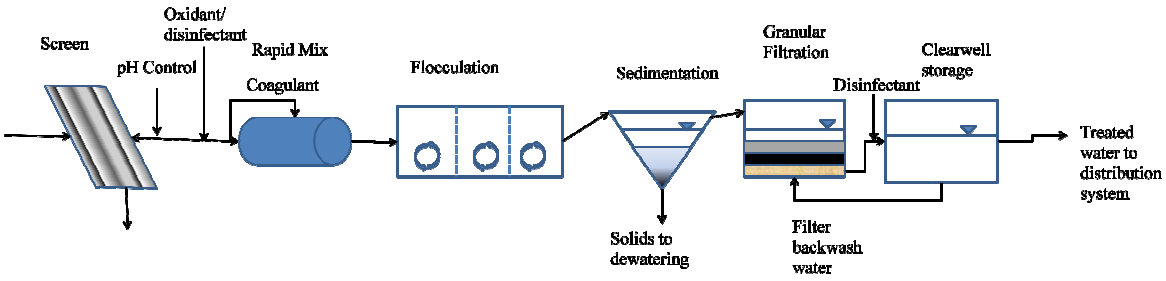


Figure 0-1: Typical process train for treatment of surface water in the United States (Water Treatment, 2005, p. 265).

In addition to concerns about public health, the aesthetic qualities of drinking water are also important. Aesthetic issues vary by source water but can include: clarity, temperature, taste and odor, and hardness. Hardness is the presence of multivalent cations, typically Ca^{2+} and Mn^{2+}; hardness is objectionable to the consumer because it decreases the effectiveness of detergents and causes scaling in pipes and faucets (Water Treatment, 2005, p. 63). Temperature is also an important aesthetic quality; consumers prefer cool or room temperature water. Clarity, taste, and odor are especially important because those factors are the key qualities that consumers use to sometimes erroneously judge the safety of their water. Water that is cloudy, odorous, or odd tasting makes consumers anxious about the safety of their water. Taste and odor problems are very closely linked to the water source. Ground waters commonly have issues with hydrogen sulfide (H_2S), reduced iron (Fe^{2+}) and reduced manganese (Mn^{2+}) (Water Treatment, 2005, p. 108).

Surface waters are frequently affected by the presence of algae and cyanobacteria which not only degrade water clarity but can also impart unpleasant tastes and odors to the water. The most common taste and odor compounds made by algae and cyanobacteria are geosmin and methylisoborneol, which cause earthy/musty odors. Algae have also been identified that produce grassy, septic, fishy and spicy tastes and odors (Water Treatment, 2005, p. 106). Geosmin is responsible for more taste and odor complaints than any other single compound (Persson et al., 1983); causing issues all over the United States (Figure 1-2). It is produced by actinomycetes and

cyanobacteria living in surface water. Geosmin has a very low odor threshold concentration of 10 ng/L (Suffet et al. 1996).

Figure 1-2: Map of the United States, with cities marked that experience severe Taste and Odor issues due to geosmin, according to their water utility webpage.

Geosmin is not removed by conventional drinking water treatment processes (Bruce et al., 2002). The main options for water utilities needing to remove geosmin from their drinking water are ozone and activated carbon (Westerhoff et al., 2006, Cook et al., 2001). Ozone is a powerful oxidant that oxidizes geosmin into non-offensive compounds primarily via an indirect mechanism (e.g., *OH). A few concerns regarding use of ozone for geosmin removal include high costs and the formation of biodegradable organic matter that can lead to biofouling and regrowth in the distribution system (Water Treatment, 2005, p. 1529). Activated carbon removes geosmin via sorption and is used in two forms: granular and powdered. Powdered activated carbon (PAC) is added directly to the water and is then removed via sedimentation and/or filtration. Granular activated carbon (GAC) is applied in a fixed or packed bed configuration. Another removal mechanism for geosmin is biodegradation. Extensive research has shown the

capacity of microbial communities to degrade geosmin in laboratory settings (MacDonald et al., 1987, Elhadi et al., 2006, Ho et al., 2007), however biodegradation has yet to be successfully implemented as a geosmin control strategy by water utilities. There is some evidence, however, that geosmin can be removed to below the odor threshold concentration using a microbial community on sand filters (McDowall et al., 2009). A few water utilities, such as Saint Paul Regional Water Services and Tulsa Water Utility, believe that biodegradation is responsible for geosmin removal on their GAC filters even though neither of these utilities designed their filtration systems for the purpose of biological removal of geosmin.

Saint Paul Regional Water Services has a history of numerous taste and odor complaints that were attributed to geosmin. In 2007, Saint Paul Regional Water Services retrofitted their sand filters into GAC filters, which resulted in a precipitous drop in consumer complaints (Figure 0-3). The GAC filters at Saint Paul Regional Water Services have already passed their estimated bed life (921 to 1241 days) yet still appear to be effective at removing geosmin. This enhanced bed life has been attributed to biological activity. The use of microorganisms in drinking water treatment is counter intuitive as historically the objective of drinking water treatment was to remove as many microorganisms as possible. Water utilities originally disinfected water at the entrance to the plant in order to control algae and bacterial growth within the plant. Recent research has indicated that the addition of a chemical oxidant at this initial stage leads to high formations of disinfection byproducts (Water Treatment, 2005, p. 1509). Many disinfection byproducts, such as haloacetic acids and halomethanes, are known to be carcinogenic; therefore, water utilities now refrain from disinfecting until after much of the natural organic matter has been removed via coagulation and sedimentation. Filters become biologically active when the point of chlorine addition is moved to post filtration or when GAC is used (GAC consumes residual oxidants) or both (Liu et al., 2001). These biologically active GAC filters contain microbial communities that are active in degrading a wide variety of organic compounds.

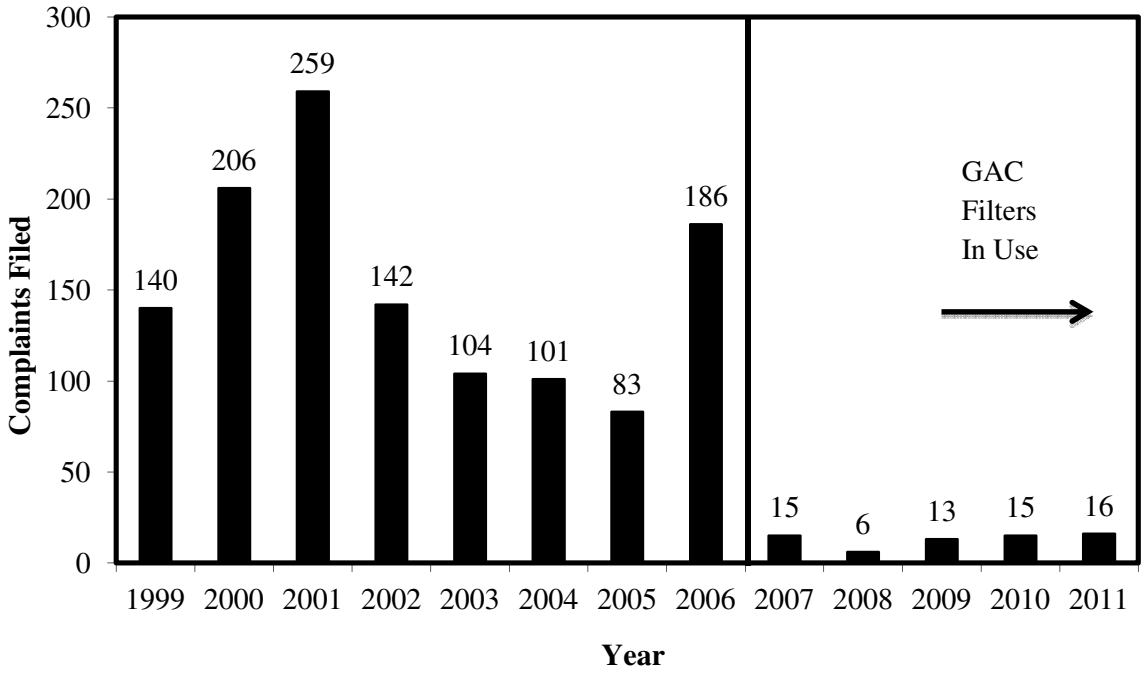


Figure 0-3: Complaint calls received by Saint Paul Regional Water Services for taste and odor issues in the last 12 years.

The goals of this research were to better understand bacterial community structure and function in full-scale biologically active GAC filters including spatial and temporal variability. An in-depth investigation into the biological activity on the GAC filters could potentially help water utilities improve water quality and decrease costs through manipulation of the community abundance and structure. To investigate these bacterial community dynamics automated ribosomal intergenic spacer analysis (ARISA) and Illumina sequencing were employed.

Another goal was to investigate two potential approaches for influencing the growth and structure of the microbial community on the GAC filters: variation of GAC type and inoculation of virgin media with biomass. Water utilities typically replace GAC every 1 - 5 years when the sorptive capacity has been exhausted. Thus, two important questions regarding GAC replacement are: (1) should the new GAC be selected solely on the basis of soprtion capacity for the

compound or compounds of interest and (2) should biomass be harvested from the old GAC media in an attempt to hasten the development of a contaminant-degrading community on new filter media. To answer these questions, a pilot-scale column system was built at Saint Paul Regional Water Services drinking water treatment plant. The pilot plant consisted of 14 columns being fed softened and settled water from the full-scale plant and spiked with geosmin for 100 days. The columns varied in type of filter media (i.e. aged GAC, two types of virgin GAC, and anthracite coal). Furthermore, six of the fourteen columns were seeded with bacteria harvested from one of the full-scale filters.

2.0 Literature Review

2.1 A brief overview of Saint Paul Regional Water Services

Saint Paul Regional Water Services provides drinking water to the residents of Saint Paul, Minnesota and surrounding suburbs. Saint Paul Regional Water Services serves almost 420,000 people over a 120 square mile area. The drinking water treatment plant was built in 1922 and has a water treatment capacity of 144 million gallons per day. Currently, Saint Paul Regional Water Services treats an average 35 million gallons per day.

Saint Paul Regional Water Services pumps water from the Mississippi River through a series of lakes (Figure 2-2-1). Water from the Mississippi River is pumped via a 60" culvert to Lake Pleasant, from which it flows into Sucker Lake and then into Lake Vadnais. Water is pumped from Lake Vadnais through another culvert to the drinking water treatment plant.

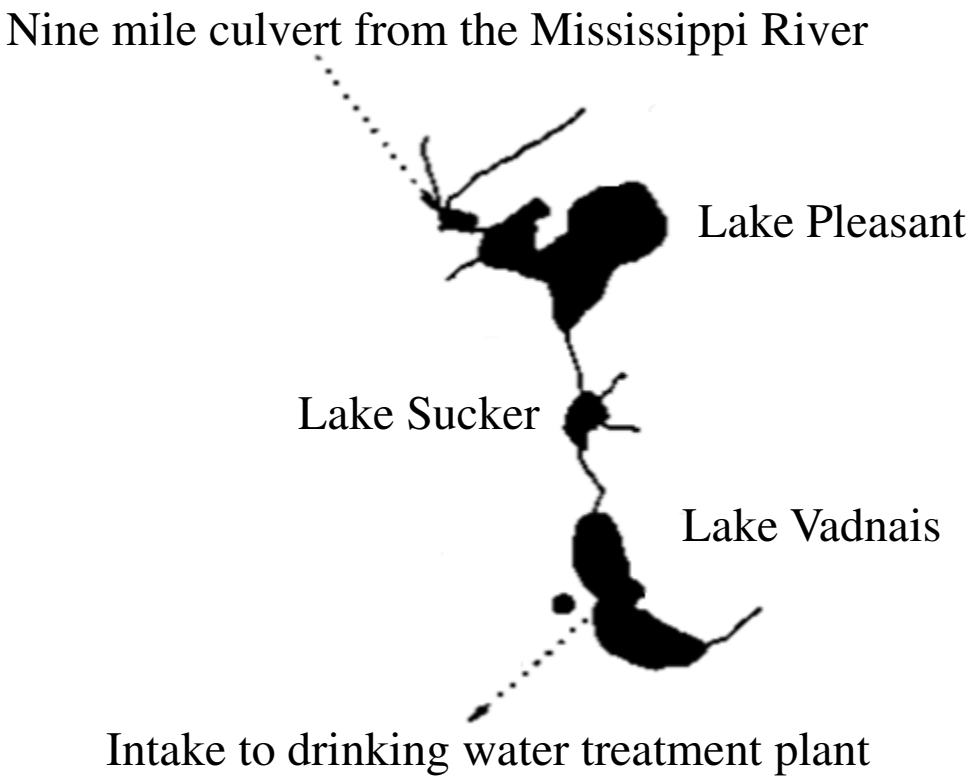


Figure 2-2-1: Map of Saint Paul Regional Water Services water sources and reservoir locations.

Water entering the plant begins treatment in a rapid mix chamber where aluminum sulfate and lime are added to encourage coagulation and remove hardness respectively. After an initial settling period, water is flocculated before sedimentation. Fluoride is then added to promote resident dental hygiene. Water is than recarbonated to decrease the pH to 8.0. Then, the water enters the GAC filters, after which chlorine is added for disinfection. Approximately seven minutes after the addition of the chlorine, ammonia is added to form chloramines, a more stable residual disinfectant. Water is then stored in a large underground reservoir before being pumped into the distribution system.

Saint Paul Regional Water Services consistently provides its consumers with high quality water. In its 85 year history, only one boil water advisory has been issued and even then water quality still met Environmental Protection Agency standards. Saint Paul Regional Water Services is well below maximum contaminant levels for trihalomethanes, nitrate, and haloacetic acids.

They meet turbidity, total chloroform bacteria, and copper level requirements throughout their system 100% of the time. Their most challenging water quality issue is taste and odor problems caused by the compound geosmin.

2.2 Geosmin

Geosmin imparts an earthy musty taste/odor that consumers find disagreeable in their water. Geosmin is a leading cause of taste and odor problems in municipal drinking water systems (Nerenberg et al., 2000). It is especially challenging to water utilities because geosmin has such a low odor threshold concentration at 10 parts per trillion (ng/L) (Young et al., 1996, Suffet et al., 1999).

2.2.1 Chemistry of Geosmin

The chemical name of geosmin is trans-1, 10-dimethyl-trans-9-decalol ($C_{12}H_{22}O$) (Figure 2-2). At room temperature the pure compound is a yellow oil. It has a molecular weight of 182.3 g/mol and a density of 0.985 g/cm^3. Geosmin is relatively hydrophobic (log K_{ow} = 3.70; Pirbazari et al., 1992) and readily sorbs onto activated carbon. It is semi-volatile and has a Henry's Law constant of 0.025 [dimensionless] at 20°C (Omur, 2004). Gesomin is relatively insoluble with a solubility of 0.55 g/L in water.

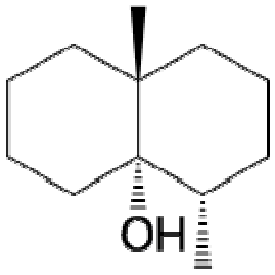


Figure 2-2 Chemical structure of geosmin, IUPAC name 1,10-*trans*-dimethyl-*trans*-9-decalol.

2.2.2 Microbiology of Geosmin

Geosmin is produced by cyanobacteria (formerly known as blue-green algae) in surface waters, as well as other nonphotosynthetic bacteria (e.g., *Streptomyces* spp.). Furthermore, fungi and some plants, such as liverworts and beets, can also produce geosmin (Giglio et al., 2008). Geosmin production rates are highest during the late spring, summer, and early fall when cyanobacterial blooms are most likely to flourish. A specific purpose for geosmin is unknown, but Utkilen and Frøshaug (1992) proposed that geosmin may act as a signaling molecule for marine fish that lay eggs in fresh water.

Three different pathways have been elucidated for geosmin synthesis (Figure 2-3). All proposed pathways end with a single Mg^{2+}-dependent protein catalyzing the cyclization of farnesyl diphosphate to a mixture of geosmin and germacradienol (Gust et al., 2003). Farnesyl diphosphate is a precursor in the production of terpenes, terpenoids, sesquiterpenes, and sterols. Terpenes are plant essential oils; their derivatives are the building blocks of steroids and other hormones.

Other organisms have now been identified that contain proteins with similar sequences (45 – 85% similarity) to the first isolated geosmin synthase. Using the universally conserved region of the geosmin synthase gene, the South Australia Water Corporation has developed a test to screen bacteria for the geosmin synthase enzyme (Giglio et al., 2008). With a test for geosmin producers, utilities may be able to predict episodes of high geosmin concentrations and implement treatment strategies to limit geosmin concentrations in the finished water.

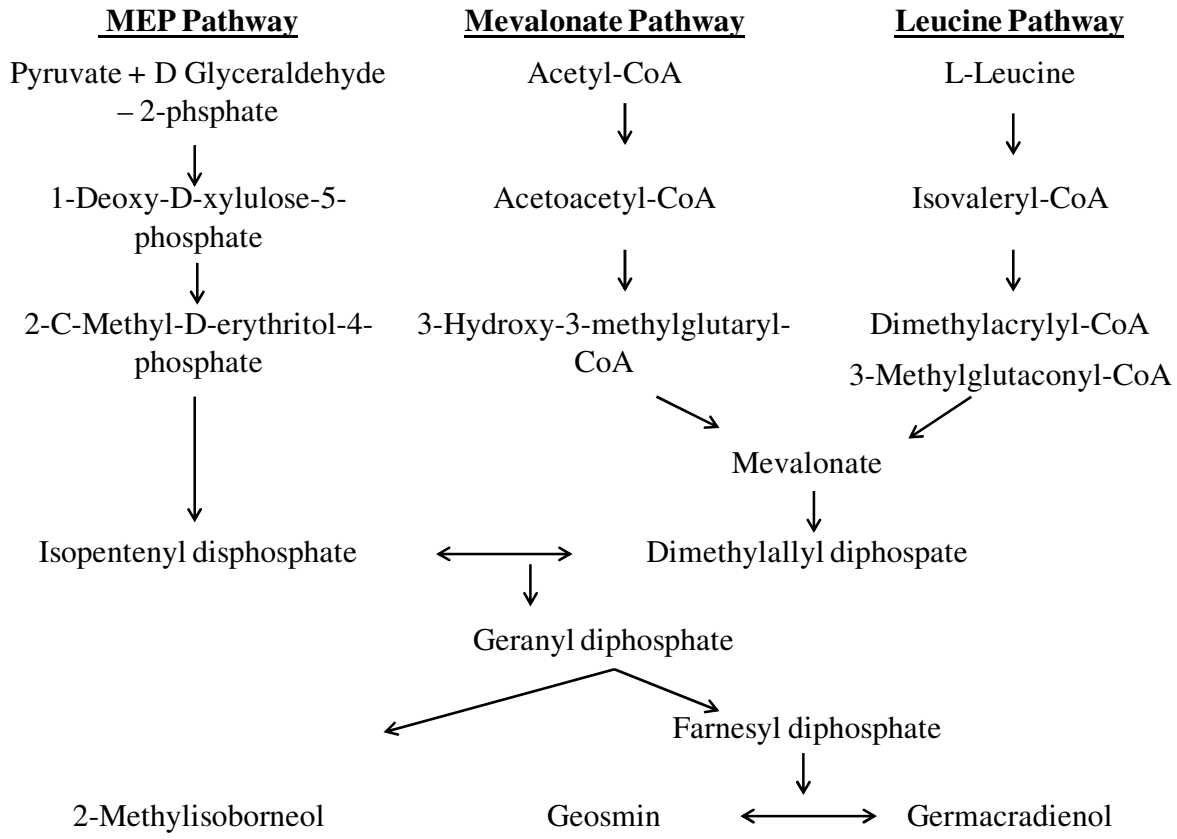


Figure 2-3: Synthetic scheme (suggested or proven for the formation of geosmin in streptomycetes and myxobacteria). (Juttner &Watson, 2007).

Compounds are biodegraded by either oxidation or reduction reactions. The bacteria known to degrade geosmin are aerobic which indicates that geosmin is most likely used as an electron donor or carbon source. The exact biodegradation pathway of geosmin is unknown and may vary between species. Geosmin may also be cometabolized – meaning the organisms obtain energy and carbon from another substrate but degrade geosmin fortuitously. Furthermore, even if geosmin is not cometabolized, the seasonal variation and the low concentrations (<200ng/L) of geosmin in surface water imply that geosmin degraders are capable of using other substrates.

Many research groups have reported biodegradation to be effective in removing geosmin from drinking water (Yagi et al., 1988, Elhadi et al., 2004, Elhadi et al., 2006, Scharf et al., 2010). Certain biofilms growing on sand, clay, gravel and GAC have been observed to remove geosmin

(Sumitomo, 1992, Yagi et al., 1988, Elhadi et al., 2004, Elhadi et al., 2006, Persson et al., 2006, Scharf et al., 2010). The Morgan Water Treatment Plant in South Australia has used biologically active rapid sand filters to consistently and effectively remove geosmin and methylisoborneol (another earthy musty odor compound) to below the odor threshold concentration for 30 years (Ho et al., 2007) even though research by Ho et al. (2002) and Elhadi et al. (2006) have reported that sand filtration is not an effective geosmin removal strategy.

A variety of organisms degrade geosmin, including both gram negative and gram positive species. Early researchers reported *Bacillus* sp. to be effective at degrading geosmin (Silvey et al., 1970, Narayen & Nunez 1974). MacDonald et al. (1987) could not replicate this success with *Bacillus* sp.. Further work by Saadoun and El-Migdadi (1997) found species of gram positive bacteria, *Arthrobacter atrocyaneus, Arthrobacter globiformis, Chloropenolicus* N-1053 and *Rhodococcus maris* capable of degrading geosmin. Their experiments, however, used naturally produced geosmin from *S. halstedii* without purification, leaving room for other influences to be affecting the biodegradation. Recently, Hoefel et al. (2006) reported that a consortium of three gram negative bacteria, most similar to previously cultured species of *Sphingopyxis alaskensis*, *Novosphingobium stygiae* and *Pseudomonas veronii* (based on 16S rRNA gene sequences) was able to effectively degrade geosmin. Each of the isolates, when cultured alone, was unable to degrade geosmin.

Saito et al. (1999) reported difficulty encouraging biological degradation of geosmin without the addition of ethanol, suggesting that degradation is cometabolic. Saito et al. (1999) identified three major byproducts of geosmin degradation (Figure 2-4). Ho et al. (2006) determined the biodegradation of geosmin to be a pseudo-first order reaction with rate constants between $0.12 d^{-1}$ and $0.24\ d^{-1}$ initially and $0.50\ d^{-1}$ and $0.58\ d^{-1}$ for a second spike. The authors hypothesized the higher rate constant for the second spike was due to a higher level of biomass in the sand column reactors.

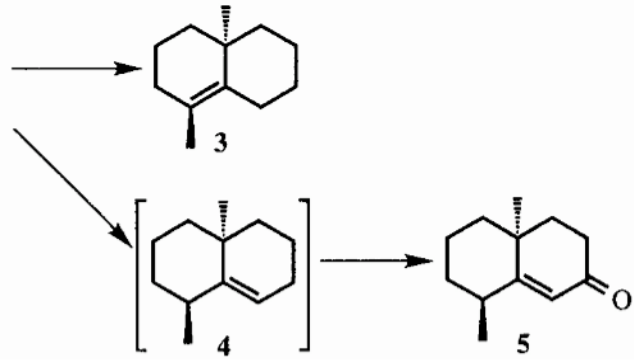


Figure 2-4: Main degradation products of geosmin (Saito et al., 1999)

2.3 Removal of Geosmin by Water Treatment Processes

There are a limited number of treatment processes that are capable of consistently removing geosmin. Conventional drinking water treatment processes (i.e. coagulation, flocculation, sedimentation, and disinfection) are ineffective at removing geosmin (Nerenberg et al., 2000, Ho et al., 2002). The most successful treatment processes for the removal of geosmin include activated carbon (granular or powdered), biological removal and advanced oxidation processes (AOP) (Nerenberg et al., 2000). AOP is the use of hydroxyl radicals to remove organic materials through oxidation reactions; hydroxyl radicals are typically formed by the use of ozone, hydrogen peroxide and/or UV light.

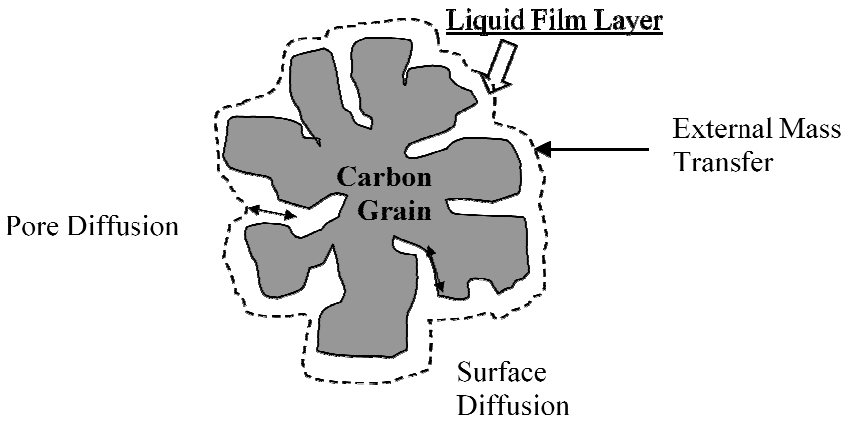
Ozone (O_3) has been shown to remove geosmin when the conditions of the source water matrix are favorable, although the tertiary structure of gesomin makes it resistant to direct oxidation by ozone (Ho et al., 2002). Ozone is a chemical oxidant, which generates hydroxyl radicals that oxidize geosmin into numerous other compounds lacking the taste and odor concerns of geosmin. Ozone generation, however, is energy intensive (Crittenden et al., 2005). Ozone also leads to the production of some disinfection byproducts that are of concern (e.g., bromate), although it produces fewer halomethanes and haloacetic acids than chlorination (Krasner et al., 2006).

The degradation rate of geosmin in the presence of ozone depends on a variety of water quality parameters (Westerhoff et al., 2005). The degradation rate tends to increase for conditions that promote radical production (i.e. high pH) and when radical scavenger (i.e. carbonate, other organics compounds) concentrations are low. Conversely, chlorine and chlorine dioxide are not effective at removing geosmin under any conditions (Glaze et al., 1990).

2.4 Sorption to Activated Carbon

Another common method for removing geosmin from drinking water is activated carbon. Water treatment plants often use either powdered activated carbon (PAC) or granular activated carbon (GAC) for resolving taste and odor issues. PAC and GAC are differentiated by size; GAC particles are on average 1 millimeter in diameter, which is 10 to 100 times the size of PAC. The use of GAC and PAC is also very different. PAC is added directly to the water and is removed from the water via sedimentation and/or filtration. GAC is used as filter media in beds and typically lasts between 1 - 4 years depending on the target compounds to be removed and the background organic matter (Liu et al., 2001). GAC can also be either regenerated or replaced.

The mechanism of geosmin removal by activated carbon is sorption. Before a compound can sorb to the activated carbon it must diffuse from the bulk solution through the liquid film layer to the carbon particle (Figure 2-5). This external mass transfer does not control sorption to GAC. The sorption of compounds to activated carbon is mainly controlled by two processes; surface diffusion and pore diffusion. Surface diffusion is the internal transfer of sorbate molecules along the walls of pores. Pore diffusion is the diffusion of particles through the liquid in the pores, which is driven by the high surface area within the pores (Sontheimer et al., 1988, p. 292).



GAC particle.

2.4.1 Surface Diffusion

Surface diffusion along the GAC surface can be modeled with Fick's Law:

$$J_{S,i} = \rho_P D_{S,i} \frac{\delta q_i}{\delta r}$$

where: $J_{S,i}$ is the mass flux in the adsorbed phase, $D_{S,i}$ is the surface diffusion coefficient; ρ_P is the density of the adsorbent; and $\delta q_i / \delta r$ represents the driving force for diffusion or the change in solid phase concentration (q_i with change in distance (i.e. radius or r). A mass balance on a spherical sorbant grain results in the homogeneous surface diffusion model (HSDM).

$$\frac{\delta q_i}{\delta t} = D_{S,i}\left(\frac{\delta^2 q_i}{\delta r^2} + \frac{2}{r}\frac{\delta q_i}{\delta r}\right)$$

Typical boundary conditions are $q_i = 0$ at r = 0 and solution concentration (c_i) of several hundred nanograms of geosmin, initial conditions $q_i = 0$ from r = 0 to r = $\frac{d_p}{2}$. Matsui et al. (2009) reported a surface diffusion coefficient of ~ 5.8×10^{-12} m^2/s. A graphical representation of the HSDM is shown in Figure 2-5.

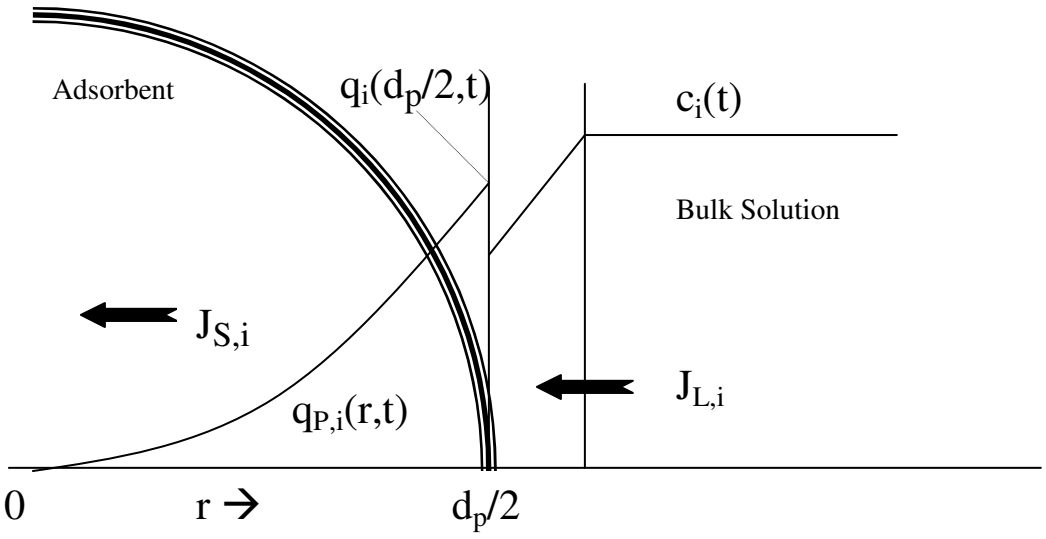


Figure 2-6: Solid-phase concentration profiles within the adsorbent according to the film-surface diffusion model (Sontheimer et al., 1988).

3.0.2 Pore Diffusion

The second process for controlling the rate of sorption is diffusion from the external surface of the activated carbon grain into the pores; it can also can be modeled with Fick's Law:

$$J_{P,i} = D_{P,i}\frac{\delta c_{P,i}}{\delta r}$$

where: $J_{P,i}$ is the pore diffusion flux, $D_{P,i}$ is the pore diffusion coefficient, and $c_{P,i}$ is the concentration of the adsorbate in the pores. The pore diffusion model assumes that $c_{P,i}$ is in equilibrium with the solid-phase concentration. A graphical representation of the pore diffusion model is shown in Figure 2-7.

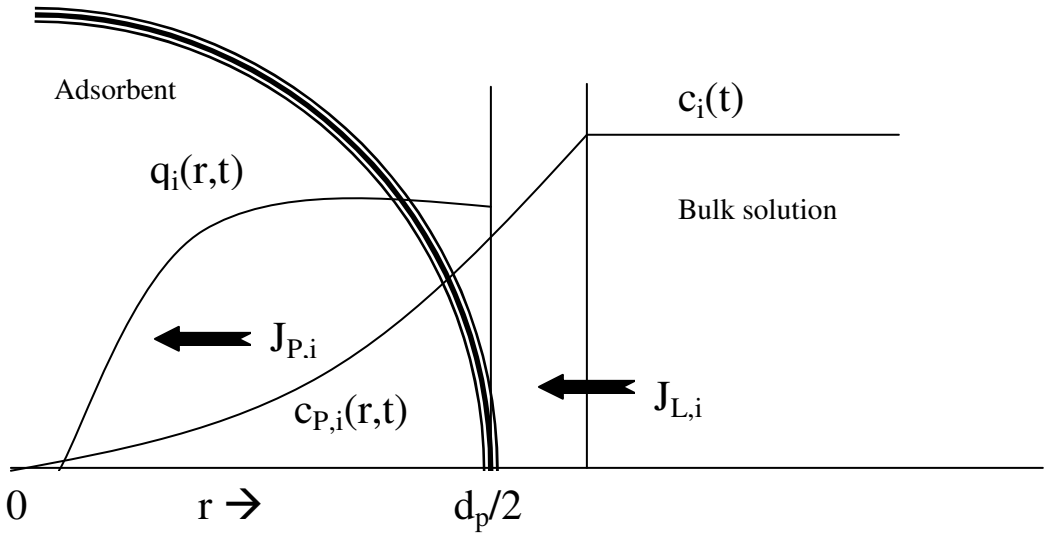


Figure 2-7: Solution concentration profiles within the adsorbent according to the film-pore diffusion model, (Sontheimer et al., 1988).

More complex models simultaneously account for both surface diffusion and pore diffusion (i.e., the pore and surface diffusion model or PSDM; Sonetheimer et al., 1988). For easily adsorbed compounds, sorption is typically controlled by surface diffusion; while pore diffusion controls for weakly adsorbed compounds in complex solutions (Sonetheimer et al., 1988).

In order to utilize the model the sorbate diffusion coefficients ($D_{Pi,}$ D_{Si}) must be estimated or measured. The diffusion coefficient of a compound is a function of the molecular size and water temperature, as demonstrated by the Wilke-Chang equation. For geosmin the diffusion coefficient has been measured as D_{Pi} 3.5 × 10-12 cm^2/s (Pirbazari et al., 1993).

2.4.3 Sorption Isotherms

Pore and surface diffusion combine to control the kinetics of sorption to GAC. The equilibrium sorption of geosmin to activated carbon has been modeled with a Freundlich Isotherm (Herzing et al., 1977, Sontheimer et al., 1988, Huang et. al., 1996). The equation for a Frendlich isotherm is:

$$q_e = K_f C_e^n$$

where: q_e is the solid phase concentration at equilibrium, K_f and n are empirical constants, and C_e is the liquid phase concentration at equilibrium.

The sorption of geosmin to activated carbon has also been modeled with a linear isotherm (Johnston, 2005, Scharf, 2007, Scharf et al., 2010). The equation for a linear iostherm is:

$$q_e = K_f C_e$$

The values of these constants are strongly dependent on the specific water conditions. Freundlich isotherm constants for geosmin on Calgon F400 were K_f = 3.969 ng/mg GAC and n = 0.74 (R^2 = 0.97) in organic free water (Pirbazari et al., 1993) where q_e and C_e are in units of µg/g and µg/L, respectively. Johnston (2005) determined the geosmin isotherm constants for Calgon F400 in Saint Paul Regional Water Services raw water (Table 2-1).

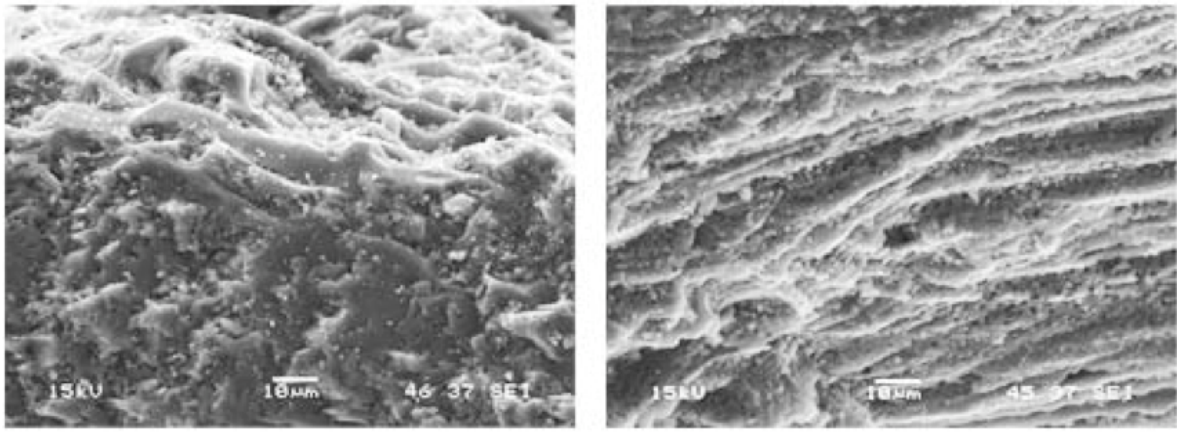
Table 2-1: Isotherm parameters for geosmin in Saint Paul Regional Water Services water on Calgon F400 GAC at pH 8.5 and room temperature.

Isotherm	K	n	R^2
Linear	0.7	-	0.95
Freundlich	1.71	0.74	0.89

Source: Johnston (2005)

2.4.4 Activated Carbon Types

Activated carbon can be made from wood, coal, nutshells, peat, and lignite. Different GAC types have different pore structures as observed via scanning electron microscopy (Figure 2-8). Pore differences and generation processes can have a large impact on sorption. At low concentrations, geosmin sorbs better to coal and bagasse (sugar cane husk) based GAC than pecan-shell based GAC, though no difference has been observed at high geosmin concentrations (Ng et al., 2002).



Wood Carbon Coconut Husk Carbon

Figure 2-8: Scanning electron micrographs of wood and coconut GAC pores (Source: BAT Science – Filters).

2.4.5 PAC

PAC is dosed as a dry powder or as a slurry. The most common points of addition in a conventional water treatment plant are plant intake, rapid mix tank, or the filter influent. Contact time varies depending on where PAC is added but is typically between 10 and 60 minutes. PAC nears equilibrium at approximately 4 hours and the adsorption rate declines with time as most geosmin sorbs in the first 60 minutes (Cook et al., 2001, Bruce et al., 2002).

The necessary PAC dose varies greatly depending on the conditions of the raw water as well as desired effluent geosmin concentration. A variety of experimental values are shown in Table 2-2-2.

Table 2-2: Geosmin removal as a function of PAC dose and contact time

PAC dose for geosmin (mg/L)	Contact Time	Final Geosmin Concentration (ng/L)	% Removal	Source
22	50 minutes	10	75	Cook et al., 2001
30	6.4 minutes	39	80	Jung et al., 2004
10	6.4 minutes	125	40.5	Jung et al., 2004
10	4 hours	18	82	Bruce et al., 2002
45	1 hour	10	90	Johnston et al., 2005

Geosmin removal by PAC is independent of the initial concentration of geosmin but the sorption capacity of the PAC is a function of the desired effluent concentration (Jung et al., 2004). The advantage of PAC is that it can be used only when required because high geosmin concentrations are often seasonal. Using jar tests, similar to those conducted for coagulation, one can optimize PAC dosages. PAC is very ineffective at high geosmin concentrations (>100 ng/L) (Bruce et al., 2002). PAC should also not be used in combination with chlorine, as the presence of even 2 mg/L of chlorine can decrease geosmin removal from 82% to 51% (Lalezary-Craig et al., 1988).

2.4.6 GAC

While PAC may be dosed at various locations within a water treatment plant, GAC often replaces the conventional filter media (i.e., sand or anthracite). The design of a GAC filter-sorber is based on the contaminant of concern, particle removal and headloss. The goal is to achieve necessary particle removal with long filter run times. Adjusting the design of a packed bed filter for GAC begins with consideration of particle removal performance and headloss. After the filter is designed to maintain particle removal, the removal of the target dissolved compound or

compounds are considered. Often small pilot studies or rapid small-scale column tests are conducted to evaluate the effectiveness of GAC at removing the compound of interest. Furthermore, comparing GAC filters with conventional sand or anthracite filters, the media is more expensive and it is important to not waste any sorption capacity of the GAC. Filters can be designed in series or in parallel. Placing two or more filters in series ensures that the entire sorptive capacity of the first filter is used without contaminants breaking through. Water treatment plants, such as Saint Paul Regional Water Services, that retrofit sand filters with GAC and are not concerned with removal of toxic compounds, often use the previously designed filters in parallel. The useful GAC bed life for geosmin can be as short as 7 weeks or as long as several years (Gillogly et al., 1999).

GAC has many advantages over PAC: ability to be regenerated, lower operation and maintenance costs, higher removal efficiencies, and ease of carbon application. The largest disadvantage of GAC over PAC is higher initial cost. Scharf et al. (2010) performed batch scale experiments, pilot studies, and model simulations to investigate geosmin removal by GAC for Saint Paul Regional Water Services; Scharf et al. (2010) reported estimated bed lives ranging from 594 to 1241 days. The removal by the pilot-scale GAC beds far exceeded predictions based on batch sorption isotherms and the full-scale GAC has been in place for 5.5 years and still appears to be working. Hence, geosmin removal could not be explained via adsorption alone and the authors demonstrated that biological activity was contributing to geosmin removal in these systems (Scharf et al., 2010).

2.5 Biological Removal

Biofiltration is a unique type of biological treatment in which the organisms responsible for the treatment exist in a different phase than the fluid (water or air) being treated. Slow sand filters are one of the oldest known forms of biofiltration applied for municipal water treatment. The conscious use of other filter mediums (anthracite, GAC) to support microbial biomass has

happened more recently; biologically enhanced granular activated carbon filters have been used since the 1970s. Biological activity was initially favored because of improvement in the useful life of GAC and increased time between regeneration and replacement (Klotz et al., 1976). Now, biofiltration is being used for targeted removal of specific compounds such as estradiols, geosmin, and volatile organic compounds.

Researchers have began to characterize the microbial communities in biologically active GAC filters because of concerns regarding the possible presence of pathogens. Klotz et al., 1976 found mostly species of *Pseudomonas* and *Bacillus* and no known pathogens; however there are some species of the observed phyla known to be opportunistic pathogens. The next most common groups were *Acinetobacter* and *Flavobacterium.* These studies were done before genetic testing was widely available. Little research has been done using new molecular techniques, molecular techniques are capable of characterizing the entire community and not just the dominant culturable species. Recently, Pinto et al. used high throughput sequencing to examine microbial communities on dual-media (GAC and sand), the dominant phyla in decreasing abundance were *Proteobacteria, Actinobacteria, Bacteroidetes, Nitrospira,* and *Planctomycetes.*

Development of a geosmin-degrading community on the filter media occurs 14 – 22 days after start up (Elhadi et al., 2006, Ho et al., 2006). The establishment of geosmin-degrading community in the filters often occurs naturally but can be enhanced by seeding with geosmin-degrading organisms (McDowell et al., 2009). A consortium of geosmin-degrading bacteria isolated by Hoefel et al. was introduced into a sand filter, after which the filter removed 75% of geosmin compared to 25% removal by unseeded sand. No other research has been done published to date on introducing microorganisms into fresh filter media. Biofilms that are capable of degrading geosmin are not fragile and can withstand resting periods up to one month without losing geosmin removal capacity. (Elhadi et al., 2006).

Biological geosmin removal is often observed in the 88 – 97% range. On exhausted GAC an established biofilm can remove 87% of 100 ng/L of geosmin. (Elhadi et al., 2006). Biologically active filters can be effective at removing geosmin from water, with reported removal efficiencies ranging from 60 to 100 % (Elhadi et al., 2006, Elhadi et al., 2004). For example, Elhadi et al. (2006) reported 87% removal of geosmin (Influent concentration = 100 ng/L) by bacteria residing on exhausted GAC. Substantial geosmin removals have also been reported for biologically active sand filters (90 to 100 %; Ho et al., 2007, McDowall et al., 2009) and anthracite filters (30% to 70 %, Scharf et al. 2010). It is difficult to compare these results directly as not only is media type varying but there also are differences in water quality (e.g., temperature).

In fact, biological removal of geosmin depends on a variety of factors including media type, temperature, biological organic matter (BOM) concentrations, empty bed contact time (EBCT), backwashing, the presence or absence of a disinfectant in the backwash water, filter bed velocity, and acclimation. Elhadi et al. (2006) listed media type and temperature as the two most important factors affecting biofiltration efficiency. For instance, biofilms grown on GAC are more effective at removing geosmin than biofilms grown on anthracite or sand (Liu et al., 2001). The enhanced performance of GAC is likely because GAC can sustain three to eight times more biomass (by mass) than other media (Wang et al., 1995). Similarly, exhausted GAC filters remove more geosmin than sand filters. (Elhadi et al., 2004). Elhadi et al. (2006) only investigated sand, anthracite, and GAC, not different types of GAC, leaving unanswered the influence of different GAC types on biodegradation. Higher geosmin removal at is seen at higher temperatures (Elhadi et al., 2006, Persson et al., 2007). The geosmin removal improves by 25 to 50% when temperature increases to from 8°C to 20°C (Elhadi et al., 2006, Persson et al., 2007). BOM Levels are very important as well, Saito et al. (1999) only witnessed geosmin degradation when a secondary substrate perhaps explaining why BOM levels improve geosmin degradation. Elhadi et al. (2006) observed higher geosmin degradation at higher BOM concentrations, though

whether BOM increases the ease of geosmin degradation or more degradation is seen because more biomass is present is not clear. The Morgan water treatment plant has effective geosmin removal with an empty bed contact time of 30 min. Most column studies are done with an empty bed contact time of 15 min. Ho et al. (2006) observed a breakthrough of geosmin when the empty bed contact time was sequentially decreased from 15 minutes to 2.5 minutes and then increased to 15 minutes over a period of 6 days. Geosmin removal was effective again when the empty bed contact time returned to 15 minutes. Disinfection and backwashing have not been studied related to geosmin removal, however, disinfection upstream of filters can kill potential biofilms or decrease the diversity of the microbial community (Liu et al., 2001).

2.6 Summary and Research Needs

Geosmin is a compound that causes earthy/musty odors in drinking water leading to consumer complaints. The most effective control processes for geosmin are ozone and activated carbon. Activated carbon is cheaper and more effective; activated carbon removes geosmin via sorption. Extensive research has shown that biodegradation of geosmin is a possible control strategy, however, biodegradation has yet to be understood well enough to used purposefully and not fortuitously.

Research is needed into the microbial ecology of biologically active GAC filters. The current level of knowledge is abysmal, leaving water utilities and plant operators to depend on the spontaneous colonization of their new filter media with helpful microorganisms. Until the microbial community of biologically active GAC filters is understood biodegradation will not be leveraged to its full potential. Engineered biologically active GAC filters have the potential to remove parts per trillion levels of contaminants. In today's world of emerging contaminants that are having effects at previously immeasurable levels the capacity of biologically active GAC filters to remove these contaminants should not be overlooked.

3.0 Bacterial Community Dynamics on Full-Scale Biologically Active Granular Activated Carbon Filters

3.1 Introduction

The purpose of drinking water treatment is to remove pathogens and other contaminants from water in order to ensure that the water is safe to consume. Clean drinking water is an important public health goal as millions of people die each year from disease caused by unsafe drinking water (WHO, 2010). Furthermore, unsafe drinking water is responsible for 9.1% of the global disease burden (WHO, 2010). In the United States, consumers rely on and expect local water utilities to provide clean safe drinking water; however, in recent years, water utilities have begun to face unanticipated competition from the makers of bottled water. Consumers displeased with aesthetic qualities of their water, like taste and odor, often select bottled water, which is potentially inferior to tap water from a public health perspective; bottled water is unregulated by the EPA for certain water constituents (e.g. fluoride, arsenic) and creates wasteful transportation and environmental costs.

In order to improve aesthetic water quality factors, drinking water utilities can install GAC filters, which are designed to remove taste and odor compounds, such as geosmin, via sorption. GAC filters, however, are now known to also be biologically active, removing compounds through a combination of sorption and biodegradation. Biologically active GAC filters conflict with the historical philosophy of drinking water treatment, which was to eliminate as many microorganisms as possible under the general assumption that any microbial growth would potentially compromise the microbiological safety of the water. Even now, GAC filter design is based on the sorption capacity of the compound(s) of concern without considering biological activity. Biological activity enhances GAC filters because it can degrade compounds of concern, degrade sorbed compounds, and biologically regenerate sorptive capacity. Biologically active GAC filters are important because they are able to remove contaminants from

water that are present at very low concentrations (i.e., ~ng/L). Additionally, biologically active GAC filters have the capacity to remove multiple compounds in tandem at no additional costs. They are versatile, inexpensive to operate and maintain, and applicable to a variety of source waters and environments.

In spite of their importance, very little is currently known about the microbial ecology of biologically active GAC filters. Biologically active GAC filters perform better than anthracite filters after backwash with chlorinated water (Kasuga et al., 2007) and removal efficiencies of organic matter by biologically active GAC filters is unaffected by air scour time (Simpson, 2008). GAC bed life calculated by computer models or rapid scale columns underpredict empirical results based on pilot studies and actual life time (Scharf et al., 2005); this indicates that biological activity is important even when it is not a design consideration. Biological activity can increase GAC filter life, saving water utilities in GAC regeneration and/or replacement costs. Biological activity can also improve removal efficiencies of specific taste and odor compounds, decreasing consumer complaints without increasing costs. In general, it takes 20 - 40 days after installation of GAC filters to become biologically active (Liu et al., 2001) and at steady state biomass levels are in the range of 9×10^9 cells/g GAC (Velten et al., 2007).

In this study I investigated the bacterial community composition in full-scale biologically active GAC filters at Saint Paul Regional Water Services. The goals of this project were to better understand bacterial community structure with respect to filter-to-filter variability and bacterial community dynamics. This study is needed to better understand the microbial ecology of a technology that is used to produce aesthetically pleasing drinking water without compromising public health. Samples were collected from 20 different filters over the course of 12 months and from three different locations within each filter in order to examine interfilter variability. Bacterial community structure was tracked by automated ribosomal intergenic spacer analysis (ARISA) and Illumina sequencing targeting the V6 hypervariable region of the 16S rRNA gene.

The community was observed to be more diverse with greater richness than expected; this helps explain its high functional stability.

3.2 Materials and Methods

3.2.1 Sample Collection and Preparation

Replicate GAC samples were collected from the top of the filters using clean and disinfected 1 L bottles and immediately transported to the University of Minnesota (< 4 hours). A fraction of the samples were immersed in 0.5 mL of 1 N NaOH and incubated at 100°C for 10 minutes to solubilize proteins; samples were then stored at -20°C until they could be quantified by the Lowry method (Lowry et al., 1951). Lysis buffer (5% SDS, 10 mM $NaPO_4$, pH=8) was added to the remaining samples, which were bead beat for 30 seconds in a FastPrep machine (MP Biomedical, Solon, OH). Genomic DNA was then extracted using the MP BioMedicals, LLC Spin Kit for Soil following the manufacturer's directions. Genomic DNA was stored at -20^{o}C until needed.

3.2.2 Automated Ribosobal Intergenic Spacer Analysis (ARISA)

Automated ribosomal intergenic Spacer Analysis (ARISA; Fisher and Triplett, 1999) was used to generate fingerprints of the bacterial community composition using primers ITSF and ITSReub (Cardinale et al., 2004). PCR was carried out in a thermocycler (Bio-Rad DNA Engine, Peltier Thermal Cycler) with an initial denaturation at 94°C for 2 minutes, followed by 30 cycles of 94°C for 15 seconds, 55°C for 15 seconds, and 72°C for 45°C seconds with a final extension at 72°C for 10 minutes. PCR products were resolved via denaturing capillary electrophoresis (ABI Genetic Analyzer, BioMedical Genomics Center, University of Minnesota) using the MapMarker 1000 size standard. Fragment lengths were calculated by using GeneMapper 4.0 (Applied Biosystems). Fragments less than 121 bp were excluded, which is less than the theoretical minimum for amplification with these primers.

3.2.3 Sample Processing for TruSeq

PCR was performed on 20 ng of template DNA three times using a master mix containing: 1× PCR buffer (with 1.5mM $MgCl_2$, Choice Taq - Denville scientific), 1 µl dNTPs (10mM), 2 µl forward primer cocktail (10µM), 2 µl reverse primer cocktail (10 µM), 29.5 µl H_2O, and 2.5 u Taq enzyme (Choice Taq - Denville Scientific). A mixture of V6 primers from Huber et al. (2007) were used (V6F_1: 5′-CNA CGC GAA GAA CCT TAN C-3′, V6F_2: 5′-CAA CGC GAA AAA CCT TAC C-3′, V6F_3: 5′-CAA CGC GCA GAA CCT TAC C-3′, V6F_4: 5′-ATA CGC GAR GAA CCT TAC C-3′ and V6F_5: 5′-CTA ACC GAN GAA CCT YAC C-3′, V6R: 5′-CGA CAG CCA TGC ANC ACC T-3′) with a 6 nucleotide barcode added to the 5′-end so that sequences could be separated during data analysis. The thermocycling protocol was 3 min at 95°C followed by 25 cycles of: 30 sec at 95°C, 30 sec at 55°C, 30 sec at 72°C. The 25 cycles were followed by 3 min at 72°C.

3.2.4 Sequence Processing

Sequencing was done on an Illumina MiSeq Personal Sequencer (Illumina, Hayward, CA). Samples were pooled into four pools of 20 samples each and library preparation and sequencing was done following manufacturer's instructions.

3.2.5 Sequence Processing

Sequence data was processed and analyzed by using the MOTHUR program (Schloss, 2009). First, sequences were screened for quality; sequences containing mismatches in the barcode, more than one mismatch in the barcoded primer, with lengths less than 50 bp or more than 125 bp, and sequences with ambiguous bases or homopolymers longer than 8 bp were removed. Sequences were removed if their MiSeq-defined average quality score was less than 25 in a sliding window of 15 bp (25 = 0.3% of incorrect basecalling). Qualifying sequences were binned by sample with primers and barcodes trimmed from the sequence reads. Sequences were

aligned to the SILVA bacterial 16S rRNA database and the UCHIME algorithm was used to detect possible chimeric sequences, which were also removed from the dataset (Edgar, 2011).

3.2.6 Data Analysis

Non-integer ARISA fragment lengths between 121 and 800 bp were binned using R (Ramete, 2009) with a window size of 1.0 and a shift of 0.1. All samples binned together were run on the same plate on the ABI Genetic Analyzer to avoid bias. Relative peak areas were ranked and fragments of less than 1% of the cumulative proportion were excluded. Community structures were then compared via non-metric multidimensional scaling (nMDS) using R ver. 2.7.0. Illumina sequences were clustered into operational taxonomic units (OTUs) at a cutoff of 90% sequence identity (Cole, 2009). Sequence frequency was ranked by abundance and OTUs less than 1% of the cumulative proportion were excluded in analyses used to directly compare with ARISA results. Finally, taxonomy information for all sequences was obtained using the RDP7 database via nMDS using R version 2.7.0. Shannon diversity indices and Chao1 richness estimates (Chao 2005) were calculated from the complete set of OTU data.

3.3 Results

Biologically active GAC filters at Saint Paul Regional Water Services were able to consistently reduce total organic carbon content (Figure 3-1). Removal efficiencies typically ranged from 5 - 15%, with subtly higher removal efficiencies (student t-test, H_0: $\mu_{summer} = \mu_{winter}$, $p = 0.036$) occurring during the warmer months (May through September). Substantial quantities of bacterial biomass were detected on the GAC, with by protein concentrations ranging from 53 mg protein/g GAC to 1725 g protein/g GAC (Figure 3-2(a)). The quantity of bacterial biomass varied with respect to backwash (Figure 3-2(b)), with biomass levels reaching steady state approximately 2 days after backwash.

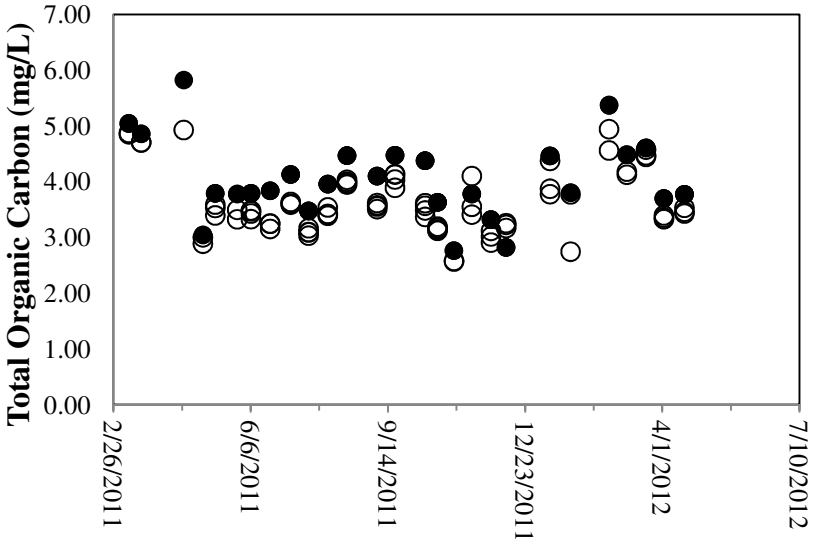


Figure 3-1: Total organic carbon removal at Saint Paul Regional Water Services, measured on filters 6, 9, 17, and 20 from February 2011 through May 2012. Solid circles: influent, open circles: finished water.

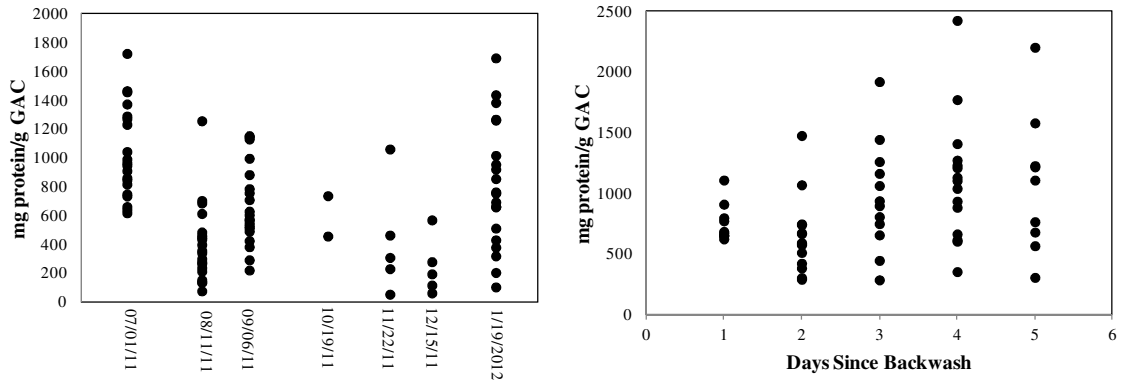


Figure 3-2: Protein content on GAC filters at Saint Paul Regional Water Services (a) over a 12 month period (triplicate samples average) (b) samples from July 1, 2011 organized by days since the filter was backwashed.

Bacterial community composition varies substantially along the water treatment system from the source water (Lake Vadnais) to the finished water within the distribution system (Figure 3-3). ARISA results revealed that the variability in community structure is greater among samples taken in the reservoir, Lake Vadnais, than the rest of the treatment process. Also, the finished water had a significantly different community structure than the lake water, influent water, or filter communities. The GAC filter community is similar to the community structure of Lake Vadnais but significantly different from the finished water. Interestingly, the raw water is significantly different (95% CI) from all other bacterial communities observed throughout the treatment system. The community composition of the filters is significantly different from that of the recarbonation chamber that immediately precedes the filters. The substantial change in pH between the Lake (pH = 7.9 ± 0.2) the clarifier (pH=11.1±0.3), and the recarbonation chambers (pH=8.6 ±0.2) does not significantly change the bacterial communities' structures.

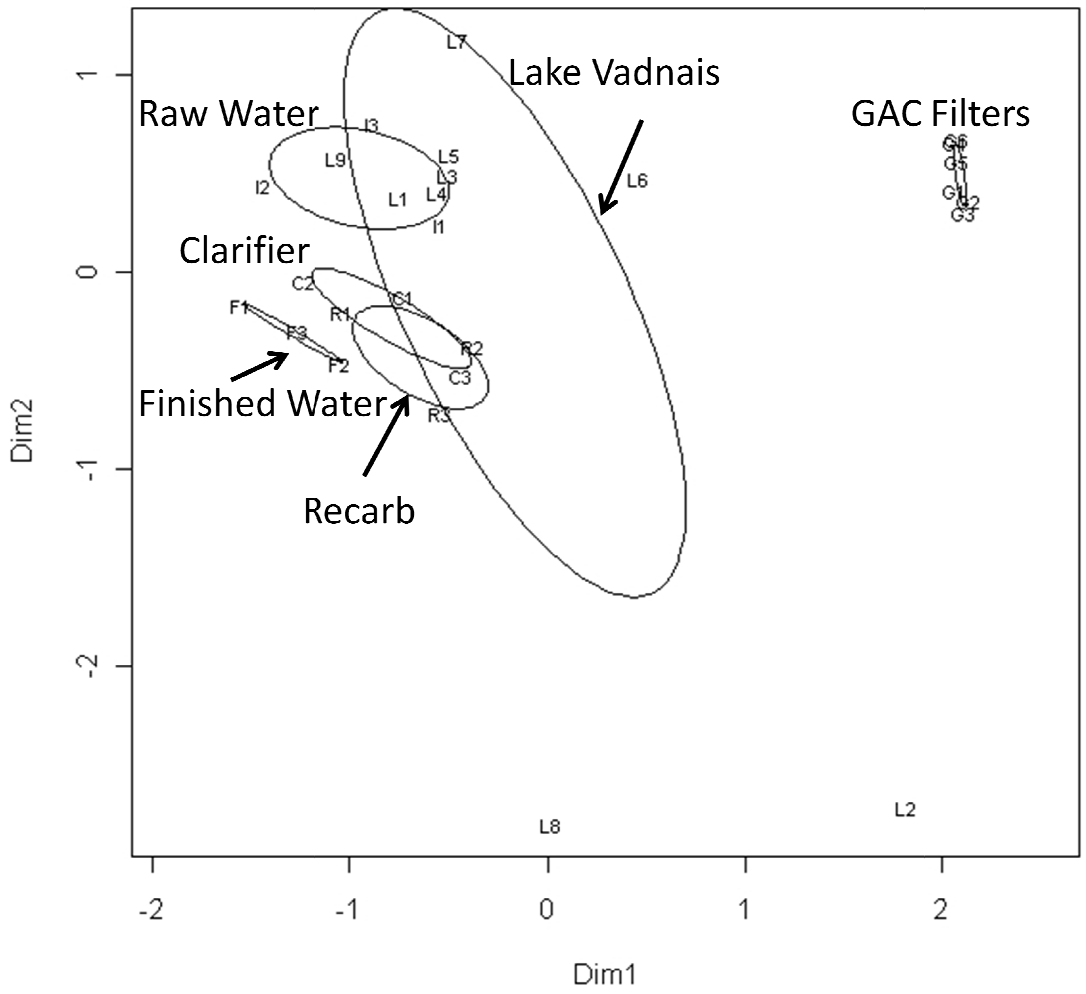


Figure 3-3: Non-metric multi-dimensional scaling plot of community structure at different points in the Saint Paul Regional Water Services McCarrons Water Treatment Plant on October 18, 2011.

Further results give greater resolution to the differences in the GAC filter communities. The filters did not present a clear pattern of change over time; each filter changed differently over time. ARISA on samples from March 2011 through 2012 show community dynamics. For filter 3 (Figure 3-4(a)), the filter communities were not significantly (95% confidence interval) different in the hottest months (August, July) and overlapped with December; in May and March, however, the communities were significantly different. On filter 6 (Figure 3-4(b)), the filter communities

were different in all six months. On filter 13 (Figure 3-4(c)), the communities were different in the four summer months sampled (March, May, July, August) but not significantly different in the winter samples (October, November, December, and February).

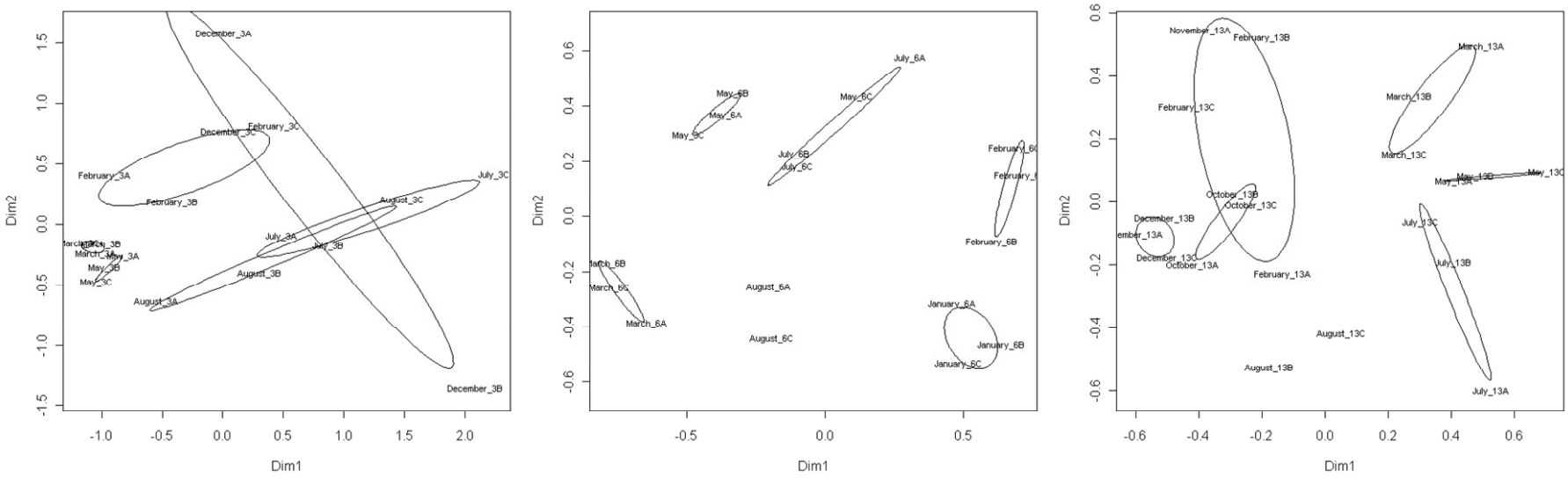


Figure 3-4: Non-metric Multidimensional Scaling plots showing community dynamics based on ARISA over a 12 month period from March 2011 through February 2012. Ellipses represent 95% confidence intervals of community structure (a) Filter 3 (b) Filter 6 (c) Filter 13. Triplicate samples were not available for August filter 6, or November and August filter 13.

The extent of filter-to-filter variability of the bacterial communities changed over time. Samples were taken from 10 to 12 filters on March 15, 2011 and July 1, 2011 (Figure 3-5) and community compositions were compared via ARISA. In March, the ellipses, indicating 95% confidence intervals for the community structure, clumped closely together (Figure 3-5(a)). In July, however, each of the 10 filters sampled had significantly different community structures (Figure 3-5(b)).

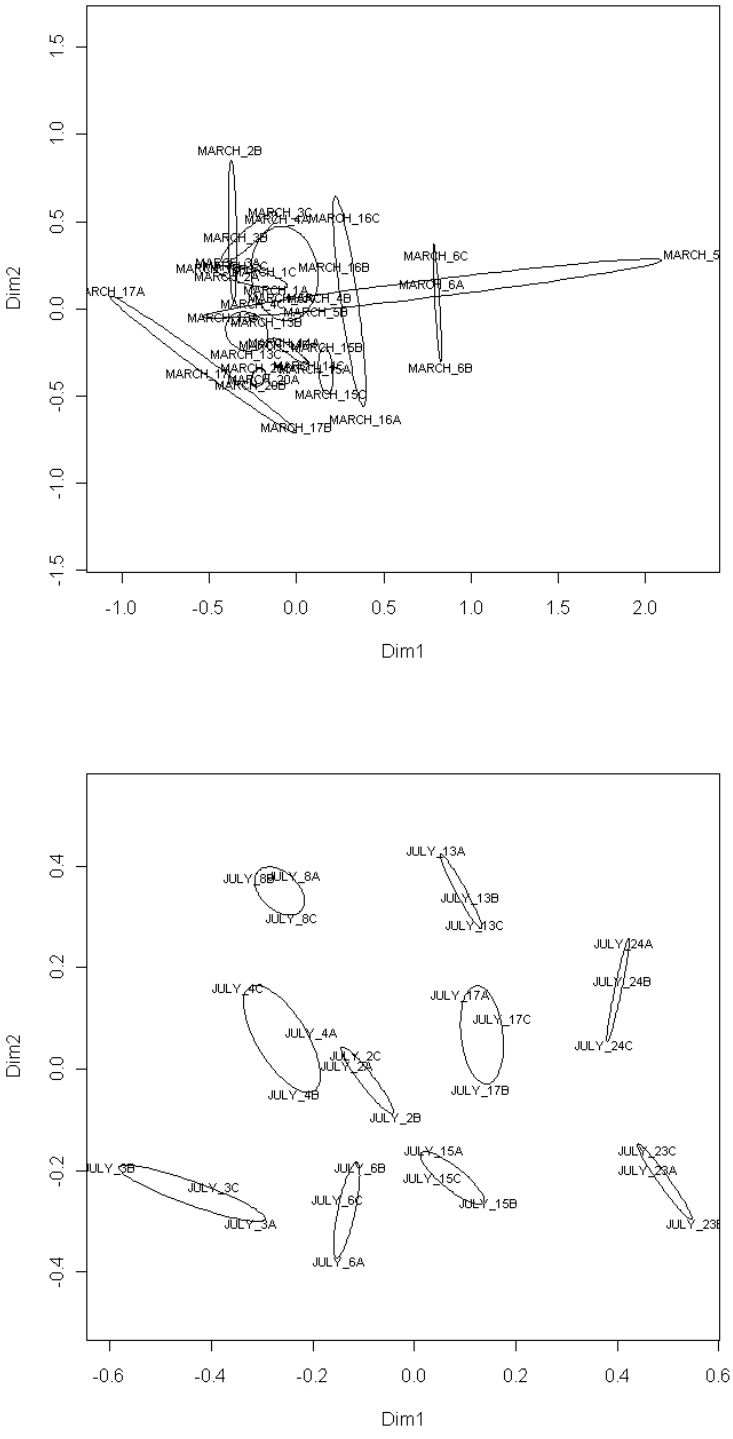


Figure 3-5: Non-metric Multidimensional Scaling Plots showing community structure based on ARISA on 10 - 12 different filters on the same time point. Ellipses represent 95% confidence intervals of community structure (a) March 15, 2011 (b) July 1, 2011.

The filter communities were very dynamic (Figure 3-6). When examined with Illumina sequencing, filter 3 (Figure 3-6(a)) had significantly different communities at each time point. Filter 6 (Figure 3-6(b)) had significantly different communities each month except July and August. Filter 13 (Figure 3-6(c)) had significantly different communities each month except March and May. These are different than the community dynamics observed with ARISA. For example, with ARISA the community structure on filter 6 is different at every time point. When the community structures observed via Illumina are compared, the communities are indistinguishable in July and August. Similarly, Illumina indicates the communities are distinct at every time point on filter 3, while with ARISA community structures were similar in July, August, and December.

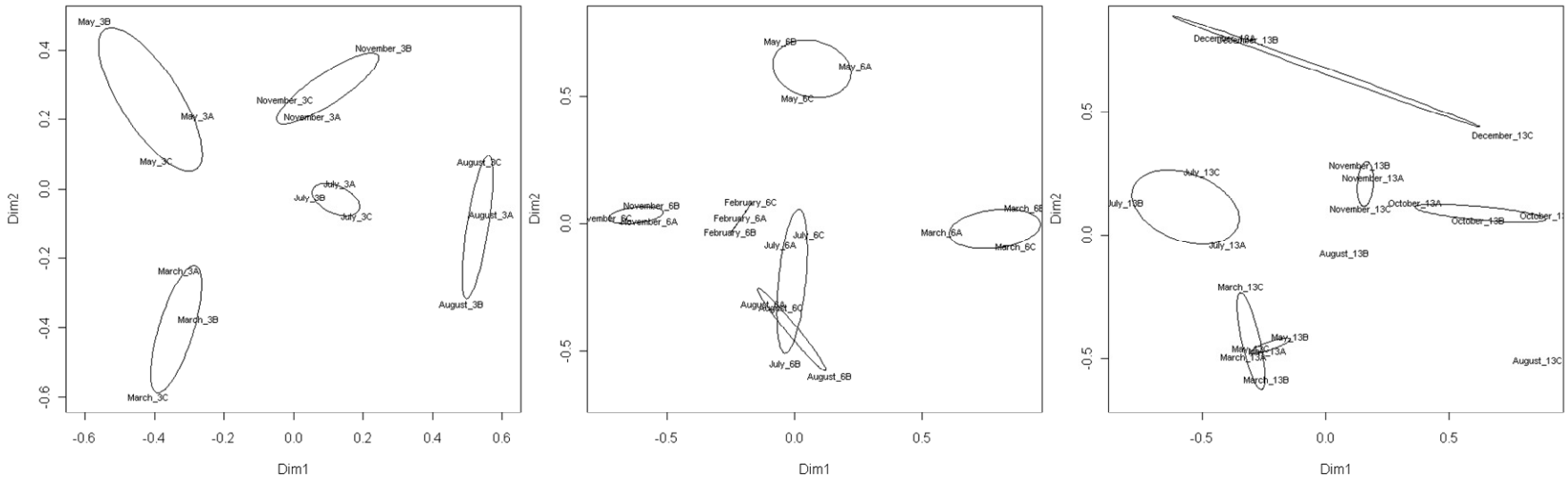


Figure 3-6: Non-metric Multidimensional Scaling Plots showing community dynamics based on Illumina sequencing over a 12 month period from March 2011 through February 2012. Ellipses represent 95% confidence intervals of community structure (a) Filter 3 (b) Filter 6 (c) Filter 13.

Illumina sequencing was also used to examine the filter-to-filter variability of bacterial community on July 1, 2011 (Figure 3-7). Based on the Illumina results, the communities on each filter were significantly different from each other. The Illumina sequence data also shows a different arrangement of filter differences than ARISA. For example, ARISA showed filters 17 and 13 to be more similar than filters 2 and 13, however Illumina shows filter 2 and 13 to be more similar than filter 17 and 13.

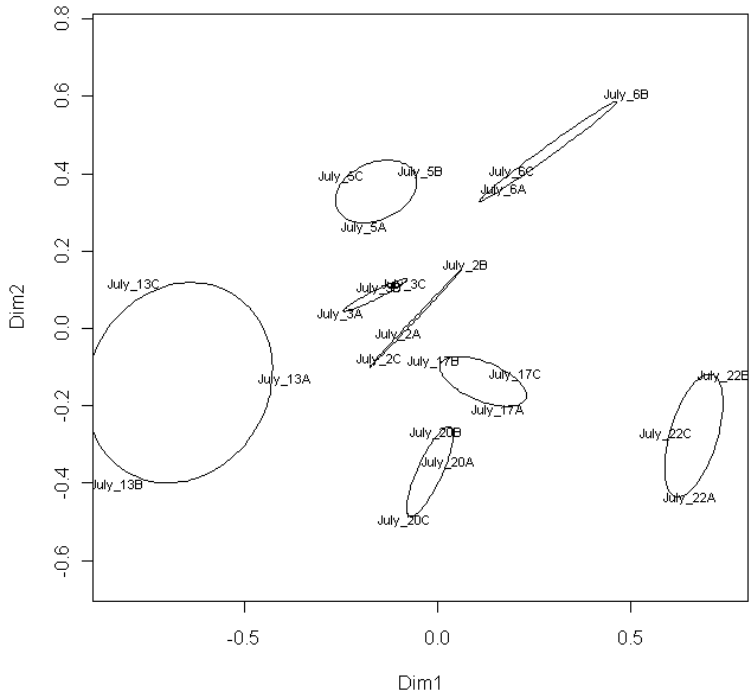


Figure 3-7: Non-metric Multidimensional Scaling Plot showing community structure based on Illumina on 8 different filters on July 1, 2011. Ellipses represent 95% confidence intervals of community structure.

Illumina sequencing revealed diverse bacterial communities in all of the GAC filters, independent of the time of year (Figure 3-8). The filter richness and diversity is much higher than anticipated. The Shannon Index was between 5.37 and 6.63 for every filter and time point, with a mean of 6.05 and a standard deviation of 0.24. The Chao Estimator had more variability, with a range from 3511 to 8198 with a mean of 5698 and a standard deviation of 915. Torsvik et al. (1989) observed 4,000 species of bacteria per gram of soil, Illumina sequencing observed 2,300

species per half gram of GAC. The three filters examined did not show a clear trend or pattern of richness or diversity. On average the filter species richness increased over the time period sampled with no correlation with water temperature. The Shannon Index had a positive slope over time for filters 3 and 6 and a negative trend for filter 13. The filter communities not only exhibit high richness but also evenness, no member of the community was more than 5.5% of the total community.

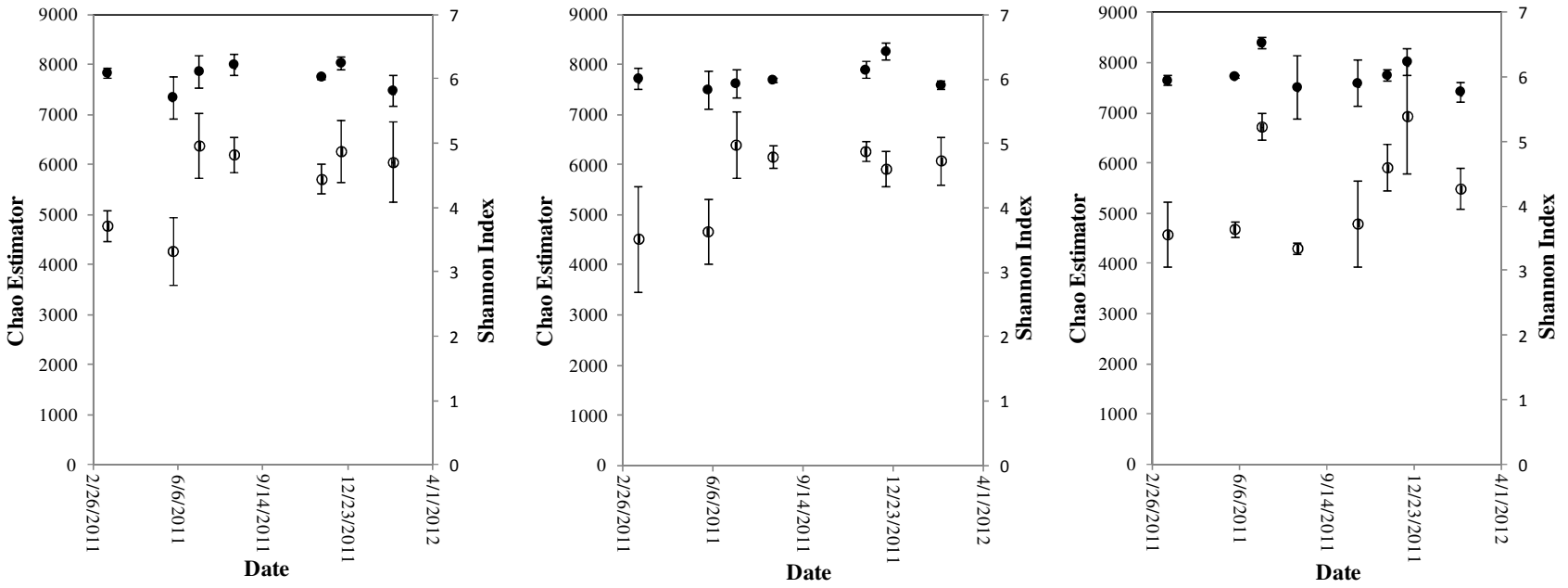


Figure 3-8: Graphs of the Chao Estimator and Shannon Index based on Illumina Sequencing over 12 months from March 2011 through February 2012 for Saint Paul Regional Water Services filters; (a) Filter 3 (b) Filter 6 (c) Filter 13. Error bars represent 1 standard deviation. Closed circles are Chao Estimator, open circles are Shannon Index.

Taxonomy data generated from the Illumina sequences shows the change in the specific community members over time (Figure 3-9, Figure 3-10, Figure 3-11). The most common phlya observed were *Acidobacteria* (3.9%), *Bacteroidetes* (4.4%), *Chloroflexi* (2.4%), *Nitrospira* (9.5%), and *Proteobacteria* (72.2%). *Nitrospira*, a nitrite oxidizing bacterium, makes up between 8 and 12% of the population throughout the study year, which is curious because ammonia oxidizing bacteria were minor (<1%) and not present in every sample. Pinto et al., (2012) also reported *Nitrospira* as a dominated phlya in full-scale dual media rapid sand filters, although *Nitrospira* were never more than 4% of the filter community. Of the dominant families (>1% of total), twelve are members of the *Proteobacteria* phylum. Four contain important in nitrogen cycling: *Nitrospiracae* (nitrite oxidizing), *Rhodocyclaceae* (some denitrifying bacteria) as well as *Rhodobacteracea* and *Oxalobacteraceae* (nitrogen fixing). Both *Methylocystaceae* and *Methylophilaceae* are methylotrophs capable of oxidizing methane. The most common genera were *Nitrospira* (8.75%), *Schlegelella* (3.0%), *Roseinatronobacter* (2.96%), *Dongia* (2.14%), *Aquimonas* (2.0%), and *Rhodophila* (1.93%). Only one member of the *Enterobacteria* family was observed but that genus does not contain any known human pathogens.

There is no pattern to the change in proportion of the dominant phyla and dominant families. For filter 3 the proportion of *Proteobacteria* remains constant between March and July (69.4%±0.5%), while for filter 6 it increases 3% and for filter 13 it decreases 3%. The proportion of *Nitrospira* is 6.28% in filter 6 in March, while it is 11.89% in filter 13. None of the phlya show a consistent trend. The family data further mimics the randomness; *Oxalobacteraceae* are 12.61% of the March filter 6 community and do not dominate at any other time point. The percentage of *Comamonadacea* among the different filters on July 1, 2011 ranges from 5.98% to 10.27%. Other phlya commonly vary by 10%; *Proteobacteria* ranges from 66.4% to 74.1%. Of the five most common phyla found on the Saint Paul Regional Water Services GAC filters, four are among the seven most common phyla found on the rapid sand filters in Ann Arbor (Pinto et al., 2012), the differing phyla was *Chloroflexi*. *Chloroflexi* are filamentous green non-sulfur

bacteria; the phylum includes aerobic thermophiles and anaerobic halorespirers. Some of the species of *Chloroflexi* are from the class *Dehalococcoides*, which dehalogneate halogenated organic compounds. It is likely that halogenated compounds are present in the filters because they are backwashed with chloroaminated finished water, however it is not likely that the filters are anaerobic.

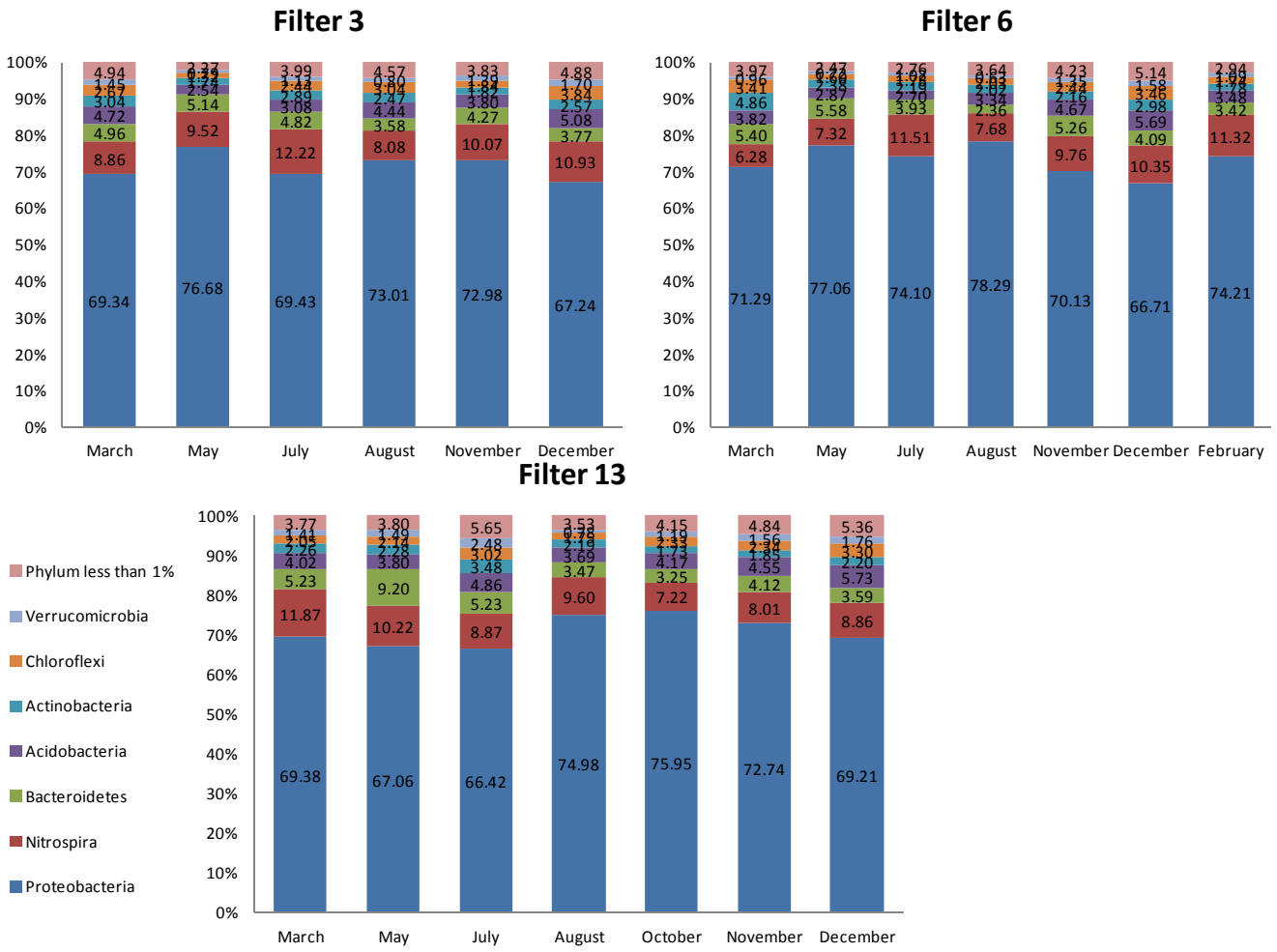


Figure 3-9: Dominant phyla present in each Saint Paul Regional Water Services drinking water treatment plant biologically active GAC filters; 3, 6, and 13.

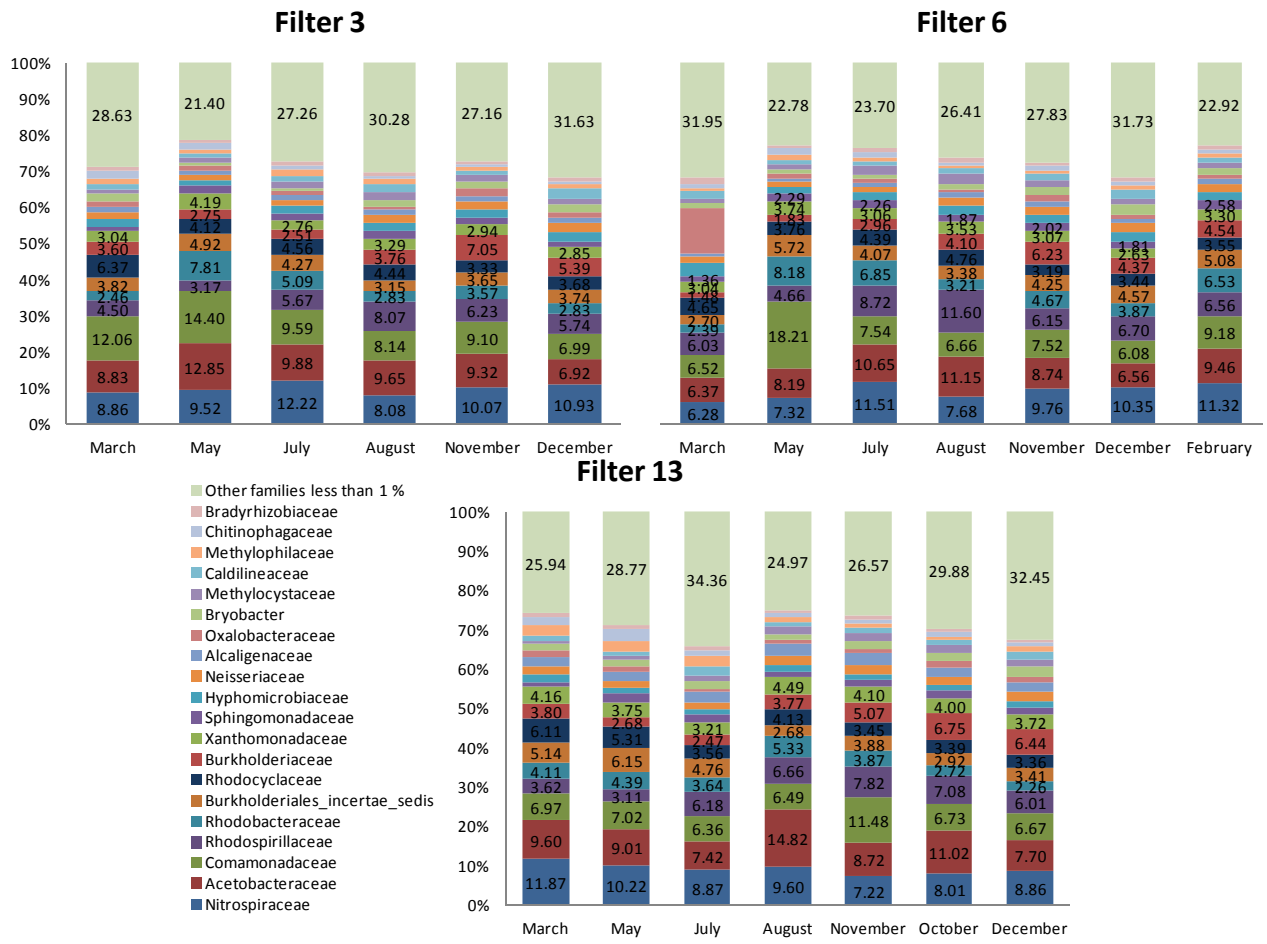


Figure 3-10: Dominant families present in each Saint Paul Regional Water Services drinking water treatment plant biologically active GAC filters; 3, 6, and 13.

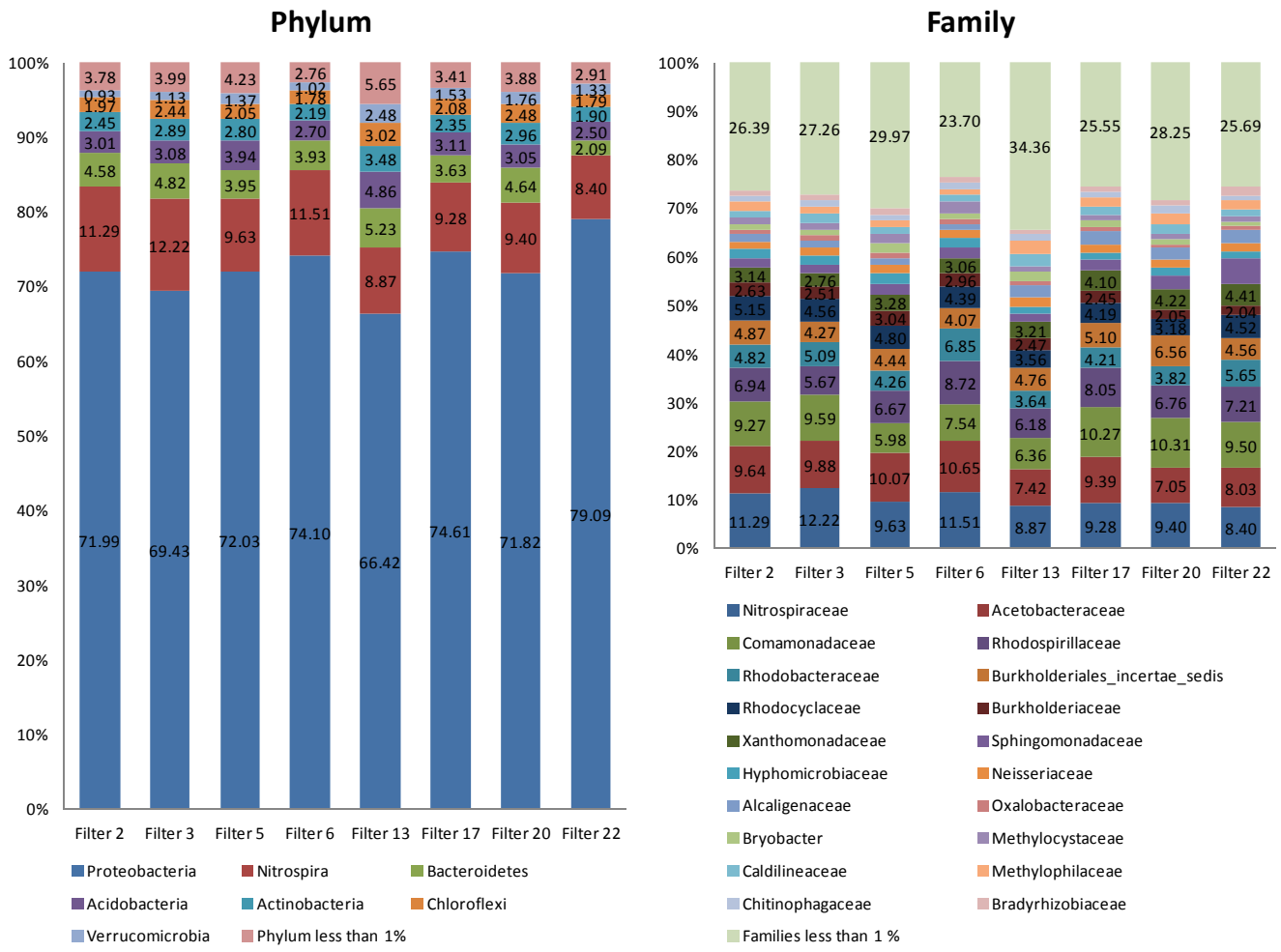


Figure 3-11: Interfilter variability on July 1, 2011 on multiple Saint Paul Regional Water Services drinking water treatment plant biologically active GAC filters.

3.4 Discussion

The biologically active GAC filters at Saint Paul Regional Water Services have robust, diverse and dynamic communities that should make them very functionally stable (Figure 3-1). Because biodiversity in terrestrial environments has been linked to higher biomass levels and stronger stability (Tilman, 1996), the high biodiversity on the GAC filters is also likely to lead to higher biomass levels and stronger stability. There are a variety of reasons that high species richness and diversity in the GAC filters would make them functionally stable. If there are more diverse organisms in an environment they should use resources more efficiently and more completely and thus be able to generate more biomass and be more productive (Tilman, 1999). Because the GAC filter communities have both high richness and good evenness they experience an averaging effect; if an external disturbance affects different organisms differently, some positively some negatively, then the average impact on a large number species will be closer to the baseline than the average impact on a few species (Doak et al., 1998). In highly diverse communities, organisms also benefit from facilitation or the use of the other species in the community for protection, waste products etc. (Turner et al., 1966). The function of the community is also more stable due to a negative covariance effect - if an increase of species A leads to an increase in species B then in a more rich community the effect is smaller than in a community with fewer members (Tilman et al., 1996). Diverse communities are also protected by an insurance effects; with more species there is a higher likelihood that species have redundant functions (Naemm and Li, 1997). Finally, communities that more completely use their resources are more resistant to invaders (Tilman, 1999). Research on activated sludge bacterial communities has shown an increase in biodiversity improves resistance to toxic shock loading (Saikaly and Oerther, 2010). Similarly, the GAC filter communities should be resistant to toxins due to their high biodiversity.

The biologically active GAC filters at Saint Paul Regional Water Services have unique community structures from filter to filter and at different time points. This suggests that the microbial community is always active, which is surprising given that the water temperature is typically ~4°C during the winter months. This interpretation is supported by the performance data, which indicates that the GAC filters consume dissolved organic carbon year round. Biological activity in the winter-time could also explain the long GAC bed life because lower influent levels of TOC could lead to desorption of organic material sustaining the biomass.

There is no pattern or seasonal relationship between species richness/diversity and time. Unlike freshwater phytoplankton which have predictable succession patterns based on environmental influences such as temperature, minerals, etc. (Lund, 1964), the three filters studied did not have a consistent trend even though each was receiving the same minerals, organic compounds, and temperature perturbations. The variation indicates the filters are not consistently heterogeneous throughout the three samples location in each filter or homogeneous throughout the three samples locations in each filter. This is corroborated by the differing sizes in the ellipses on the nMDS plots of the ARISA and Illumina sequences with larger ellipses showing greater differences between the triplicates.

The communities are much richer than anticipated; other engineered systems such as activated sludge have reported Shannon Indices of between 3.0 and 5.6 and with 30 to 1000 operational taxonomic units (Pommier et al., 2010, Saikaly and Oerther, 2010). In contrast, an average of 2,300 operational taxonomic units were observed on the biologically active GAC filters and Shannon indices were between 5.4 and 6.6. *Nitrospira* are nitrite-oxidizing bacteria common in marine and other environments, however their presence on the filters is curious because there are not high concentrations of ammonia-oxidizing bacteria found via Illumina sequencing. Nitrite is of concern in drinking water because it can cause methanemoglobinameia, which causes impaired oxygen delivery, typically in newborns. The absence of ammonia oxidizing bacteria combined with the abundance of *Nitrospira* suggests that ammonia-oxidizing

archaea might be important members of the filter biome. Unfortunately, we did not test for the presence of archaea on the filters.

A minor limitation to this study is the inability to completely saturate the rarefaction curves generated from data gathered. In order to identify all of the genara present on the filters more sequences are needed. The operational taxonomic units observed via Illumina sequencing represent only 65% of the total indicated by the Chao Estimator.

Biologically active GAC filters should be better understood because they have high potential to improve water quality and decrease treatment costs. Drinking water utilities typically replace GAC in stages because it is cost prohibitive to replace all at once. Biological activity not only improves GAC removal it also increases GAC life. Furthermore, the richness and diversity of biologically active GAC filters leads to communities that are robust, functionally stable, and active at all times of the year.

4.0 Pilot Scale Investigation of GAC Type and Inoculation Strategy on Geosmin Removal Efficiency in Biologically-Active Activated Carbon Filters

4.1 Introduction

Water utilities spend on average 4.5% of their budgets on abatement of taste on odor issues and 43% of water utilities using surface waters report significant taste and odor episodes lasting longer than 2 weeks (Suffet et al., 1996). Aesthetic concerns are of increasing importance as consumers have other drinking water options (i.e., bottled water).

Geosmin is a leading cause of taste and odor problems in municipal drinking water (Persson et al., 1983). Geosmin imparts a disagreeable earthy/musty odor. Geosmin is especially problematic because it has a low odor threshold concentration of 10 parts per trillion (ng/L) (Young et al., 1996, Suffet et al., 1999). Gesomin is not effectively removed by conventional drinking water treatment. The main options for water utilities needing to remove geosmin from their water are ozone and activated carbon.

Activated carbon is the most effective process for taste and odor removal (Suffet et al., 1996). Activated carbon removes geosmin via sorption. Currently water utilities choose PAC or GAC based on sorption isotherms and cost. Brands of activated carbon are made with different source material and are activated either chemically or physically; therefore each type of activated carbon typically has a unique sorption capacity in a specific source water matrix. Water utilities test types of GAC for their target compound in their source water to determine which brand has the best cost-to-benefit ratio. In the case of GAC filters, this testing scheme is deficient; biological activity on the carbon can lead to increased bed life and increased removal of target compounds. Saint Paul Regional Water Services installed GAC filters in 2007. Based on AdDesignS modeling, the estimated bed life of the GAC filters at Saint Paul Regional Water Services was 594 days (Scharf et al., 2009). That bed life has already been exceeded three-fold

and Saint Paul Regional Water Services continues to have no break-through of geosmin. Biologically active GAC can biodegrade geosmin, increasing geosmin removal; furthermore, biological activity can regenerate GAC increasing bed life.

My experimental goals were to investigate removal of taste and odor compounds by biologically active GAC filters and determine the influence of GAC type and inoculation strategy on filter performance. For this purpose, a column study was designed to compare two different types of commercially-available GACs as well as anthracite (which served as a non-sorptive control media). The study also compared the effects of inoculation of the columns with a suspension of bacteria obtained from the full-scale filters. The geosmin dosing scheme consisted of a 100 ng/L target for the first eight weeks, then a target of 0 ng/L for six weeks, followed by a large spike (2 µg/L) and a continued dosing target of 100 ng/L. During the study period influent and effluent geosmin concentrations and attached biomass (i.e., ATP) levels were measured for the pilot-scale columns.

4.2 Materials and Methods

4.2.1 Column system

The columns were constructed of 2 inch (0.0508 m) inner diameter acrylic tubes. Each column was first filled with 1.25 inches of sand and then filled to the top of the bottom 12 in section (an additional 10.75 inches) with the specified media type. The columns were dry packed except for two columns, which were filled with GAC that was aseptically removed from Filter 6, 6 hours after backwash, called Aged GAC. Of the remaining columns: 4 were filled with virgin Calgon F-400 (a coal-based GAC), 4 contained Nuchar WV-A 900 (a wood-based GAC), and 4 contained anthracite (a non-sorptive control media). Pictures are shown in Appendix C: Photos of Column Set-Up.

The pilot-scale column system was fed filter influent from the full-scale Saint Paul Regional Water Services that was softened, settled, and recarbonated to pH ~ 8. Water was pumped from the full-scale filter influent line to a 55 gallon drum reservoir. During the first 105 days of experiment when the influent water was cold, the water was heated to 19±1 °C; using an in-line heater (Chromolax Circulation Heater (Cat No NWH-31525XX)). The pilot plant water temperature exceeded 20°C during the late spring and summer months (days 105 - 122) as there was no equipment available to cool the water (Figure 4-2, Appendix E: Temperature of Saint Paul Regional Water Services raw water during the column study.). Geosmin was then added to the water at the specified concentration, and the water was pumped to each column at 110 mL/min for a hydraulic loading rate of 1.30 gpm/ft^2 (Figure 4-1).

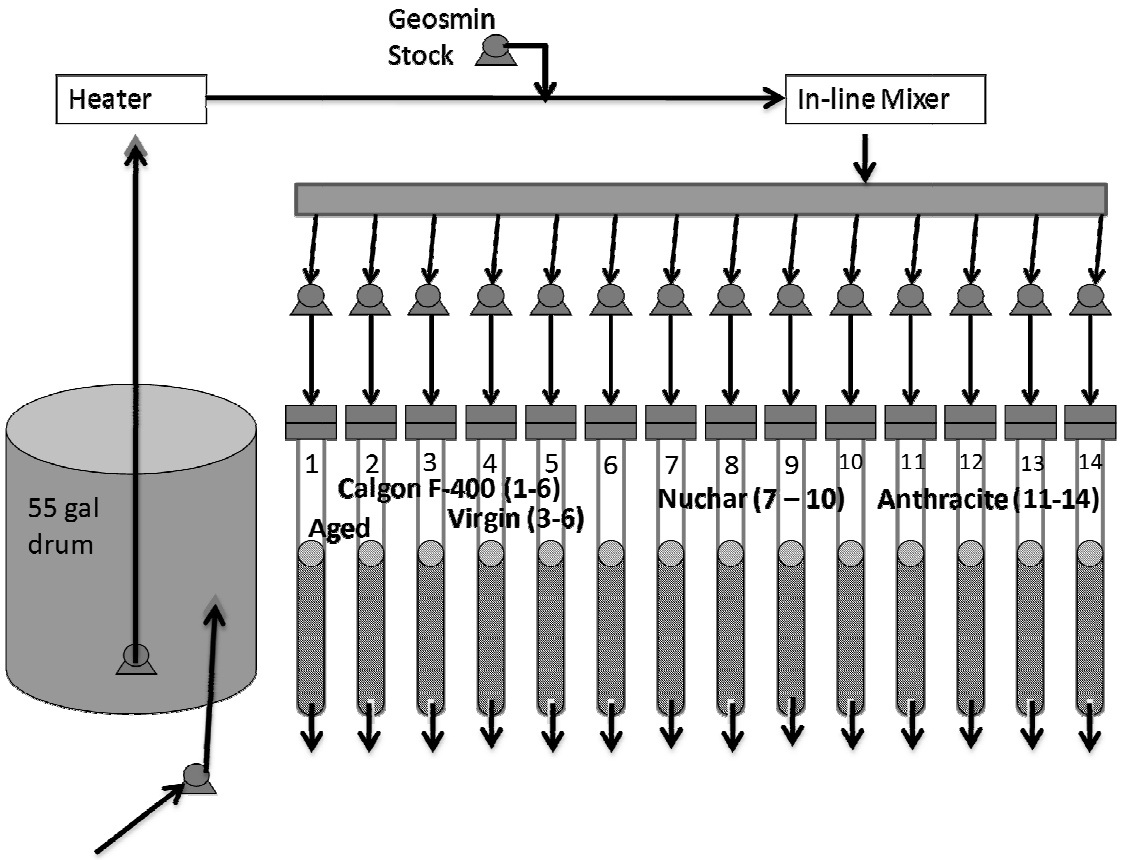


Figure 4-1: Diagram of pilot GAC filtration system at Saint Paul Regional Water Services.

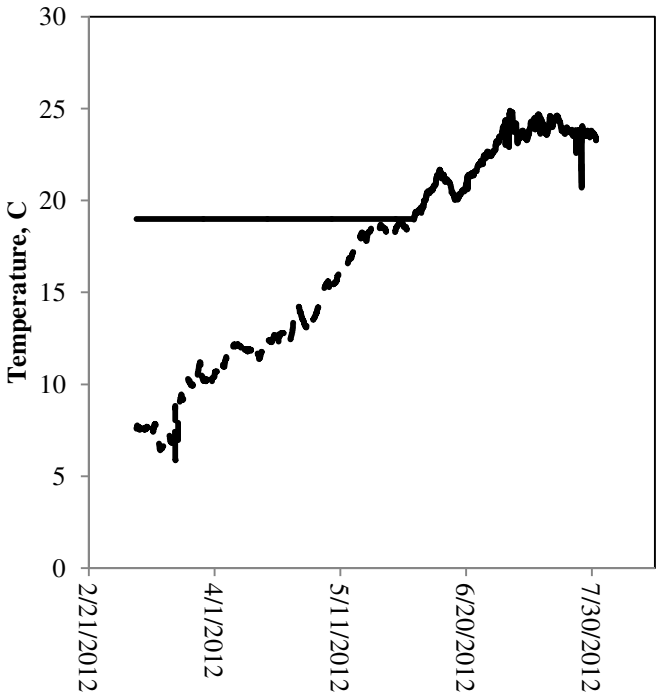


Figure 4-2: Temperature of filter influent water during column study, solid line = temperatures to the pilot-scale column study, dashed line = temperatures to the full-scale filters

Table 4-1: Characteristics of granular activated carbons used in the pilot-scale column study.

Media Characteristic	Calgon F-400	Nuchar WV-B900
Apparent Density (g/cc)	0.48	0.24 - 0.30
Iodine Number (mg/g)	1000 min	900 min
Effective Size (mm)	0.55 - 0.75	0.8 - 1.1
Uniformity Coefficient (max)	1.9	1.8
US Mesh	12 x 40	8 x 25
Oversize (%)	5	8
Undersize (%)	4	5

4.2.2 Column Inoculation

Half of the 14 columns were inoculated with organisms from a full-scale filter. GAC was removed from Filter 6, (37 hours post backwash). Equal volumes of GAC and Camper Solution (0.01% peptone, 10^{-6} M Zwittergent 3-12, 10^{-3} M EGTA, 10^{-1} M Tris buffer (pH = 7)) (Camper

et al., 1985) were mixed in 400 mL volumes. The GAC suspension was sonicated (Branson Digital Sonifier 250) for 1 min @ 60 Hz. The desorbed biomass solution (100mL per filter) was added to 6 columns; 2 coal, 2 wood, and 2 anthracite (Columns numbers: 4, 5, 7, 8, 13, and 14). The columns were then backwashed to remove fines. During a backwash cycle the following day column 8 broke and spilled some media. The inoculation procedure was repeated on February 27th.

4.2.3 Column Operation

The columns were operated from March 6, 2012 until July 13, 2012. Backwash dates and any perturbations to the columns are listed in Appendix D: Log of Column Study Events.

4.2.3.1 Geosmin Dosing

The columns were dosed at an average of 200 ng/L of geosmin for 8 weeks followed by 6 weeks without geosmin (Figure 4-3). After the period without geosmin in the feed, a large geosmin spike (2000 ng/L) was dosed followed by an additional 4 weeks at 100 ng/L.

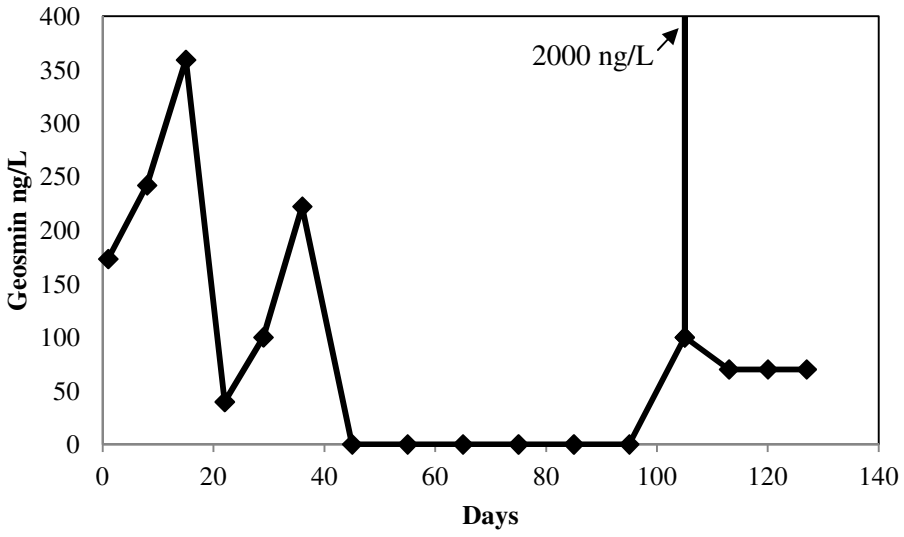


Figure 4-3: Influent geosmin concentration versus time for pilot-scale column study at Saint Paul Regional Water Services.

4.2.3.2 Backwashing

The columns were backwashed with finished water from SPRWS. The full-scale filters are backwashed every 6 days or when headloss reaches 72". Typically filters are backwashed every 6 days; reaching critical headloss very rarely**.** For the pilot-scale columns, a backwashing protocol was designed to effectively remove debris from the filter media and mimic the full-scale procedure (i.e., reach 30% bed expansion). Each media type has a different specific weight and needed to be backwashed at a different flow rate (Table 4-2) in order to reach 30% bed expansion.

Table 4-2: Pilot-scale column study backwashing flow rates.

Media Type	gal/min	gpm/ft^2	m/h
Aged Calgon GAC	0.221	10.1	24.9
Virgin Calgon GAC	0.400	18.3	45.0
Virgin Nuchar	0.166	7.6	18.8
Anthracite	0.257	24.2	59.5

4.2.3 Geosmin Analysis

Geosmin was analyzed by a head-space solid phase microextraction (HS-SPME) method adapted from Watson et al. (2000) by Johnston (2005). Sodium chloride was added (3 g) to a 10 mL sample in 20 mL vials, with 20 µL of internal standard (250 µg/L naphthalene, D-8 in methanol). An autosampler (Overbrook, USA) heated the sample to 65°C and shook the sample for 5 minutes. A PDMS-DVB fiber (Supelco, Australia) was then extended into the headspace and incubated for 60 minutes at 65°C. The geosmin and naphthalene were desorbed from the fiber in the large volume injection port of a gas chromatograph (GC) (Hewlett Packard 5890 Series II) for 1.0 minutes at 270°C. The fiber was cleaned between each sample in an oven at 250°C for 2 minutes. The GC temperature program was 40°C for two minutes with a ramp up of 11°C /minute to 240°C followed by a 5 minute hold. Geosmin and naphthalene were detected by

mass spectrometer (Hewlett Packard 5972) in selective ion monitoring mode (geosmin:112 m/z, naphthalene: 136 m/z). Geosmin standards were run every five samples and a standard curve was made using DI water and geosmin standards (Sigma Aldrich) (Appendix F: Geosmin Analysis and Standard Preparation).

4.2.4 ATP measurement

Adenosine tri-phosphate (ATP) is often described as the energy currency of the cell and is found universally in organisms from humans to bacteria. Thus, ATP levels can be used to estimate quantities of cells or biomass. ATP was measured following the protocol of Velten et al. (2007) using the Promega BacTiter-Glo™ Microbial Cell Viability Assay. First, 100 µL of phosphate buffer was added to 200 mg of GAC (wet weight) and the GAC was incubated for 3 minutes at 30°C. Concurrently, 300 µL of Bactiter-Glo reagent was also incubated for 3 minutes at 30°C. After 3 minutes, the 300 µL of BacTiter-Glo reagent was added to the GAC; after 90 seconds, the relative light units were read on a Luminometer with at 30 second delay. A calibration curve was made using the same procedure and a Sigma-Aldrich ATP standard. Additionally, a series of negative control samples consisting of virgin anthracite, virgin Calgon GAC, and virgin Nuchar GAC, were also analyzed in the same manner as the media samples from the filters. GAC samples were saved and dried and values are reported as ng ATP/g GAC (dry weight).

4.2.5 Chemicals

Pure geosmin was purchased in 50 mg amounts from Dalton Pharma Services (Canada). A stock solution of 1 ug/mL was prepared by dissolving in Milli-Q water and storing in a freezer protected from light. Geosmin calibration curves (Appendix F: Geosmin Analysis) were created using geosmin solution in methanol from Wako Chemical (#072-03421). Naphthalene-d8 (Supelco #442716) was used as an internal standard for geosmin analysis.

4.3 Results

All of the GAC columns effectively removed geosmin throughout the study period (Figure 4-4). Even the aged GAC from the full-scale filters at Saint Paul Regional Water Services removed high level of geosmin (>80%) during the entire study period. In contrast, the anthracite columns did not begin removing significant amount of geosmin until day 35. After the six-week period without geosmin, the anthracite columns still removed 40% of geosmin and two weeks later removal efficiencies exceeded 95%. The virgin Nuchar and Calgon GACs had very similar geosmin removal efficiencies. In order to confirm that geosmin was being biologically degraded and not being lost to other removal mechanisms a bottle test was done (Appendix I: Test for Biological Activity).

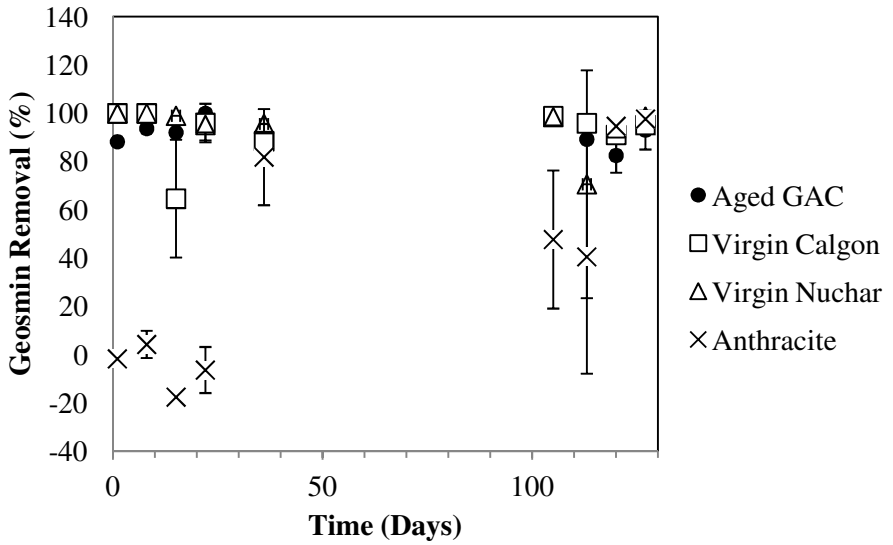


Figure 4-4: Effect of media type on geosmin removal efficiency. Data points represent the average of the four columns with the same media. Error bars show 1 standard deviation when greater than 5%.

Inoculation did not appear to have any effect on geosmin removal in the columns as the removal efficiencies for the inoculated and non-inoculated columns were similar (Figure 4-5).

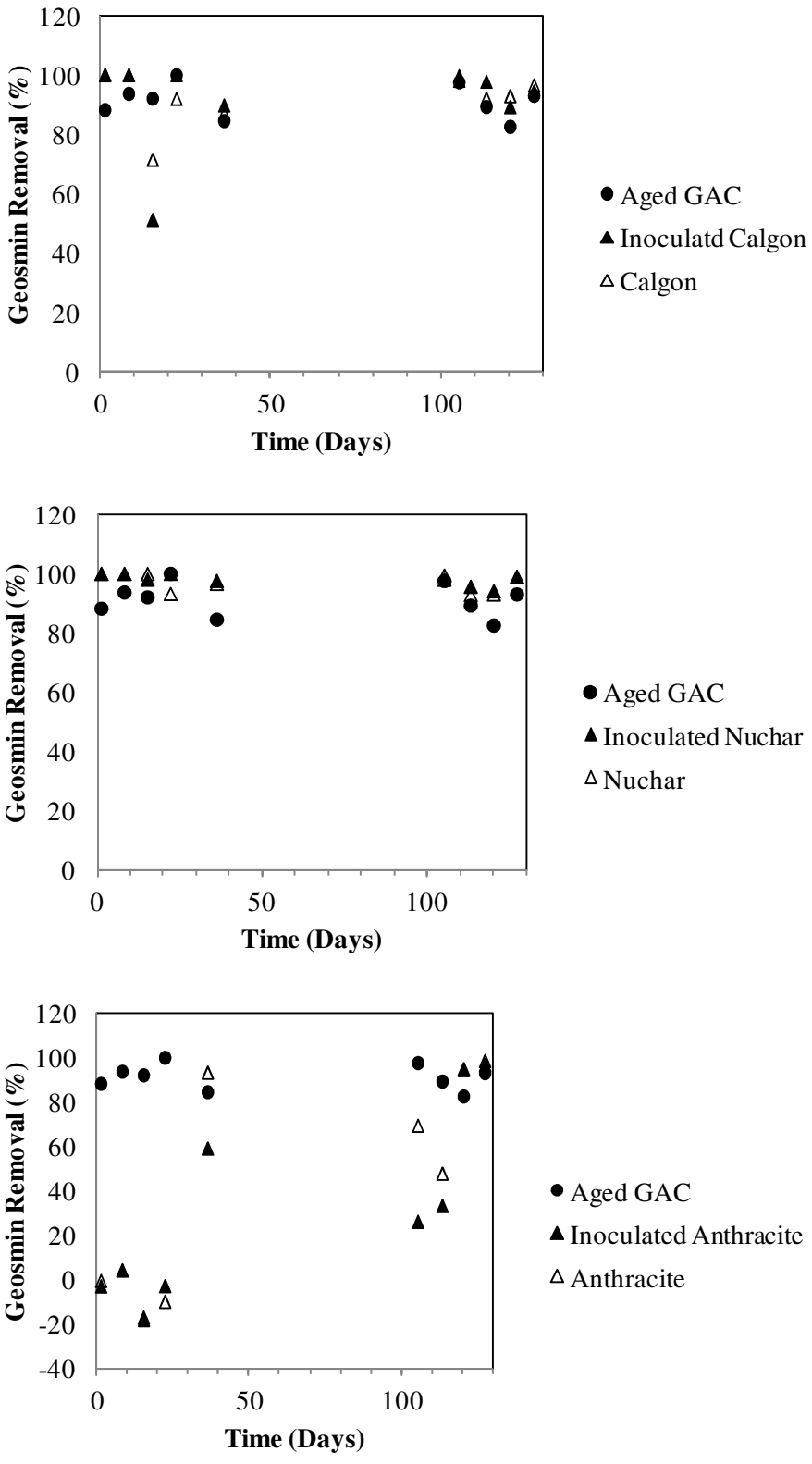


Figure 4-5: Effect of inoculation on geosmin removal efficiency. Each data point represents the average of the two columns.

Biomass levels increased during the study period for the pilot-scale filters and full-scale filter 6 (Figure 4-6). The average ATP level between the media types at the end of the experiment was 140 ng/g GAC. Interestingly, ATP levels on the aged GAC columns, which was obtained from filter 6, were very different then levels on filter 6 GAC. The aged GAC columns, however, received heated water versus filter 6 which was receiving 5 - 15°C colder water until day 105. The first data point on day 9 already shows much higher ATP levels on the old GAC than on filter 6. As expected, the anthracite columns consistently had the lowest levels of ATP. Anthracite has been previously shown to support lower levels of biomass than GAC (Wang et al., 1995). Finally, the wood (Nuchar) GAC had higher ATP levels than the virgin coal (Calgon) GAC but not the aged coal (Calgon) GAC.

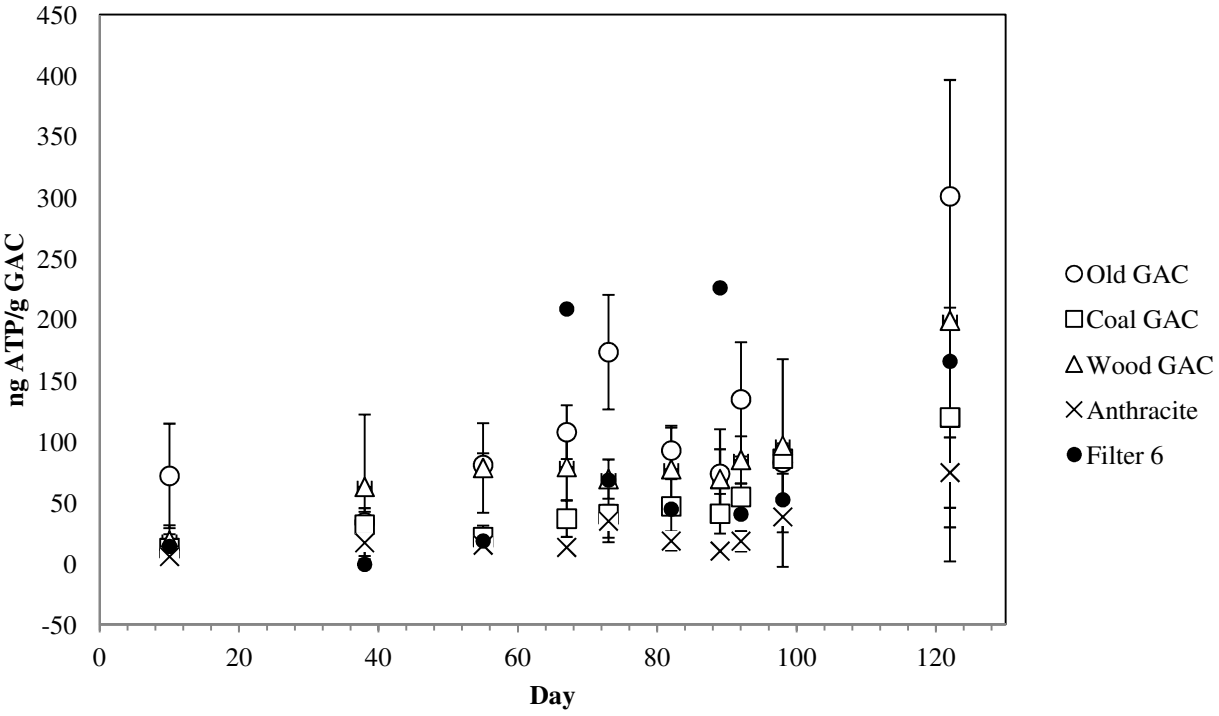


Figure 4-6: Mean ATP levels in the pilot-scale filters and full-scale filter 6. Data points represent the average of the four columns with the same media. Error bars represent 1 standard deviation when the standard deviation exceeded 5 ng ATP/g GAC.

The effect of inoculating the columns on ATP levels varied for each type of media (Figure 4-7). For the Calgon GAC there is no significant difference ($p = 0.44$, paired t-test) between the seeded and unseeded column the average difference in quantity of ATP is -18.6%. For the Nuchar GAC the unseeded column has significantly more ATP per g of GAC, by 57.6% on average ($p=0.02$, paired, t-test). For both GAC types, it would appear that inoculation is counterproductive and decreased the amount of biomass present on the GAC even after 100 days.

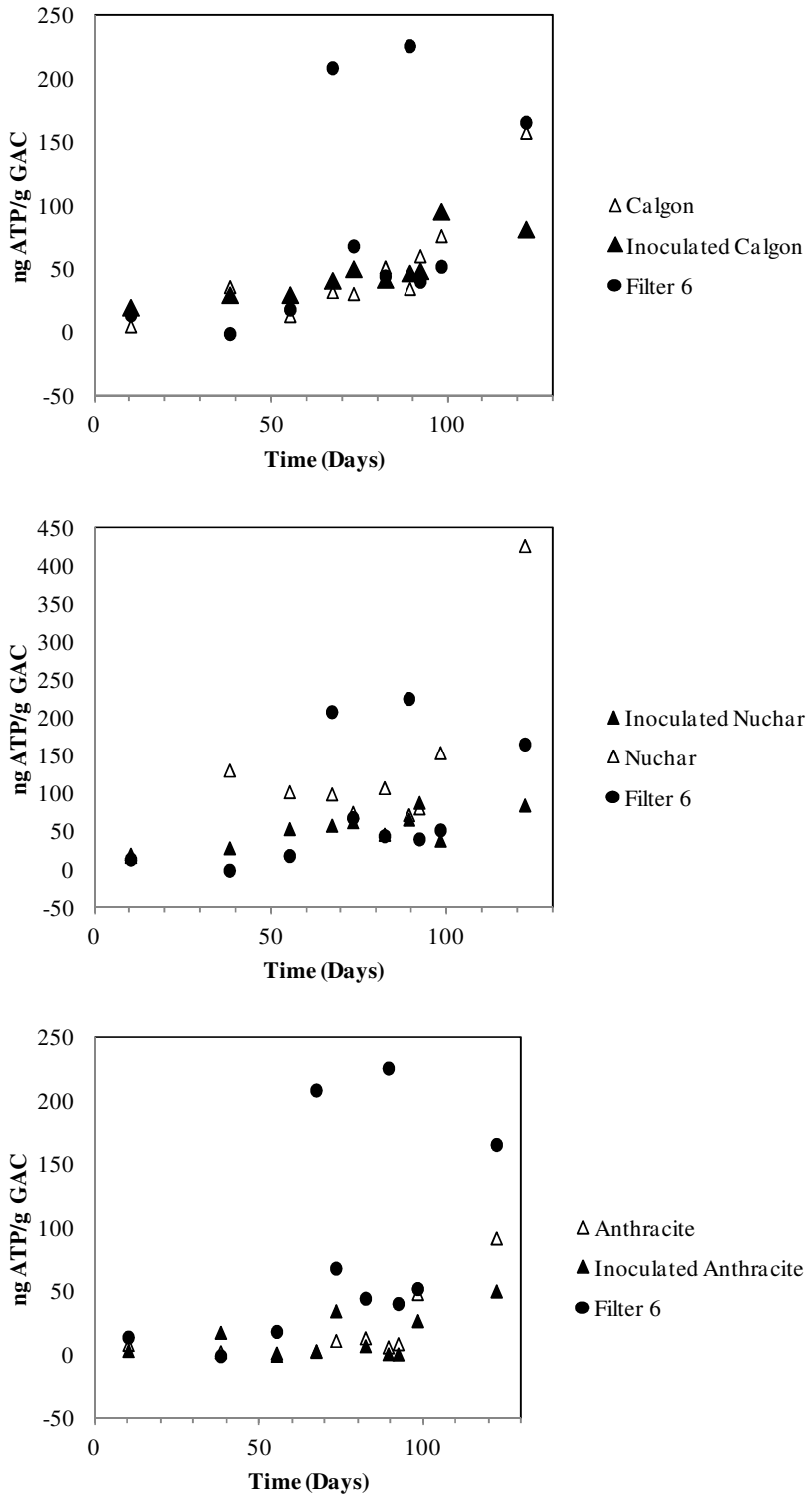


Figure 4-7: Effect of inoculation on ATP levels in the pilot-scale filters and full-scale filter 6. Each data point represents the average of the two columns.

4.4 Discussion

Biological degradation appears to be an important contributor to geosmin removal in the Saint Paul Regional Water Services GAC filters. Enriching for geosmin degrading organisms was successful after only five weeks of adding parts per trillion levels of geosmin to the filter influent water. Furthermore, this geosmin-degrading community after a 6 week no- geosmin period was still able to remove 40% of geosmin when dosing resumed.

The removal of geosmin by the anthracite columns indicates biological removal of geosmin after 40 days (Appendix I: Test for Biological Activity), similar to that reported by other researchers (Liu et al., 2001). Geosmin-degrading organisms are present in the raw water at Saint Paul Regional Water Services and are capable of growing on GAC. Those organisms are present in March, when water temperatures are around 4°C; this is surprising because geosmin is not present in Saint Paul Regional Water Services source water at this time. The organisms are therefore likely present in Saint Paul Regional Water Services source water year round. The aged GAC that was past its estimated sorptive bed life, continued to remove geosmin as well as the virgin GAC. This suggests that microorganisms living on the GAC are sustaining geosmin removal either via direct biodegradation or via bioregeneration of the GAC sorption capacity.

Curiously, inoculation of the filters did not help in establishing a biologically active GAC filter community. Inoculation of the columns was expected to increase the speed with which a functioning biofilm could be formed. Inoculating the virgin media with a large population of species suited for living fixed on GAC would give the columns a head start on columns that were only being inoculated with the low levels of bacteria in the filter influent water (HPC = [10 - 555 cfm/mL]). There are three probable reasons for the lack of difference between the inoculated and non- inoculated columns: either the inoculation method was ineffective at transferring viable biomass, the microbial community that the columns were inoculated with did not impart any advantage, or the continuous re-inoculation with influent water is a more important source of

biofilm growth. The biomass desorption method (Camper et al., 1985) was chosen because based on heterotrophic plate counts it removes viable biomass from GAC. The columns inoculation may also have been unsuccessful because the February filter community was not adapted to water simulated from summer water temperature (> 19°C).

Typically biofilms on filters for drinking water treatment take 1 1/2 months to develop (Liu, 2001); however in this case after 3 months a plateau in biomass levels is not observed. It is most likely that this is due to the increase in water temperature during that time. Previous research has shown that biological removal of taste and odor compounds improves at 25°C versus 20°C (Ho et al., 2011). As shown in Appendix E: Temperature of Saint Paul Regional Water Services raw water during the column study., during the first 8 weeks water temperatures averaged 19°C, after that water temperatures averaged 26°C. ATP levels are an order of magnitude lower than those found by Velten et al. (2007). Velten et al. measured average ATP levels of 1820 ng/g GAC. This is another indication the ability of GAC to support organisms changes as the GAC ages. GAC typically becomes finer after years of backwash. The virgin Coal GAC is approximately 250 backwash cycles younger than the Aged GAC.

A pilot study is very limited to comparing a small number of variables. Only two types of GAC were compared. The two used in this study, Calgon F-400 and Nuchar WVB, were chosen by Saint Paul Regional Water Services based on their interest on using those GACs in the future. Furthermore, only one inoculation method was tried. Lastly, adding geosmin to filter feed water is not a viable option for water utilities hoping to grow filter communities capable of degrading geosmin.

Organisms capable of degrading geosmin spontaneously grew on pilot scale columns when encouraged by the addition of geosmin to the feed water, therefore species capable of degrading geosmin are present in the source water. When these species establish themselves as members of the community they are robust enough to survive geosmin-free periods. Inoculation pilot scale columns with biomass desorbed from full-scale filters does not improve geosmin

removal or decrease time to steady state biomass concentrations. Water utilities should evaluate the biological activity of different GAC types when making large investments in GAC brands.

5.0 Conclusions

The microbial dynamics of biologically active GAC filters is not well understood. This study examined GAC filter communities, especially their filter-to-filter variability and seasonal community dynamics. The study was needed to better understand the microbial ecology of a technology used to produce aesthetically pleasing drinking water without compromising public health. Water treatment plants currently only choose GAC based on cost and sorption. Water treatment plants also only hope for the colonization of GAC with good microorganisms; treatment plants do not have any control over who becomes members of the GAC microbial community. This study showed that GAC filters support diverse communities that are dynamic and functionally stable and active at all times of the year. The study showed that GAC filter communities can be enriched in 40 days. The study also showed that GAC installed for the removal of a specific taste and odor compound can still remove that compound after 5 years.

For the first high throughput sequencing techniques were used to identify organisms living on biologically active GAC filters. High numbers of OTUs and unprecedented levels of diversity were observed. High diversity is important because this means that the filter communities are active and robust throughout the year. Large differences in the bacterial community between filters was unexpected because the filters have the same temperature, feed water and similar operation parameters. The filters are biologically active throughout the year, evidenced by their distinct and changing community structures. The microbial communities are very diverse and should be highly robust and functionally stable. The organisms living on the filters include high percentages of *Nitrospira*, a nitrite-oxidizer not expected to play a large role in GAC filter communities.

Natural geosmin degraders are present at Saint Paul Regional Water Services and can be enriched by the addition of parts per trillion levels of GAC. The natural geosmin degraders turned non-sorptive anthracite into an efficient geosmin removal after 40 days. Once established

the community is robust enough to survive 6 weeks without the continued dosing of geosmin. The biologically activity also allowed the 5 year old GAC to perform as well as virgin GAC. Inoculation had little effect on the total biomass growth on the GAC or the time it took for the GAC to become biologically active. An expensive columns study was not a cost effective way to evaluate the influence of GAC on biological activity.

Future research should be done on inoculating virgin media with a known geosmin degrading culture at the full-scale. McDowall et al. (2009) successfully inoculating virgin media with a known geosmin degrading culture on the pilot scale but this research will only benefit water utilities if it can be replicated at the full-scale. Future research should determine the best way to evaluate the impact of GAC type on biological activity.

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6.0 Appendices:

Appendix A: Lowry Method Calibration Curve and Analysis

Lowry's method is a colorimetric technique that is time dependent, BSA standards were run each time sample concentrations were measured. The standards were used to generate curves like that seen in Figure 6-2. The r-squared valves were always greater than 0.90. Additionally, because the method measures aromatic amino acids background aromatic compounds will confound the quantification. GAC is made by the partial combustion of organic material that potentially contains aromatic compounds. In order to measure the background concentration, the 'protein content' of 10 samples of virgin GAC was measured. The mean was 714 mg protein/g of GAC with a standard deviation 209.5 mg protein/g GAC. Based on the normal probability plot, (Figure 6-2), the distribution appears to be normally distributed so the mean (714 mg/g) was used as a background concentration.

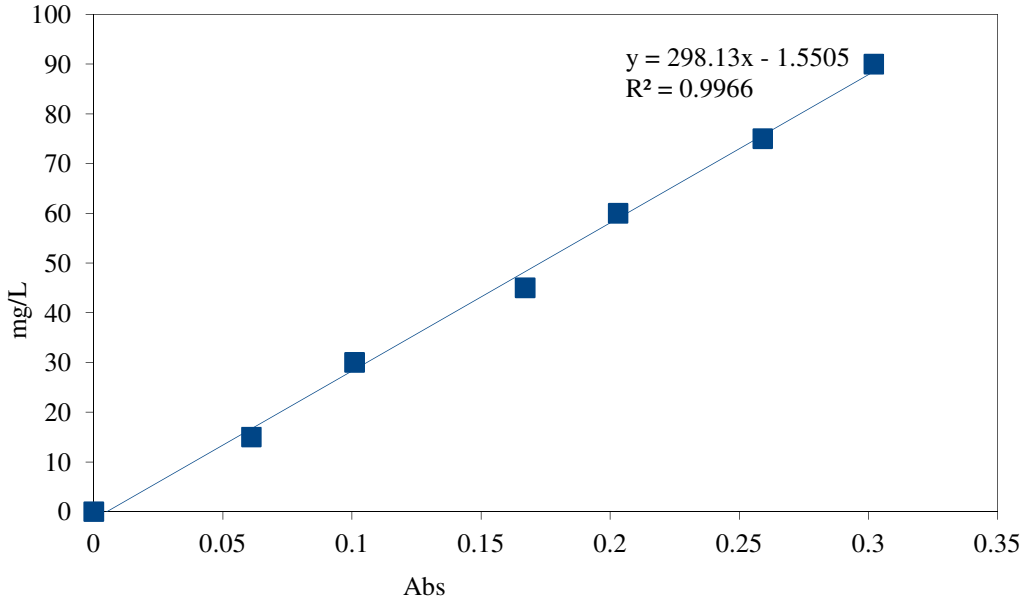


Figure 6-1: Example standard curve, January 2012.

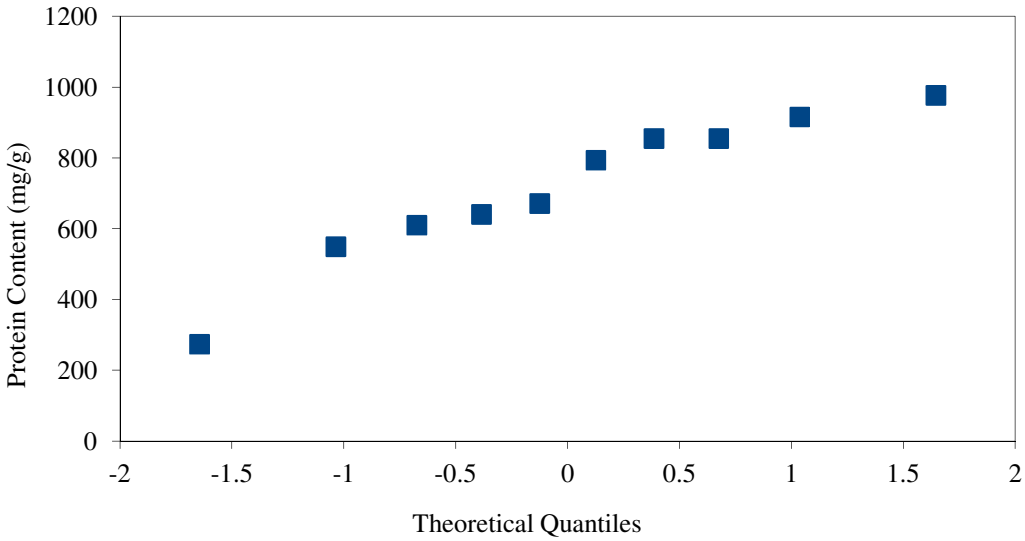


Figure 6-2: Normal quantile plot of background 'protein' levels on Calgon F400 GAC.

Appendix B: Nitrogen and Phosphorus Levels in Saint Paul Regional Water Services Raw and Finished Water

Table 6-1: Nitrogen Levels in raw and finished water at Saint Paul Regional Water Services between March 2011 and February 2012.

	Parameter	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Raw	Total Phosphorus, mg/L	0.076	0.025	0.015	0.017	0.024	0.038	0.016	0.024	0.039	0.018	0.026	0.018
	Ammonia-N, mg/L	<0.010	0.042	<0.010	<0.010	0.010	<0.010	0.015	<0.010	<0.010	<0.010	<0.010	<0.008
	Nitrate-Nitrite-N, mg/L	0.652	0.505	0.594	0.246	0.232	0.182	0.186	0.271	0.407	0.446	0.457	0.454
	Total Nitrogen-N, mg/L	1.086	1.205	1.143	0.993	0.660	0.845	0.796	0.455	0.845	2.206	0.836	N/A
Finished	Total Phosphorus, mg/L	0.043	<0.001	0.004	0.003	0.004	0.005	0.001	0.005	0.035	0.001	0.026	0.018
	Ammonia-N, mg/L	0.633	0.755	0.725	0.764	0.800	0.785	0.793	0.823	0.771	0.764	<0.010	<0.008
	Nitrate-Nitrite-N, mg/L	0.733	0.610	0.598	0.310	0.271	0.193	0.193	0.312	0.418	0.533	0.457	0.454
	Total Nitrogen-N, mg/L	1.389	1.365	1.323	1.415	0.978	1.175	1.174	1.348	1.252	1.280	0.836	N/A

Appendix C: Photos of Column Set-Up

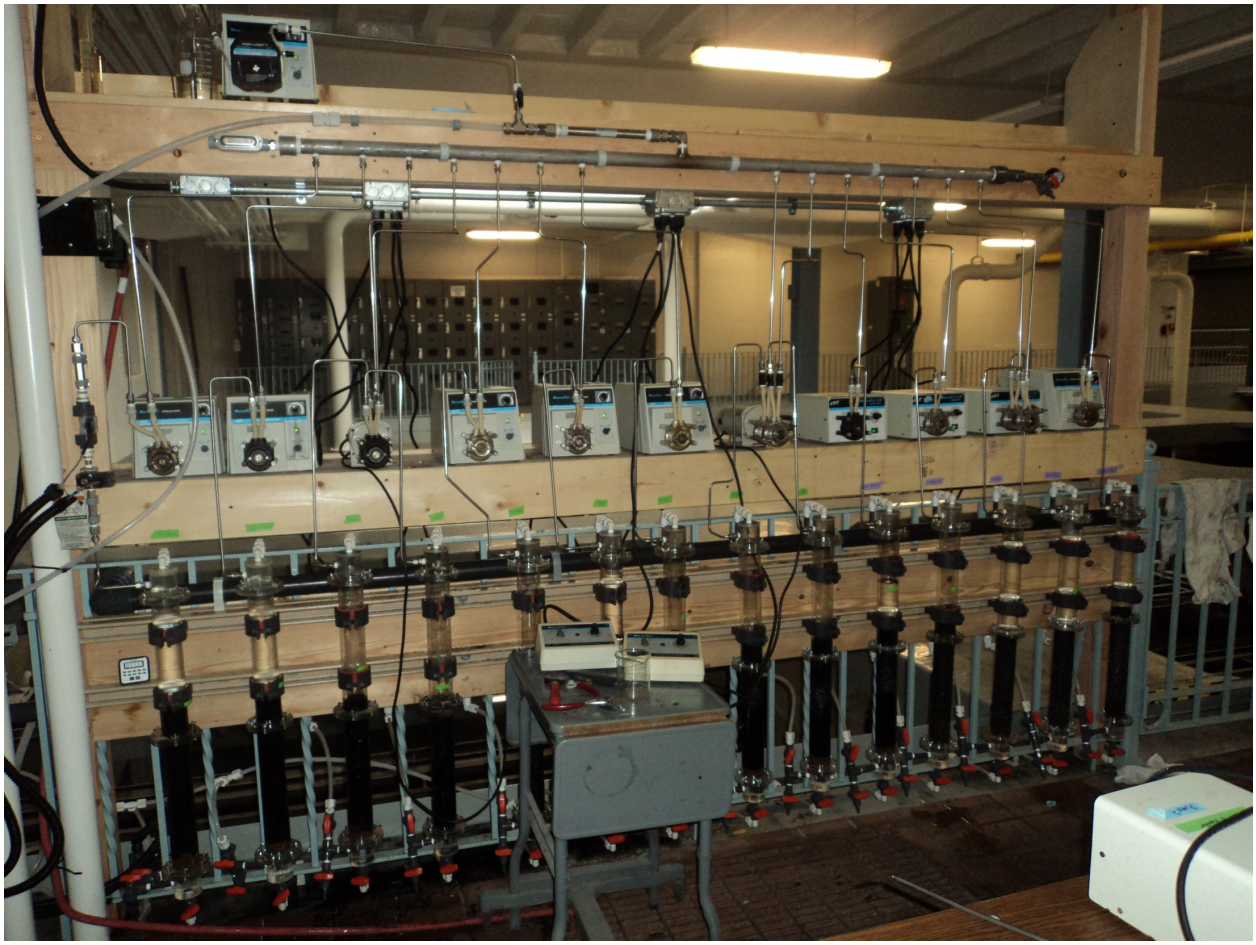


Figure 6-3: Column Set-Up at Saint Paul Regional Water Services.

Figure 6-4: Set-Up of column feed water before addition of geosmin.

Figure 6-5: Close-up of two columns, notice bottom layer of sand supporting GAC media.

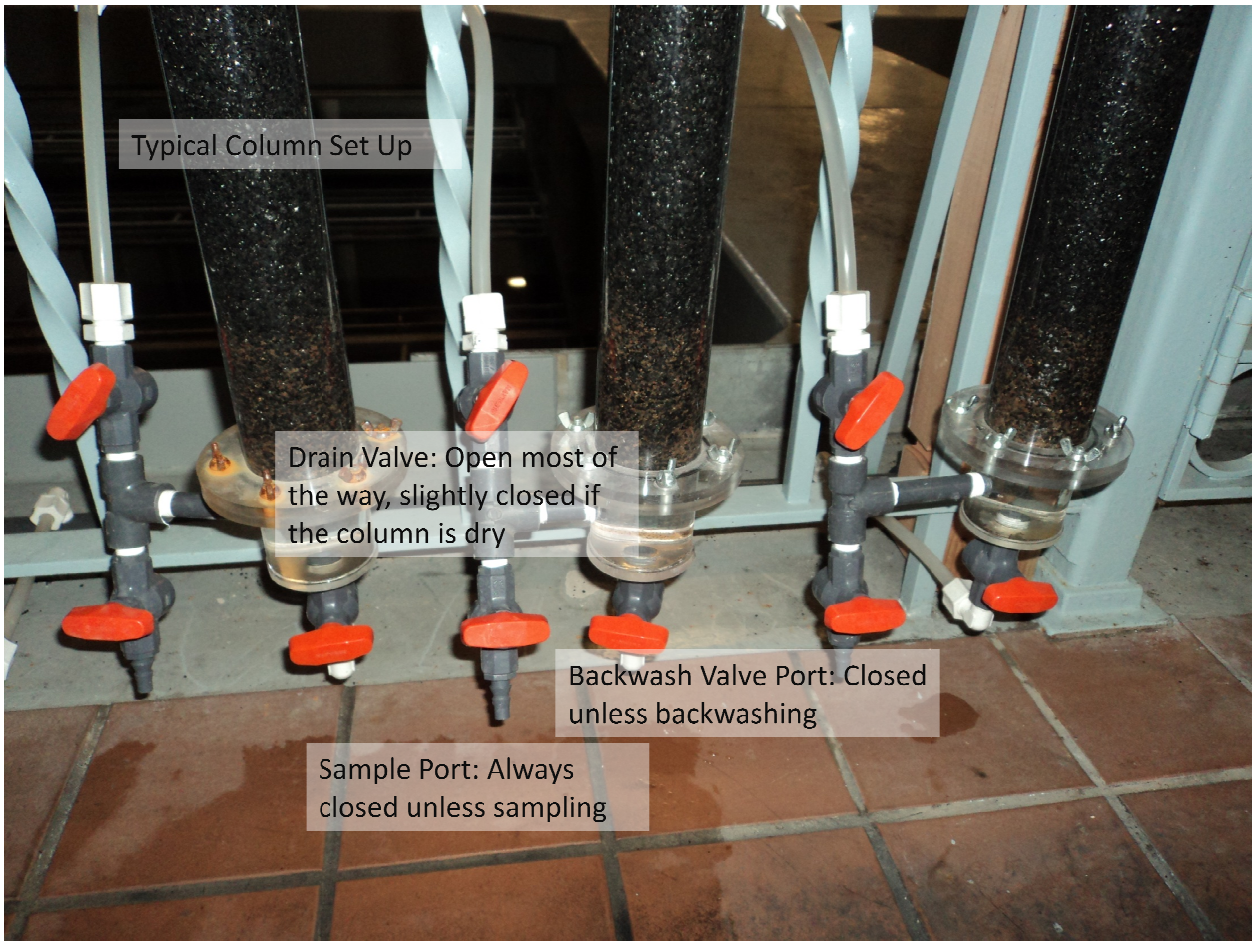


Figure 6-6: Column backwash and drain valves.

Appendix D: Log of Column Study Events

Day	Date	Event
-13	2.23.2012	Filled Columns with GAC/Anthracite. Backwashed to remove fines - broke columns 12, 13, 14.
-12	2.24.2012	Replaced columns 12, 13, 14. Seeded columns 4, 5, 7, 8, 12, 13
-11	2.25.2012	Broke columns 12, 13 again during backwash
-9	2.27.2012	Reseeded all columns.
-9	2.27.2012	20:00 Column 6 tubing split and pump was turned off
-8	2.28.2012	10:00 Column 6 tubing replaced Backwashed seeded columns, air scour 30 sec, 2min30seconds water to 30% expansion to remove fines that were added with seeding.
-7	2.29.2012	Backwashed all columns 30 sec air, 2min 30 sec water Broke Caps on Columns 4 and 5 - replaced from stock.
-5	3.01.2012	Begin Backwashing with Plant Replicating Sequence (1 minute air scour, followed by 10 minutes at 30 expansion), all backwashing past this date is this sequence unless otherwise noted.
-4	3.02.2012	Backwash Removed screens because clogging with fines.
-3	3.03.2012	Backwash
-2	3.04.2012	Backwash
-1	3.05.2012	Backwash, Columns 13 broke. Installed pressure breaker on backwash line
0	3.06.2012	Replaced Syringe Pump with high pressure pump head and console drive pump. Geosmin Feeding begins = 135 ng
1	3.07.2012	Backwash
1	3.07.2012	12:00 Shelf installed - geosmin dosing pump switched off
2	3.08.2012	9:00 Geosmin pump turned on Backwash
3	3.09.2012	Needed to re-organize drain lines because hydraulic issues were stopping columns 1 and 2 from draining.
5	3.11.2012	Backwash, Column 11 broke
7	3.13.2012	Circuit Blows - Pumps 1,3,4,5,7,12,13,and 14 off for 2 - 5 hours.
8	3.14.2012	Backwash
9	3.15.2012	Geosmin dosage pump didn't run for 24 hours
11	3.17.2012	Backwash
12	3.18.2012	Circuit Tripped again, columns 1, 3, 4, 5, 7, 12, 13, and 14 off for 0 - 24 hours.

15	3.21.2012	Backwash
18	3.24.2012	Columns 11 and 14 off for 24 hours
19	3.25.2012	Backwash
22	3.28.2012	No Geosmin Dosed for 24 hours Backwashed
26	4.01.2012	Backwash
29	4.04.2012	Backwash
29	4.04.2012	Backwash
34	4.09.2012	Installed new vessel for geosmin stock
35	4.10.2012	No Geosmin Dosed for 24 hours Backwashed
38	4.13.2012	Backwash
42	4.17.2012	Backwash
45	4.20.2012	Backwash
50	4.25.2012	No Geosmin Dosed 48 hours Backwashed - increased time to 20 minutes
51	4.26.2012	Hose on Column 6 shredded - found and repaired by operator within 3 hours
52	4.27.2012	1900 Hose on Column 6 shredded again
53	4.28.2012	Cleaned and re-installed hose on column six Backwash
55	4.30.2012	Backwash Realized that columns 12, 13, 14 had been continually backwashed since 4.28.2012
57	5.02.2012	Column 5 filling from drain line - reorganized drain lines
58	5.03.2012	Backwash
61	5.06.2012	Backwash
66	5.11.2012	Backwash - Geosmin Dosing Stopped
71	5.16.2012	Backwash
73	5.18.2012	Column 8 feel off screen into bottom cap - clogging drain line and backwash line
75	5.20.12	Backwash
79	5.24.12	Backwash
84	5.29.12	Backwash
89	6.03.12	Backwash
95	6.09.12	Backwash
100	6.14.2012	Backwash
101	6.15.2012	Column 11 broke, lost 1/4" to 1/2" of GAC
105	6.19.2012	Backwash
108	6.22.2012	Backwash Column 7 broke
113	6.27.2012	Backwash

		Pumps 12 and 13 malfunctioning, replaced pump
117	7.01.2012	Column 2 pump hose shredded - replaced by operator in 14 hours or less
118	7.02.2012	Backwash
121	7.05.2012	Backwash Replaced Hose on Pumphead of Column 12
125	7.09.2012	Backwash
128	7.12.2012	Backwash

Appendix E: Temperature of Saint Paul Regional Water Services raw water during the column study.

Date	Hourly Average Plant Influent Water Temperature (^{0}C)
3/7/12 7:00 AM	7.68
3/7/12 7:00 PM	7.66
3/8/12 7:00 AM	7.63
3/8/12 7:00 PM	7.60
3/9/12 7:00 AM	7.61
3/9/12 7:00 PM	7.56
3/10/12 7:00 AM	7.58
3/10/12 7:00 PM	7.70
3/11/12 7:00 AM	7.72
3/11/12 7:00 PM	7.53
3/12/12 7:00 AM	7.49
3/12/12 7:00 PM	7.73
3/13/12 7:00 AM	7.77
3/14/12 7:00 PM	6.48
3/15/12 7:00 AM	6.58
3/15/12 7:00 PM	6.68
3/16/12 7:00 AM	6.70
3/16/12 7:00 PM	6.80
3/17/12 7:00 AM	6.69
3/17/12 7:00 PM	6.99
3/18/12 7:00 AM	6.82
3/18/12 7:00 PM	6.84
3/19/12 7:00 AM	6.84
3/19/12 7:00 PM	5.92
3/20/12 7:00 AM	6.19
3/20/12 7:00 PM	8.76
3/21/12 7:00 PM	9.15
3/22/12 7:00 AM	9.49
3/22/12 7:00 PM	9.82
3/23/12 7:00 AM	10.17
3/23/12 7:00 PM	10.16
3/24/12 7:00 PM	9.99
3/25/12 7:00 AM	9.78

Date	Hourly Average Plant Influent Water Temperature (^{0}C)
3/25/12 7:00 PM	10.29
3/26/12 7:00 AM	10.44
3/26/12 7:00 PM	10.72
3/27/12 7:00 AM	11.17
3/27/12 7:00 PM	10.59
3/28/12 7:00 AM	10.24
3/28/12 7:00 PM	10.23
3/29/12 7:00 AM	10.20
3/29/12 7:00 PM	10.22
3/30/12 7:00 AM	10.50
3/30/12 7:00 PM	10.55
3/31/12 7:00 AM	10.35
3/31/12 7:00 PM	10.39
4/1/12 7:00 AM	10.68
4/1/12 7:00 PM	10.60
4/2/12 7:00 AM	10.69
4/2/12 7:00 PM	10.90
4/3/12 7:00 AM	11.11
4/3/12 7:00 PM	10.97
4/4/12 7:00 AM	11.23
4/4/12 7:00 PM	11.39
4/5/12 7:00 AM	11.60
4/5/12 7:00 PM	11.81
4/6/12 7:00 AM	11.92
4/6/12 6:00 PM	11.90
4/7/12 7:00 AM	12.15
4/7/12 7:00 PM	12.10
4/8/12 7:00 AM	12.12
4/8/12 7:00 PM	12.11
4/9/12 7:00 AM	12.07
4/9/12 7:00 PM	11.90
4/10/12 7:00 AM	11.93
4/10/12 7:00 PM	11.93
4/11/12 7:00 AM	11.89
4/11/12 7:00 PM	11.84

Date	Hourly Average Plant Influent Water Temperature (^{0}C)
4/12/12 7:00 AM	11.87
4/12/12 7:00 PM	11.74
4/13/12 7:00 AM	11.89
4/13/12 7:00 PM	11.78
4/14/12 7:00 AM	11.80
4/14/12 7:00 PM	11.62
4/15/12 7:00 AM	11.40
4/15/12 7:00 PM	11.75
4/16/12 7:00 AM	12.06
4/16/12 7:00 PM	12.36
4/17/12 7:00 AM	12.49
4/17/12 7:00 PM	12.41
4/18/12 7:00 AM	12.39
4/18/12 7:00 PM	12.31
4/19/12 7:00 AM	12.43
4/19/12 7:00 PM	12.60
4/20/12 7:00 AM	12.74
4/20/12 7:00 PM	12.71
4/21/12 7:00 AM	12.18
4/21/12 7:00 PM	12.65
4/22/12 7:00 AM	12.80
4/22/12 7:00 PM	12.79
4/23/12 7:00 PM	12.61
4/24/12 7:00 AM	12.61
4/24/12 7:00 PM	12.57
4/25/12 7:00 AM	12.58
4/25/12 7:00 PM	12.99
4/26/12 7:00 AM	13.67
4/26/12 7:00 PM	13.80
4/27/12 7:00 AM	14.09
4/27/12 7:00 PM	14.21
4/28/12 7:00 AM	13.93
4/28/12 7:00 PM	13.60
4/29/12 7:00 AM	13.42
4/29/12 7:00 PM	13.19

Date	Hourly Average Plant Influent Water Temperature (^{0}C)
4/30/12 7:00 AM	13.21
4/30/12 7:00 PM	13.34
5/1/12 7:00 AM	13.45
5/1/12 7:00 PM	13.41
5/2/12 7:00 PM	13.63
5/3/12 7:00 AM	13.90
5/3/12 7:00 PM	14.13
5/4/12 7:00 AM	14.18
5/4/12 7:00 PM	14.50
5/5/12 7:00 AM	14.91
5/5/12 7:00 PM	15.20
5/6/12 7:00 AM	15.38
5/6/12 7:00 PM	15.48
5/7/12 7:00 AM	15.51
5/7/12 7:00 PM	15.20
5/8/12 7:00 AM	15.40
5/8/12 7:00 PM	15.44
5/9/12 7:00 AM	15.56
5/9/12 7:00 PM	15.63
5/10/12 7:00 AM	15.89
5/10/12 7:00 PM	16.05
5/11/12 7:00 AM	16.10
5/11/12 7:00 PM	16.38
5/12/12 7:00 AM	16.40
5/12/12 7:00 PM	16.56
5/13/12 7:00 AM	16.55
5/13/12 7:00 PM	16.84
5/14/12 7:00 AM	16.83
5/14/12 7:00 PM	17.10
5/15/12 7:00 AM	17.18
5/15/12 7:00 PM	17.47
5/16/12 7:00 AM	17.61
5/16/12 7:00 PM	18.12
5/17/12 7:00 AM	18.03
5/17/12 7:00 PM	18.26

Date	Hourly Average Plant Influent Water Temperature (^{0}C)
5/18/12 7:00 AM	18.01
5/18/12 7:00 PM	17.90
5/19/12 7:00 AM	17.79
5/19/12 7:00 PM	18.23
5/20/12 7:00 AM	18.31
5/20/12 7:00 PM	18.47
5/21/12 7:00 AM	18.45
5/21/12 7:00 PM	18.34
5/22/12 7:00 AM	18.20
5/22/12 7:00 PM	18.39
5/23/12 7:00 AM	18.39
5/23/12 7:00 PM	18.66
5/24/12 7:00 AM	18.57
5/24/12 7:00 PM	18.48
5/25/12 7:00 AM	18.44
5/25/12 7:00 PM	18.20
5/26/12 7:00 AM	18.19
5/26/12 7:00 PM	18.09
5/27/12 7:00 AM	18.26
5/27/12 7:00 PM	18.29
5/28/12 7:00 AM	18.28
5/28/12 7:00 PM	18.52
5/29/12 7:00 AM	18.64
5/29/12 7:00 PM	18.79
5/30/12 7:00 AM	18.78
5/30/12 7:00 PM	18.75
5/31/12 7:00 AM	18.53
5/31/12 7:00 PM	18.57
6/1/12 7:00 AM	18.39
6/1/12 7:00 PM	18.57
6/2/12 7:00 AM	18.64
6/2/12 7:00 PM	18.90
6/3/12 7:00 AM	18.90
6/3/12 7:00 PM	19.27
6/4/12 7:00 AM	19.32

Date	Hourly Average Plant Influent Water Temperature (^{0}C)
6/4/12 7:00 PM	19.45
6/5/12 7:00 AM	19.46
6/5/12 7:00 PM	19.49
6/6/12 7:00 AM	19.59
6/6/12 7:00 PM	20.02
6/7/12 7:00 AM	20.19
6/7/12 7:00 PM	20.49
6/8/12 7:00 AM	20.40
6/8/12 7:00 PM	20.60
6/9/12 7:00 AM	20.55
6/9/12 7:00 PM	20.80
6/10/12 7:00 AM	20.90
6/10/12 7:00 PM	21.39
6/11/12 7:00 AM	21.41
6/11/12 7:00 PM	21.60
6/12/12 7:00 AM	21.19
6/12/12 7:00 PM	21.40
6/13/12 7:00 AM	21.10
6/13/12 7:00 PM	21.16
6/14/12 7:00 AM	21.01
6/14/12 7:00 PM	20.94
6/15/12 7:00 AM	20.45
6/15/12 7:00 PM	20.37
6/16/12 7:00 AM	20.11
6/16/12 7:00 PM	20.19
6/17/12 7:00 AM	20.09
6/17/12 7:00 PM	20.21
6/18/12 7:00 AM	20.31
6/18/12 7:00 PM	20.55
6/19/12 7:00 AM	20.50
6/19/12 7:00 PM	20.70
6/20/12 7:00 AM	21.12
6/20/12 7:00 PM	21.39
6/21/12 7:00 AM	21.33
6/21/12 7:00 PM	21.51

Date	Hourly Average Plant Influent Water Temperature (^{0}C)
6/22/12 7:00 AM	21.37
6/22/12 7:00 PM	21.59
6/23/12 7:00 AM	21.61
6/23/12 7:00 PM	21.93
6/24/12 7:00 AM	21.91
6/24/12 7:00 PM	22.14
6/25/12 7:00 AM	21.97
6/25/12 7:00 PM	22.46
6/26/12 7:00 AM	22.31
6/26/12 7:00 PM	22.60
6/27/12 7:00 AM	22.45
6/27/12 7:00 PM	22.55
6/28/12 7:00 AM	22.59
6/28/12 7:00 PM	22.70
6/29/12 7:00 AM	22.77
6/29/12 7:00 PM	23.16
6/30/12 7:00 AM	23.13
6/30/12 7:00 PM	23.48
7/1/12 7:00 AM	23.53
7/1/12 7:00 PM	23.95
7/2/12 7:00 AM	24.16
7/2/12 7:00 PM	24.26
7/3/12 7:00 AM	24.31
7/3/12 7:00 PM	24.74
7/4/12 7:00 AM	24.62
7/4/12 7:00 PM	24.46
7/5/12 7:00 AM	23.84
7/5/12 7:00 PM	24.20
7/6/12 7:00 AM	23.12
7/6/12 7:00 PM	23.40
7/7/12 7:00 AM	23.49
7/7/12 7:00 PM	23.74
7/8/12 7:00 AM	23.66
7/8/12 7:00 PM	23.53
7/9/12 7:00 AM	23.26

Appendix F: Geosmin Analysis

Geosmin was analyzed via Headspace Solid Phase Micro Extraction . A calibration curve was made with geosmin standards in methanol (Sigma-Aldrich, St. Louis, MO) in DI water. Standards were run ever five samples. If the standards were with 15% of the full calibration curve the curve was used, new calibration curves were generated every time fibers were replaced or other significant parameters on the GC-MS were changed. An example calibration curve is shown, Figure 6-7.

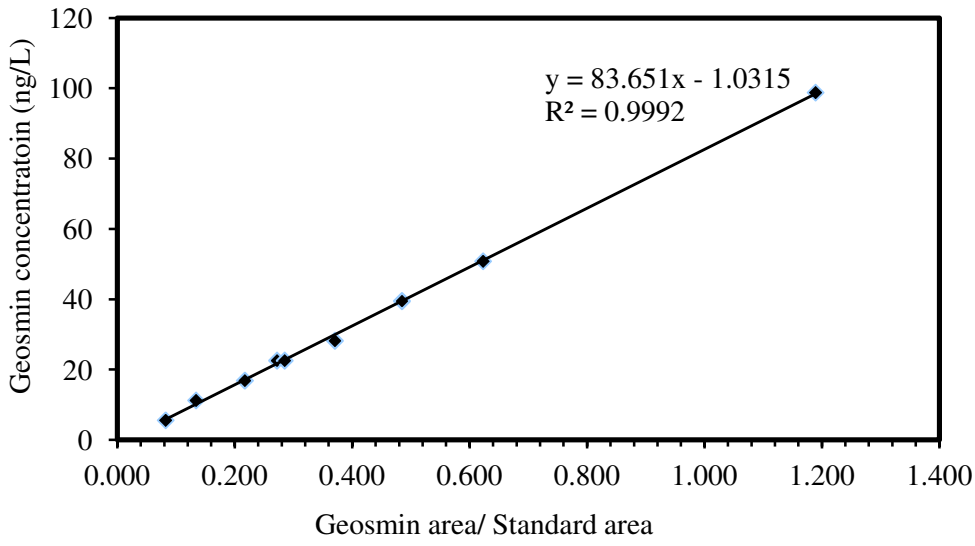
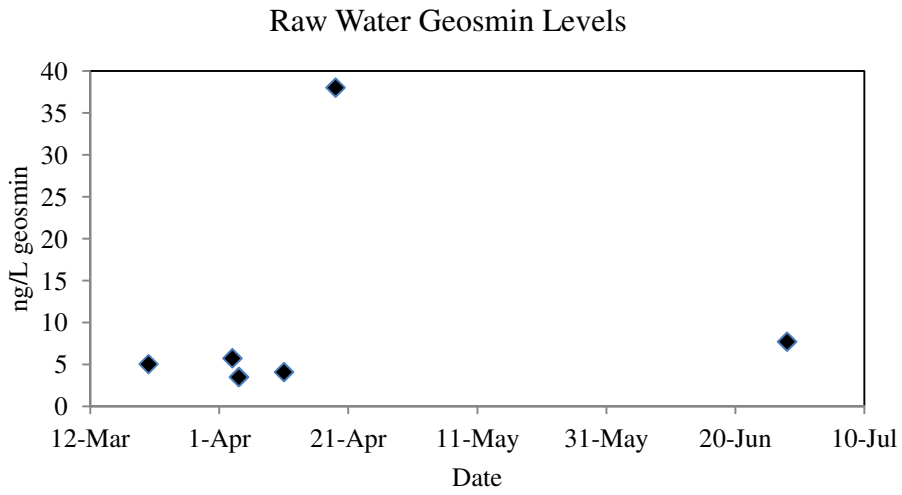


Figure 6-7: Example calibration curve for geosmin detection by head space solid phase micro-extraction.

Appendix G: Raw Water Geosmin Levels



Appendix H: ATP Data

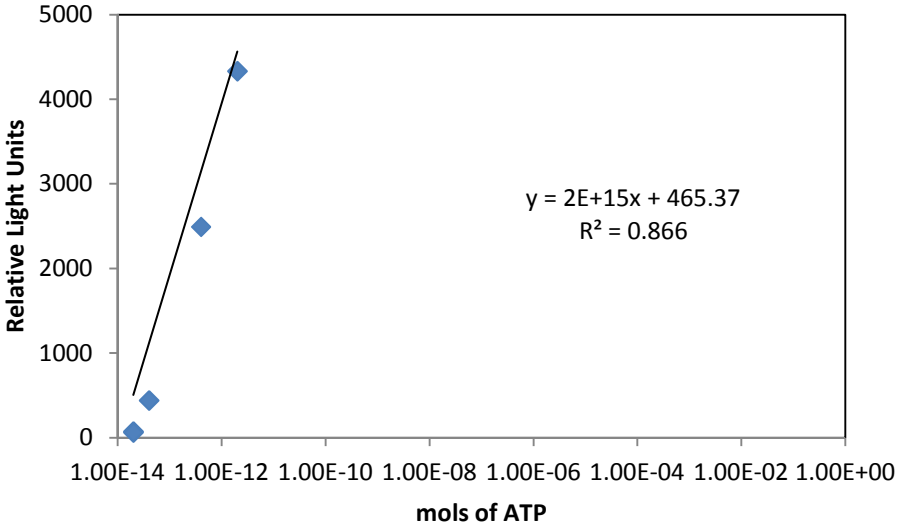


Figure 6-8: ATP Calibration Curve

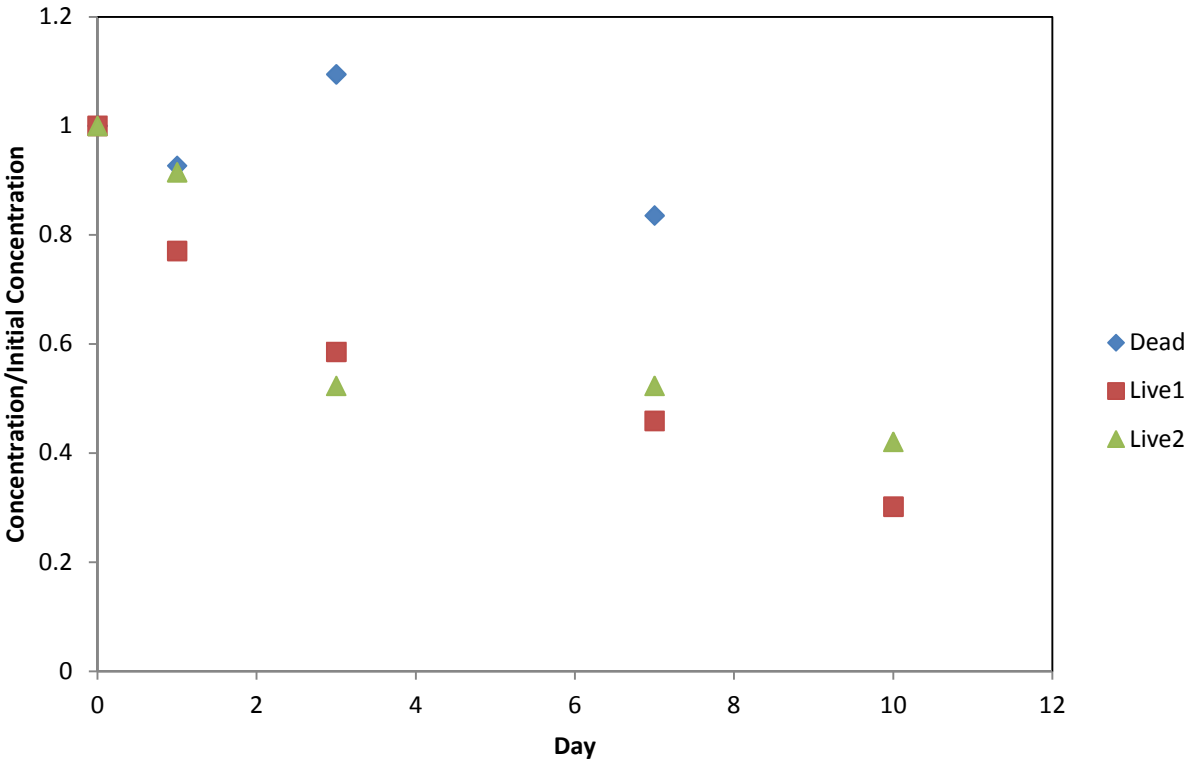
	ATP level; ng ATP/g GAC (dry weight)														
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**Filter 6**
10	102.36	41.82	5.61	10.76	29.64	5.56	16.01	19.18	35.67	5.32	11.93	3.57	5.28	3.85	14.30
38	40.14	27.89	36.46	17.08	43.08			131.41	26.73	31.18	2.68	18.04			-0.40
55	74.28	87.82	13.33	29.18	31.11	14.12	124.31	81.55	34.80	73.90	3.63	-0.18	-0.27	-0.21	18.78
67	92.35	123.48	29.24	24.53	58.32	36.09	115.74	84.37	62.69	54.64	5.64	5.30	0.18	1.75	208.88
73	206.74	140.30	17.93	29.87	71.15	44.09	58.94	92.62	67.99	58.43	11.65	35.65		34.34	68.64
82	106.18	79.58	40.37	18.51	66.00	63.60	109.00	107.48	41.98	51.58	18.47	14.33	9.01	0.51	44.90
89	99.66	48.01	32.66	29.42	64.85	37.53	72.68	72.40	96.13	37.12	11.42	1.35	1.72	0.84	226.21
92	167.91	101.69	66.89	40.54	57.38	54.70	98.36	64.83	104.68	73.07	17.61	1.34	0.50	0.54	40.75
98	80.57	85.59	77.85	88.85	102.74	75.50	124.90	184.36	37.88	40.51	94.15	13.15	3.19	41.27	52.54
122	301.18		62.51	71.30	92.38	253.68	427.04		82.85	88.04	109.46	59.62	75.32	41.57	165.98

Column Number	Description
1	Aged Calgon GAC
2	Aged Calgon GAC
3	Virgin Calgon GAC
4	Inoculated Virgin Calgon GAC
5	Inoculated Virgin Calgon GAC
6	Virgin Calgon GAC
7	Inoculated Virgin Nuchar GAC
8	Inoculated Virgin Nuchar GAC
9	Virgin Nuchar GAC
10	Virgin Nuchar GAC
11	Anthracite
12	Inoculated Anthracite
13	Anthracite
14	Inoculated Anthracite

Appendix I: Test for Biological Activity

Batch Geosmin Biodegradation Experiment

A 10 mg (wet weight) sample of anthracite was placed into each of three organic carbon-free sterile 1 L brown glass bottles. The bottles were then filled 1 L of filter influent that was spiked with 2 µg/L of geosmin. Sodium azide was added to one of the bottles to create a killed controls. Each bottle had a stir bar and was placed on a stir plate at level 7 to ensure good mixing. pH, dissolved oxygen, temperature, and geosmin concentrations in each bottle were measured three times a week.



Appendix J: Tulsa

The Tulsa water utility experiences seasonal geosmin episodes that they treat with GAC filters. At the same time as this study the Tulsa water utility conducted a similar column experiment. Samples were obtained for the Tulsa pilot scale GAC filter. The protein content and ARISA between the Tulsa samples and the full-scale Saint Paul Regional Water Services GAC filters were compared.

The samples from the three pilots columns in Tulsa were also used to compare reasonable protein contents (Figure 6-9). The mean of the Tulsa data was 494 mg/g of GAC compared to a December SPRWS mean of 326 mg/g of GAC. The average temperature in Tulsa in December is 39 degrees Fahrenheit compared to an average temperature of 27 degrees Fahrenheit in Saint Paul.

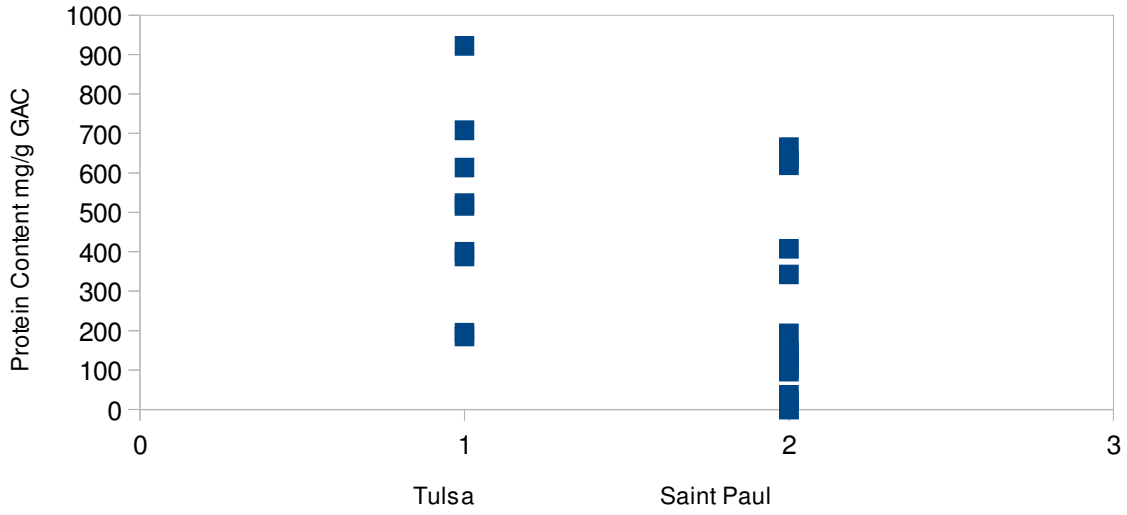


Figure 6-9: Protein content on GAC from Tulsa water utility and Saint Paul Regional Water Services in December 2011.

The differences between the samples from the Tulsa columns and the SPRWS full-scale filters are larger than any variability internally in each sample set (Figure 6-10). Closer analysis

shows that the two groups share no keystone populations. There are 8 species that are present it every one of the Saint Paul samples and not present in any of the Tulsa samples. There are also 4 species present in all of the Tulsa samples that aren't present in any of the Saint Paul samples. There are no species that occur in all of the samples. This would appear to indicate that biologically active filters are significantly influenced by the influent water population and that even at steady state there are no species universal to all biologically active Activated Carbon filters.

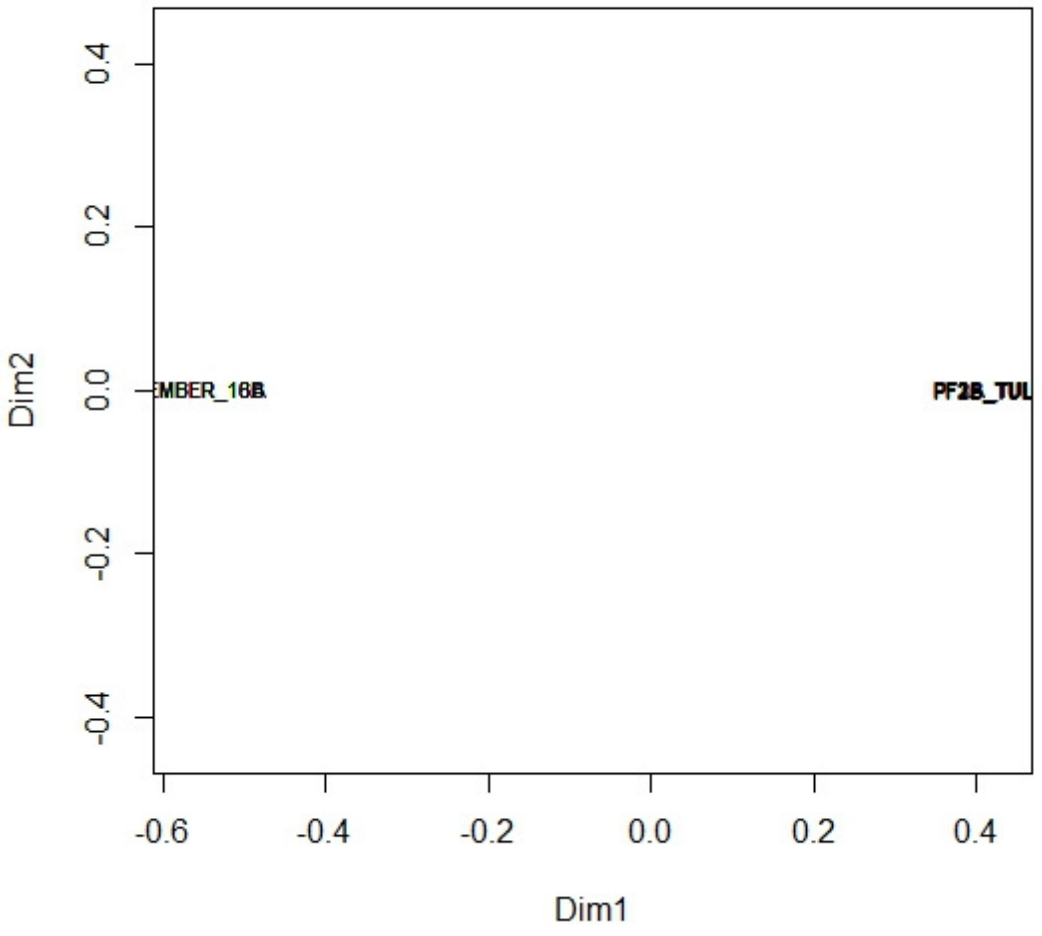


Figure 6-10: nMDS of ARISA on GAC samples from Tulsa water utility and Saint Paul Regional Water Services, December 2011.