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Trace Organic Contaminant Removal in Drinking Water Biofilters Under Carbonaceous and Nitrogen-Supplemented Conditions and Evaluating Biomass with ATP and Phospholipid Methods

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**Trace Organic Contaminant Removal in Drinking Water Biofilters under
Carbonaceous and Nitrogen-Supplemented Conditions and Evaluating Biomass
with ATP and Phospholipid Methods**

By: Katherine Dowdell

Concurrent B.S. M.S, University of Colorado 2012

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirements for the degree of
Masters of Science
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2012

This thesis entitled:

Trace Organic Contaminant Removal in Drinking Water Biofilters under Carbonaceous and Nitrogen-Supplemented Conditions and Evaluating Biomass with ATP and Phospholipid Methods

written by Katherine Dowdell

has been approved for the
Department of Civil, Environmental, and Architectural Engineering

R. Scott Summers

Karl Linden

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Date December 7, 2012

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

ABSTRACT

Dowdell, Katherine (M.S., Civil Engineering)

Trace Organic Contaminant Removal in Drinking Water Biofilters under Carbonaceous and Nitrogen-Supplemented Conditions and Evaluating Biomass with ATP and Phospholipid Method

Thesis directed by R. Scott Summers, Professor, Department of Civil, Environmental and Architectural Engineering, University of Colorado at Boulder

Biofiltration is a growing field within drinking water treatment. Biological filters, or biofilters, are capable of removing organic matter, including a variety of trace organic contaminants. Using biomass attached to media from a source water that was wastewater-impacted and media from an unimpacted source, bench-scale filters were run under carbonaceous and nitrogen-supplemented conditions to determine the impact on the removal of trace organics. The majority of contaminants evaluated in the study were best removed by the wastewater-impacted biomass filter media that was operated under carbonaceous conditions. Unimpacted biomass media showed comparable removals to the impacted biomass media under nitrogen-supplemented conditions. Filter media with higher biomass levels was also generally more effective at removing trace organics. Results of this study can be used in conjunction with Zearley and Summers (2012) and future work to enable engineers and operators to better predict how biofilter removal of trace organic compounds will change over time.

An important factor in the operation of biofilters is the biomass, which can often be linked to the filter's ability to remove organic compounds. This second study was designed to develop a relationship between an ATP based method and a phospholipids based method, two common biomass analysis techniques. The two biomass analysis methods were linearly related with a correlation coefficient of 0.91 when applied to media from similar source waters. Media samples taken from filters

exposed to pre-oxidized water and media from new filters exhibited lower ATP per nmol of phospholipids ratios. Using the ATP and phospholipids cell count approximation methods yields cell ATP cell counts that are only one-quarter of the count predicted by the phospholipids approximation method. Both ATP and phospholipids relationships with depth are best fit using logarithmic regressions. However, the slopes of the depth versus biomass curves may depend on media type or filter influent. The holding time study results showed that if samples cannot be immediately tested for ATP, the cell activity remains closer to initial levels when media is held unextracted.

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I would like to thank the staff at the following utilities for their assistance and for all generously providing media samples for these studies; in particular the Greater Cincinnati Water Works – Richard Miller Water Treatment Plant (OH) and the City of Aurora Peter Binney Treatment Plant (CO), as well as the North Bay Regional Water Treatment Plant (CA), the City of Longmont Water Treatment Plant (CO), and the City of Tulsa Drinking A.B. Jewell Water Treatment Plant (OK). I would especially like to thank Pam Benskin, City of Aurora, for her assistance in training me on the luminescence ATP method, taking numerous samples for me, and allowing me to use her lab. I would also like to thank Dr. Mike Thurman and Dr. Imma Ferrer of the Center for Environmental Mass Spectrometry in Boulder, Colorado for analyzing my trace organic contaminant samples. I would also like to thank my committee for their guidance. I would like to thank Dr. Karl Linden and the members of the CCL3 Compound Toxicity Change during UV/AOP project for providing funding for my biofiltration work.

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Chapter 1: Introduction

1.1 Motivation

Drinking water filters with biomass attached to the filter media are often referred to as biofilters. These filters are widely used for the removal of particulates and dissolved organic matter (DOM) during drinking water treatment (Zhang & Huck, 1996; Chaudhary et al., 2003). The ability of biofilters to remove DOM has been widely studied (Amy & Minear, 1995). However, the ability of biofilters to remove trace organic contaminants, and the optimal operation conditions for this removal to occur, are not well understood (Snyder et al., 2003). Compounds occurring in the influent at low micrograms per liter concentrations or less are often referred to as micropollutants or trace organic contaminants (Virikutyte et al., 2010).

Few tools exist to assess potential of a biofilter to removal organic contaminants. One proposed measure is the biomass accumulated on the biofilters. One main issue is the extraction of the biomass or biomass indicators from media surface. Thus, a need exists to assess different measures of filter biomass. Two potential measures are ATP and phospholipids. ATP, or adenosine triphosphate, is a molecule used for energy transport in cells. Phospholipids are molecules comprised of long lipid tails and phosphate heads and are contained in the cell membrane.

Detailed background and literature reviews for the trace organic contaminant and biomass studies are given in Chapters 2 and 3, respectively. The goal of this work was to develop a better understanding of biofilters, through the ability of these filters to degrade trace organic contaminants under a range of conditions, and furthering understanding of biomass measurement techniques.

1.2 Research Objectives

The first objective was to evaluate trace organic contaminant removal with biomass that had been exposed to a wastewater-impacted source water and compare the performance to that with biomass that had been exposed to a source water with little anthropogenic influence. In addition, the influence under both nitrogen-supplemented and carbonaceous conditions was evaluated. The second objective was to evaluate a new attached biomass measurement approach based on ATP. Relationships between the ATP and phospholipids biomass measurements were developed and the impact of sample holding time on these measures evaluated.

1.3 Approach

For the trace organic contaminant removal study, four biofilters were run in parallel each under different conditions. Two of the columns had been exposed to wastewater-impacted source waters and two non-wastewater impacted. Ammonia was added to the influent of two filters with different exposures to create nitrogen -supplemented conditions. For the biomass study, samples were taken from a wide variety of drinking water biological filters throughout the US. These samples were shipped to the Summers lab at the University of Colorado and analyzed for ATP and phospholipids.

1.4 Thesis Organization

Chapter 2 evaluates biofilter conditions for the removal of 29 micropollutants by comparing filter media at differing levels of acclimation under nitrogen supplemented and carbonaceous influent conditions. Chapter 3 develops evaluates the relationship between the ATP and phospholipids methods of quantifying biomass. Chapter 4 summarizes results and conclusions of these studies.

Chapter 2: Trace Organic Contaminant Removal in Drinking Water

Biological Filters: The Impact of Acclimation Conditions and Substrate Utilization

2.1 Introduction

The use of biologically active filters for the removal of unwanted trace organic contaminants in drinking water has become increasingly prevalent over the past few decades (Juhna & Melin, 2006). Biofilters are similar to traditional drinking water rapid-filters in operation and filter media, which usually consists of sand, anthracite, or GAC. However, the filter influent water is not chlorinated, which allows for colonization by naturally occurring microorganisms. Biofilters remove a fraction of the easily assimilable organic carbon (AOC) and have the ability to remove other organic compounds (Chien et al., 2008). However, little is known as to the performance of biological filters under various metabolic conditions and varied wastewater exposures. Removal of organic compounds occurring in concentrations of micrograms per liter or less, termed trace organic contaminants, is of particular interest as they are of growing concern. Their detection in source water and treated drinking water increases and their health impacts are still uncertain. These trace organic compounds come from a wide variety of sources, including runoff, domestic and industrial wastewater discharge, and algal blooms (Focazio et al., 2008).

In this experiment, the removal of micropollutants in wastewater-impacted biomass and unimpacted biomass taken from full-scale filters was studied using bench-scale filters. Research has shown that microorganisms previously exposed to certain trace organics are more able to remove these compounds when they are reintroduced (Alexander, 1999). Depending on previous exposure, filter microorganisms may take six months or longer to adapt to metabolizing MIB and geosmin (Meyer,

2005). Zearley and Summers (2012) found that trace organic contaminant removal may take as long as five months to reach steady state.

The other filter operating condition analyzed in this experiment was the type of metabolic condition. Trace organic compound removal under carbonaceous metabolic and nitrogen supplemented conditions was analyzed. Carbonaceous conditions in this context are when microorganisms use available organic carbon for energy and cell growth. Under these conditions, cells obtain energy by converting organic carbon to inorganic carbon via cell respiration. Carbon is the electron donor and oxygen is the electron acceptor. Under nitrogen-supplemented conditions, ammonia may be converted to nitrite, then to nitrate, for cell energy or utilized for cell growth. During nitrification, ammonia is the electron donor, and oxygen and water are electron acceptors (Henze, 2008). In the carbonaceous biomass filters, the lack of ammonia in the influent prevents the growth of nitrifying bacteria and may inhibit cell growth if nitrogen is a limiting nutrient.

Another metabolic factor in the removal of organic compounds in biofilters is the type of substrate utilization. Cells produce enzymes that are designed to catalyze reactions that allow cells to obtain energy from the substrate. Primary substrate utilization is when enzymes produced by the cell are used to degrade a non-limiting substrate (Odenrantz, 1990). In carbonaceous microorganisms, this is generally an organic carbon source. In this study, therefore, the primary substrate was the natural organic carbon fed to the filters. Secondary substrate metabolism occurs when cells utilize substrates that occur at limiting concentrations. This form of metabolism may or may not yield energy for the cell (Rittmann et al., 1995). The third form of substrate utilization is cometabolism. This occurs when enzymes produced by the cell to degrade the primary substrate encounter another compound that can also be degraded. This process is not energetically favorable for the cells (Nava et al., 2007). The trace organic compounds used in the study could be degraded via these secondary metabolic processes.

Zearley and Summers (2012) studied the removal of 34 trace organic compounds over a period of one year with an impacted biomass filter under carbonaceous conditions. The study found that removals followed four general trends: increasing removal reaching steady-state with time, steady-state removal, decreasing removal, or no removal. The authors were able to model the removal of the non-recalcitrant compounds once they reached steady-state using first order kinetics. The kinetics were modeled using pseudo-first order rate constants and were dependent on EBCT and biomass concentration (Zearley, 2012). Zearley and Summers (2012) found that 19 compounds of the 34 could be degraded in impacted biomass carbonaceous biofilters.

Traditional drinking water filters that receive low concentrations of ammonia are considered carbonaceous. There are few published studies on the removal of trace organic compounds in drinking water filters under nitrifying conditions. However, previous research on trihalomethanes (THMs) has found that these compounds are removed under nitrifying biofilter conditions in drinking water (Wahman et al., 2006). Further research in the realm of trace contaminant removal has been conducted with regard to nitrifying wastewater biofilters. Radjenovic et al. (2008) evaluated the removal of 31 compounds in traditional activated sludge and membrane bioreactor systems. Ibuprofen, naproxen, acetaminophen, sulfamethoxazole, ofloxacin, and bezafibrate were all removed at 70 percent or above under both conditions. In another study evaluating the removal of trace contaminants in membrane bioreactors, Reif et al. (2007) found that naproxen and ibuprofen were highly removed, while carbamazepine and diclofenac were recalcitrant. Iopromide and trimethoprim have also been shown to be removed to above 70 percent in traditional wastewater activated sludge systems (Batt, Kim, & Aga, 2006). Several studies have linked the removal of trace organics in nitrifying wastewater conditions to higher sludge ages, which they speculate may be due to the age of the microorganisms, increased diversity of the populations, higher concentrations of nitrifying bacteria, or sludge sorption (Ifelebuegu, 2011; Ternes et al., 2004; Ternes, 2006; Batt et al., 2006).

The objective of this study was to characterize the initial removal of 29 trace organic contaminants under varying biomass conditions. Filters containing wastewater-impacted biomass were compared to filters containing non-wastewater impacted biomass under carbonaceous and nitrogen-supplemented conditions, with the goal of determining the impact of these conditions on the initial exposure removal of micropollutants.

2.2 Materials and Methods

2.2.1 Biofilter Design and Operation

Sand and anthracite media from two full-scale biologically active filters with very different source waters were used for this experiment. The wastewater-impacted biomass on sand media was from the Richard Miller Plant at Greater Cincinnati Water Works (GCWW) in Cincinnati, Ohio, which treats Ohio River water. The Ohio River at this location has been impacted by municipal and industrial treated wastewater discharges, as well as urban, industrial and agricultural runoff. Prior to filtration, the water is treated by alum coagulation and sedimentation and the filter is backwashed with unchlorinated water. Once the media was received in the laboratory it was recirculated in a PVC upflow reactor for two months prior to use. The PVC reactor was constructed entirely of three inch Schedule 40 PVC and consisted of a PVC tube and threaded caps on both ends. Stainless steel connectors were tapped into the caps to attach plastic tubing and stainless steel mesh was placed at the bottom of the filter to prevent media loss. The reactor was run upflow at a flow rate of 2 mL/ minute. Three liters of filter influent water used to maintain the media was held in an amber glass bottle and changed weekly. The filter influent water used to maintain the media was shipped from GCWW in a 200 gallon plastic barrel. The barrel was kept refrigerated at 4 °C once received. The biomass was considered wastewater-impacted due to its previous exposure to water from the Ohio River.

The anthracite media taken from the City of Longmont (CO) Water Treatment Plant anthracite filter will be referred to as unimpacted biomass media. The influent to this plant is a mountain source that is not impacted by wastewater, so the filter biomass had received little prior exposure to most trace organic contaminants. Prior to filtration the water is treated by alum and cationic polymer coagulation and sedimentation. The filter is backwashed using chlorinated water.

This experiment used six glass columns. A diagram of the filter column set up appears in Figure 1. The impacted biomass sand filters were packed in 11 millimeter diameter glass chromatography columns and sealed with Teflon caps (Ace Glass, Vineland, NJ). The sand had a uniformity coefficient of approximately 1.3 and an effective size of 0.45 mm. Swagelok stainless steel metal fittings (Cleveland, OH) were used for all tubing connections. Due to the larger particle size (0.9 to 1.1 mm) of the anthracite media, it was packed in 25 millimeter diameter columns to minimize short-circuiting. The sand media columns were packed to a height 31.6 cm and the anthracite columns were packed to a height of 30.6 cm. These packing heights targeted an empty bed contact time (EBCT) of 7.5 minutes and a loading rate of 2.5 m/hr (1 gpm/ft²). The sand media (impacted biomass) was run at a flow rate of 4 mL per minute. The anthracite media filter (unimpacted biomass) was run at a flow rate of 20 mL per minute.

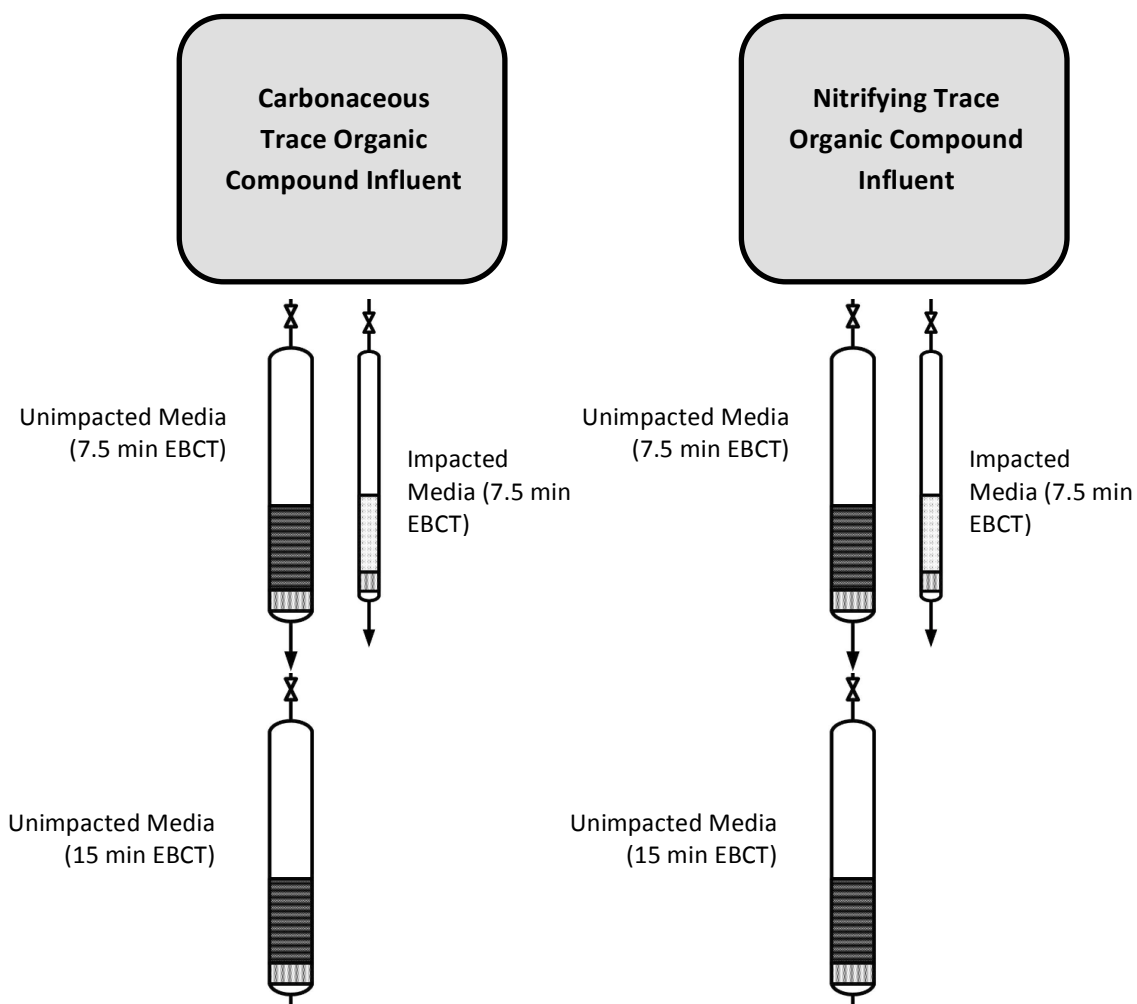


Figure 1 Diagram of impacted biomass and unimpacted biomass filters

Due to the low biomass concentrations of the unimpacted biomass media (17 nmol PO_4 / g dry media), little trace organic contaminant removal was anticipated. To compensate for this, two columns were packed in series for both the carbonaceous and nitrogen-supplemented unimpacted biomass media filters. This allowed for sampling of the unimpacted biomass media columns at EBCTs of 7.5 minutes and 15 minutes.

The bottom of each column was packed with two inches of 2 millimeter diameter glass beads encased in wire mesh to prevent media loss and clogging. The columns were run using Masterflex

pumps (Models 7553-30 and 7518-10) Masterflex tubing, and Teflon coated tubing. The columns were covered to minimize the growth of photosynthesizing microorganisms and minimize photo degradation of the trace organics.

The influents solutions were mixed in 225 liter blue polyethylene barrels. The barrels were kept covered to decrease light exposure and prevent contamination. Trace organic contaminants were dosed into City of Boulder tap water that was dechlorinated by dosing the water with an organic carbon solution 24 hours prior to use. The organic carbon solution was produced by concentrating water taken from a mountain lake in Big Elk Meadows, Colorado (TOC concentration of 17 mg/L) using a reverse osmosis membrane. The column influents were dosed to a target TOC concentration of 2 mg/L. The trace organic contaminants mix fed to each column was comprised of 29 pesticides, pharmaceuticals, and personal care products. Table 1 shows the target influent concentrations for each compound.

Table 1 List of 29 trace organic compounds used in the study

Compound	Target Influent Concentration (ng/L)
2,4-dichlorophenoxyacetic acid (2,4-D)	100
Acetaminophen	200
Acetochlor	200
Aldicarb	200
Atrazine	10
Caffeine	100
Carbamazepine	100
Carbaryl	200
Clofibric acid	200
Cotinine	100
Diazinon	10
Diclofenac	200
Dimethoate	100
Diuron	100
Erythromycin	100
Gemfibrozil	200
Ibuprofen	500
Iopromide	500
Malaoxon	200
Methomyl	200
Metolachlor	200
Molinate	200
Naproxen	200
Prometon	100
Simazine	50
Sulfamethoxazole	200
Tributyl phosphate	100
Trimethoprim	100
Warfarin	100

2.2.2 Analytical Methods

Trace organic contaminant testing was conducted at the University of Colorado Center for Environmental Mass Spectrometry in Boulder, Colorado. Biomass analysis was conducted using a phospholipids chemical extraction method first developed by Findlay et al. (1989) and adapted by Wang et al. (1995). Chapter 3 contains a detailed explanation of this method.

TOCs were run using a Shimadzu TOC-V_{CSH} analyzer using the non-purgable organic carbon (NPOC) method. Dissolved oxygen and temperature were monitored using a Thermo Orion 3 Star DO probe. pH was tested using a Denver Instruments Model 220 pH and conductivity meter. Alkalinity was tested using a HACH sulfuric acid digital titration kit. Ammonia, nitrate, and nitrite were run using HACH test kits TNT 830, TNT 835, and TNT 839. Vials were analyzed on a HACH DR 500 UV/Vis spectrophotometer.

2.3 Results and Discussion

The four filter experiment was run for 14 days. The first samples were taken after 18 hours of operation. The second sampling event occurred after seven days. The third sampling event occurred after 13 days. The biomass was sampled at the beginning and end of the experiment.

Biomass results showed that the impacted biomass media had an average biomass concentration of 50 nmol PO₄/ dry gram of media (85 nmol PO₄/ mL dry media), while the unimpacted biomass media averaged only 17 nmol PO₄/ dry gram of media (13 nmol PO₄/ mL dry media). There was little change in biomass in any of the filters during the course of the experiment. Due to the low biomass in the unimpacted media filters, it was anticipated that removals in an EBCT of 7.5 minutes would be relatively limited. Therefore, in an attempt to quantify some level of compound removal, only the effluent samples at an EBCT of 15 minutes of this column were analyzed. Also, due to the low biomass levels, the unimpacted media filters were only sampled during the first and third sampling events.

Though TOC analysis was run on influent and effluent samples, results are not included due to instrument malfunction. Figure 2 shows the influent ammonia concentration and the two effluent ammonia concentrations for the nitrogen-supplemented filters over the course of the experiment. The ammonia removal in the nitrogen-supplemented filters was fairly limited, with the unimpacted media exhibiting negligible removals and the impacted media exhibiting an average removal of 25-30 percent. The low ammonia removal resulted in nitrate and nitrite levels in the effluents that were below the

0.015 mg/L detection limit. Though previous lab experience indicated that filters would exhibit nitrification at similar ammonia concentrations, the dose in this experiment was deemed too low to significantly stimulate the growth of nitrifying bacteria. The pH in all columns ranged from 7.5 to 7.9 throughout the course of the study. The dissolved oxygen concentration ranged from 6.7 to 7.8 mg O₂ per liter. The temperature of the filters ranged from 15.5 to 19.3 °C. The alkalinity in the influent and effluents ranged from 46 to 55 mg CaCO₃ per liter.

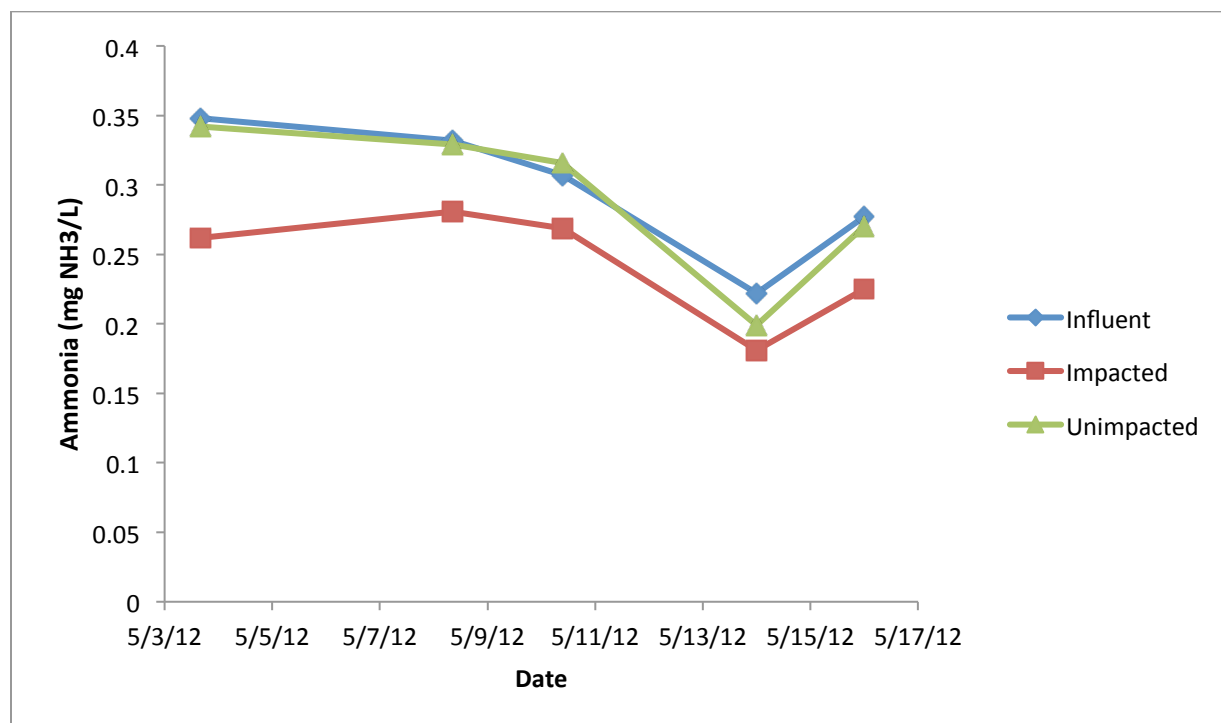


Figure 2 Plot of ammonia concentration versus time for influent, impacted media, and unimpacted media nitrogen-supplemented filters

Column performance was compared by type of acclimation (impacted and unimpacted biomass) and substrate condition (carbonaceous and nitrogen-supplemented), in order to determine what conditions would yield the highest removals for each compound. The minimum statistically significant removal, based on detection limits, was set to 15 percent (Ferrer et al., 2010; Zearley and Summers, 2012). Table 2 shows the average removals of the trace organic compounds during the study. Due to the detection limit of 15 percent, the percent removals that averaged below this level were set to 7.5

percent, half of the detection limit, to convey the uncertainty in measuring removals below this level.

The numbers in bold in all following tables indicate trace organic removal percentages that were above the detection limit.

Table 2 Average trace organic compound percent removal

Compound	Impacted		Unimpacted	
	C	N	C	N
2,4-D	7.5 ^a	7.5	7.5	21
Acetaminophen	27	38	7.5	21
Acetochlor	7.5	7.5	7.5	7.5
Aldicarb	7.5	7.5	16	7.5
Atrazine	7.5	7.5	7.5	7.5
Caffeine	37	38	38	42
Carbamazepine	7.5	7.5	7.5	7.5
Carbaryl	7.5	7.5	7.5	7.5
Clofibric Acid	7.5	7.5	7.5	7.5
Cotinine	7.5	7.5	7.5	7.5
Diazinon	7.5	7.5	7.5	7.5
Diclofenac	7.5	7.5	7.5	7.5
Dimethoate	7.5	7.5	7.5	7.5
Diuron	7.5	7.5	7.5	7.5
Erythromycin	7.5	7.5	7.5	7.5
Gemfibrozil	26	23	7.5	7.5
Ibuprofen	38	38	7.5	15
Iopromide	7.5	7.5	7.5	7.5
Malaoxon	7.5	7.5	7.5	7.5
Methomyl	7.5	7.5	16	7.5
Metolachlor	7.5	7.5	7.5	7.5
Molinate	30	27	7.5	7.5
Naproxen	7.5	7.5	7.5	7.5
Prometon	7.5	7.5	7.5	7.5
Simazine	7.5	7.5	7.5	7.5
Sulfamethoxazole	7.5	7.5	7.5	7.5
Tributyl phosphate	18	7.5	7.5	7.5
Trimethoprim	24	19	7.5	7.5
Warfarin	15	7.5	7.5	7.5

^aDue to sensitivity limitations, removals less than 15 percent were changed to 7.5 percent, or half of the detection limit.

Trace organic removal was compared to the removal of the 29 compounds found in the Zearley and Summers (2012) study. Due to relatively low removals observed over the two week study, the compounds were separated into three removal levels: less than 15 percent (recalcitrant), 15 to 30 percent, and above 30 percent. Table 3 shows the results of this comparison.

Table 3 Average removals by media and removal range

Average Removal Range	Trace Organic Removal (# compounds/category)				Zearley and Summers (2012) Steady State Removals
	Impacted Biomass C	Impacted Biomass N	Unimpacted Biomass C	Unimpacted Biomass N	
0-14%	21	23	26	25	14
15-30 %	6	3	2	3	2
Above 30%	2	3	1	1	13

As shown in Table 3, the majority of the 29 compounds were not removed in any of the filters. The carbonaceous impacted biomass media removed the most compounds above the detection limit, eight. Though the nitrogen-supplemented impacted biomass media removed two fewer compounds than the carbonaceous, it removed more at levels above 30 percent. The unimpacted biomass media filters showed very similar behavior despite the differing influent feeds. The nitrogen-supplemented impacted biomass media filter removed one more compound in the 15 to 30 percent range than the carbonaceous filter. Comparing the removal of the carbonaceous impacted media filter to Zearley and Summers (2012) shows that more compounds were removed at higher levels in the yearlong study. This is most likely due to acclimation behavior in the filter, which leads to increased removal over time. The short, two week duration of this study was most likely not sufficient for acclimation behavior to be found in the filters. Therefore, it is likely that if this two-week study had been run long enough for removals to reach steady state, the removals would better match those found by Zearley and Summers (2012).

2.3.1 Nitrogen-Supplemented v. Carbonaceous

Impacted Biomass

Though the impacted biomass media columns removed most of the compounds to similar levels, two compounds were removed to a higher extent in the carbonaceous filter than the nitrogen-supplemented filter. Tributyl phosphate and warfarin were removed above the 15 percent detection limit threshold in the carbonaceous filter, while removal remained below detection in the nitrogen-supplemented filter. Of the 29 compounds, 21 were found to be recalcitrant under both conditions.

Unimpacted Biomass

The nitrogen-supplemented and carbonaceous unimpacted biomass media columns exhibited less capacity to remove most compounds than the impacted biomass media filters. This can most likely be attributed to the low biomass and lack of previous exposure in the media. In the carbonaceous filter, aldicarb, caffeine, and methomyl were removed above the detection limit. 2,4-D, acetaminophen, caffeine, and ibuprofen were removed to reportable levels in the nitrogen-supplemented filter. Unlike the impacted biomass media filters, the nitrogen-supplemented filter was found to remove more compounds than the carbonaceous filter. Of the 29 compounds, 25 were found to be recalcitrant under both conditions.

2.3.2 Impacted v. Unimpacted Biomass

The performance of the 7.5 minute EBCT impacted biomass media filter was compared to the performance of the 15 minute EBCT unimpacted biomass media filter due to much lower biomass in the NOM filter.

Carbonaceous

Eight compounds were removed in the carbonaceous impacted biomass media column, while only three were removed above detection limits in the unimpacted biomass media

columns. Both columns removed caffeine between 35 and 40 percent. This was the only compound removed significantly in both media types. The unimpacted biomass media filter showed higher removals of aldicarb and methomyl than the impacted biomass media filter. Of the 29 compounds, 19 were found to be recalcitrant under both conditions.

Nitrogen-Supplemented

Under nitrogen-supplemented conditions, the impacted and unimpacted biomass media filters showed similar removals. The impacted biomass media filter removed gemfibrozil, molinate, and trimethoprim to higher levels than the unimpacted biomass media filter. However, the unimpacted biomass media filter showed higher removals of 2,4-D. Both removed acetaminophen, caffeine, and ibuprofen. Of the 29 compounds, 21 were found to be recalcitrant in both media.

2.3.4 Comparison of Results to Previous Studies

These results were compared to those found in a study conducted by Zearley and Summers (2012). However, Zearley and Summers (2012) studied trace organic contaminant removal for one year, while this study focused only on the first two weeks of filter operation. Table 4 shows the carbonaceous impacted biomass media removals for this two week study compared to the steady-state removals of Zearley and Summers (2012).

Table 4 Carbonaceous impacted biomass media removals compared to the long term results of Zearley and Summers (2012)

Compound	Average Removal Over 2 Weeks	Average Steady State Removal	Change in Removal with Exposure	Zearley and Summers (2012) Acclimation Behavior
2,4-D	7.5	68 ± 11	Increase	Increase
Acetaminophen	27	59 ± 11	Increase	Steady State
Acetochlor	7.5	8 ± 11	Recalcitrant	Recalcitrant
Aldicarb	7.5	49 ± 8.2	Increase	Increase
Atrazine	7.5	0.2 ± 0.4	Recalcitrant	Recalcitrant
Caffeine	37	67 ± 18	Steady-State	Steady State to Decrease
Carbamazepine	7.5	0.5 ± 1.1	Decrease	Recalcitrant
Carbaryl	7.5	3.3 ± 5.2	Recalcitrant	Recalcitrant
Clofibric Acid	7.5	35 ± 5.9	Increase	Increase
Cotinine	7.5	23 ± 21	Increase	Steady State
Diazinon	7.5	12 ± 12	Decrease	Recalcitrant
Diclofenac	7.5	21 ± 2.1	Increase	Steady State
Dimethoate	7.5	75 ± 2.7	Increase	Increase
Diuron	7.5	0.3 ± 0.8	Recalcitrant	Recalcitrant
Erythromycin	7.5	15 ± 27	Recalcitrant	Decrease
Gemfibrozil	26	70 ± 7	Increase	Steady State
Ibuprofen	38	95 ± 3.3	Increase	Steady State
Iopromide	7.5	13 ± 18	Increase	Recalcitrant
Malaoxon	7.5	16 ± 9.1	Steady-State	Steady State
Methomyl	7.5	12 ± 11	Recalcitrant	Recalcitrant
Metolachlor	7.5	6.6 ± 11	Recalcitrant	Recalcitrant
Molinate	30	85 ± 6.7	Increase	Steady State
Naproxen	7.5	72 ± 2	Increase	Increase
Prometon	7.5	2.5 ± 2.2	Recalcitrant	Recalcitrant
Simazine	7.5	6.8 ± 9.4	Recalcitrant	Recalcitrant
Sulfamethoxazole	7.5	2.4 ± 4.1	Recalcitrant	Recalcitrant
Tributyl phosphate	18	16 ± 12	Steady-State	Steady State
Trimethoprim	24	83 ± 13	Increase	Steady State to Decrease
Warfarin	15	39 ± 11	Increase	Steady State

Over two weeks, many compounds already exhibit behavior expected in long term. Notable exceptions are caffeine and trimethoprim.

The removals found in the nitrogen-supplemented filters can be compared to the removals found in the wastewater literature mentioned previously. Table 5 compares the trace organic

compound removal of the impacted and unimpacted biomass media filters to those found in Radjenovic et al. (2008), Reif et al. (2007), and Batt et al. (2006).

Table 5 Experimental removals of trace organic compounds under nitrogen-supplemented conditions compared to reported literature values

Compound	Trace Organic Compound Removal (% Removed)		
	Nitrogen-Supplemented Impacted Biomass media	Nitrogen-Supplemented Unimpacted Biomass Media	Literature
Acetaminophen	38	21	>70 ^b
Carbamazepine	7.5	7.5	<9 ^c
Diclofenac	7.5	7.5	<9 ^c
Ibuprofen	38	7.5	>70 ^b 98 ^c
Iopromide	7.5	7.5	>70 ^d
Naproxen	7.5	7.5	>70 ^b 84 ^c
Sulfamethoxazole	7.5	7.5	>70 ^b
Trimethoprim	19	7.5	>70 ^d

^b Radjenovic et al. (2008)

^c Reif et al. (2007)

^d Batt et al. (2006)

As shown in Table 5, compounds that were degraded in the nitrogen-supplemented filters, such as acetaminophen, ibuprofen, and trimethoprim, were also found to be removed in other studies under nitrogen-supplemented conditions. However, the removal levels were much lower than those found in literature. This may be attributed to the short duration of the study, the low influent concentration, and the lack of influent microorganisms, all of which may impede the development of nitrifying microbial communities. Also, two of the compounds found to be recalcitrant, carbamazepine and diclofenac, were also found to not be readily removed in other studies under similar conditions (Reif et al., 2007). Several compounds, however, were found to be highly removed in literature but were not degraded in this study. These compounds include iopromide, naproxen, and sulfamethoxazole (Batt et al., 2006; Radjenovic et al., 2008; Reif et al., 2007). This may be due to the low influent ammonia concentration used in the study, which likely prevented the development of robust nitrifying bacterial populations. It

may also be attributed to the need of the microbial populations to acclimate to degrading these compounds. Further, the lack of new microorganisms in the influent may have hindered removal if there were few nitrifying bacteria present in the media initially. Also, a significant factor in studies that use activated sludge is the sorption of compounds to biomass in the sludge. It is likely at least some of the removal in these studies may be attributed to losses due to sorption to biomass.

The results of this study show that biofilter removal of trace organics is highly dependent on the specific organic compound and filter operating conditions. In general, the impacted biomass media was better able to remove trace organic compounds. However, certain compounds were better degraded under nitrogen-supplemented conditions.

2.4 Conclusions

The importance of trace organic compound removal is growing as their occurrence increases and analytical methods improve. However, the ability of biological filters to remove these compounds is highly dependent on the specific compound, the biomass present in the filter, filter operating conditions, and influent composition. Media with higher biomass and greater exposure to trace organic compounds is generally more able to degrade these compounds. Of the trace organic compounds studied, the majority of those removed to a significant extent were removed more effectively by a filter operating under carbonaceous conditions. Notable exceptions to this trend were aldicarb, caffeine, and methomyl, which were removed as well or better in the lower biomass unimpacted biomass media filters. This study can be used in conjunction with Zearley & Summers (2012) to predict how biofilter removal of these 29 compounds will change over time. This study provides an estimate of removals over the first two weeks of exposure, and Zearley & Summers (2012) provides information on steady-state removal. Though there is still the need for further research, especially for biofilters operating under

nitrogen-supplemented conditions, these studies provide a starting point for operators that desire to target the removal of trace organic compounds.

Chapter 3: Evaluating Biomass using ATP and Phospholipid Methods

3.1 Introduction

Numerous methods have been developed over the last 20 years for the quantification of biomass on biological filter media. Common methods for assessing bacterial concentrations include cell counts, plate counts, adenosine triphosphate (ATP), phospholipids, and biomass respiration potential (Velten et al., 2007). The concentrations of biomass on media are desirable for operators because higher levels of biomass are correlated to better removal of organic compounds (Wang et al., 1995). The concentrations also provide information to the operator on the health of the filter microorganisms.

Of the many techniques, two reliable and straightforward methods are phospholipid analysis (Wang et al., 1995) and ATP analysis (Magic-Knezev & van der Kooij, 2004). The phospholipid technique was developed because phospholipids are compounds common to all cells that are found only in the cell membrane. When cells die, the phospholipids hydrolyze within several hours (Findlay et al., 1989; Wang et al., 1995). These characteristics of phospholipids result in a technique that measures only living cells attached to biofilter media and can be correlated to total biomass. The phospholipids chemical extraction procedure that was used for this study was based on a method developed by Findlay et al. (1989) for use with soils and adapted for biofilter media by Wang et al. A major shortfall of the phospholipids method is that it requires a minimum of three days to analyze a sample, making it very time-intensive and slow to yield results. ATP is a molecule used within all living cells to transport energy. It occurs only in live cells and its ability to be correlated to cell counts makes it an appealing method of biomass concentration analysis (Abelho, 2005). There are two types of ATP, extracellular, which is outside of cells, and intracellular, which is found within cells and is referred to as cATP. ATP is tested through the addition of luciferase enzyme. Luciferase, which is an enzyme originally derived from fireflies but now synthetically produced, reacts with ATP to produce light. This light can then be measured using a luminometer and correlated to the microbial population in the media sample. One

common problem with traditional ATP analysis methods is that it requires physical removal of the biomass from the media via sonification, which decreases biomass results due to incomplete removal of cells from the surface and cell damage (Velten et al., 2007).

LuminUltra Technologies (Fredericton, NB, Canada) has developed what it refers to as a “second-generation ATP test” (LuminUltra Technologies Ltd., 2011). What differentiates the company’s ATP test from traditional analyses is that it does not require physical extraction of the biomass. Instead, it uses a chemical extraction step. Also, the test is designed so that it measures only cATP. A full test can be completed and yield results in less than one hour. The new LuminUltra test is appealing when compared to the phospholipid method because it is less complex and faster. However, institutions using phospholipids results will have no way to correlate past results to ATP results because there is no established method to convert between the two results. Similarly, studies using ATP have no way to relate their findings to biomass values reported using phospholipids.

Studies have found that the microbial populations in biofilters may change with depth, primarily due to the uptake of a majority of the easily biodegradable carbon near the top (Gebert et al., 2004; Moll et al., 1998). The microbes that survive farther down in the filter tend to be those that can metabolize more complex molecules (Moll et al., 1999). It was suspected that this change in substrate availability and potential change in microbial communities could impact the ATP production per microorganism.

One general problem with all biomass methods is the impact of holding time on the results, as samples cannot always be analyzed on location. Though the LuminUltra procedure for the Deposit and Surface Analysis (DSA) test states that extracted samples will keep in a refrigerator for up to 7 days, there is some anecdotal evidence that the cATP reading may vary even in short holding times (LuminUltra Technologies Ltd., 2010).

The overall goal of this study is to develop a relationship between biomass measured by the ATP and phospholipids methods. The first objective was to apply both methods to media taken from drinking water plant filters with varying influents and treatment processes. The second objective was to apply both methods to filter core samples to evaluate the biomass distribution with filter depth. The third objective was to assess the impact of holding time on the ATP analysis.

3.2 Materials and Methods

The phospholipids method used for the biofilter media samples was developed by Wang et al. (1995) after being adapted from a soil assay method developed by Findlay et al. (1989). In the method, 0.5 grams of media is extracted by adding 2 mL of ultrapure water, 5 mL of methanol, and 2.5 mL of chloroform in 20 mL glass vials sealed with Teflon coated caps. After 2 to 24 hours, 2.5 mL of chloroform and 2.5 mL of 0.0306 M sulfuric acid water was added. The capped vials were allowed to settle overnight. The chloroform was then extracted using glass Pasteur pipettes into 5 mL glass ampules. These ampules were dried under nitrogen gas at 37 degrees Celsius and 0.9 mL potassium persulfate solution was added. These were sealed using a Bunsen burner and heated for 8 hours at 100 degrees Celsius. After the heating period, vials were cooled and opened. Next, 0.2 mL of ammonium molybdate solution was added to the vials, followed by 0.9 mL of malachite green solution 10 minutes later. These were allowed to react for 30 minutes. During the 30 minute reaction period, ampule contents were pipetted using Pasteur pipettes into one cm path length plastic cuvettes. These cuvettes were read at a wavelength of 610 nm in a HACH DR 4000 Spectrophotometer. A standard curve was created using a phosphate solution to correlate absorbances to phospholipid molar concentrations. Results are reported as nmol PO₄/mL. Three blanks were run with every test. The detection limit of this method is the absorbance of blanks, which averages approximately 0.080. Anthracite, GAC, and sand samples were also autoclaved and analyzed to serve as media blanks.

The LuminUltra Deposit and Surface Analysis test was used for all ATP testing. A Kikkoman C-110 luminometer was used to read light output from samples. Each media sample was first drip-dried using a vacuum filter with a 0.45 or 1.5 micron filter. Approximately one gram of media was then weighed into a test tube. 5 mL of UltraLyse 7 was then added to the tube and briefly vortexed. The sample was allowed to extract for a minimum of 5 minutes. After the extraction period, one mL of liquid was removed and pipetted into a second test tube. 9 mL of UltraLute was then added to dilute the extracted liquid and the mixture was vortexed. 100 μ L of the dilution liquid was then added to a 12 x 55 mm test tube. Two drops of Luminase was then added, and the test tube was briefly vortexed. This test tube was then inserted into the luminometer and read. Results were given in relative light units (RLU's). The RLU's are converted to pg cATP/g using the ratio of the RLU's of the sample to the blanks and the mass of the sample. The detection limit varies slightly by the age of the luciferase, but generally lies at about 10,00 RLU's. This is determined by running a blank with the luciferase enzyme and a LuminUltra blank solution. Results are reported in units of pg cATP/mL. Results were converted from pg cATP/ g to pg cATP/mL using the dry: wet ratios of the media and the media densities. As with phospholipids, anthracite, GAC, and sand samples were autoclaved and analyzed to serve as media blanks.

Samples used for the general relationship study and depth study were taken from five water treatment facilities: the Peter Binney Water Treatment Plant in Aurora, CO, the Richard Miller Plant of Greater Cincinnati Water Works in Cincinnati, OH, the North Bay Regional Water Treatment Plant in Fairfield, CA, the City of Longmont Treatment Plant in Longmont, CO, and the A.B. Jewell Drinking Water Treatment Plant in Tulsa, Ok. The media included sand, GAC, and anthracite. The source water for these utilities also represented a range of influent conditions, from relatively pristine mountain water to heavily wastewater-impacted river water.

The Binney Plant influent is South Platte River water that is treated with riverbank filtration. The influent is then treated with lime/ferric softening and UV/AOP before it is fed to the biological GAC filter. The Miller Plant treats its influent with traditional coagulation and sedimentation before the filter, as does the Longmont treatment plant. In addition to coagulation and sedimentation, one of the GAC filters at the Jewell plant is pre-treated with ClO_2 . One sample from the Jewell plant was taken from the pre-oxidized filter. The other sample from the plant was taken from a new filter that had only been in operation for three weeks prior to sampling. The GAC samples from the North Bay plant were taken from a filter that is not pre-oxidized. Because the Binney Plant, Longmont Plant, the North Bay Plant, and the Jewell Plant backwash biofilters with chlorine, samples were taken prior to backwashing.

Core samples were taken from the Binney Plant, the Longmont Plant, the Miller Plant, and the North Bay Plant. Cores were divided into the following depth intervals: 0 to 3 inches, 3 to 6 inches, 6 to 12 inches, and 12 to 18 inches. The purpose of these intervals was to obtain a profile of the changing filter biomass. Samples from outside of Colorado were shipped overnight in coolers at ambient temperatures to the University of Colorado. All samples were analyzed one day after sampling. Portions of each sample were taken and analyzed for both ATP and phospholipids. More than 75 percent of the ATP tests were run in duplicate. All phospholipid samples were run in quadruplicate.

Media used for the ATP holding time study was taken from the top of a biological GAC filter receiving AOP-treated influent at the Binney Plant in Aurora, CO. All holding time samples were run in duplicate. For each sample, six portions were taken and immediately extracted. Of these extracted portions, two were analyzed immediately. The other four were capped and refrigerated either one day or three days. Samples were refrigerated to comply with LuminUltra sample holding instructions (LuminUltra Technologies Ltd., 2010). The remaining portion of the sample was not extracted and kept refrigerated. Unextracted media was run in parallel with the extracted samples at one and three days.

This experiment was run using samples from the Binney GAC filter collected in two consecutive weeks. The first experiment's fresh media was stored in dechlorinated tap water dosed to a concentration of 2 mg/L TOC. During the second experiment the fresh media was stored in filter influent water that was collected at the same time as the sample.

All results were normalized to biomass per mL of dry media, which was determined by calculating the dry: wet ratio for the media and the bed density. The dry: wet ratio was found for each media sample by weighing a known mass of wet media into a vial of known mass. The vials were dried in a 100 degree Celsius oven for a minimum of 24 hours. The vials were then placed in a desiccator for a minimum of 24 hours. The dry vials were then weighted and the dry: wet ratio was calculated by subtracting the weight of the glassware from sample weights. Dry: wet ratios were run in duplicate for every biomass media sample. Bed densities for the media samples were determined by filling a 100 mL graduated cylinder of known mass with 100 mL of wet media. The media-filled cylinder was then dried in a 100 degree Celsius oven. The dry media-filled was then removed and weighed. Subtracting the weight of the glassware yielded the media dry weight and the density results were reported as grams per liter.

3.3 Results and Discussion

3.3.1 General Relationship Results

The 30 top of filter samples were analyzed for biomass by the ATP and phospholipid methods. The results of these analyses appear in Figures 3, 4, and 5. All results are shown in Figure 3 and several notable trends are seen. The error bars for both methods are one standard deviation from the mean.

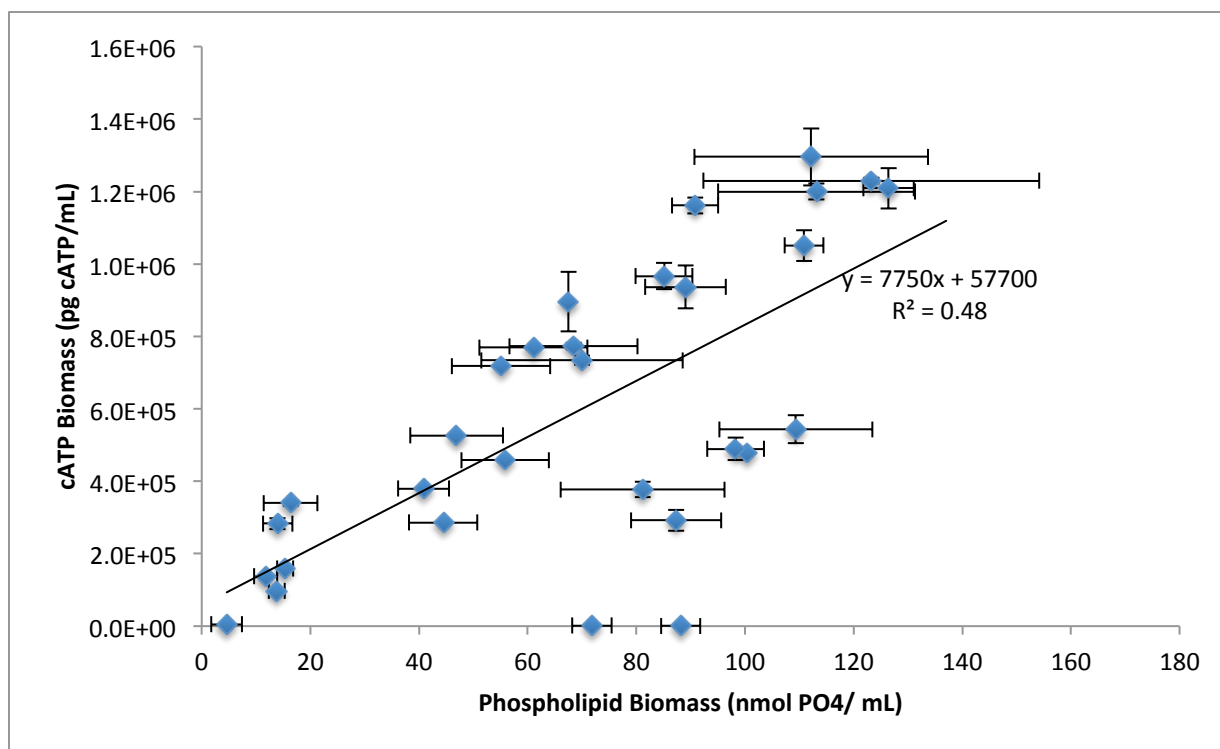


Figure 3 Plot of ATP v. phospholipid biomass for top of filter samples including outlier (n=30)

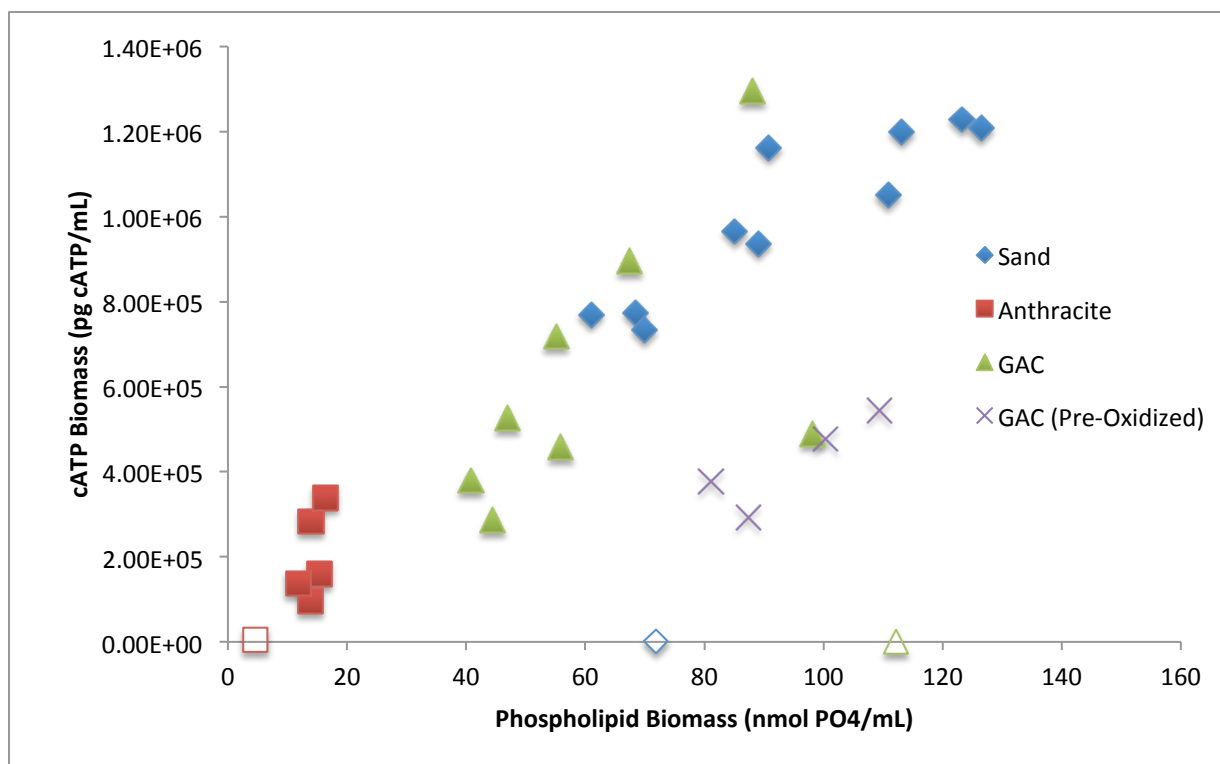


Figure 4 Plot of ATP v. phospholipid biomass for top of filter samples separated by media type (n=30)

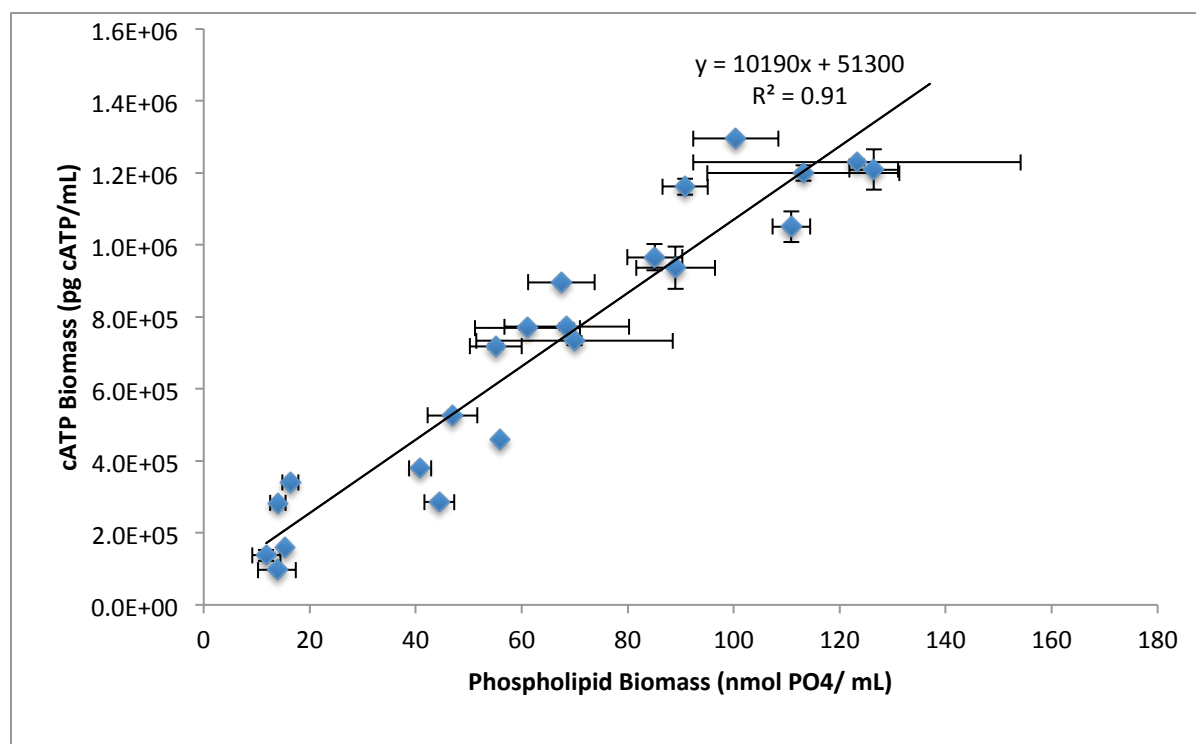


Figure 5 Plot of ATP v. phospholipid biomass for top of filter samples without outliers (n=22)

The blank media samples yielded values of near zero ATP, but substantial phospholipids values. We think that this occurred because the samples were analyzed immediately after autoclaving. The autoclave destroyed the ATP, but phospholipids take longer to degrade. Figure 4 shows the biomass as a function of media type and pre-oxidation. Samples taken from filters that received pre-oxidation (some Binney and Jewell Plant GAC samples) showed lower ATP biomass values compared to the other results. Also the other sample taken from the Jewell Plant was taken from a filter that was only in operation for three weeks prior and also showed a lower ATP biomass value. The results from two GAC samples and one sand sample were removed due to presumed experimental error. The ATP biomass versus phospholipid biomass plot is shown without outliers in Figure 5. Plots 3 and 5 also show the linear regression best fitting the data and the correlation coefficient. Error bars are one standard deviation from mean. In general, the phospholipid samples exhibited higher variation than the ATP samples. This is most likely due to the degree of precision in each method.

As shown in Figure 5, removing the blank media samples, the samples taken from pre-oxidized filters, the new media sample yields a statistically significant linear relationship between ATP and phospholipids. Results of both regression analyses appear in Equations 1 and 2.

Linear Regression with Outliers:

$$ATP \left(\frac{pg}{mL} \right) = 7750PO4 \left(\frac{nmol}{mL} \right) + 57700 \quad (1)$$

$$R^2 = 0.48$$

Linear Regression Without Outliers:

$$ATP \left(\frac{pg}{mL} \right) = 10190PO4 \left(\frac{nmol}{mL} \right) + 51300 \quad (2)$$

$$R^2 = 0.91$$

Removing the outliers results in a regression with a correlation coefficient of 0.91, indicating a high level of correlation between ATP and phospholipids. The slope of the line physically means that if the phospholipids concentration of a sample increases by one nmol PO₄ per mL of dry media, an increase of approximately 10,200 pg cATP can be expected. Notable exceptions are samples taken from filters that receive a pre-oxidized influent and the sample taken from a newly operational filter. This indicates that pre-treated filter influent water may result in lower ATP levels per nmol of phospholipids than in filter receiving influent not treated with AOP. Newly operation filters may also exhibit a different relationship between ATP and phospholipids than the relationship found in more established filters.

3.3.2 Estimation of Cell Counts Using ATP and Phospholipids

Both ATP and phospholipid biomass measures can also be converted to cell counts. In Findlay et al. (1989), the authors used a sample containing naturally occurring, mixed population cells to determine that 100 nmol of phospholipids equated to 3.4×10^9 cells. In the ATP test, E. coli is used as the representative cell and 1000 cells is equated to 1 pg of cATP. Using these conversions, top of filter data

was converted to cell counts and plotted. The results, with the same outliers removed as in Figure 5, appear in Figure 6.

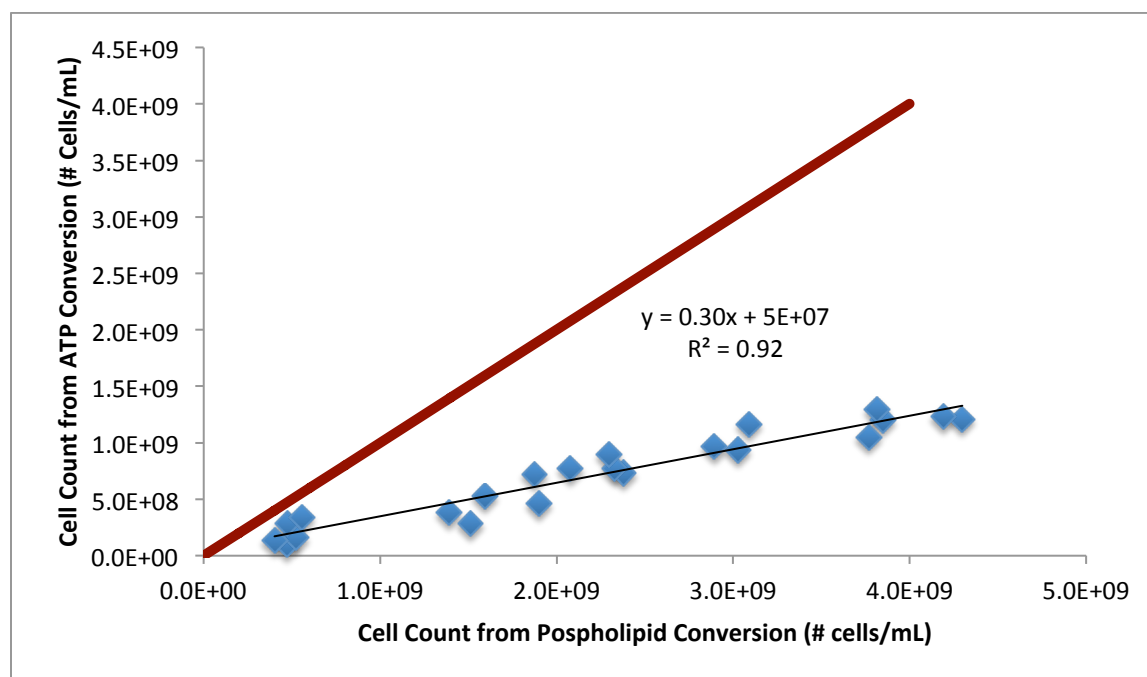


Figure 6 Plot of cell concentration based on ATP versus cell concentration based on phospholipid

Figure 6 shows that the cell counts are not equally predicted by both methods. The 0.3 cells ATP per cell phospholipids slope of the regression indicates that for every cell predicted by the phospholipids method, only 0.3 cells are predicted by the ATP method. At least some of this difference is likely due to the use of a mixed culture for cell conversion in the phospholipid method and the use of *E. coli* in the ATP method. Depending on the population, the cells may have phospholipid and ATP values that are very different from the cells used to determine the conversion factors. The discrepancy in cell counts may also indicate that the ATP method underestimates the total cell count in the sample, or the phospholipid method overestimates the cell count, or potentially both. Given the slower decay rate of phospholipids in dead cells, as seen in the autoclaved media blank samples, it seem more likely that the phospholipid method may be overestimating the total number of live cells in samples.

Relationships between biomass values and the influent TOC and the influent water temperature were also explored, but significant relationships were not found.

3.3.2 Impact of Sample Depth On Relationship

ATP and phospholipid biomass were also analyzed in four core samples collected from drinking water treatment plant biological filters across the country. The sand samples were taken from The Miller Plant in Cincinnati, Ohio (GCWW), the anthracite was taken from the City of Longmont's drinking water plant in Longmont, Colorado, one GAC core was taken from the UV-AOP treatment train at the Binney Water Plant in Aurora, Colorado, and the other GAC core was taken from the North Bay Regional Treatment Plant in Solano County, California (Solano). The results of ATP and phospholipid biomass methods, as well as the linear regression from the top of filter samples (Figure 4) are plotted in Figure 7.

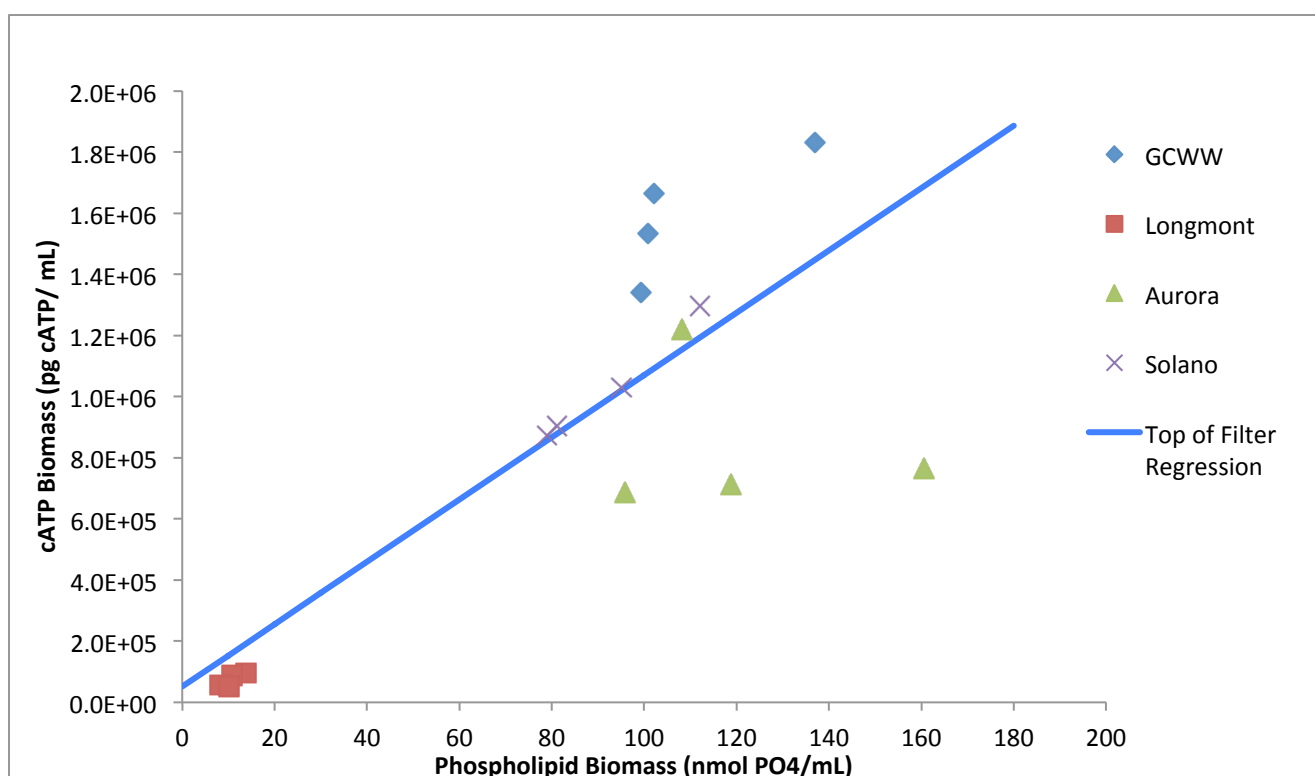


Figure 7 Plot of ATP v. phospholipid biomass for core samples and the top of filter samples linear regression from Figure 4

The core sample analysis showed a wide variation in biomass levels by core, and varying differences in biomass levels within each core. In an effort to better understand the relationship between biomass and depth, sample depth was plotted versus both ATP and phospholipids for the three cores. The results of this analysis appear in Figures 8 and 9.

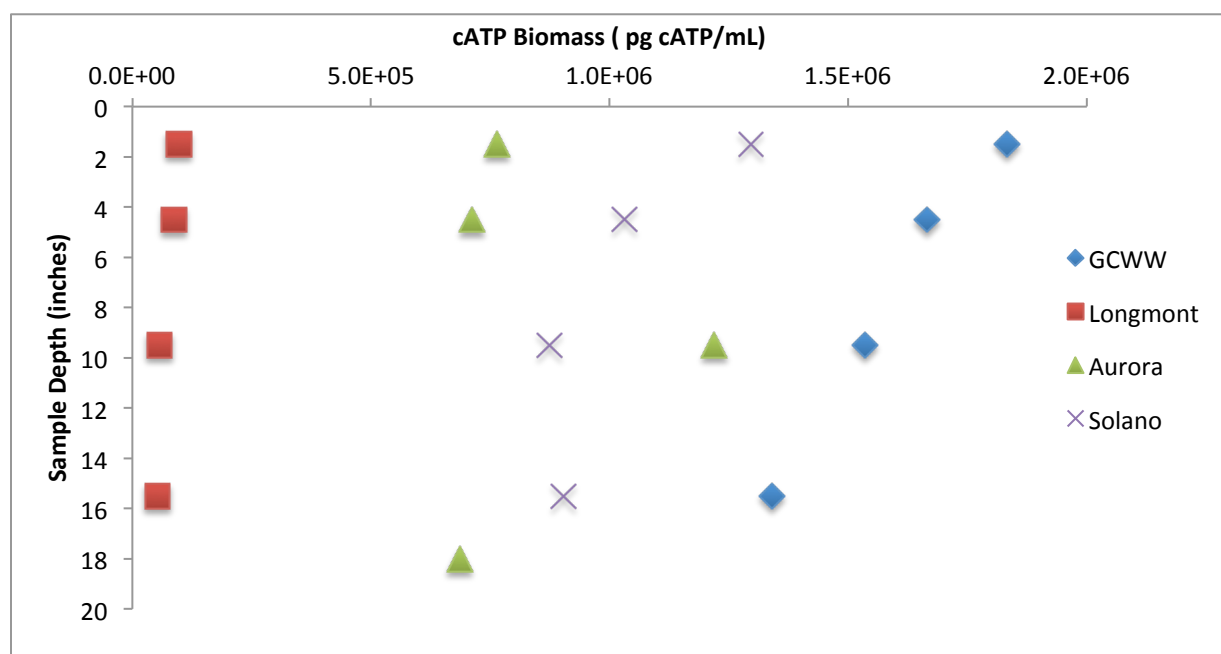


Figure 8 Plot of sample depth v. ATP biomass for the core samples

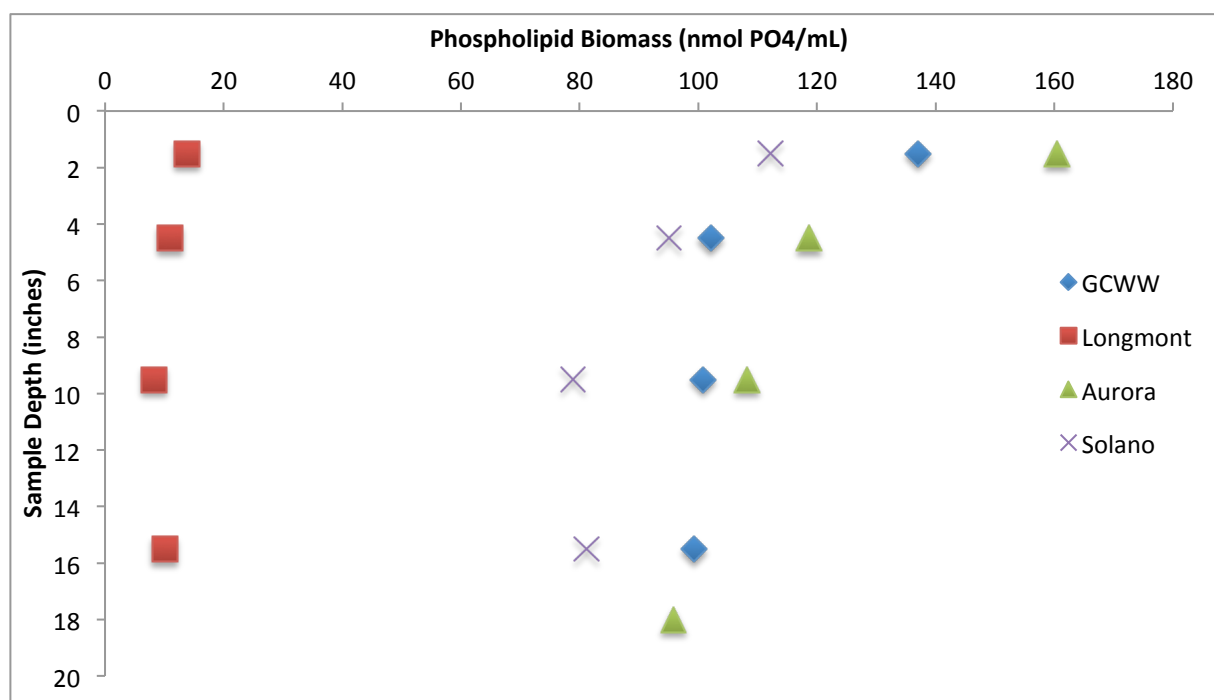


Figure 9 Plot of sample depth v. phospholipid biomass for the core samples

Results show there appears to be a logarithmic decline in biomass concentration with depth. However, the shape of the curves varies by core sample for both biomass analyses.

Next, the biomass values were normalized to the top of filter biomass concentration and plotted versus sample depth. The biomass concentrations were normalized to the top of filter concentration because this sample usually contains the highest biomass due to the fact that it is the first layer of media to receive the influent, and the primary substrate for filter microorganisms is most readily available. In the ATP data there is one Aurora GAC sample that appears to be an outlier (See Figure 10). When fitting a regression the normalized ATP versus sample depth data, this one point was omitted. The regression curve fit to the data appears in Figure 11.

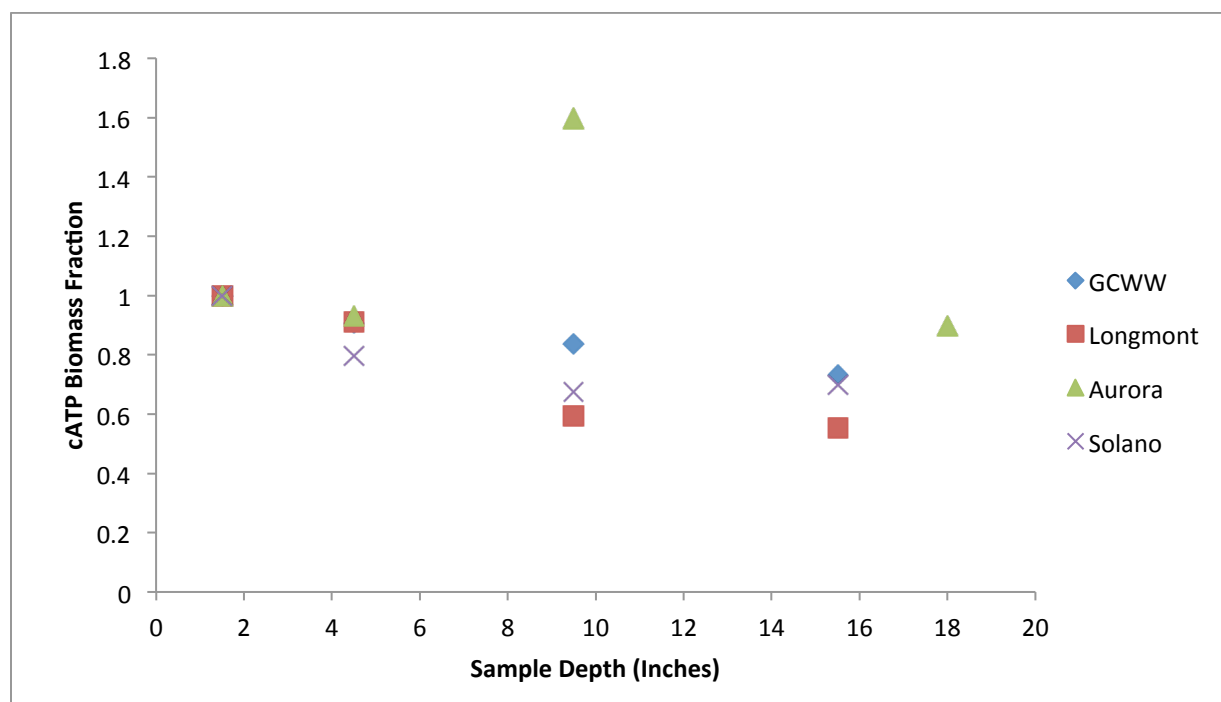


Figure 10 Fraction of ATP biomass in relationship to top of filter ATP biomass v. sample depth

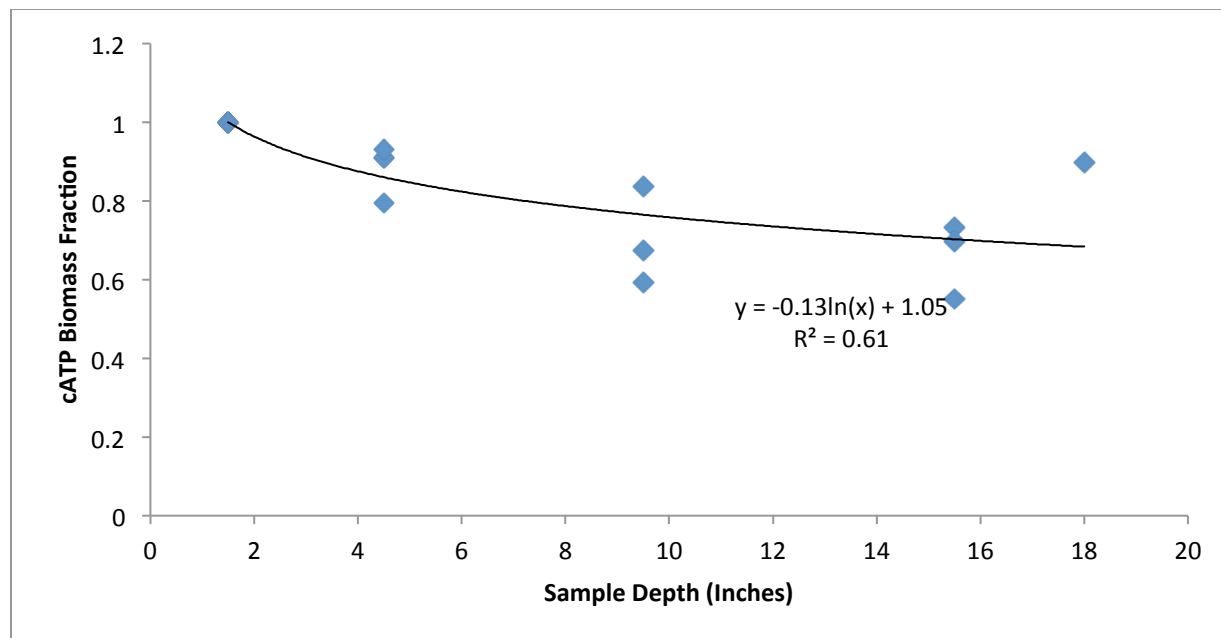


Figure 11 Plot of fraction of ATP biomass in relationship to top of filter ATP biomass v. sample depth with one outlier removed

The best fit for the data was a logarithmic regression, which yielded a correlation coefficient of 0.61. Though this does not show a strong correlation, there appears to be a consistent trend. With more samples this correlation could possibly be improved.

This normalization of the biomass data was then repeated for the phospholipids core sample results. The results appear in Figures 12 and 13.

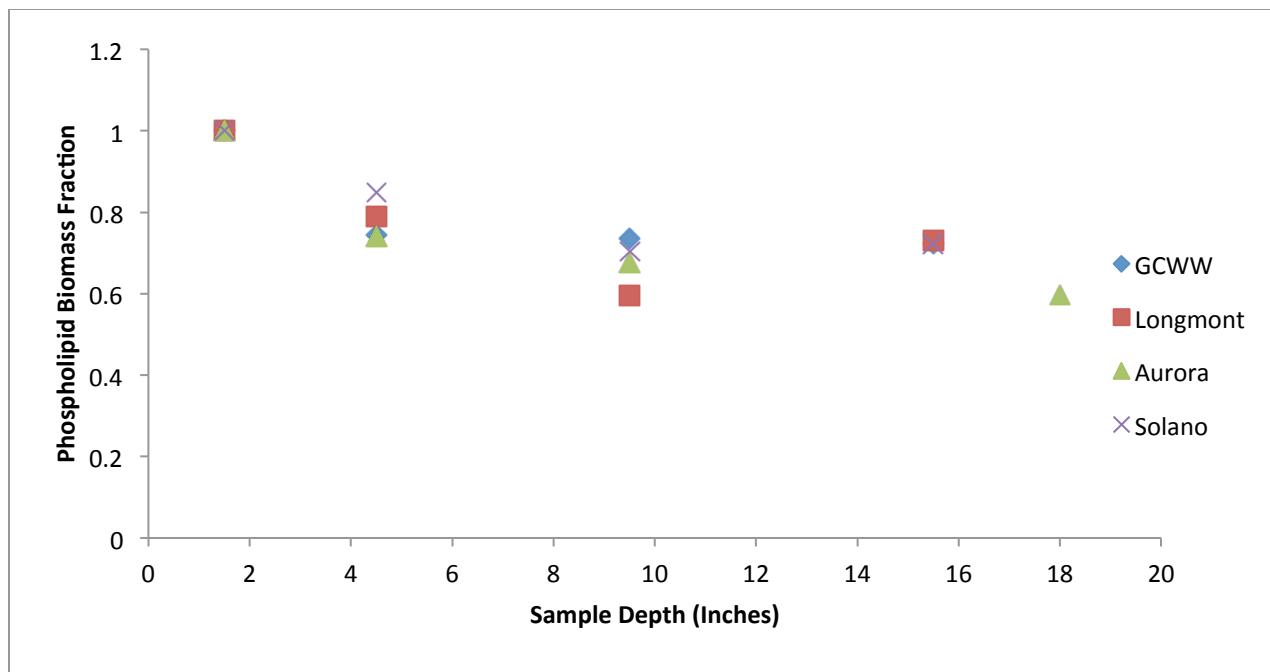


Figure 12 Plot of fraction of phospholipid biomass in relationship to top of filter phospholipid biomass v. sample depth

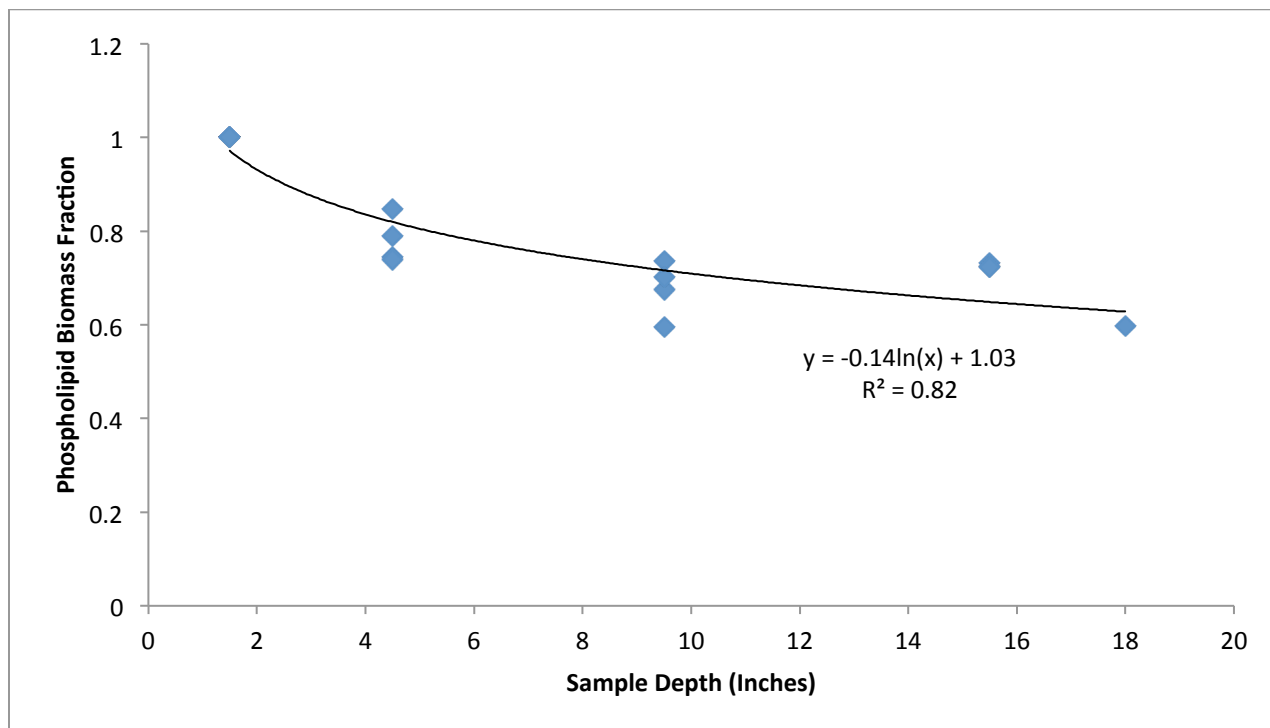


Figure 13 Plot of fraction of phospholipid biomass in relationship to top of filter phospholipid biomass v. sample depth

The normalized plot of phospholipid biomass data is also best fit by a logarithmic regression. The regression has a higher correlation coefficient than that of the ATP regression, with a value of 0.82. The normalized plots shown in Figures 12 and 13 have very similar shapes to those of the ATP data in Figures 10 and 11. This indicates that the relationship between biomass at the top of the filter and at depth is similar for both ATP and phospholipids.

These plots show that both ATP and phospholipids decrease with depth. This is logical because as the influent, which contains primary substrate, passes through layers of biological filter media, the easily accessible substrate is removed. Therefore, farther down in the filter, there is less available substrate to use for energy and growth. The result is that the biomass in biological filters is concentrated towards the top of the filter. The logarithmic shapes of the biomass curves indicate that biomass concentrations do not decay linearly in filter. Rather, the concentrations initially decrease rapidly, then more gradually, with increased depth. However, the varying shapes of the curve indicate that the biomass distribution may also be linked to media type and filter influent.

The relationship between biomass and empty bed contact time (EBCT) was also explored. But the hydraulic loading rates of the filters were all very similar, only varying from 3 to 3.8 gpm/ft². Thus there was little difference in the depth profile.

3.3.3 Effect of Holding on Measure of ATP

To evaluate the change in ATP with holding time, samples were extracted and held for several time periods prior to ATP analysis. At the same time, fresh samples of the same media were kept moist and run alongside the extracted samples. The extracted media samples showed a significant decline in ATP after 1 day in both experiments. Figures 14 and 15 show the change in ATP in the extracted and unextracted samples over 3 days for the two experiments. Figure 14 shows the experiment where the media was held in water dosed to a concentration of 2 mg/L TOC. Figure 15 shows the experiment with

fresh media held in filter influent water. The initial cATP was much higher in the second sample. This is due to the longer run time of the filter from which the sample was taken. The error bars are the standard deviations of the samples, which were run in quadruplicate.

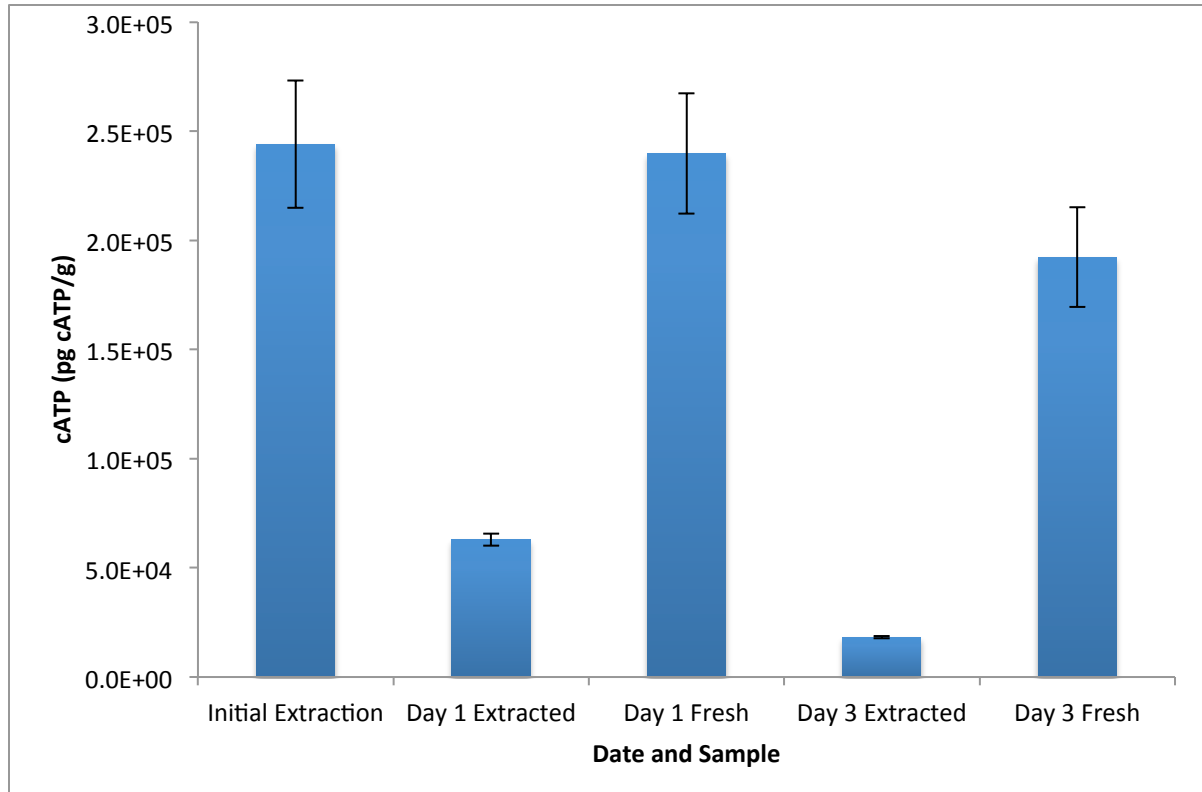


Figure 14 Change in ATP in extracted and unextracted samples over three days. The fresh media was refrigerated in water with a TOC of 2 mg/L.

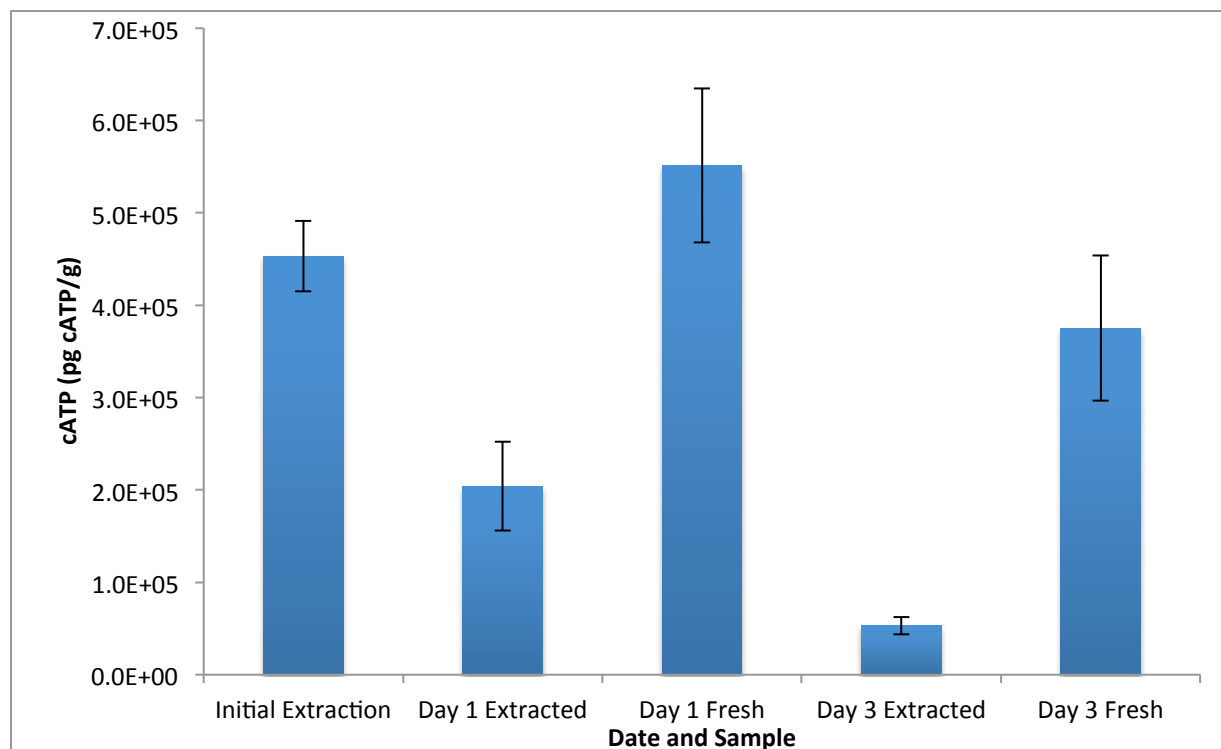


Figure 15 Change in ATP study repeated with the fresh media refrigerated in filter influent water

The error bars show one standard deviation from the mean. Both experiments resulted in significant declines in extracted media ATP over one day. There was little difference in the decrease in ATP between the experiments, indicating that holding water did not significantly impact ATP levels in the fresh media. The fresh media maintained sample ATP levels over one day, with only small decreases after 3 days. This is most likely due to the sensitivity of ATP, which rapidly degrades when removed from the cell. The extraction process does not sufficiently stabilize the ATP to prevent loss post-extraction, during the holding period. The ATP is likely better maintained in the fresh media due to the preservation of the cell structure, protecting cellular ATP from hydrolysis and degradation.

3.4 Conclusions

The results of this study indicate that there is a correlation between ATP and phospholipid measured biomass. Excluding the blank media samples and the media samples that were taken from

filters receiving a pre-oxidized influent, the results form a linear relationship with a correlation coefficient of 0.91. This correlation appears to be independent of media type. The samples that were taken from pre-oxidized filters and newly operational filters exhibited lower ATP per nmol of phospholipids ratios. Using the ATP and phospholipids cell count approximation methods yields cell counts that are off by a factor of 3, with the ATP cell count only one-third of the count predicted by the phospholipids approximation method. This likely due, at least in part, to the types of cells used when determining cell conversion factors. The difference in predicted cells is also potentially due to overestimation of cell counts by phospholipids due to slow decay. Both ATP and phospholipids relationships with depth are best fit using logarithmic regressions. In all media, the biomass drops quickly over the first 6 inches, then more gradually over the next 12 inches. However, the slopes of these curves varied with the samples, indicating they may depend on media type or filter influent. The holding time study results showed that if samples cannot be immediately tested for ATP, the cell activity remains closer to initial levels when media is held unextracted. This is most likely due to the instability of ATP molecules when removed from cells.

Chapter 4: Conclusions

Biofiltration is still a growing field within drinking water treatment. However, biofilters have been shown to be capable of removing organic matter and a variety of trace organic contaminants. For the majority of contaminants evaluated in the trace organics study, removal was highest in wastewater-impacted biomass media filter that was operated under carbonaceous conditions. However, the unimpacted biomass media showed comparable removal to the impacted biomass media under nitrifying conditions. Filter media with higher biomass levels were also generally more effective at removing trace organics. Notable exceptions to these trends were aldicarb, caffeine, and methomyl, which were removed as well or better in the lower biomass unimpacted biomass media filters. This study can be used in conjunction with Zearley and Summers (2012) and future work to enable engineers and operators to better predict how biofilter removal of trace organic compounds will change over time.

An important factor in the operation of biofilters is the biomass, which can often be linked to the filter's ability to remove organic compounds. The results of this study indicate that there is a strong correlation, correlation coefficient of 0.91, between ATP and phospholipid methods of measuring biomass. This relationship allows for the conversion between the two measures. However, samples that were taken from filters that treated pre-oxidized water exhibited lower ATP per nmol of phospholipids ratios. The ATP and phospholipid biomass measures were converted to cell concentrations. The cell count approximation methods yields cell ATP cell counts that are only one-quarter of the count predicted by the phospholipids approximation method. Both ATP and phospholipid biomass relationships with depth are best fit using logarithmic regressions. In all media, the biomass dropped quickly over the first 6 inches, then more gradually over the next 12 inches. However, the slopes of these curves may depend on media type or filter influent. The holding time study results showed that if samples cannot be immediately tested for ATP, the cell activity remains closer to initial levels when media is held unextracted.

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