ANALYSIS OF ULTRA-VIOLET DISINFECTION ON MICROBIAL ACTIVITY AND VALIDATION OF RAPID TESTING METHODS

by

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Submitted in partial fulfilment of the requirements

for the degree of Master of Applied Science

at

DALHOUSIE UNIVERSITY

Halifax, Nova Scotia

For my father
Who always kept me interested in how things work

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Abstract

Monitoring disinfection at wastewater treatment plants typically involves quantifying fecal and total coliforms, the results of which take 24 hours to produce. A faster method is proposed and validated in this research. The proposed method uses ATP analysis in conjunction with an incubation process to encourage life cycling of the microbes to better represent the inactivation of the UV processes.

Three WWTP were sampled over the course of the sampling schedule; the average disinfection efficiency of the Dartmouth plant using the HPC method was 84%. ATP testing gave an average disinfection efficiency of -10%, a false negative that shows immediate analysis is a poor approach but applying the grow out method gives an average disinfection efficiency of 77%.

NOM (humic acid) had a notable effect on disinfection performance. A dose of 2 mg/L yielded 4.22 log reduction in E. *coli* concentration; but a concentration of 20 mg/L gave no reduction. Turbidity and amino acid studies showed that little to no effect on disinfection performance. Turbidity of 100 NTU and 1000 NTU with respective E. *coli* concentrations experiencing 3.67 log reduction and 3.58. Amino acid dosed at 2 mg/L and 20 mg/L yielded reductions in E. *coli* concentrations of 3.94 log and 3.89 log respectively.

The proposed method was applied in parallel to standard tests and the results affirmed the applicability and value of the research. Immediate ATP testing results showed no disinfection was achieved but the grow-out method yielded measurable and accurate disinfection results when compared to the standard methods.

List of Abbreviations and Symbols Used

ATP	Adenosine Tri-phosphate
BUV	Before Ultra-violet
cATP	Cellular Adenosine Tri-phosphate
CFU	Colony Formation Unit
cm^2	Centimetre Squared
COD	Chemical Oxygen Demand
DI	De-ionized
DNA	Deoxyribonucleic Acid
E. *coli*	Escherichia coli
HPC	Heterotrophic Plate Count
MAC	Maximum Allowable Concentration
MCLG	Maximum Contaminant Level Goal
MF	Membrane Filtration
Mg	Milligram
MGD	Mega Gallons per Day
mJ	Millijoule
mL	Millilitre
MTF	Multiple Tube Fermentation
mW	Milliwatt
nm	Nanometer

NOM	Natural Organic Matter
NTU	Nephelometric Turbidity Unit
η	Disinfection Efficiency
P-A	Presence-Absence
PBS	Phosphate Buffer Solution
pg	picogram
PUV	Post Ultra-violet
QGA	Quench-gone Aqueous
RLU	Relative Light Unit
RTCR	Revised Total Coliform Rule
TSS	Total Suspended Solids
UV	Ultra-violet
UV-T	Ultra-violet Transmittance
V_x	Volume of x
VBNC	Viable but not Culturable
WTP	Water Treatment Plant
WWTP	Wastewater Treatment Plant

Acknowledgements

I would like to thank first and foremost Dr. Graham Gagnon for giving me the opportunity to study with him and make use of the water research laboratory at Dalhousie University. Having the chance to collaborate with a renowned water expert and make use of the amazing lab was paramount to my success in this academic endeavour.

I would also like to thank LuminUltra for the project inspiration and the research partnership they have with Dalhousie's water group that allowed me to complete my masters.

Finally, a sincere and deep thank you to my colleagues, fellow grad students, co-op students, staff, and of course friends that helped me along the way. Discussing research issues and coming up with innovative ways to study water keeps us all interested in bettering the community; not to mention keeping morale up when things got tough, thank you so much.

Chapter 1 – Introduction

Access to clean water is a necessity for life but its diminishing availability is increasingly becoming a global concern. 783 million people do not have access to clean water and 2.5 billion do not have access to adequate sanitation (UN Water, 2014)

Water treatment processes vary by region (and ergo source water quality) but also by desired end usage. In other words, drinking water and wastewater require different treatment trains but also must adhere to varying water quality parameters and quality control guidelines. A common treatment concern for water and wastewater treatment is a disinfection process which leads to the inactivation of pathogenic and/or invasive microbiological species (Owoseni, Olaniran, & Okoh, 2017) (Environmental Protection Agency, 1999).

Treatment plants that employ chemical disinfection, i.e. the use of chlorine, will often purposely maintain residual levels of the disinfectant to ensure continued proper disinfection as the water passes through the distribution system (Roopali & Patel, 2015). There is concern for disinfection by-product formation, or DBPs when chemical disinfection is used. Since disinfectants like chlorine are oxidizers if they come into contact with organic matter (such as fulvic, humic acids, and/or amino acids) they can produce hazardous compounds like trihalomethanes and haloacetic acids (Richardson, Plewa, Wagner, Schoeny, & DeMarini, 2007).

For this reason, many wastewater treatment facilities in Canada disinfect using UV systems, which actually holds true for most of North America. This technology is most

often employed because of its advantages over the alternatives in that there is no residual, therefore it is non-toxic (CCME, 2014), and eliminated risk of by-product formation.

That said, one of the biggest drawbacks of this technology is its significantly high level of energy consumption (Dabkowski, et al., 2011). Even here in Nova Scotia, the cost of UV disinfection is a significant one; so much so that the UV systems at WWTPs are shut down during the winter months to save on these significant energetic costs (CBC News, 2016). The main concern with this is how do we determine the continued effectiveness of the wastewater treatment, specifically the disinfection, if the power of the UV systems was reduced or turned off completely?

Testing the efficacy of these disinfection processes is not is not as simple as some other water quality parameters. Tests are time consuming, often expensive, and require specific training. The usual case for wastewater treatment plants is that they test for fecal and total coliform presence and concentration (Government of Prince Edward Island, 2011) as they are indicator organisms, meaning they may indicate the presence of other pathogenic bacteria (Oram, 2014).

While these tests do provide sufficient information on the general safety of the water sample, they are neither helpful for identifying other microbiological contaminants nor are they quick; taking 24 hours to yield reliable results (American Public Health Association, et. al., 1992). There are more robust methods for qualifying and quantifying microbiological activity in a water sample, but these tests require even more training and

time results. The standard method for quantifying bacterial contamination is heterotrophic plate count (HPC) but this method demands a fair amount of knowledge in microbiology as well as a full week of incubation time for results to be available.

Even though wastewater treatment plants typically use the faster testing procedure of only quantifying fecal and total coliforms the results of your disinfection process still arrive the following day; when the water that was treated has certainly been discharged from the plant. Meaning that if a problem had been detected the water in question is long gone (Dickerson, n.d.).

A faster testing method for detecting and quantifying microbiological contamination in water is required for ensuring safe discharge from water treatment plants as well as mitigating the down time between detecting a problem with the discharged water quality and conducting reparative actions. The waiting time required by these tests is unacceptable for compliance monitoring of wastewater discharge as well as routine testing for ensuring efficacy.

Chapter 2 – Research Objectives

2.1 Rationale

Monitoring the performance, or efficiency, of the disinfection process of a wastewater treatment plant (WWTP) is not an easy task.

The tests take days to yield results and as such utilities usually apply more disinfectant loading than may be necessary to ensure proper inactivation of microbiological contaminants.

The aim of this research to develop a method that is not only fast but accurately describes disinfection performance in order to benefit water treatment utilities in their ability to disinfect properly and to reduce both their direct and indirect consumption of energy and resources.

Determining which water quality parameters have an effect on the performance of UV disinfection systems will also be analyzed to better understand what indicators should be monitored for proper disinfect intensity.

2.2 Objectives

a) Analyze the UV disinfection performance of three wastewater treatment plants; Halifax, Dartmouth, and Herring Cove plants in the Halifax Regional Municipality
b) Quantify the disinfection performance and efficiency of these plants using standard heterotrophic plate count as well as ATP methods

c) Compare the results from methods in part b with the proposed incubation grow-out method for ATP analysis
d) Perform initial observations into the effects specific water quality parameters may have on disinfection performance

2.3 Organisation of Thesis

This thesis consists of six chapters relating to the study of disinfection and augmenting its functionality

Chapter 1: Introduces the subject and the background information pertaining to the research. A brief history of the methods used and the potential benefit of the proposed new method

Chapter 2: Identifies the importance of the research and the goals to be met to finalize it

Chapter 3: Details the mechanisms and function of the related topics to this research. How disinfection is applied to water treatment, the resulting effect on the microorganisms found in the water being treated, the current regulations on effluent biomass concentrations and how adenosine triphosphate methods are being applied to quantify it. Finally, it details the effect of several water quality parameters on the disinfection process and how they can be used to better the performance of that process.

Chapter 4: Explains the steps taken to accomplish the research; equipment used and methods applied to generate data to be analyzed

Chapter 5: Presents the results obtained from the experiments and explains their importance to the water community. Contains the significant information pertaining to improving water treatment processes

Chapter 6: Discusses possible future research and how to expand on the findings of this research. Identifies holes in knowledge that this research dug up and suggests options for filling them in

Chapter 3 – Background

3.1 Disinfection Mechanisms; Chemical and Ultraviolet

Since disinfection processes themselves and the available options for treatment trains are not in the scope of this research only the most common methods will be discussed.

Disinfecting water whether it be wastewater or intended for drinking almost universally involves chlorine and/or ultraviolet radiation. The usage of chlorine remains prevalent mainly due to its relatively predictable behaviour, and its efficiency as a disinfectant (Calderon, 2000).

Typically, chlorination is applied at the beginning of the treatment train (i.e. pre-chlorination) and again at the end of the treatment train (i.e. post-chlorination). It is done in duality like this to disinfect the raw water entering the treatment plant and then again to ensure high enough chlorine residuals in the distribution system to residential users.

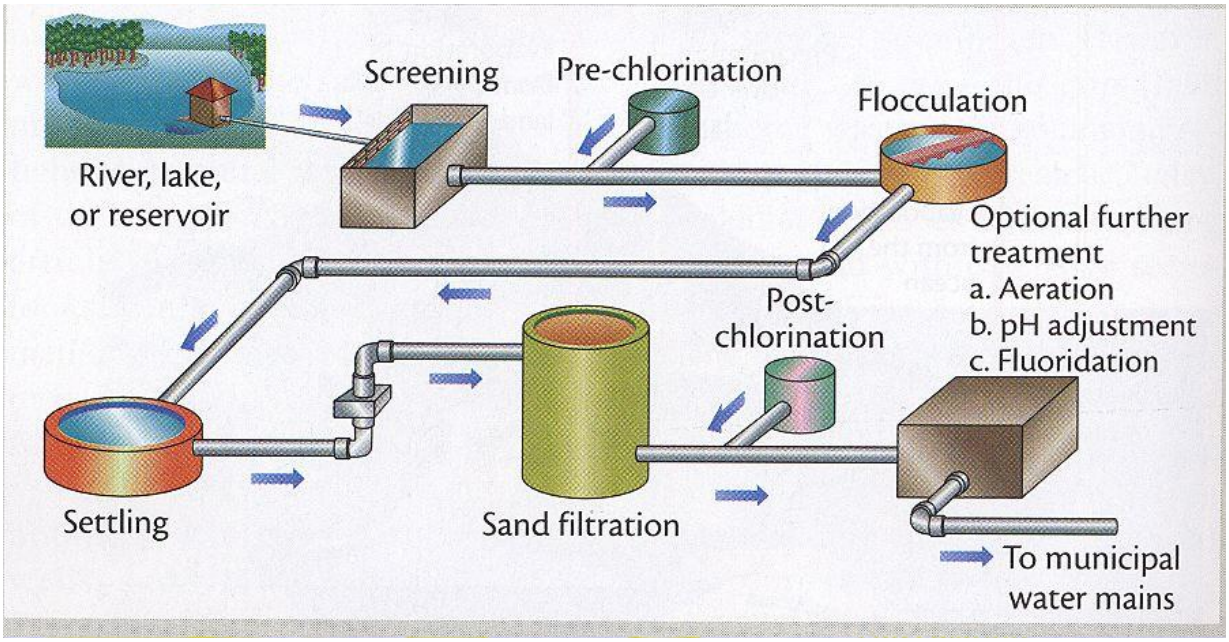

Figure 1 Typical Water Treamtent Processes (American Chemical Society, 2002)

For water treatment chlorine can be added in pure gaseous form, as sodium hypochlorite solution (NaOCl), or solid calcium hypochlorite ($Ca(OCl)_2$) (Centre for Disease Control and Prevention, 2015).

As a halogen chlorine is a strong oxidizing agent and by stripping electrons from the organic molecules it is capable of disinfecting pathogens and bacteria found in water (Calderon, 2000) (Sedlak & von Gunten, 2011).

The purpose of both pre-chlorination and post-chlorination is similar but they vary slightly in intended end result. Chlorine is added at the inlet of the raw water to kill any biological contaminants entering the treatment system. This is necessary to restrict bacterial growth with the treatment train itself which could negatively impact its functions (e.g. algae growth on filters and tanks). Presence of biological contaminants within the treatment

train could easily result is taste and odor issues further down the distribution system as well (Westerhoff, n.d.).

Post-chlorination is used at the end of the treatment train in order to ensure proper chlorine residuals in the distribution system. These chlorine residuals are crucial for restricting regrowth of bacteria and mitigation of algal development just as with pre-chlorination except in the case of the distribution system piping and the end user (e.g. residences) (American Water Works Association, 2012) (Center for Disease Control and Prevention, 2014).

In the case of wastewater treatment however ultraviolet radiation is typically used instead of chlorination (especially post-chlorination) since the outlet of the plant is typically a large body of receiving water rather than households. There's no need to maintain disinfectant residual levels in this case since there is no distribution system and moreover having excess disinfectant could negatively affect the receiving waters by killing the naturally occurring and often crucial micro ecosystem (Environmental Protection Agency, 1999).

Ultraviolet radiation disinfection functions by exposing the water to light emitted wavelengths specifically tuned to damage DNA (Rastogi & al., 2010)(Trojan UV, 2016) (Environmental Protection Agency, 1999). This is typically set to 254nm as wavelengths between 200nm and 300nm are deemed germicidal in addition to the fact that 254nm is an optimal resonation wavelength for nucleic acids (Oram, Brian; Water Research Center, 2014).

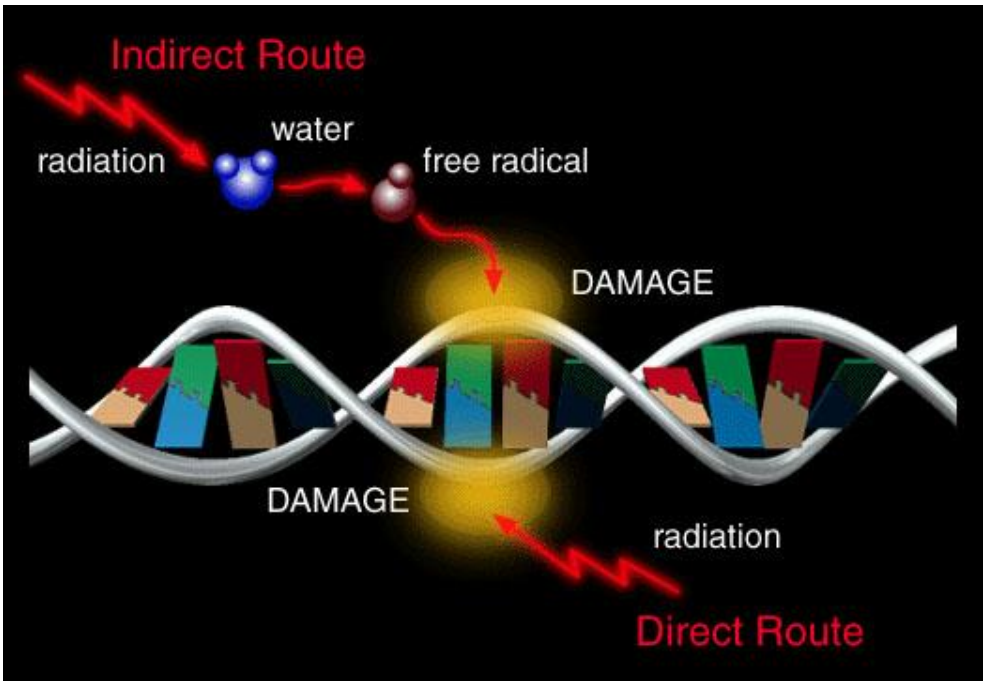

Figure 2 Ultraviolet Disinfection Mechanism (Bouquet, 2015)

There is also the possibility of additional damage being done to the structure of the microbe's DNA by free radicals. Free radicals are molecules with a free, unpaired, electron and ergo are very reactive (Walling, 2016). In the case of ultraviolet radiation in water, if a photocatalyst is present, the radicals produced are hydroxyl radicals (•OH).The particular photocatalyst used will alter the mechanism of creating the radicals, but hydroxyl radicals are often produced using titanium dioxide, ozone, hydrogen peroxide, or ferrous iron (Kavitha & Palanivelu, 2004) (Kent, et al., 2011).

When a proton (hydrogen atom) is removed from the water molecule by the radiation and a neutrally charged hydroxide ion is left, named hydroxyl radical for differentiation. This highly reactive radical disrupts the nucleic acid chain in the microbe's DNA and renders it unable to reproduce (Sunil Paul, Aravind, Pramod, & Aravindakumar, 2013).

3.2 Viable but not Culturable (VBNC) State

Killing microorganisms by disinfection could be considered a bit of a misnomer; instead the term inactivated is often used. This term is preferable since the organisms are often still alive, but their DNA has been altered in such a way that they can no longer reproduce. This is important to differentiate since the organisms continue to be present in the water that has been treated and will continue to carry out their metabolic activities until their lifecycle is complete and they perish without having reproduced (Zhang, Ye, Lin, Lv, & Xin, 2015).

This state is referred to as Viable but not Culturable (VBNC) and as the name suggests if one were to try to grow these organisms, on agar for example, no growth would be seen since they cannot reproduce but they are still alive immediately following disinfection.

This is significant for water treatment utilities in that if a test were to be carried out for quantifying microbiological contamination (or disinfection performance) the results may be skewed one way or another. A fast test may give a false poor disinfection performance result as the organisms that have been inactivated are still alive and depending on the contaminant in question a standard quantifying method (i.e. heterotrophic plate count (HPC)) may give a false satisfactory disinfection performance since the VBNC organisms may have done damage whilst living out their life cycle. This is possible because VBNC organisms may maintain pathogenicity and additionally there is the risk of regrowth or recovery from the stressed state that the disinfection caused (Lleo, et al., 2001). Note that

regrowth and recovery from the VBNC state is outside of the scope of this research and the focus will instead be on properly quantifying microbiological activity in water samples.

3.3 Current Regulations and Quantifying Methods

Regulations for effluent water quality vary across the globe and their enforcement varies even between states and provinces in the United States of America and Canada respectively (Payne, 2007). Though the enforcement of the regulation varies state by state the US follows the Revised Total Coliform Rule (RTCR) where Maximum Contaminant Level Goal (MCLG) for *Escherichia coli* is set to 0. This rule has been revised from measuring total coliforms to only *E. coli* since the former does not necessarily present a hazard to human health but *E. coli* is pathogenic to humans (Environmental Protection Agency, 2016).

Canada requires monitoring of total coliform concentrations of the effluent water and has a Maximum Allowable Concentration (MAC) of 0/100 mL (Government of Canada, 2013).

There have been numerous methods developed for enumerating and identifying microbiological organisms but for the sake of relativity and conciseness only those that are pertinent to water treatment utilities will be discussed here.

There are three methods accepted by the Government of Canada for monitoring total coliform concentrations. They are the Presence-Absence (P-A), Membrane Filter (MF), and Multiple Tube Fermentation (MTF) tests. Detailed descriptions of these methods can be found in Standard Methods for Examination of Water and Wastewater (APHA, 2012). All

three of these tests require a wait time of at least 24 hours; both the P-A and MTF tests even suggest waiting 48 hours for a more reliable result (Hach Company, 2013) (Hach Company, 2012) (Eckner, 1998).

These methods for detecting coliforms and/or *E. coli* have been verified and are standard methods for analysis but their drawbacks are that they only yield results on coliforms and *E. coli* while this is valuable information as far as pathogenicity of the water sample in question to human health, they do not give a full picture of microbiological activity in the water sample (i.e. there are other organisms living in the sample that will not show up in these tests). A method that was developed for quantifying microbial organisms non-selectively was developed by LuminUltra using adenosine triphosphate (ATP) quantification (LuminUltra, 2016).

3.4 ATP Mechanisms and Quantification

Adenosine triphosphate is the energy molecule found in all living cells; used to effectuate metabolic activities (Solomon, Berg, & Martin, 2005). When ATP is used by a living cell typically a phosphate group is removed for the energy in the bond, returning it to a precursor form, and feeding the organism (e.g. microbial cell, human body) new bonds are formed and energy is stored in the ATP molecule once more. This means that ATP is recycled within the organism time and time again as the main source of energy for its metabolism (Knowles, 1980) (Biology Pages, 2012).

As with most ATP monitoring technologies, the techniques used for this research developed by LuminUltra makes use the ATP molecules reactivity with luciferase. Luciferase is the enzyme responsible for bioluminescence (Ohmiya, Hirano, & Ohashi, 1996); light being produced by animals like fireflies and some algaes (Gould & Subramani, 1988) (Callaway, 2013). The luciferase reacts with the ATP to produce light and this light output is measured with a luminometer.

$$ATP + O_2 + Luciferin \xrightarrow[Luciferase]{Mg++} AMP + PPi + oxyluciferin + light$$

Figure 3 ATP Reaction with Luciferase to Produce Light (LuminUltra, 2016)

ATP can be found within the cells of the microorganisms as well as outside in the bulk solution mainly from dead microbes that have released their ATP. Since in most water treatment analyses the current level of microbiological activity is the desired measurement the cellular ATP concentration is the unit of importance.

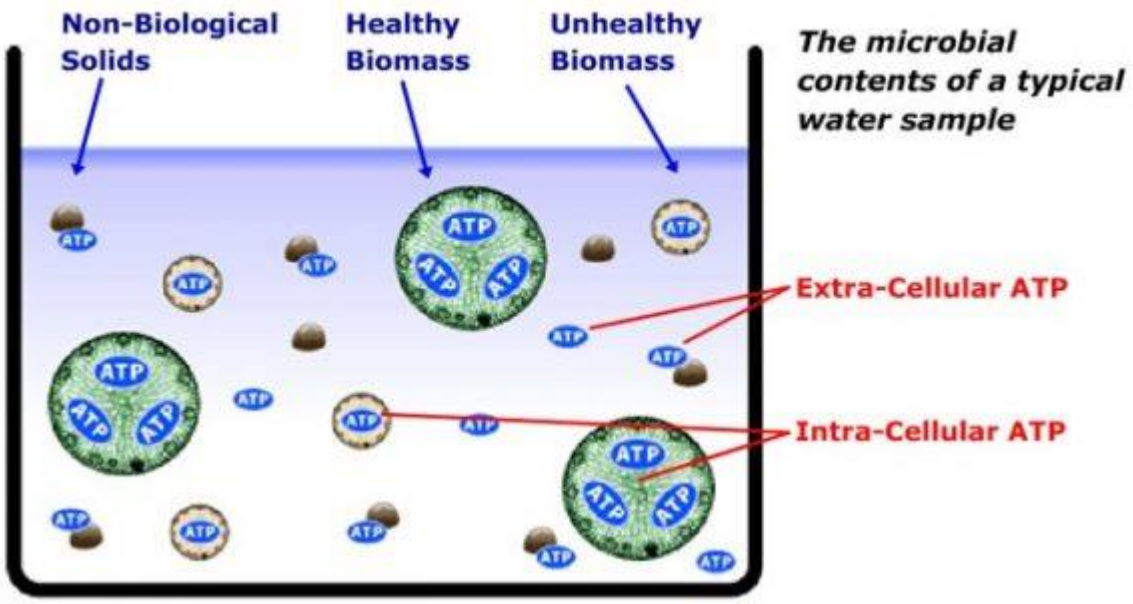

Figure 4 Simplified Visual of ATP in Water (LuminUltra, 2016)

In order to measure solely the cellular ATP and not the total ATP the Quench-Gone Aqueous (QGA) method is used. The sample in question is filtered, lysed, and reacted. Pushing the sample through a syringe filter traps the microbes and allows the bulk solution along with the free ATP to flow through. A lysing agent is then pushed through the same filter and the ATP rich solution that is released is collected.

To properly harness the potential of all ATP present in the cells lysing is a crucial step. A lysing agent is a compound capable of deteriorating cell walls (or membranes) usually by enzymatic or osmotic means (Jolles, 1996) (ThermoFisher Scientific, 2016). After having released the cellular ATP the solution can be reacted with the luciferase enzyme and the light production quantified. This amount of light can be converted directly to a concentration of microbiological organisms in the water sample using the equation below.

$$cATP\left(pg\ ATP/mL\right) = \frac{RLU_{cATP}}{RLU_{ATP1}} \times \frac{10{,}000\left(pg\ ATP\right)}{V_{Sample}\left(mL\right)}$$

Figure 5 Calculation of Cellular ATP Concentration (LuminUltra, 2013)

This method requires a fraction of the time required for the previous quantification methods mentioned. The time required to obtain results using the QGA test is limited mainly by the operator; experienced technicians can have a result in as little as five minutes. This is the main appeal of this type of test, as the water utility is able to obtain feedback on the functionality of their disinfection processes in as little as 3 thousandths of the time as with conventional testing methods (5 mins compared to 24 hours).

A drawback of this method is its inability to differentiate between inactivated microorganisms and healthy, unaffected microorganisms.

Since ultraviolet radiation can induce VBNC states in microorganisms these bacteria or pathogens may be unable to reproduce but are still alive and carrying out metabolic activities in your water. These organisms have successfully been 'treated' in the sense of disinfection processes but since they have not yet died they still contain ATP. For this reason, a normal ATP quantification test will yield results that are a false low for disinfection performance. A new method is proposed that deals with this issue by encouraging life cycling of the microorganisms in the sample ergo a die off of the inactivated organisms but leaving the healthy (i.e. not affected by the disinfection) organisms to be measured.

3.5 Proposed Rapid ATP Analysis

The proposed method in this research expands on the research done by Xie, 2014. Xie looked into testing methods that were not only faster but also took into account the inactivated but still living microbiology in a treated water sample. In other words, a method that was quick and gave results on disinfection performance without false negatives from VBNC microorganisms.

The method consisted of introducing the treated water sample to a nutrient rich broth and incubating it to encourage metabolic activities of the microorganisms. As the

microbes continued carry out life functions those that were unaffected by the disinfection process simply remained but those that were VBNC died off.

The method development focussed on promoting life cycling of the microorganisms to ensure the VBNC organisms were removed from the QGA testing procedure to better represent disinfection performance.

Xie (2014) found a nutrient broth and incubation temperature that looked promising and this research explores that method and populates the dataset to determine whether or not the method is valid and practical.

3.6 Water Quality Factors on Disinfection Performance

Water treatment plants struggle with monitoring disinfection performance mainly due to the required down time between testing and obtaining results for microbiological quantification. For this reason, proxy parameters must be used as a "best guess" approach for how rugged their disinfection treatment must be.

Typical UV-T readings are obtained by using 254nm light. A light source is shone through the water sample and the amount of light received at the other end of the sample is used to determine the percentage of light that was able to pass through unimpeded (UV Pure, 2012) (RealTech Incorporated, 2015). Ultraviolet Transmittance (UV-T) is most commonly used as the indicator for disinfection intensity requirement. The reason is two-fold; firstly, a water sample that has high microbiological loading will likely become cloudier and ergo reducing the UV-T value, secondly, since 254nm light is the optimal resonant wavelength

for nucleic acids microorganisms will absorb the incident light preventing if from reaching the receiving end of the UV-T equipment.

However, UV-T is not as reliable an indicator as these reasons may make it seem. Some pathogens and bacteria do not resonate at 254nm so they may not be affected by the incident radiation, or low UV-T values may simply be due to suspended solids in the water (Grun, Bowles, Gillis, & Wang, 2010) (Kunapareddy, et al., 2015).

For this reason, many water treatment plants use a very high dose of UV light to ensure proper disinfection; higher even than may be suggested by UV-T – contamination correlations. There is a 'better safe than sorry' mentality to some disinfection processes since, short of this research, real-time performance monitoring is as of yet not possible.

Since UV-T may not be as reliable a water quality parameter as hoped for indicating required disinfectant dosage it is intended to examine several other parameters for correlations.

Three water quality parameters were chosen for their hypothesized interference with ultraviolet disinfection. These parameters were amino acid, organic matter content (or Natural Organic Mater, NOM), and turbidity. Amino acid is the main constituent of proteins and are a crucial part of all living organisms (Reece, et al., 2013).

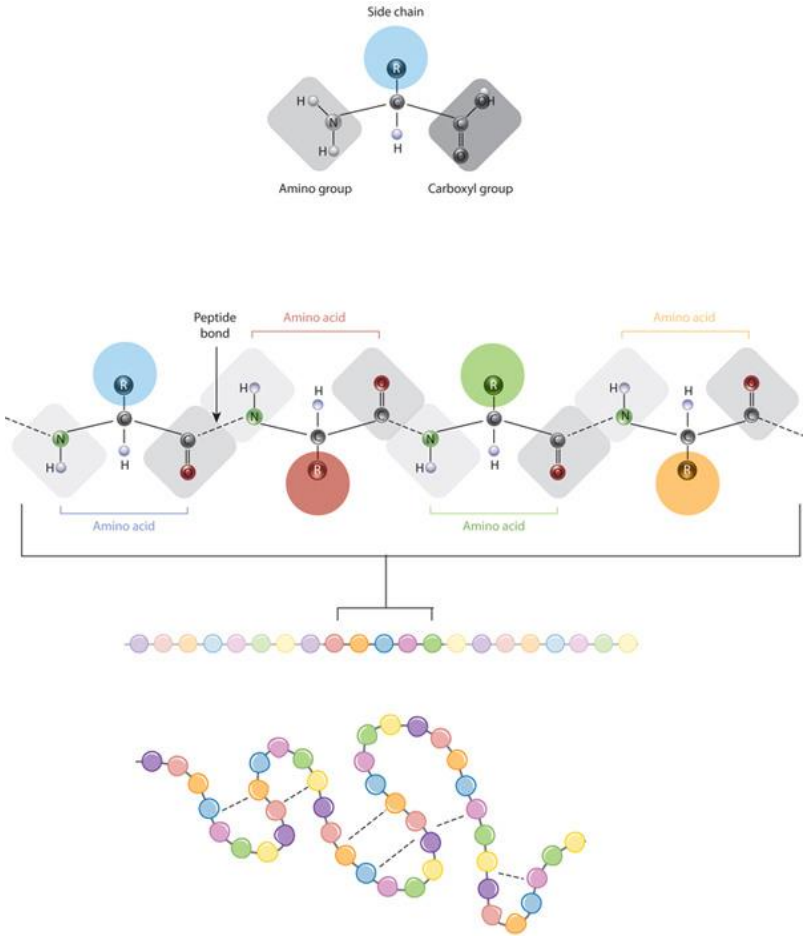

Figure 6 Amino Acid Structure and Protein Formation (Scitable by Nature Education, 2014)

Since amino acids form proteins, which are found in living cells, it is worth investigating whether the presence of the acid alone will affect the disinfection by taking the place of the intended recipient of the radiation (the contaminant microbes).

NOM was chosen for its structure. One of the main components of organic matter found in surface waters is humic acid. This is important because humic acid is comprised mostly of aromatic rings and these also happen to resonate with light emitted at 254nm (Pettit, 2004) (Yu, Kim, Han, & Kim, 2005) (Rodrigues, Brito, Janknecht, Proenca, & Noguiera, 2009).

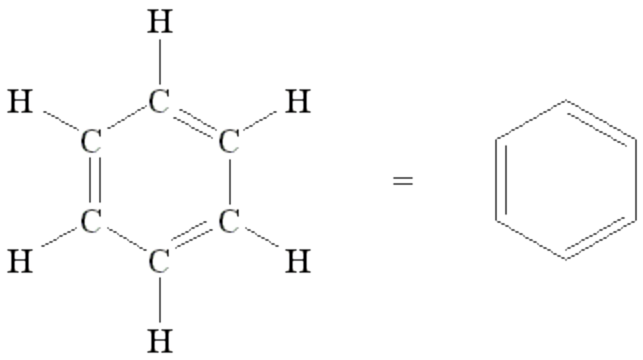

Figure 7 Structure of a Typical Aromatic Ring (Benzene) (Angelo State University, 2016)

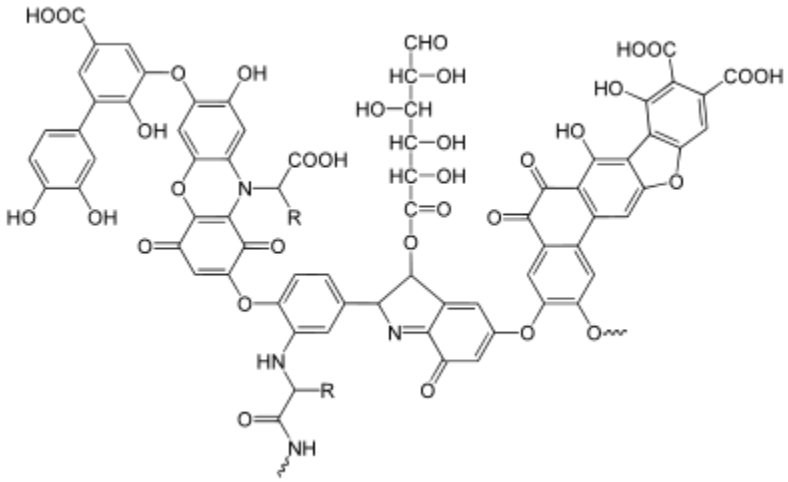

Figure 8 Structure of Humic Acid (Stevenson, 1994)

Naturally as the structure of humic acids absorb the same wavelength of UV radiation as the disinfectant radiation then a reduction in successful disinfection should be seen.

Finally, turbidity was chosen as an indicator parameter to be studied for its potential to shield the microorganisms. Shielding refers to the protection of microbes by getting in the way of the incident radiation (Winward, Avery, Stephenson, & Jefferson, 2008). The microbes are able to survive by being in the 'shadow' or behind the particulate matter that may be in the water samples and so are not affected by the radiation (Kollu & Ormeci, 2012). The mechanism of shielding may involve simple reflection of the incident

radiation back towards the source, or as complex as microorganisms being able to protect themselves in the nooks and crannies of the surface of the solids (Government of Canada, 2014).

Chapter 4 - Materials and Methods

4.1 Experimental Design

All testing to fulfill the research objectives was completed at Dalhousie University, Halifax, Nova Scotia in the Water Quality Research Laboratory. The measurement and testing of water quality parameters was carried out in the main section of the laboratory while the biological tests (i.e., ATP and HPC procedures) were carried out in the secured microbiology section of the lab.

Real water samples were used rather than synthetic water (DI water spiked with a contaminant) in order for the method validation to more accurately represent practical applicability. However, for the parameter study on disinfection performance synthetic water was used. This was favourable over real water samples in order to verify that any change in disinfection performance was due solely to the parameter in question and there was no interference or contribution from another component in the water matrix.

Throughout the experimental processes deionized water was obtained from an ultrapurification system from Millipore with a resistance of 18.2 MΩcm. All glassware was thoroughly cleaned in a sanitizing dishwasher as well as autoclaved if their intended uses brought them in contact with microbiological organisms.

4.2 Sample Collection

Wastewater samples were collected twice weekly from February of 2015 until May of 2015. Three wastewater treatment facilities in the Halifax Regional Municipality in Nova

Scotia, Canada were sampled from. These treatment plants were in Herring Cove, downtown Halifax, and Dartmouth.

All three facilities use a bank of UV lights suspended into the effluent flow, Halifax using dual banks in parallel to split the volume served. Herring Cove is relatively small operation having a capacity of 20 MGD; a median UV-T of 78, and average flowrate of 87 L/s during the sampling schedule. Dartmouth's capacity is 50 MGD; over the same time period their median UV-T was 60, and their average flowrate was 449 L/s. Halifax serves the largest population with a capacity of 90 MGD; again over the sampling schedule their median UV-T was 60, and the average flowrate was 749 L/s.

The effluent from all three plants had undergone screening, coagulation, and clarification with the UV disinfection process being the final treatment before discharge to the receiving waters. One litre of effluent immediately prior to the ultra-violet disinfection process and one litre of effluent directly after UV treatment was collected in sterile glass bottles.

The samples were returned to the laboratory and tests were performed on the same day to avoid significant changes in biological activity.

4.3 Water Quality Measurements

Each sample had its ultra-violet transmittance (UV-T), pH, chemical oxygen demand (COD), turbidity, total suspended solids (TSS), and temperature measured in the laboratory. These are common parameters used across the industry for comparative purposes and quantitative reference.

Ultra-violet transmittance (UV-T) data were recorded in triplicate for each sample to ensure accuracy. They were measured using a spectrophotometer (HACH DR 5000, London, Ontario) following their programmed method 10243.

An Fisher Scientific accumet Excel XL50 pH probe was used to measure pH of each sample. The samples were continuously stirred using a magnetic stir bar and the value was read when the readout reached a stable value. The probe had a built-in temperature gauge and ergo the same unit was used to measure temperature of each sample.

COD measurements were taken following the standard method 5220 D found in American Public Health Association's Standard Methods (American Public Health Association, 2012).

Turbidity was measured on a HACH Turbidimeter (2100AN, London, Ontario). Approximately 10 mL was decanted into a glass cuvette immediately after thoroughly stirring the sample to fully suspend any particulate matter. The highest value was recorded as the true turbidity as settling of the particulates will cause a decrease in turbidity as time progresses.

Standard method was followed for measuring TSS (American Public Health Association, 2012) wherein a specific volume of sample was filtered through glass microfiber filter paper under vacuum. The filter paper was oven dried overnight at 120°C and weighed prior to filtration of the sample. Following filtration, the paper was oven dried a second to remove adsorbed water and weighed again. The difference in weight gives the mass of suspended solids in the volume of sample filtered expressed as a mass per unit volume.

In addition to the parameters measured in the laboratory; in-line pH, turbidity, temperature, and TSS values measured at the point of sampling by the treatment plants were recorded. Lastly the disinfection parameters at each plant were tabulated. They included UV dose (in millijoules per centimeter squared, mJ/cm^2), effluent flow rate and retention time through the UV system (litres per second, and seconds respectively), and UV light intensity (in milliwatts per centimeter squared, mW/cm^2).

The disinfection efficiency of the plants was determined by comparing the number of surviving microbiological organisms to the original population. This efficiency value was obtained using the standard HPC methods and the efficiency found using the cellular ATP ratio was used afterwards to ensure applicability of the method.

The disinfection efficiencies were evaluated against other process information such as turbidity, UV-T, and Dose. These potential relationships would help in understanding disinfection performance and water quality following treatment.

4.4 Biological Activity Measurements

The main concern of this research is with biological testing and monitoring. The water quality parameter testing detailed previously was conducted mainly for correlative purposes and for database building.

The disinfection efficiency of the plants was determined by comparing the number of surviving microbiological organisms to the original population. This efficiency value was obtained using the standard HPC methods and the efficiency found using the cellular ATP ratio was used afterwards to ensure applicability of the method.

The disinfection efficiencies were evaluated against other process information such as turbidity, UV-T, and Dose. These potential relationships would help in understanding disinfection performance and water quality following treatment.

Heterotrophic plate count is the microbiological concentration quantification method most standardly used and was ergo used in this research to compare the new method to.

The new method consists of measuring ATP levels but with a novel procedure that reduces the wait time for results and is meant to increase applicability to water treatment utilities for compliance monitoring. It subjects the samples in question to a four hour incubation time at 40°C in a quarter strength TSB (1 part TSB, 3 parts milli-Q water).

4.4.1 Heterotrophic Plate Count

In order to determine concentrations of microbiological organisms in both the before UV treatment (BUV) and post UV treatment (PUV) samples standard method HPC was used. This method was employed because of its pervasiveness in the industry as well as its virtually universal acceptance as a strong indicator for actual microbiological activity (World Health Organization, et. al., 2003) (American Public Health Association, 2012).

All equipment involved was sterilized prior to use by using a Steris AMSCO small steam sterilizing autoclave. In a biosafety cabinet, serial dilutions were performed on all samples down to the desired dilution factor. The magnitude of dilution for the BUV samples was 10^{-3} while for the PUV it was 10^{-2}. Initially a dilution range of 10^{0} (raw sample) to 10^{-5} was used in order to determine the typical range of microbiological concentrations found in the samples we were examining. The serial dilutions were performed by pipetting one millilitre of the raw sample into 9 mL of phosphate buffer solution (PBS) and then one millilitre of this ten-fold diluted solution into another tube of 9 mL of PBS and repeating. While performing the dilutions the top section of the tube was subjected to flame from an ethanol burner to ensure sterilization as well as discourage air currents from entering the tube while it was uncapped. The tubes were mixed thoroughly via vortexer after each dilution for maximum dispersion of wastewater sample in the buffer solution.

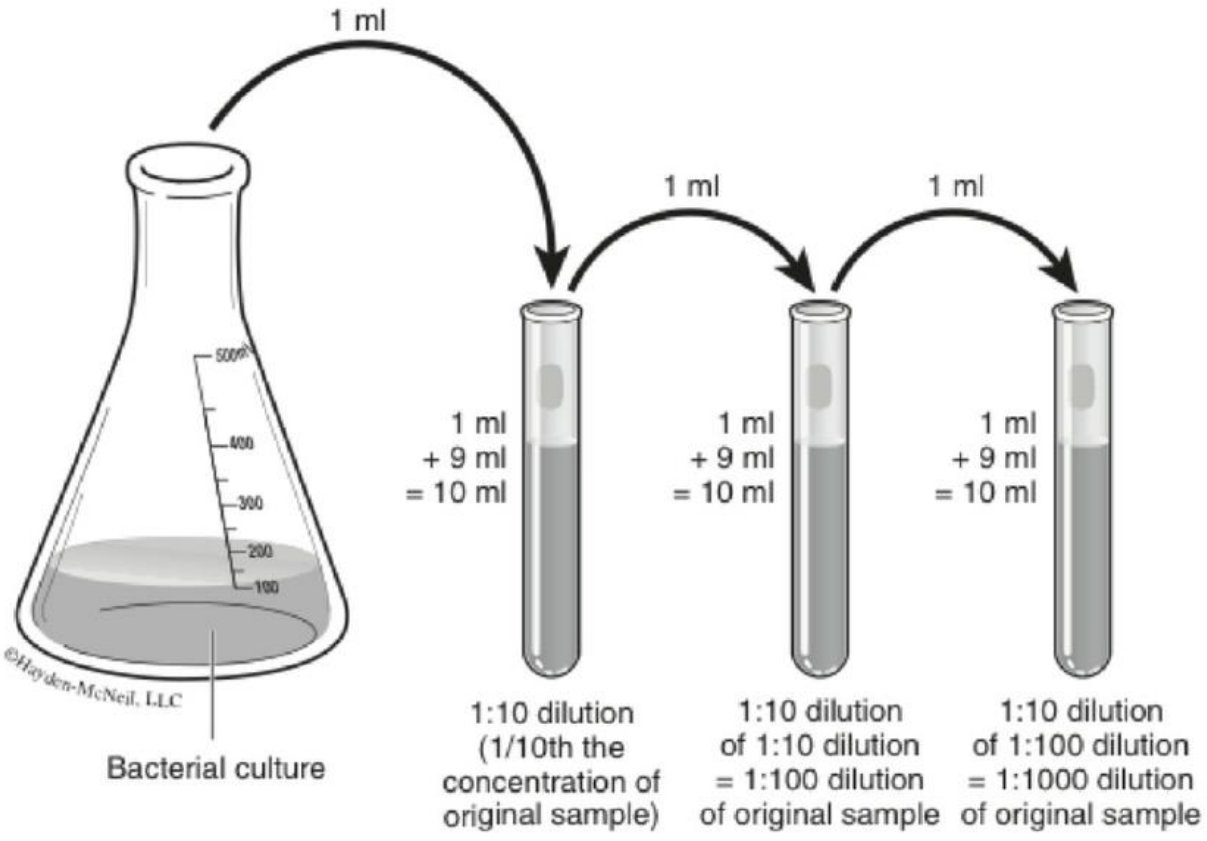

Figure 9 Serial Dilution (Hester, Sarvary, & Ptak, 2014)

Once the dilutions were complete 0.1 mL of raw sample was pipetted onto R2A agar plates (triplicates were made) and spread with a sterilized glass spreader. Then 0.1 mL of the first dilution was pipetted onto R2A agar plates and continued for each dilution of each sample. The plates were incubated at 28°C for seven days and then counted.

4.4.2 Adenosine Triphosphate Testing

ATP concentrations in the BUV and PUV samples were measured using LuminUltra's© Quench-Gone Aqueous (QGA) testing kits (LuminUltra, 2015). These kits arrive with all the materials required to perform the test already sterilized, as well as the chemical reagents.

QGA tests were done on all raw samples collected from the WWTPs and again on the samples that were subject to the new incubation method by following the procedure outlined in the instructional insert included in each kit.

A known volume of sample (dependant on the quality of the water being tested, as outlined in the test kit instruction booklet) is pushed through the syringe with a luerlock filter attached which retains the biomass. A lysing agent is then pushed through that same filter to rupture the trapped cells and release their ATP into a receiving dilution tube. 0.1 mL of this ATP solution is added to an assay tube and then 0.1 mL of the luminase enzyme is combined in the assay tube, swirled to mix, and put into a Kikkoman Luminometer to measure released light.

4.5 Parameter Specific Testing

In an effort to understand which physical characteristics of a water matrix affect disinfection, three separate parameters were studied. These parameters were amino acids, organic matter, and turbidity. These parameters were synthesized in the laboratory using tryptophan, humic acid, and kaolin clay respectively. These parameters in particular were chosen based on their hypothesized relationship to disinfection efficacy. The presence of amino acids can indicate pre-existing microbiological contamination since amino acids are a key component in the structure of proteins. Having an elevated 'background' concentration of microbes would naturally affect your disinfection process as it would increase the required dosage of ultraviolet light to sufficiently inactivate the contaminants to outlet water regulation levels. In addition, if there were not a pre-existing

microbial contamination the presence of amino acids may hinder disinfection performance still by simply absorbing the incident radiation itself and ergo keeping it from reaching the intended microbes.

As for organic matter its make-up of aromatic rings is what makes this constituent important. Humic acid is a principle constituent of organic matter and can be comprised of phenolic and carboxylic groups by mainly by large amounts of linked aromatic rings. It is well known that aromatic rings absorb light at 254nm very easily and since this is the typical wavelength used for disinfection is has a significant effect on the process. Since the organic matter and intended targets of the disinfecting ultraviolet light are in direct competition the efficiency of the process is significantly reduced with high concentrations of humic acid. This effect is well documented but it is intended to quantify the effect and compare it to the other parameters for reference (Johnson, Bao, & John, 2002) (Rodrigues, Brito, Janknecht, Proenca, & Noguiera, 2009).

Turbidity is expected to have similar effects as humic acid however instead of absorbing the incident radiation, it is expected that particulate matter suspended in a water sample (in this case the kaolin clay) will reflect the light back. In addition to the reflection of the incident radiation, it is likely that shielding of the microbes occurs simply by having them 'hide' behind the particulate matter. Since high levels of turbidity will cloud the water and reduce the UV-T of the sample, naturally the disinfection efficiency will decrease accordingly. The transmission of ultraviolet light is after all how the microbes in the bulk sample receive the radiation and are inactivated.

Testing these parameters consisted of spiking deionized water with *E. coli* then adding known concentrations of each substance prior to disinfecting the sample. Sufficient volume from vials of *E. coli* K 12 (ATCC#47076 Strain Designations: MG1655) that had been prepared by Xie (Xie, 2014) were removed from the -80°C freezer and allowed to return to room temperature. Once thawed the vials were incubated overnight at 35°C to encourage metabolic activity and return the bacteria to a neutral state. Following incubation, the vial was centrifuged to pelletize the bacteria. The supernatant was decanted, the pellet was rinsed with PBS to ensure there was no remaining TSB, then ~15 mL of PBS was pipetted into the tube and vortexed to resuspend the bacteria. This 15 mL of concentrated *E. coli* culture was diluted by adding it to 500 mL of PBS (or 3 mL per 100 mL, depending on volume needs) and this was the stock solution used for spiking.

For each test done for the parameter test, 30 mL of the stock *E. coli* solution was added to a petri dish and the parameter in question was added to that dish. Tryptophan, as well as humic acid, was added at 2mg/L and, an order of magnitude more, 20mg/L to ensure a effect would be noticeable if there was one. In the same vein kaolin clay was added to create a solution of 100 NTU and 1000 NTU.

The tryptophan was pipetted to the sample from a stock solution of 1000 mg_{Tryp}/L. Similarly, the humic acid was added from a solution of 400 mg_{HA}/L. Kaolin clay was slowly added to a bulk solution while turbidity readings were continuously read until the desired levels were reached.

In order to make appropriate observations of the performance of the water treatment facilities disinfection studies were carried out on synthetic water samples. In this case

synthetic water refers to the stock *E. coli* solution described previously. This way the 'standard' performance of disinfection processes can be determined due to the absence of interference from a real water matrix. In other words, eliminating the noise in data sets by guaranteeing the absence of unwanted particulate matter, chemical compounds, organic matter, etc. that may interfere with the disinfection of the microorganisms.

These experiments were conducted using a collimated beam unit from Calgon Carbon containing a 40W low pressure mercury UV bulb. This unit was chosen since it was desirable to subject the samples to a constant and quantifiable UV intensity.

The unit was engaged, allowed to warm up, and the intensity of the UV light emitted was measured using an ILT-1400 radiometer to ensure consistency throughout the experimental process. Petri dishes containing a magnetic stir bar and the sample being treated were placed on a stir plate within the irradiation area of the unit. While being constantly stirred the samples were treated for various lengths of time depending on the desired dose of UV radiation. The required length of time for each dose was calculated with the following set of equations:

$$E_{avg} = E_{cal} \times I_F \times P_F \times \frac{1 - 10^{-A_{1cm} \times d}}{2.303 \times A_{1cm} \times d}$$

$$t = \frac{D}{E_{avg}}$$

Where E_{avg} is the average irradiance (mW/cm^2), E_{cal} is the radiometer reading (mW/cm^2), I_F and P_F are the integration and petri factor respectively, A_{1cm} is the absorbance of the liquid per 1cm thickness, which is intrinsic to the fluid, d is the depth of the sample (cm).

The second equation denotes the exposure time, t (s), equalling the UV Dose, D (mJ/cm^2) divided by the previously calculated E_{avg} (Bolton & Linden, 2003) (Kuo, Chen, Nellor, & Kuo, 2003) (Blatchley, 1997).

Chapter 5 – Results and Discussion

5.1 Disinfection Baseline

Several UV doses that fell within the range of zero dose to the typical wastewater treatment plant application, were used to populate a performance curve based on incident radiation to the sample.

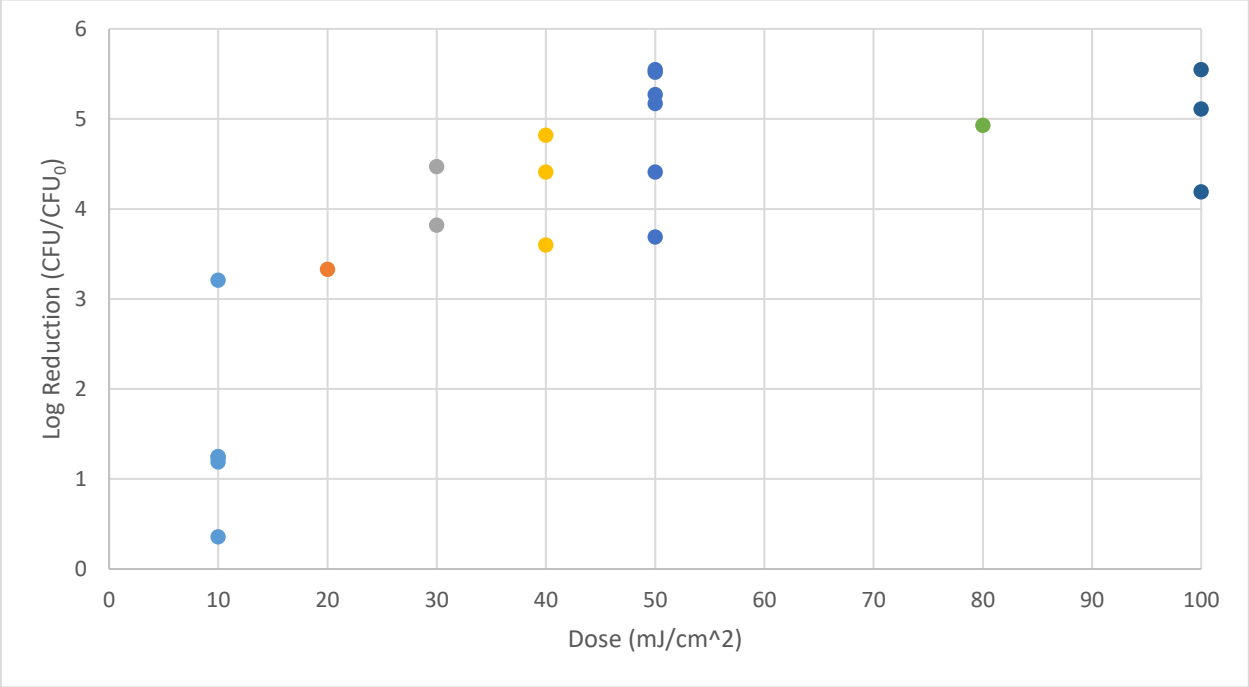

Figure 10 Standard disinfection performance of E. coli

Plotted above are the disinfection results at each dosage applied; 10, 20, 30, 40, 50, 80, and 100 mJ/cm^2. Each dosage was applied to five samples to observe a satisfactory trend, however from spoiling of agar, contaminated petri dishes, or from sample spillage some of the duplicates were discarded.

It can be seen from the trend that the optimum UV dose for disinfection lies around 50 mJ/cm^2 as the disinfection performance plateaus at this level and the higher dosages that follow, and ergo higher energy demand, does not improve the disinfection results. This dose yields a log reduction of E. *coli* of close to 5. However as stated this was synthetic water and ergo it should be expected that a higher dose would be required in practice to achieve the same log reduction. In addition, this result is for E. *coli* alone whereas water treatment plants would naturally be dealing with a myriad of microbiological concentrations. However, this dosage and disinfection relationship for E. *coli* is used in the research that follows for the parameter specific testing.

The heterotrophic plate count data for the water treatment plants corroborates with the expected need for higher dosage. In the following figure, it can be seen that the log reduction in contaminant concentration is nearly constant across the data set.

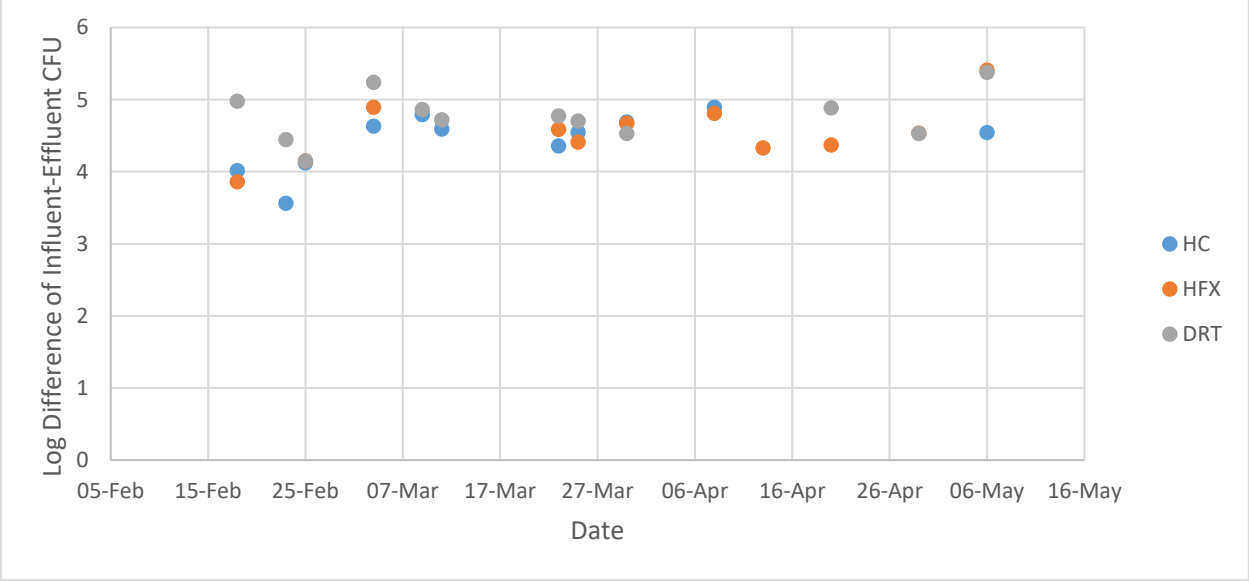

Figure 11 Disinfection Performance of Wastewater Treatment Plants (HC – Herring Cove WWTP, HFX – Halifax WWTP, DRT – Dartmouth WWTP)

The decrease in microbiological activity hovers around 4.5 for all three of the treatment plants across the data set. This is nearly the same value as the "optimal" disinfection value found for the synthetic water spiked with E. *coli*. However, the dosages required to achieve this reduction was much higher across the board.

	Ultra-Violet Dose Applied (mJ/cm^2)	
Water Treatment Plant	Mean	Median
Herring Cove (HC)	117	118
Halifax (HFX)	186	180
Dartmouth (DRT)	86	74

Figure 12 Ultraviolet Dosage of Wastewater Treatment Plants

5.2 Analysis of the Proposed Rapid Testing Method Data

These log difference values in HPC data were calculated by plating the 'before UV' exposure and the 'after UV' exposure samples individually. These difference in concentrations between the samples was determined and logged in the base of ten.

These data were compiled mainly as a comparison tool for the validation of the proposed ATP testing method. The HPC method has been exhaustively used for microbiological quantification and research and is a widely accepted standard. For this reason, it will be our benchmark for ensuring the new method is performing correctly.

Using ATP to test microbiological activity has been used in the past but combining the testing with our proposed incubation method has not been done. To qualify and quantify the method's value ATP testing was done on the samples without being subject to the innovative method, and the same tests were done while in combination with the proposed method to better understand it's contribution.

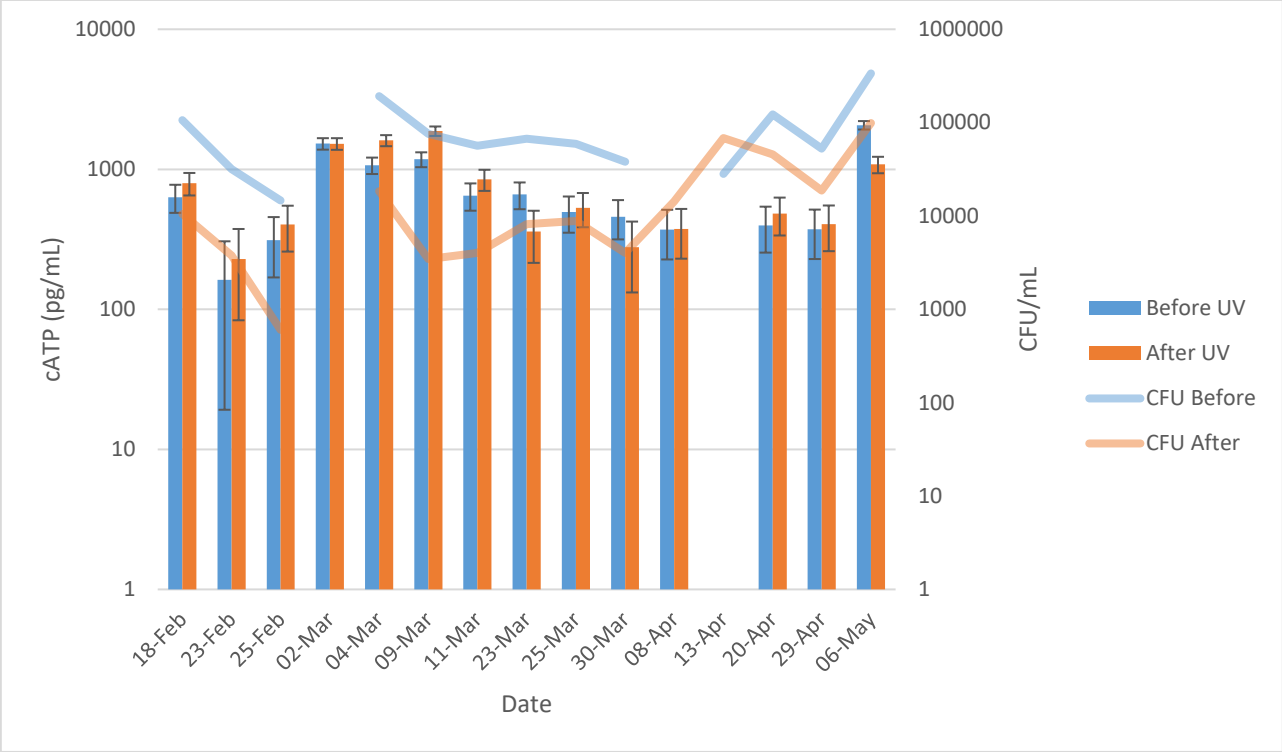

Figure 13 ATP Testing - No Incubation, Dartmouth Plant

Critical observations from this set of data is that the ATP values both before and after UV are relatively equal. This was expected as explained by the VBNC research that has been carried out, as well as general knowledge of the function of ultraviolet radiative disinfection; the microbiological organisms have been inactivated but are still living and ergo still release ATP when their cells are lysed. Note that data from the thirteenth of April

is missing as the agar that was prepared for the culturing of this day's samples had been contaminated.

In addition, it is important to note that the 'after UV' ATP data do not follow the same trend as the HPC results. This suggests that simply testing ATP levels post-disinfection will not yield results that accurately described the efficiency the process.

Encouragingly, when we introduce the same samples to the incubation grow-out method the results change to a more representative set.

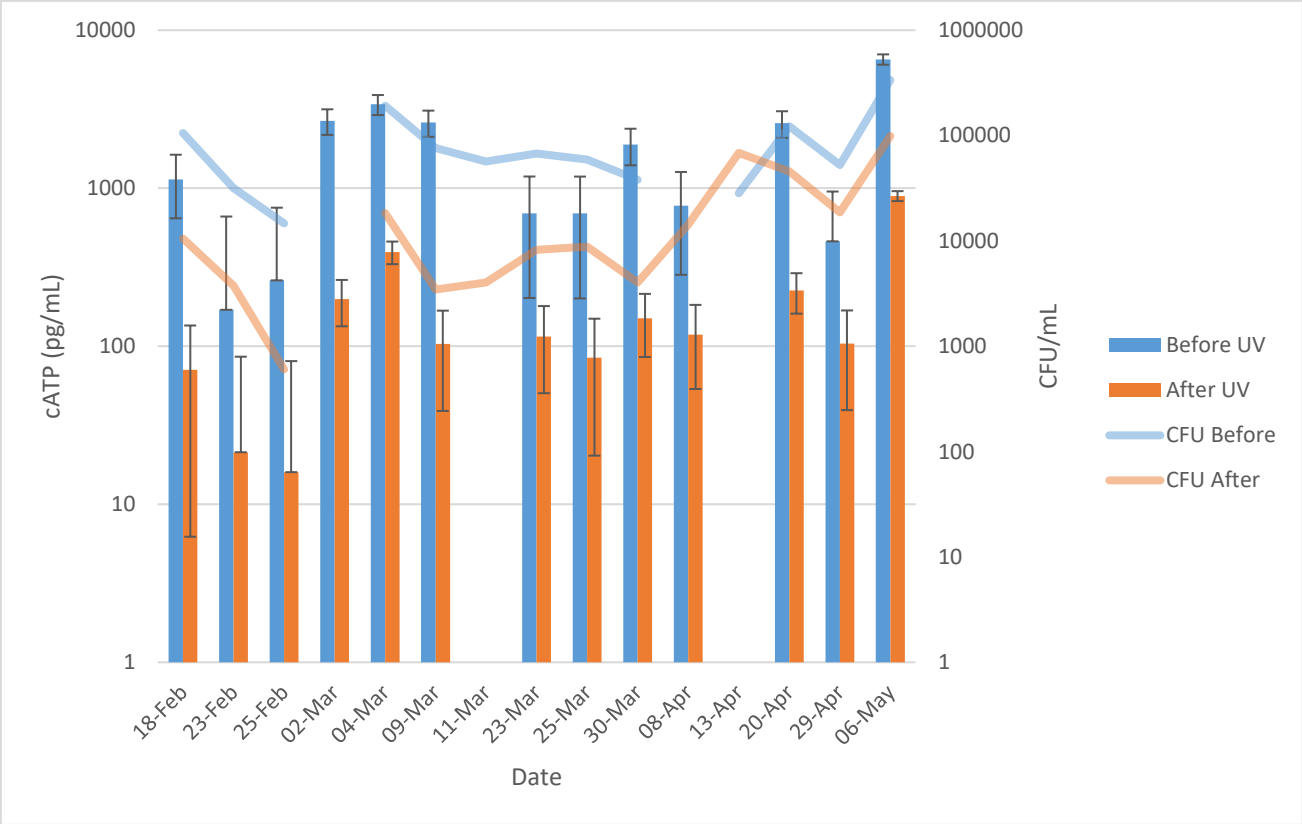

Figure 14 ATP Testing - Incubated, Dartmouth Plant

The first significant change in the data set is the drop in ATP concentrations in the 'after UV' samples. This would seemingly better represent the actual disinfection performance of the plant. This is reinforced by the fact that the 'after UV' ATP data now follow the same trend line as the HPC data whereas without the incubation method they did not.

The same data set was collected and analyzed for the Herring Cove and Halifax plants with the same statistical analysis being performed to evaluate the applicability of the method at each plant individually.

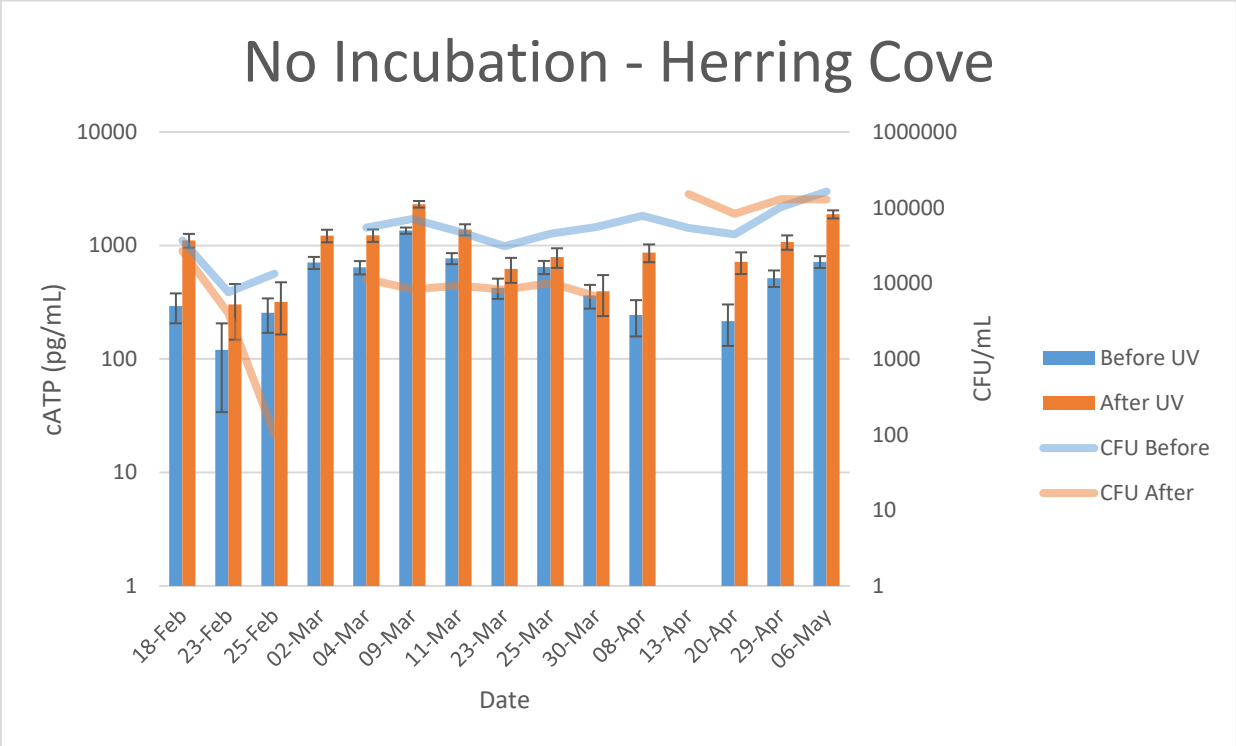

Figure 15 ATP Testing - No Incubation, Herring Cove Plant

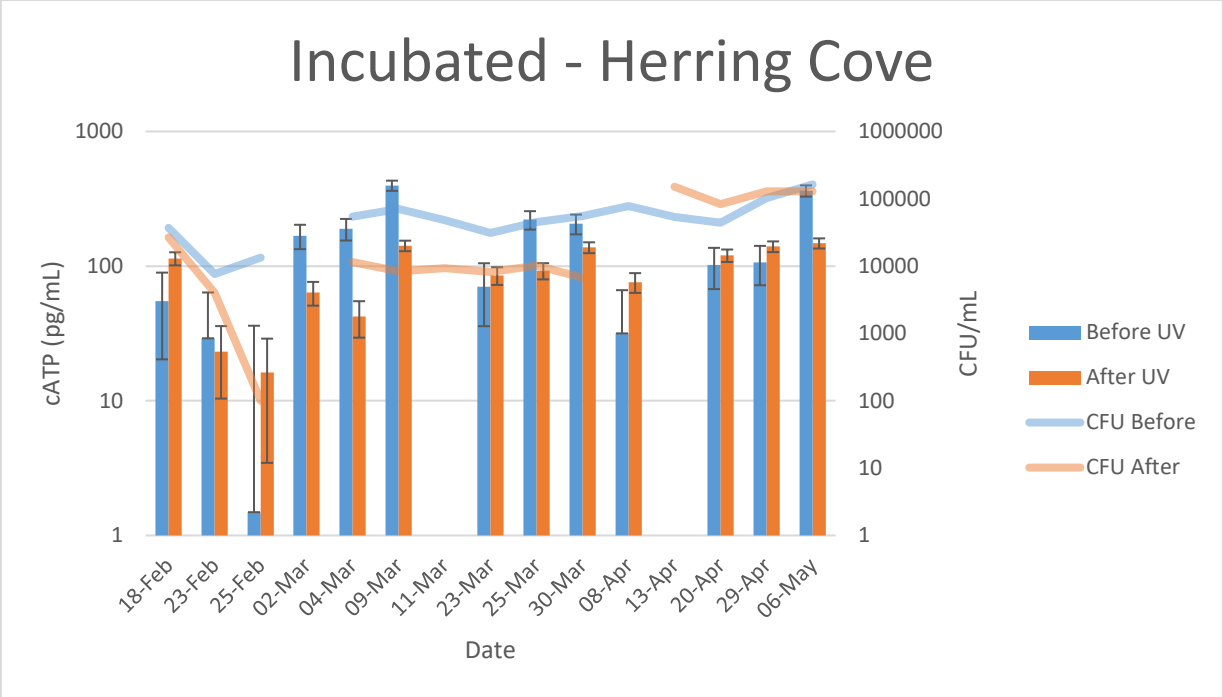

Figure 16 ATP Testing - Incubated, Herring Cove Plant

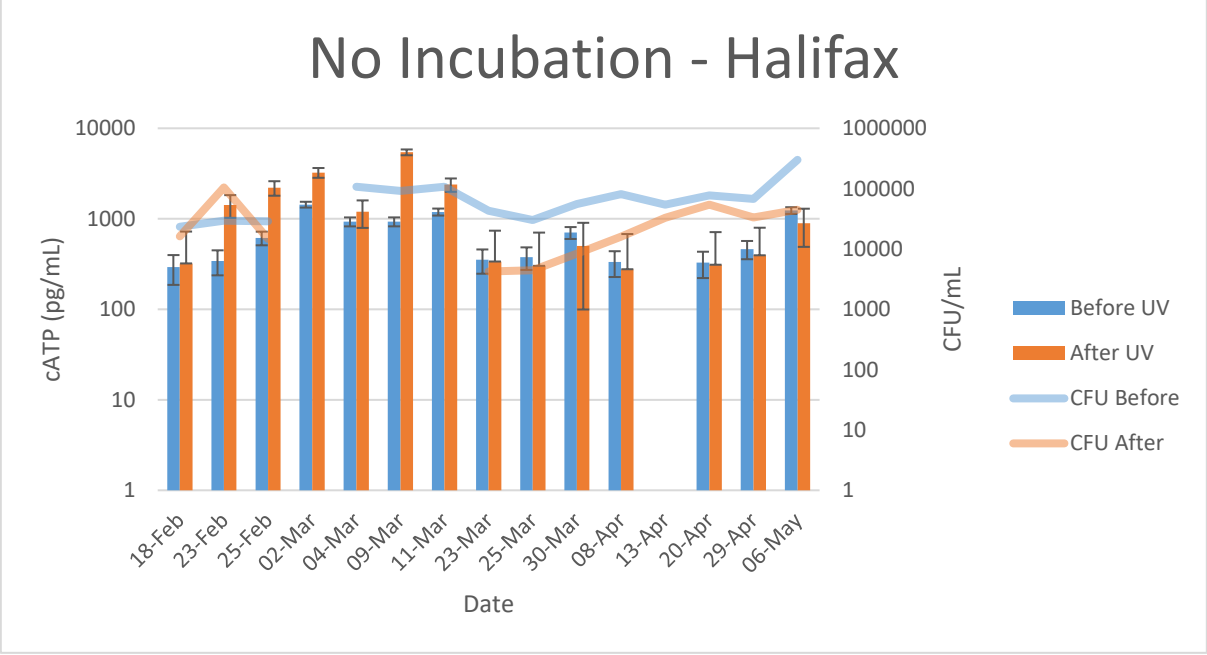

Figure 17 ATP Testing - No Incubation, Halifax Plant

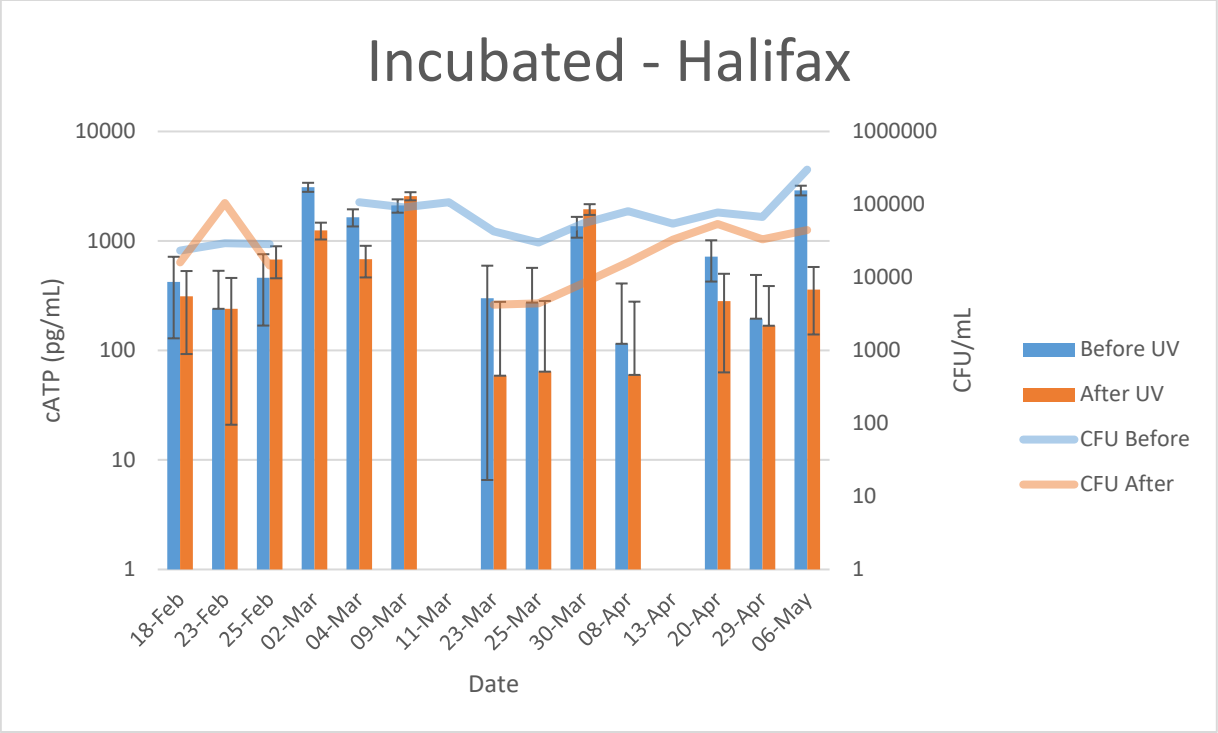

Figure 18 ATP Testing - Incubated, Halifax Plant

To better demonstrate the validity and applicability of the proposed method a direct comparison of influent biomass loading to effluent biomass was conducted. On this graph, the logarithmic value of the influent cellular ATP levels were plotted along the x-axis and the effluent (after UV disinfection) cellular ATP is plotted along the y-axis.

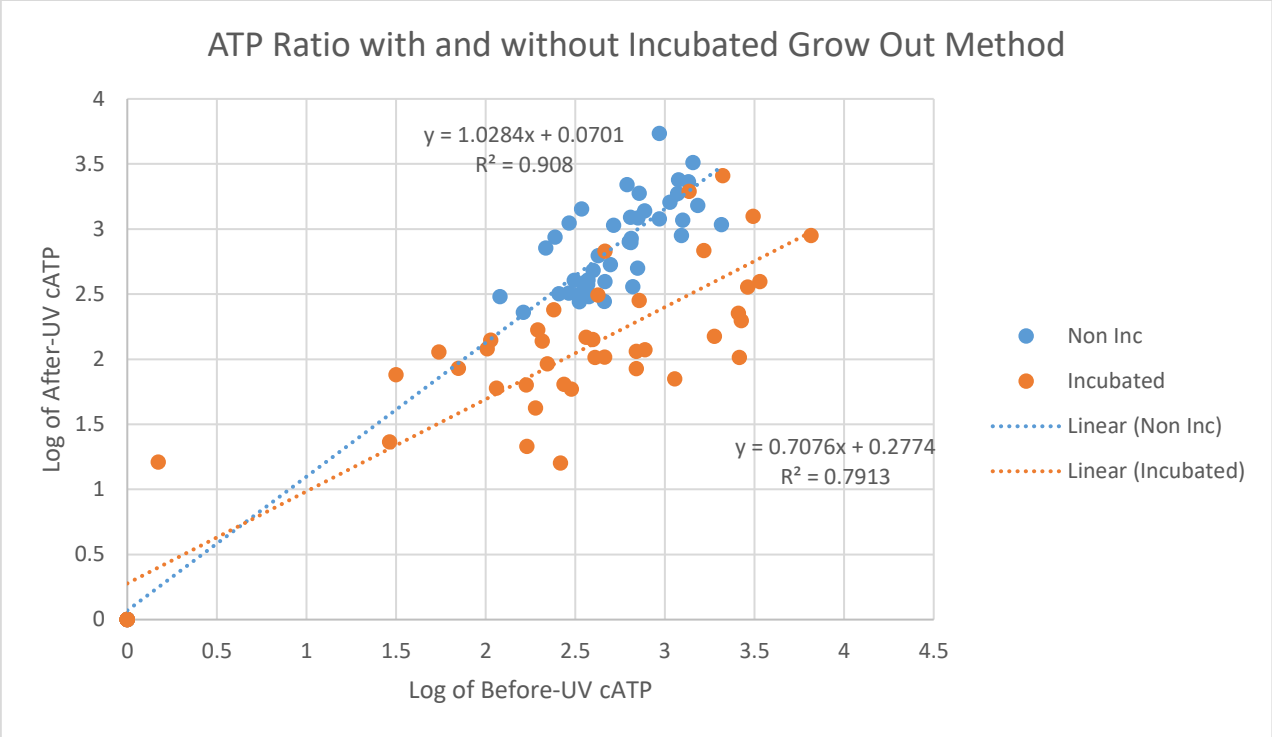

Figure 19 Validation of Proposed Grow Out Method

This graph is very encouraging as it clearly shows the incubation method deviating from the traditional method in an informative way.

Without the incubated growout method the before UV and after UV data are in essence equal. The data follow a y=1.028x line. This means that if the ATP data were used to monitor disinfection performance the results would show “no disinfection is being performed”. However, with the incubated grow out method the effluent, after UV, data deviate from that y=x line and show instead that the treated water has less living biomass than the influent water which is the desired result. Applying the method yields results where the effluent is 30% less biologically active than the influent, y=0.708x suggesting disinfection actually did occur.

An important note as well is that the observable difference in the grow out method becomes more and more pronounced and informative as the influent biomass loading increases. At a 3-log influent concentration the difference in the methods is almost a full log and only increases as influent loading increases.

5.3 Water Quality Effects on Disinfection

The disinfection performance of the waste water treatment plants was calculated as an efficiency based on the ratio of CFU of the 'after UV' samples to the CFU of the 'before UV' samples. The surviving concentration of microbes divided by the initial concentration would give a survivability ratio so subtracting that from unity gives a 'kill' ratio or disinfection efficiency.

$$\eta = 1 - \frac{CFU_A}{CFU_B}$$

Efficiencies were calculated for each set of samples (i.e. each day the plants were visited) and plotted against several of the recorded water quality parameters to check for correlations or causal relationships.

It would be expected from literature, as well as from the synthetic water spiked with E. *coli* tests, that higher ultra-violet doses would result in higher disinfection efficiency. This was however not the case for all three of the treatment plants.

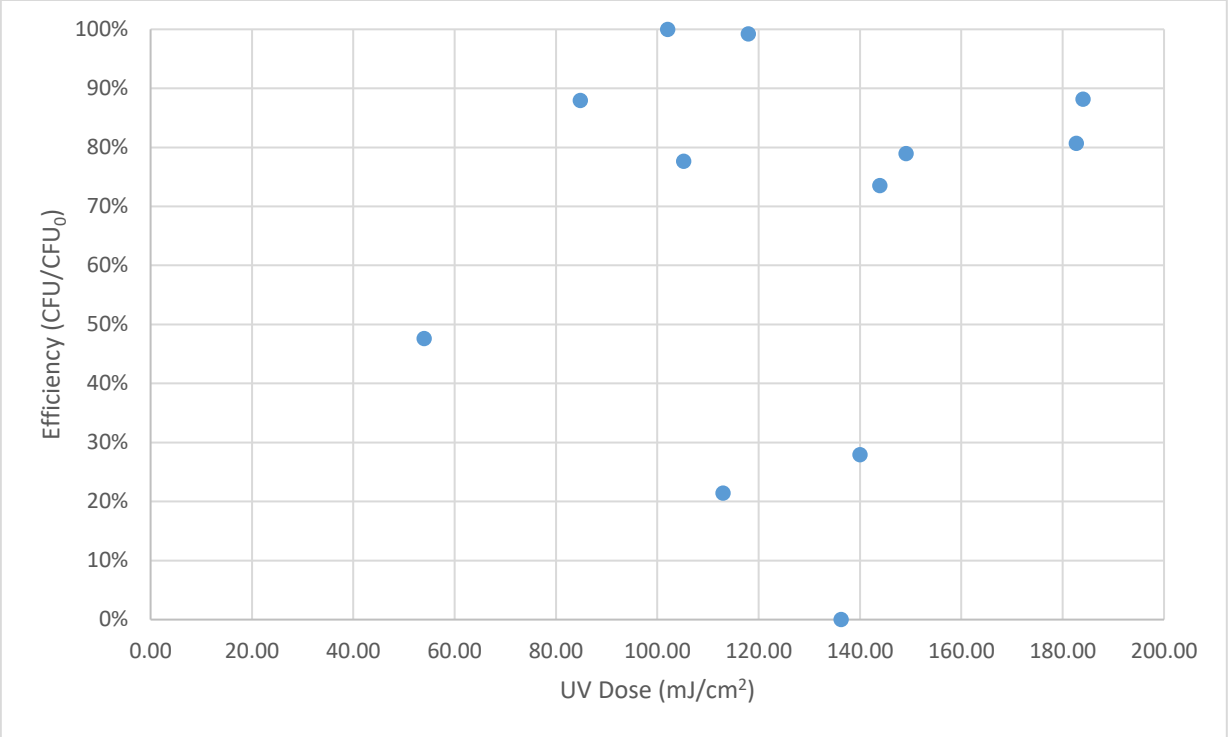

Figure 20 UV Dosage Effect on Disinfection Performance at Herring Cove WWTP

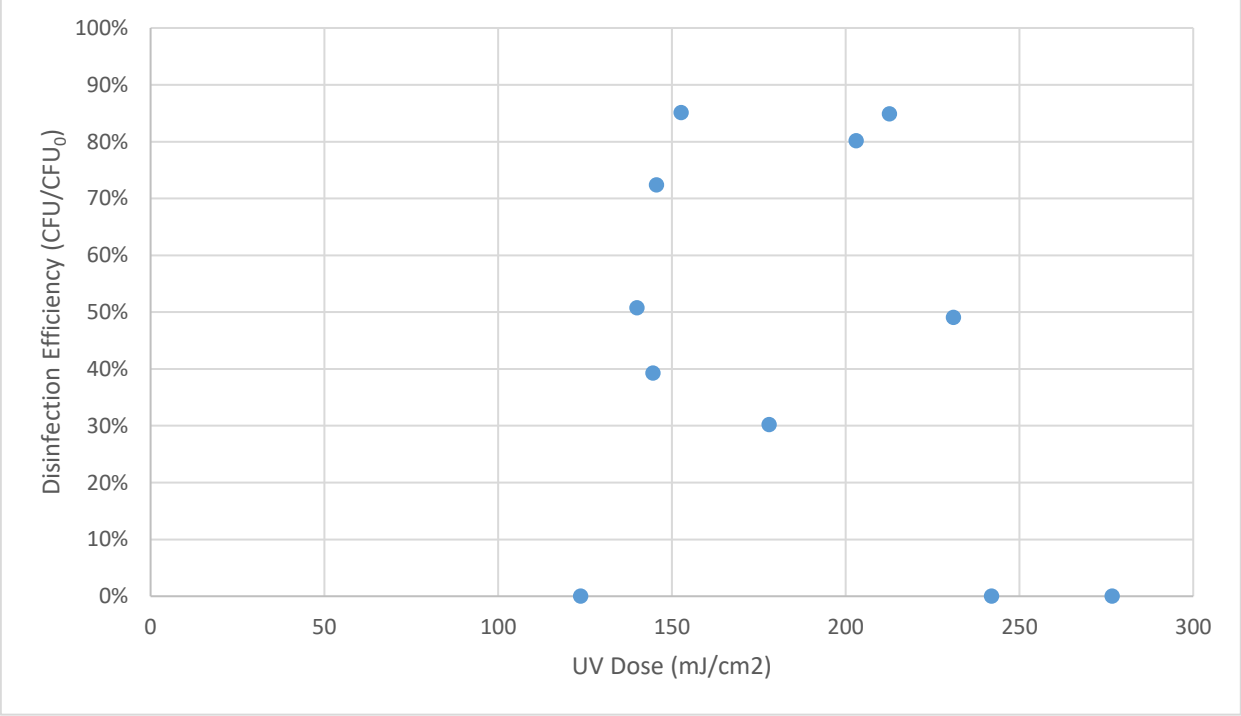

Figure 21 UV Dosage Effect on Disinfection Performance at Halifax WWTP

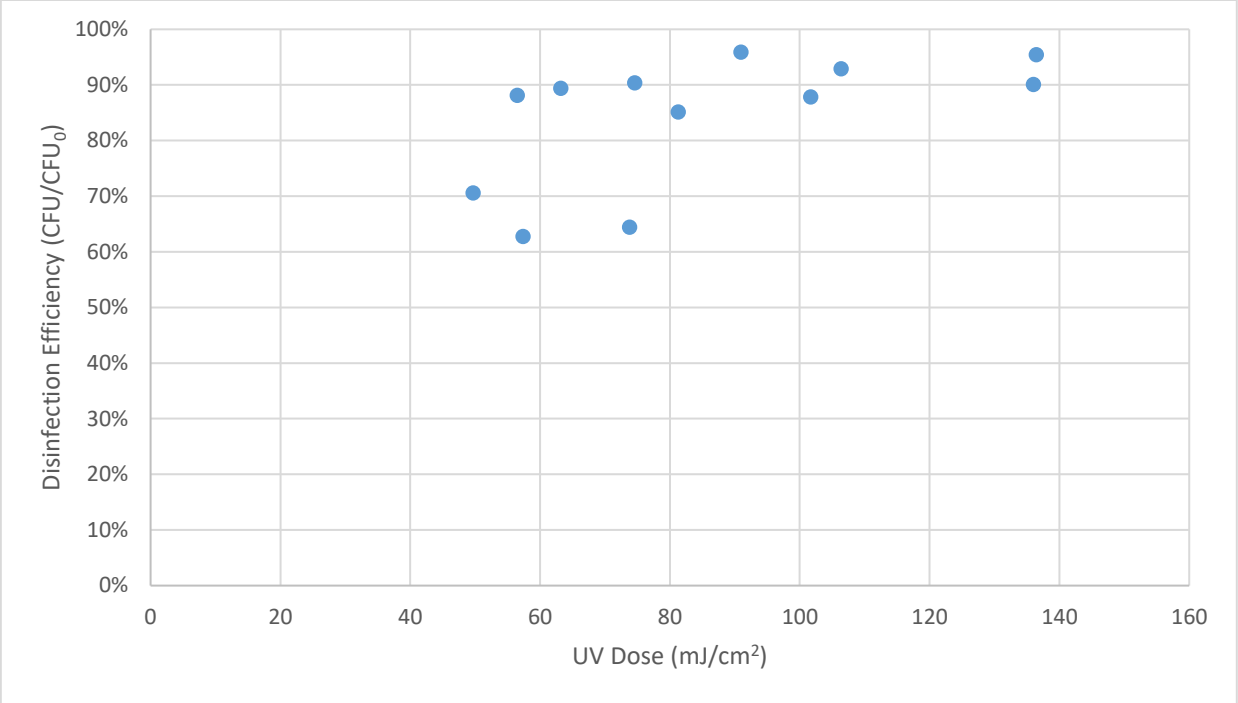

Figure 22 UV Dosage Effect on Disinfection Performance at Dartmouth WWTP

As can be clearly seen none of the plants exhibit the same relationship between dose and disinfection efficiency as was found with the synthetic water. This was not altogether unexpected as real water matrices contain innumerable other compounds that may interfere with the disinfection process. This interference can be effectuated in many ways as previously discussed.

Since experiments were carried out to analyze the effect of turbidity and absorbance (UV-T) using kaolin clay and humic acid these water quality parameters were graphed against the disinfection performance of the disinfection process. The parameter values were recorded at the water treatment plant at the time of sample collection. Since the plant

can’t collect amino acid, protein, or any similar concentration in line that parameter was studied solely in the lab.

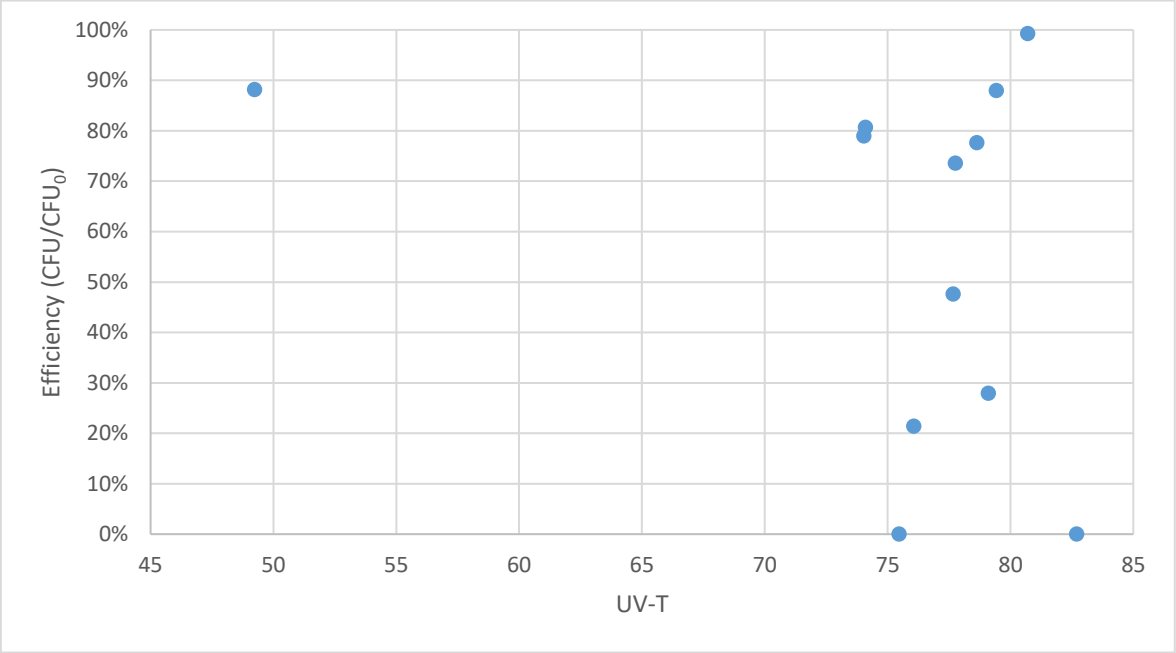

Figure 23 Disinfection performance as compared to ultraviolet transmittance at the Herring Cove WWTP

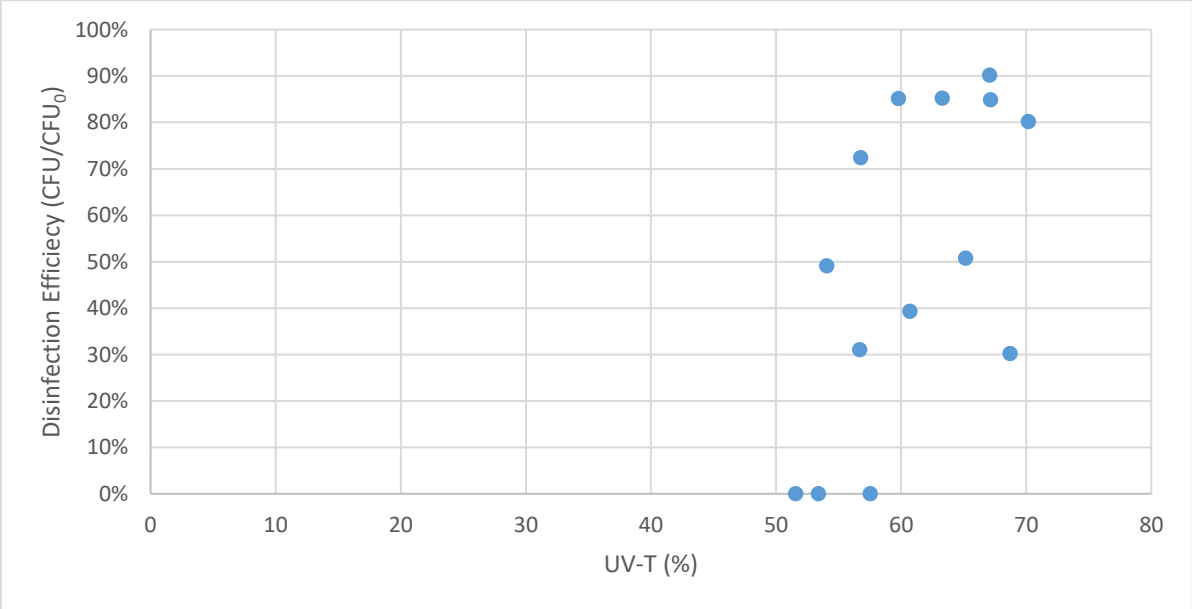

Figure 24 Disinfection performance as compared to ultraviolet transmittance at the Halifax WWTP

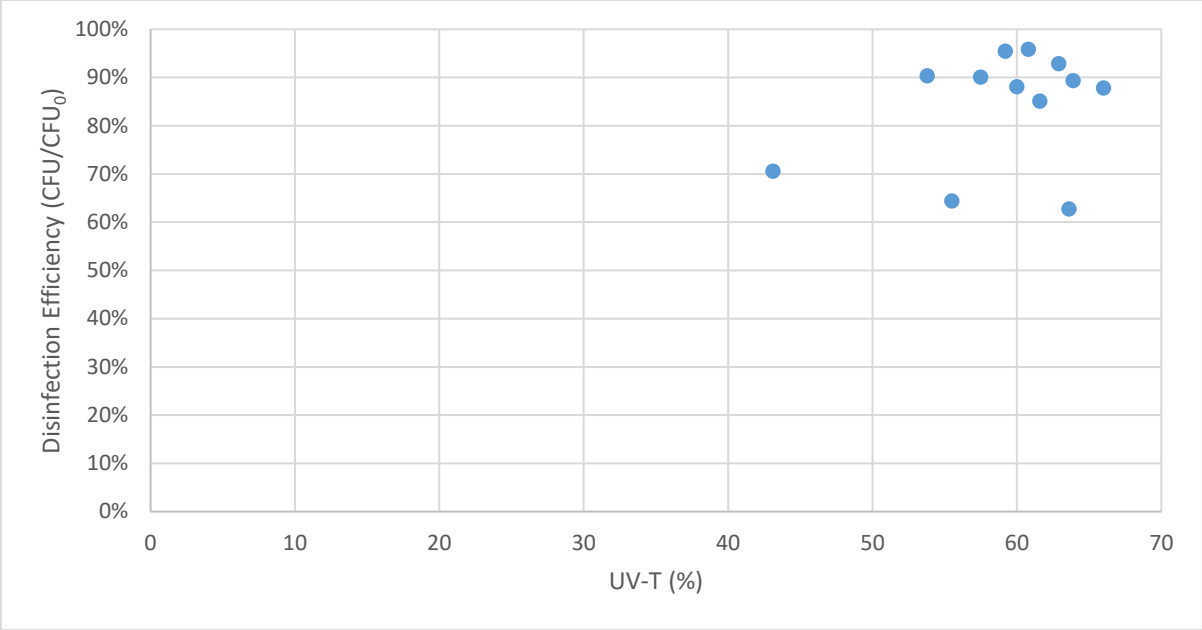

Figure 25 Disinfection performance as compared to ultraviolet transmittance at the Dartmouth WWTP

It is clear from the compiled data that UV-T had a limited effect on the disinfection performance. The hypothesized downward trend of microbiological kill to transmittance levels was not seen in any of the WWTP. Even though the transmittance did not seem to play a role in the performance of the disinfection process at the water treatment plants there are several factors to be considered, primarily the effects of the water matrix; how the other constituents in the water being treated interacted with each other and the incident radiation to affect the disinfection. For this reason, the role of UV-T alone will be examined later, as with the presence of amino acid and humic acid.

For the sake of completeness and for reference, the trend between disinfection performance and turbidity levels was also analyzed. This was done since the turbidity data is constantly monitored at the WWTPs as well as the fact that turbidity is the main cause of decreased UV-T values. The question was whether suspended solids in the water

affected the disinfection more than other constituents in the matrix and these other constituents were the reason for the lack of trend between disinfection performance and UV-T.

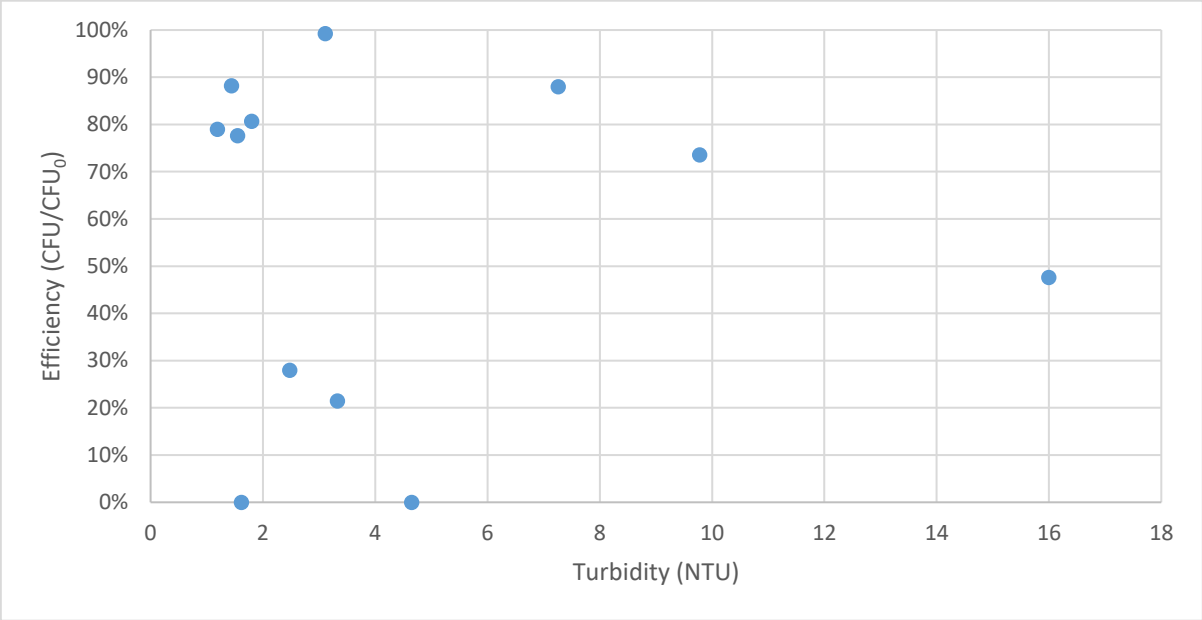

Figure 26 Disinfection performance as compared to turbidity levels at the Herring Cove WWTP

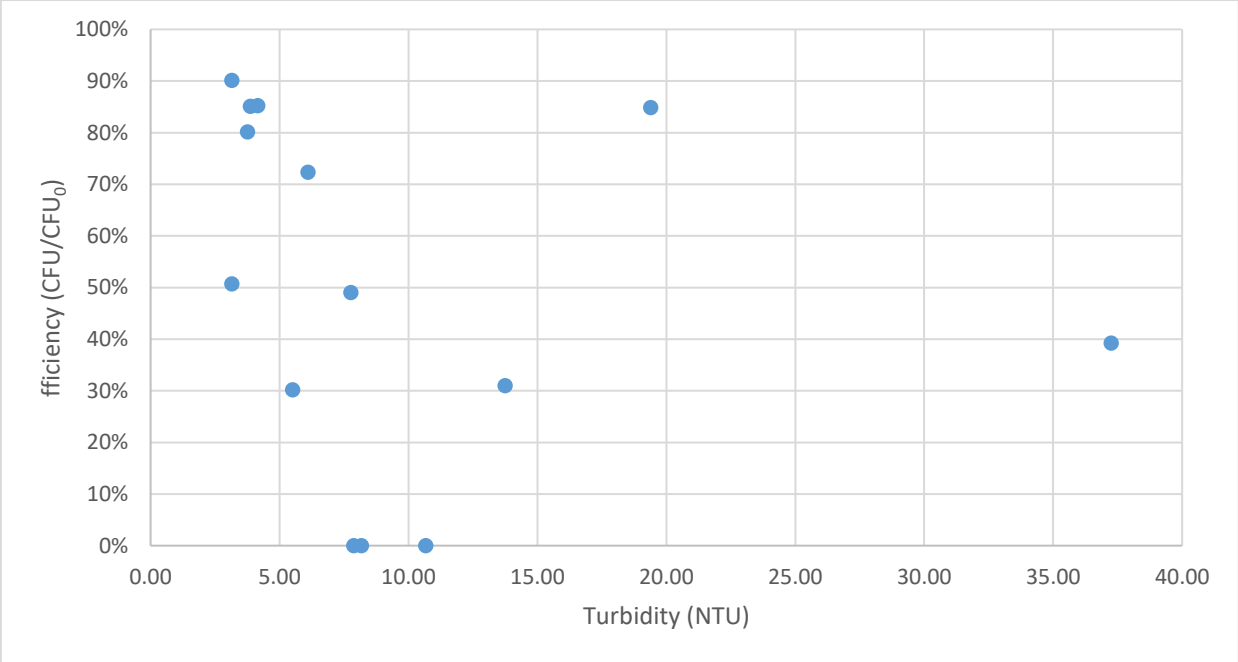

Figure 27 Disinfection performance as compared to turbidity levels at the Halifax WWTP

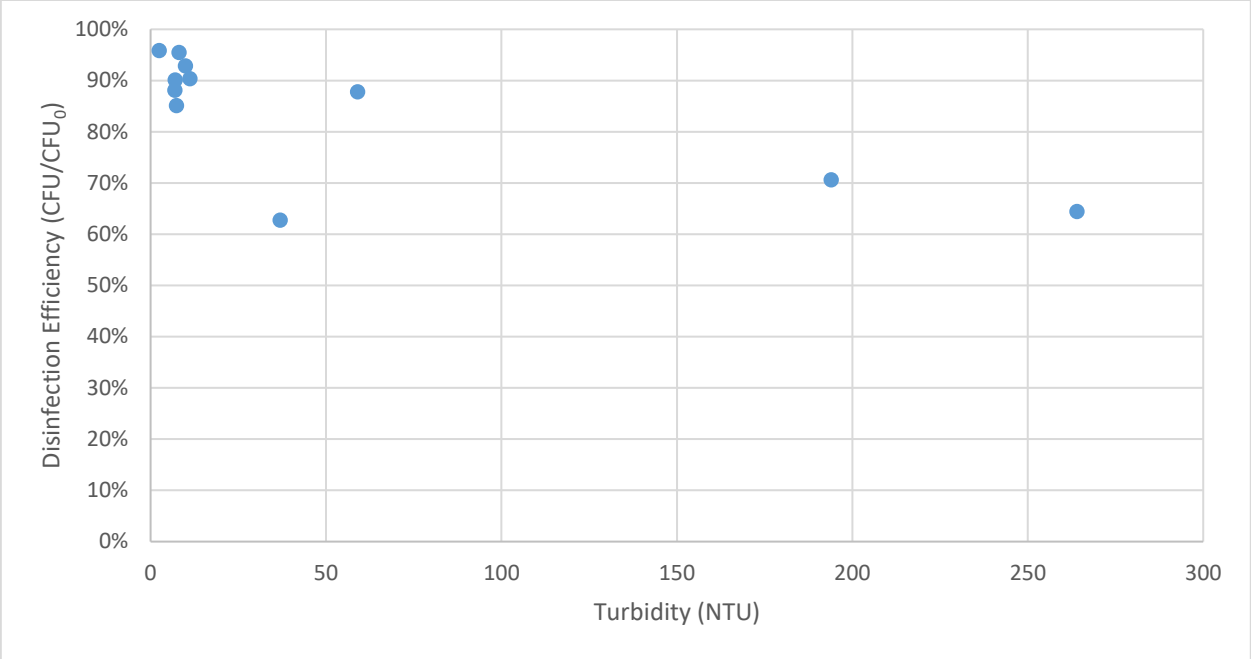

Figure 28 Disinfection performance as compared to turbidity levels at the Dartmouth WWTP

From these graphs, it could be said that there is a downward trend in disinfection performance with increasing turbidity, but with other factors playing a significant role at lower turbidity levels. A piecewise function may be able to model the behaviour well but as this is solely observational testing modeling of the relationship is out of the scope of this research.

For these lab scale experiments, it is seen that at low turbidity levels disinfection performance is very successful with all three plants seeing an 80% or reduction in viable microorganisms. The Dartmouth WWTP in particular saw very successful disinfection at low turbidity levels suggesting that particulate matter in their treatment train may be a significant barrier to efficiency, but the other two WWTPs saw some noise in this area. Though there was still successful disinfection there is a notably steep downward trend in some of the data that suggests that even at low turbidity other water quality parameters

like the presence of NOM would affect the disinfection efficiency moreso than the lack of TSS. Huck and Coffey found that while analyzing *Cryptosporidium* removal, treatment efficiency was decreased with higher turbidity values, namely in the absence of relative increase in coagulant dosage (Huck & Coffey, 2004).

Additionally, Gullian et. al. found that UV disinfection was inefficient at treating HB above a certain turbidity threshold. Their research analyzed treating HB from 0 to 24 hours at turbidity level increments up to 31 NTU and found that UV systems were ineffective at treating these waters above a turbidity threshold of less than 9.9 NTU.

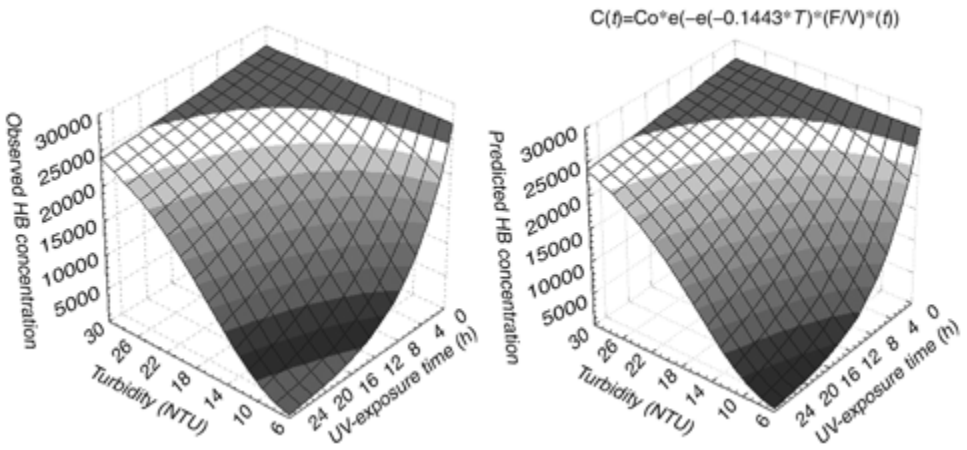

Figure 29 Disinfection performance in recirculating aquaculture systems (Gullian, Espinosa-Faller, Nunez, & Lopez-Barahona, 2012)

The results found in these two studies corroborates the findings of this research in that turbidity does play a significant role in disinfection performance, especially in that even NTU values as seemingly low as 10 a measurable negative relationship will be observed with respect to disinfection efficiency.

5.4 Lab Scale Individual Parameter Testing

The parameter specific testing was conducted following the tabulation of the data above in order to have a better understanding of what may occur from the synthetic parameter spiking.

Humic acid was spiked at 2 mg/L and 20 mg/L, disinfected, and then tested for ATP concentrations with and without incubation as with the water treatment plant samples.

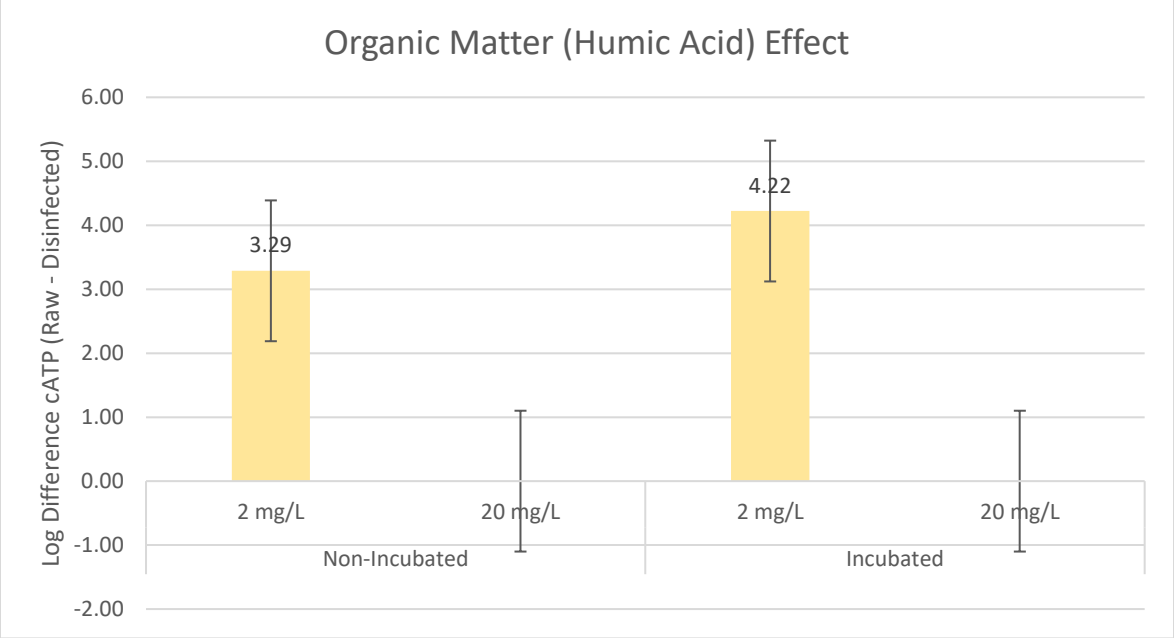

Figure 30 Natural Organic Matter Effect on Disinfection Performance

The results show that the addition of organic matter (humic acid in this case) affects disinfection performance quite significantly. Compared to the E. *coli* disinfection study the 2 mg/L dose had a small effect but a reduction nonetheless. However, the higher dose of 20 mg/L had such an effect that no measurable disinfection was actually performed; the

disinfected sample had the same bacterial concentration, or higher, as the raw samples. The negative difference in bacterial concentration is what produces the erroneous “zero” in logarithmic difference.

This means that any real water being disinfected with a high amount of organic matter will see vastly reduced effectiveness. It is for this reason that water treatment plants work diligently to remove as much organic matter as possible through the pre-disinfection processes i.e. coagulation and settling.

The next parameter of concern is turbidity. To study this effect, the samples were dosed with kaolin clay (and continuously stirred to ensure maximum dispersion) and disinfected as before.

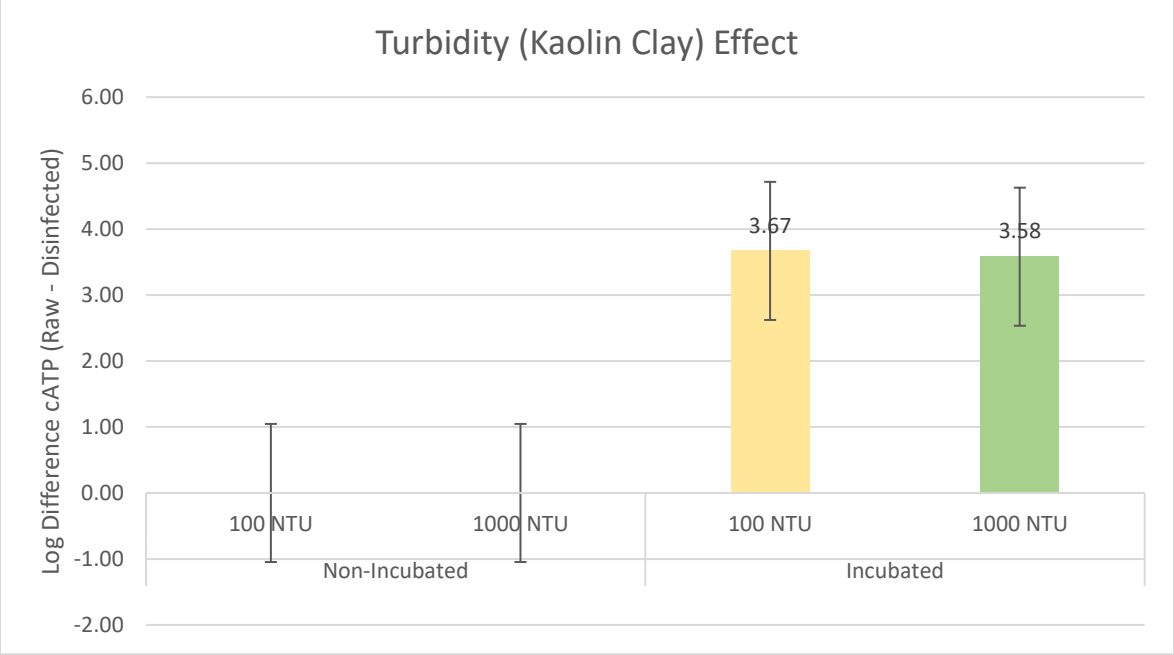

Figure 31 Turbidity Effect on Disinfection Performance

Similarly, as before the erroneous ‘zeroes’ in the graph above are a result of a negative difference in the disinfected samples. Meaning that there was no recorded disinfection without the incubation method. The negative difference resulting in an incomputable log. It is clear from this result that turbidity had only a small effect on the disinfection process. This was somewhat unexpected as shielding and reflection of incident UV radiation was expected to interfere with the performance. It is also worth noting that without undergoing the ATP grow-out method no change in bacterial concentration was measured, but after being subject to the innovative method we can see that indeed the disinfection process succeeded in inactivating the contaminants.

It may be that having a water sample with extremely high levels of turbidity (i.e. 1000 NTU) may have a more pronounced effect on disinfection but our petri dish was too small. Though the sample had high turbidity it may be that the depth of the sample was too small to realistically affect the transmission of UV light. However, this turbidity level would virtually never be seen at a WTP so at realistic levels for drinking water it can be assumed that the turbidity would have little to hindrance on disinfection.

Finally, amino acid effects were studied using tryptophan as the indicator compound.

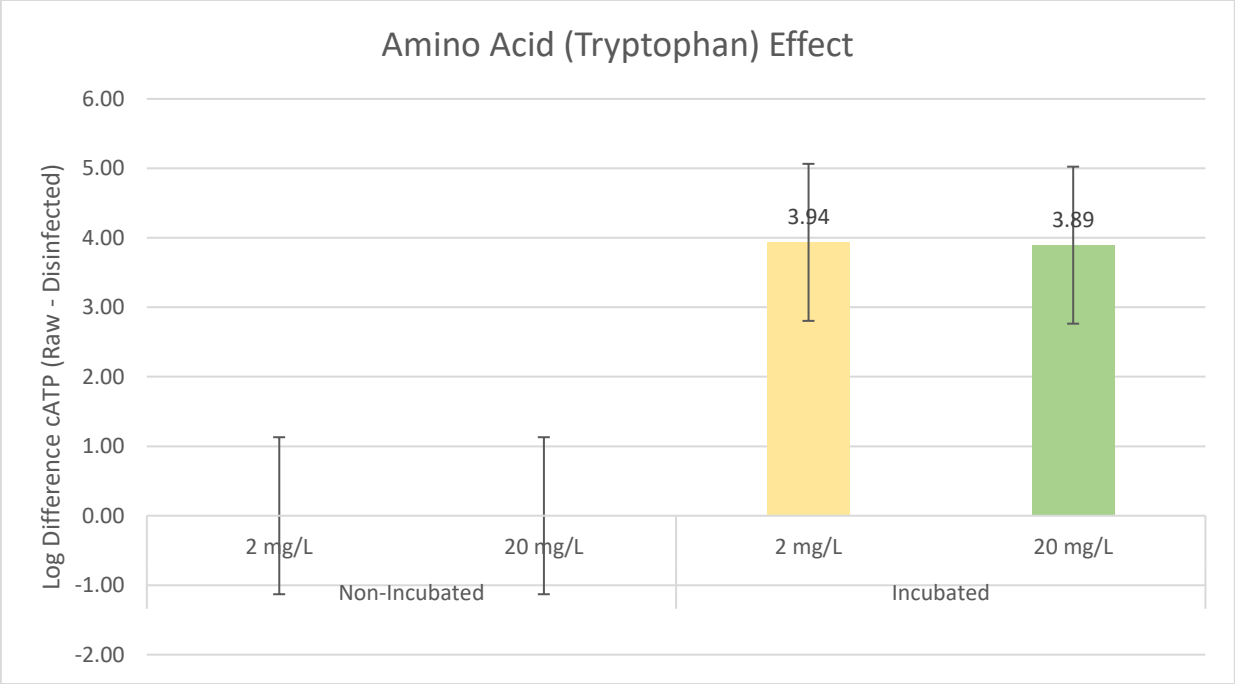

Figure 32 Amino Acid Effect on Disinfection Performance

These results are very similar to the turbidity results in that even increasing the tryptophan concentration by an order of magnitude had no effect on the efficiency of the disinfection. In addition, the negative difference, resulting in an incomputable log, in the non-incubated samples was seen here as well.

They are also another indicator of the validity of the grow-out method, showing that without the incubation the samples showed no disinfection was performed but in reality, the contaminants simply had not had the time to life-cycle and had not yet perished without replicating.

Chapter 6 - Conclusions

6.1 Conclusions

The research showed strong applicability for the proposed method in industrial wastewater treatment facilities to monitor their disinfection performance. The verified relationships, especially at higher influent biomass loadings, of the grow-out method to actual disinfection performance show that the method could be employed to more quickly and more proactively alter the disinfection process to reflect the ultraviolet dosage need. The ability to alter the applied dosage more quickly allows for less discrepancy between required disinfection dosage and applied dosage. This tighter relationship reduces energetic costs since UV processes are highly energetically demanding.

The disinfection performance of solely E. *coli* in a water sample approximated that the optimal dosage for a 5-log reduction in microbiological activity was 50 mJ/cm^2. It was suggested that this dosage would be low relative to a WWTP's as there would be multiple other factors to consider that needed compensating for. This was verified by the sampling and data collection schedule; all three WWTP applied a higher dose than was found to be optimal for the indicator organism since they were also dealing with particle shielding, absorption by NOM, reflection of incident radiation from turbidity/TSS, etc.

The optimal dosage for disinfection synthetic water with E. *coli* was used for the parameter specific disinfection study that followed the grow-out method study.

ATP testing in general is a great approach to immediate biological activity quantification but does not adequately reflect the performance of the disinfection process if analyzed directly post exposure.

The proposed incubation grow-out method yielded very promising results. Showing a clear relationship that more closely aligns with actual disinfection performance than that of ATP testing without use of the proposed method. The benefit of the method is two-fold in that it takes a fraction of the time required for traditional biological quantification methods and accurately describes disinfection performance.

It was found that testing the ATP concentrations of the disinfected water samples immediately after exposure to UV light showed little to no change in microbiological loading. However, conducting the same ATP test after subjecting the samples to the grow-out method the results much more closely resembled those of the standard HPC methods. Subjecting the disinfected samples to the grow-out method hastened life-cycling of the microbes and those that had been inactivated by the radiation died without reproducing and the ATP test reflected that. The average disinfection efficiency over the course of the sampling schedule of, for example, the Dartmouth WWTP using the HPC method was 84%, analysing disinfection using ATP testing gave an average disinfection efficiency of -10%. This false negative shows that immediate analysis is a poor approach since the microbes have not had the time to life cycle. Applying the grow out method gives an average disinfection efficiency of 77%. Similarly, for the Herring Cove plant the HPC disinfection efficiency was 68%, ATP testing immediately after UV exposure gave an efficiency of -115% but with the grow-out method this result changed to a more significant result of

76%. As for the Halifax plant, HPC results gave an efficiency of 63%, ATP testing gave -86%, while the grow-out method gave an efficiency of 57%.

When analyzing the disinfection performance of the WWTPs with respect to individual water quality parameters CFU data were used since they are an accepted standard. It was found that UV-T had no affect on disinfection performance, but that turbidity did show a slight downward trend with respect the disinfection. However, that trend was very slight with much noise at lower turbidity values. Though there was no significant relation between disinfection performance at the industrial scale with UV-T or turbidity, this could easily be attributed to the interference from other water quality parameters and the correlation may still be there however hidden behind the noise of the other factors. For this reason, these parameters were examined at the lab scale in the set of experiments that followed for observational purposes.

ATP testing was done on the parameter specific study with and without the grow out method and it was found that humic acid, model compound for NOM, had a significant effect on disinfection performance. At the low dose of 2 mg/L there was an acceptable 4.22 log reduction in E. *coli* concentration; but at the high dose of 20 mg/L of humic acid there was no reduction seen, sometimes conversely resulting in higher ATP concentrations after disinfection. This increase in concentration following disinfection yielded negative differences and ergo an incomputable log difference. This increase in ATP concentration following disinfection is potentially due to the subjection of stress to the microbes causing them to attempt to repair the damage, increase their metabolic activities, or simply engage survival mechanisms.

As for the turbidity and amino acid, it was found that they had little to no effect on disinfection performance. When turbidity was dosed at 100 NTU and 1000 NTU using kaolin clay the disinfection performance did not change, going from 3.67 log reduction to 3.58. Amino acid dosed at 2 mg/L and 20 mg/L yielded reductions in E. *coli* concentrations of 3.94 log and 3.89 log respectively, showing no effect.

These tests were conducted using ATP testing immediately prior to disinfection as well as using the grow out method and they reaffirmed the value of the proposed method. There was no disinfection reported at all for tests, with the exception of the low dose of humic acid which was an outlier, but when the grow out method was applied there were measurable and accurate disinfection results.

6.2 Recommendations

It is recommended that investigations into reducing that time requirement further with altering the nutrient broth, incubation temperature, or simply analysing the samples sooner than four hours be conducted in an effort to benefit water utilities even more.

Water treatment utilities could very well make use of ATP testing to estimate their influent biomass loading to better judge the required disinfection intensity but the direct relationship between influent ATP concentration and applied UV dose at a WWTP has not yet been analyzed and would be a worthwhile endeavour for water research groups.

As for the water quality parameters affecting disinfection it was seen that UV transmittance had little relationship with disinfection at all. It is hard to say why this particular parameter has become the industry standard for indicating required

disinfection intensity. This research showed that of the three parameters analyzed (amino acids, natural organic matter, and turbidity) only organic matter content of the water sample had an affect on disinfection performance.

It should be restated that there may be a more noticeable effect from turbidity on disinfection performance and that in this research the physical depth of the water sample being treated was too small to accurately reflect that effect. It is suggested that turbidity specific studies on disinfection performance be more thoroughly examined. However, from this research alone it is clear that organic matter content should be analyzed for disinfection intensity requirement.

Since organic matter is removed in the coagulation process it should be quantified post coagulation and pre-UV process to properly indicate intensity requirements and research into the applicability of this should be conducted (Matilainen, Vepsalainen, & Sillanpaa, 2010).

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Appendix A – Raw Water Quality Data

	Date	Feb 11 2015	Feb 18 2015			Feb 23 2015			Feb 25 2015			March 2 2015			March 4 2015		
	Raining or Snowing	No	No			No (Rained day before)			No			Snowing			No		
	Amount	0	0			0			0			10 cm			0		
	Plant	HC	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
Before UV	UV-T (%)	71.5 71.9 72.1	79.1	56.4	57.5	77.4 77.8 77.8	65.9 63 66.6	60 60.9 62.3	81 80.5 80.6	58.7 58.1 58.9	60.8 61.8 62	75.9 75.2 75.3	56.8 55.8 55.9	53.5 53.7 53.5	74.5 73.8 73.8	57.2 56.8 56.6	53.8 53 52.2
	pH	6.8	6.39	6.79	6.9	6.85	6.91	6.51	6.38	6.96	6.76	6.7	6.66	6.72	6.81	6.73	6.84
	COD (mg/L)								79	127	107				Bad Samp	113	93
	peCOD (mg/L)	21.1 >25 >25															
	Temperature (°C)	21.3	18.6	18.1	17.8	22.5	21	19.9	18	19.9	17.9	17.8	17.5	16.3	20.4	20.8	20.6
	Turbidity (NTU)	1.62	2.48	5.5	2.23	16	8.44	30.7	3.11	5.34	3.94	1.62	6.24	7.15	1.19	5.87	6.27
	TSS (mg/L)	1.6	2.2	8	5.6	6.4	7.2	49.3	2.6	6	7.6	1.8	5	1.5	1.4	11.2	4.4
After UV	UV-T (%)		16.3	57	58.2	75.7 76 76.5	60.9 59.8 60.1	65.9 63 65.1	79.2 80.1 80.4	50.1 49.6 49	61.8 62 62	76.4 76.8 76.7	49.1 51.3 51.4	53.4 50.9 5.9	73.5 73.7 73.7	57.2 56.5 56.3	53 52.1 52.4
	pH	6.7	5.38	6.75	8.81	6.35	6.77	6.48	6.55	7.16	6.79	6.63	6.89	6.77	6.8	6.82	6.98
	COD (mg/L)								121	128	60				30	112	81
	peCOD (mg/L)	24.3 >25 >25															
	Temperature (°C)	21.4	19.4	18.1	18.3	19.9	20.1	20.2	17.4	19.4	17.5	15.2	18.3	15.9	21.4	21.2	21.3
	Turbidity (NTU)	1.85	5.5	22	4.52	12.9	14.5	24.3	3.82	10.2	3.85	0.981	9.52	8.13	1.72	6.34	5.75
	TSS (mg/L)	2.4	236	47.2	6.4	14	10.6	34	6.4	14.7	8.4	0.8	18	15.5	3.8	7.6	6.4
Plant Data	pH	6.6	5.46	6.3	6.5	6.4	7.14	6.22	6.5	6.62	6.56	6.6	6.45	6.67	6.71	6.66	6.65
	Turbidity (NTU)	17.3	254	2.88	7	22.1	2.21	6.92	9.34	2.83	2.5	8.8	7.28	6.02	7.29	2.08	11.25
	Temperature (°C)	9.3	8.5		7	7.5	18.7	6.2	7.8	11	6.8	8.3	11.4	7.1	8.3	11.4	7
	UV Dose (mJ/cm2)	130	140		136	54	183.26	56.5	118	231	90.98	136.3	242	160	149.12	145.6	74.6
	Flow at UV (L/s)	60	57		68	146.5	913	530	66	725	363	57	695	404	52	1160	447
	TSS Before (mg/L)	164.4								105							
	TSS After (mg/L)	3.2								26							
	Retention Time (s)																
	Intensity (mW/cm2)											5.9	15	9.4	5.9	15.1	9.4

	Date	March 9 2015			March 11 2015			March 23 2015			March 25 2015			March 30 2015		
	Raining or Snowing	No			No			No			No			No		
	Amount	0			0			0			0			0		
	Plant	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
Before UV	UV-T (%)	69.7	57.9	59.2	74.5	61.4	62.9	78	68.4	66	78.7	63.8	61.6	79.6	68.9	63.9
		70.9	57.5	58.8	73.9	60.3	62	77.8	67.9	65.6	78.7	63.8	61.4	79.3	68.1	64.3
		7.1	57.8	59.1	73.9	60.7	61.6	77.5	68	65.5	78.5	64.1	61.2	79.4	68.3	64
	pH	7.06	6.73	6.95	6.72	6.83	7.02	6.69	6.78	6.77	7.22	6.78	7.03	5.52	6.74	6.99
	COD (mg/L)				37	98	66				19	89	74	39	81	132
	peCOD (mg/L)															
	Temperature (°C)	21.5	20.6	19.8	20.8	19.9	19.6	20.4	19.1	18.8	21.5	20.5	20.3	20.8	19.4	19.1
	Turbidity (NTU)	1.44	3.67	5.78	1.8	2.54	3.25	9.78	2.86	2.67	1.55	3.62	3.65	7.26	18.3	9.11
	TSS (mg/L)	0.75	2.4	7.2	1	2.8	6.4	10.25	4	5.33	-	3.33	6.33	10.5	16	53.2
After UV	UV-T (%)	70.5	45.9	58.6	74.6	53.1	61.7	70.9	65.9	66.5	79.7	63	61.6	76.4	64.7	59.3
		63.9	45.9	57.8	74.3	55.3	61.6	65.8	66.2	66	79.5	62.3	60.4	76.5	67.1	60.5
		71.3	44.4	57.7	74.2	54.4	61.1	69.9	66.1	64.1	79.8	62.8	60.4	76.3	65.9	65.9
	pH	7.44	6.96	7.03	7.13	7.04	7.12	6.75	6.87	6.82	7.33	6.92	7.05	6.98	6.86	7.11
	COD (mg/L)				39	111	67				28	97	75	28	98	114
	peCOD (mg/L)															
	Temperature (°C)	21.9	20.6	20	20.9	19.6	20.2	20.5	19	19.2	21.4	20.5	20.3	20.5	19.3	19.4
	Turbidity (NTU)	2.81	12.7	6.66	1.81	18.8	3.37	13.1	3.46	3.24	2.57	4.7	3.84	5.7	20.5	34.3
	TSS (mg/L)	5.25	12.8	5.6	0.75	22.8	6	24.75	4.33	5.67	2.5	5.67	7	17.5	40.4	52.4
Plant Data	pH	6.62	6.88	6.45	6.44	7.34	6.52	5.9	6.61	6.32	6.53	6.64	6.31	6.34	6.51	6.19
	Turbidity (NTU)	9.06	1.8	8.12	9.43	2.26	9.95	25.96	3.9	59.03	9.04	3.75	7.38	10.6	6.41	
	Temperature (°C)	8.8	12	15.6	8.5	20.4	7	8.1	10.3	6.8	8	11	7.4	7.2	10.1	6.4
	UV Dose (mJ/cm2)	184	276.72	136.46	182.72	123.76	106.4	143.96	450	101.7	105.24	460.2	81.28	84.8	212.6	63.22
	Flow at UV (L/s)	43.4	597	244.5	40.1	560	311.4	54.1	371	324	75	360	415	94	788	521
	TSS Before (mg/L)															
	TSS After (mg/L)															
	Retention Time (s)	15.1	9.313	7.15	14.88	8.1	5.7	12.1	15	5.4	8.892	15.26	4.34	7.064	7.058	3.38
	Intensity (mW/cm2)	5.9	15	9.4	5.9	7.65	9.4	5.9	15.1	9.4	5.9	15	9.4	5.9	15	9.3

	Date	April 8 2015			April 13,2015			April 20,2015			April 29 2015			May 6 2015		
	Raining or Snowing	No (melting snow)			No (melting snow)			No (Melting Snow)			No			No		
	Amount	0			0			0			0			0		
	Plant	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DT	HC	HFX	DT
Before UV	UV-T (%)	83	70.7	62.9	82.3	63.1	60.5	79.4	69.7	63.6	74.5	65.8	55.5	76.8	61.2	43.1
		82.3	70.7	54.3	79.6	63.1	62.7	81	69.2	59.6	76.3	65.5	58.5	75.7	61	46.7
		82.8	70.9	64.4	81.6	62.1	63.9	82.6	69.2	61.3	76.2	65.5	59.8	75.7	61	45.1
	pH	5.13	6.51	6.78	6.66	6.48	6.4	6.6	6.71	6.81	6.48	6.54	6.79	6.59	6.64	6.91
	COD (mg/L)	33	111	141	26	107	88				40	87	92			
	peCOD (mg/L)															
	Temperature (°C)	20.5	18.4	18.2	25	25	25	15.7	15.4	14.8	21.8	21.7	21.8	22.3	21.8	22
	Turbidity (NTU)	4.65	3	19.1	3.78	37.3	38.6	7.29	5.21	29	11.3	3.22	33.1	3.33	3.34	68.1
	TSS (mg/L)	5.25	3.5	56	6	52	77	11.2	15	122	23	12	49	ND	ND	127
After UV	UV-T (%)	75.2	70.4	60	78.9	59.1	66.3	77.9	68.5	57	45.8	64.9	58.4	76.6	58.7	44.6
		76.5	68.2	58.1	78.7	59.1	62.6	77.4	68.2	60.2	45.1	64.8	57.7	76.6	58.5	46.1
		75.8	70.1	61.7	78.7	57.8	63	78.4	67.5	60.6	44.8	64.5	57.7	76.2	58.4	43.9
	pH	6.82	6.61	6.89	6.48	6.58	6.42	6.89	6.74	6.86	5.95	6.76	6.8	6.86	6.79	6.88
	COD (mg/L)	64	121	>150	32	91	113				99	83	77			
	peCOD (mg/L)															
	Temperature (°C)	18	18.7	18.7	25	25	25	15.4	15.4	15.1	22.2	21.8	20.8	22.3	22	21.9
	Turbidity (NTU)	7.4	4.52	26	8.88	37.2	38.2	12.7	5.81	28	60.9	3.09	20.8	1.82	4.4	69.4
	TSS (mg/L)	17.75	10.5	79	18.8	66	61	12	16	75	101	4	39	ND	ND	114
Plant Data	pH	6.37	6.48	6.6	6.45	6.93	6.27	6.54	6.67	6.39	6.04	6.69	6.5	6.67	6.89	6.54
	Turbidity (NTU)	8.95	7.25	44.93	8.8	1.85	28.88	10.54	2.02	36.92	31.5	3.74	264	10.14	3.58	194
	Temperature (°C)	7.5	10.1	6.6	6.8	13.4	6.5	7.4	10	8.7	8.1	11.2	7.8	9.4	12.8	9.6
	UV Dose (mJ/cm2)	102.06	203	64	37.4	144.6	36	54.6	178	57.4	154	140	73.8	113	152.68	49.72
	Flow at UV (L/s)	76.5	827	515	209	1155	900	144	942	592	149.6	785.5	444	69	605	657
	TSS Before (mg/L)															
	TSS After (mg/L)															
	Retention Time (s)	8.54	6.71	3.43	3.17	4.75	2	4.6	5.9	3.13	4.4	5.34	4	9.6	9.1	2.67
	Intensity (mW/cm2)	5.9	15.1	9.3	5.9	15.1	9.3	5.9	15.1	9.3	5.9	15.2	9.3	5.9	8.4	9.3

Appendix B – Raw ATP Data

Date	11-02-2015			18-Feb-15			23-Feb-15			25-Feb-15			02-Mar-15		
Plant			Herring Cove	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
ATP		Calibration	6391	24865	24865	24865	23176	23176	23176	19488	19488	19488	14634	14634	14634
			7184	22856	22856	22856	25349	25349	25349	20823	20823	20823	16816	16816	16816
	No Incubation	Before UV	8777	6999	7110	14470	2922	8647	4037	5857	12879	6317	11128	21366	22306
			8331	6954	6809	15691	2904	7988	3857	4457	11925	6280	11111	23803	25659
		After UV	9328	27567	7664	18732	7924	34125	5355	6887	44393	7888	17640	50768	24192
			6596	25394	7669	19274	6777	35021	5769	5986	44300	8405	20777	51118	23714
		Calibration	6391	24865	24865	24865	23176	23176	23176	19488	19488	19488	14634	14634	14634
			7184	22856	22856	22856	25349	25349	25349	20823	20823	20823	16816	16816	16816
	Incubated	Before UV	2803	1282	10119	26418	734	5449	3810	30	9252	5233	2511	47398	41315
			2725	1338	10068	27855	679	6197	4444	30	9400	5297	2780	50203	42545
		After UV	1081	2438	7449	1747	550	4952	512	330	12772	375	1007	18815	2922
			318	3001	7435	1631	572	6702	524	323	14446	269	994	20502	3315

04-Mar-15			09-Mar-15			11-Mar-15			March 23 2015			March 25 2015		
HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
21684	21684	21684	13754	13754	13754	28464	28464	28464	19361	19361	19361	24805	24805	24805
19228	19228	19228	18279	18279	18279	29373	29373	29373	18984	18984	18984	23396	23396	23396
13061	18504	22225	21032	14009	17560	22050	32251	19915	7833	6093	12978	14876	9049	12024
13225	19546	21506	22324	15810	20205	22516	36672	17669	8440	7439	12415	16235	9163	11888
24419	23116	31070	35833	86881	30066	40805	63890	23563	17010	8546	9513	23249	8718	14893
25941	25762	34728	38217	87303	30054	39175	74236	25416	19046	10970	11331	22434	8758	15855
21684	21684	21684	13754	13754	26292	28464	28464	28464	16517	16517	16517	24805	24805	24805
19228	19228	19228	18279	18279	25931	29373	29373	29373	13226	13226	13226	23396	23396	23396
3996	31652	66457	6611	31621	43018	over	over	over	1272	6513	11843	4882	6239	16356
3756	35863	72466	6098	35820	40488	over	over	over	1427	5012	14780	5782	6956	17037
807	15034	7389	2002	39931	1594	over	over	over	1692	1195	2351	2077	1447	1948
918	12932	8782	2541	42325	1722	over	over	over	1578	1060	2060	2377	1639	2143

March 30 2015			April 8 2015			April 20 2015			April 29 2015			May 6 2015		
HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
16038	16038	16038	19226	19226	19226	26434	26434	26434	18830	18830	18830	16249	16249	16249
13357	13357	13357	20030	20030	20030	26236	26236	26236	19133	19133	19133	20148	20148	20148
5373	10304	6651	4313	5813	6950	5501	8978	10690	8294	8569	6725	11522	23262	39124
5318	10401	6849	5268	7254	7600	5893	8247	10255	11321	9013	7406	14653	21787	36109
11496	13842	7626	24410	8348	10935	19025	8146	13725	26934	11129	11979	54522	25635	31942
11273	15139	8438	25717	7677	10794	22341	9835	14185	35088	11725	11502	54517	25918	30699
16038	16038	16038	21020	21020	21020	26434	26434	26434	18830	18830	18830	16249	16249	16249
13357	13357	13357	23032	23032	23032	26236	26236	26236	19133	19133	19133	20148	20148	20148
3091	20429	29081	672	2076	14840	2714	18473	68062	2074	3479	8600	6281	56282	111827
2985	19704	26421	571	2439	15594	2664	19436	67706	1977	3926	8913	6935	49293	126249
2017	28417	2141	1596	1136	2371	3263	7314	5818	2520	3109	1930	2568	6056	16144
2025	28832	2270	1386	1212	2269	3061	7551	6055	2788	3274	2018	2815	7015	16391

Appendix C – Raw Heterotrophic Plate Count Data

Date			11-02-2015	18-Feb-15			23-Feb-15			25-Feb-15			02-Mar-15		
Plant			Herring Cove	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
HPC	Before UV	10^0	TNTC		TNTC										
			TNTC		TNTC										
			TNTC		TNTC										
		10^-1	TNTC	TNTC	TNTC	TNTC	67	TNTC	TNTC	107	155				
			0	TNTC	206	TNTC	88	TNTC	TNTC	101	168				
			311	TNTC	238	TNTC	74	TNTC	TNTC	110	143				
		10^-2	32	30	24	117	17	27	29	18	21	15			
			37	39	21	110	11	30	38	10	39	17			
			44	42	25	91	10	31	28	12	26	12			
		10^-3	3	2	2	17			0			1	Ruined agar, no HPC today		
			8	7	1	18			0			3			
			4	3	2	16			2			1			
		10^-4	0		0	1			0						
			0		2	1			0						
			0		0	2			0						
		10^-5	0		1	0									
			0		0	0									
			0		0	0									
	After UV	10^0	352	TNTC	TNTC	TNTC	TNTC		TNTC			57			
			100	TNTC	TNTC	TNTC	TNTC		TNTC			62			
			250	TNTC	TNTC	TNTC	TNTC		TNTC			62			
		10^-1	50	205	175	100	36	TNTC	32	1	144	6			
			56	TNTC	150	110	36	TNTC	40	2	146	5			
			16	210	158	film	48	TNTC	41	0	148	6			
		10^-2	2	22	20	10		95	4	0	11				
			1	28	19	47		110	3	0	19				
			1	30	26	15		109	1	0	20				
		10^-3	0		5	2									
			0		1	2									
			0		5	1									
		10^-4	1		0	0									
			0		0	2									
			0		0	0									
		10^-5	0		0	0									
			0		0	0									
			0		0	0									

Date			04-Mar-15			09-Mar-15			11-Mar-15			March 23 2015			March 25 2015		
Plant			HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
HPC	Before UV	10^0															
		10^-1	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC
		10^-2	62 52 49	105 115 103	193 192 190	79 62 film	94 104 76	76 film 76	49 45 51	103 111 film	50 50 70	36 23 34	42 46 41	78 62 62	50 43 43	30 24 36	55 72 51
		10^-3															
		10^-4															
		10^-5															
	After UV	10^0	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC	TNTC TNTC TNTC
		10^-1	106 122 115	295 TNTC 300	189 180 film	85 85 80	TNTC TNTC TNTC	33 36 22+film	60 111 109	TNTC TNTC TNTC	47 41 33	68 16+film 96	36 44 47	44+film 85 79	105 103 96	38 50 45	93 93 79
		10^-2															
		10^-3															
		10^-4															
		10^-5															

Date			March 30 2015			April 8 2015			April 13 2015			April 20 2015			April 29 2015			May 6 2015		
Plant			HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT	HC	HFX	DRT
HPC	Before UV	10^0																		
		10^-1	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC												
			TNTC	TNTC	TNTC	TNTC	TNTC	TNTC												
			TNTC	TNTC	TNTC	TNTC	TNTC	TNTC												
		10^-2	53	55	36	82	65	0	49	54	20	40	77	95	96	63	45	Ruined	TNTC	TNTC
			58	51	38	63	81	0	60	53	33	43	75	137	100	73	60	Ruined	TNTC	TNTC
			56	60	40	90	96	0	53	56	32	50	80	136	110	67	30+film	Ruined	TNTC	TNTC
		10^-3							6	7	3	8	11	10	16	7	3	18	31	33
									6	5	2	1	8	11	17	11	4	18	28	35
									3	11	1	5	9	12	13	9	5	13	31	33
		10^-4																		
		10^-5																		
	After UV	10^0	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC												
			TNTC	TNTC	TNTC	TNTC	TNTC	TNTC												
			TNTC	TNTC	TNTC	TNTC	TNTC	TNTC												
		10^-1	60	75	41	TNTC	150	145	TNTC	TNTC	60	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
			74	80	34	TNTC	160	143	TNTC	TNTC	70	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
			TNTC	96	46	TNTC	170	142	TNTC	TNTC	75	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
		10^-2							140	31	11	82	45	43	130	33	20	126	50	103
									153	35	9	92	61	49	115	32	20	130	51	95
									160	33	10	75	56	45	143	35	16	129	33	66+film
		10^-3																		
		10^-4																		
		10^-5																		

Appendix D – Raw Parameter Study Data (ATP Tests)

ATP Tests (RLUs)				
Tryptophan				
2 mg/L			20 mg/L	
Check	23475		Check	23475
	23359			23359
Average	23417		Average	23417
Raw	419414		Raw	449597
	429480			442468
Average	424447		Average	446032.5
Disinfected (1)	537976		Disinfected (1)	565139
	520213			580631
Average	529094.5		Average	572885
Disinfected (2)	459066		Disinfected (2)	507394
	418878			505038
Average	438972		Average	506216
Disinfected (3)	512110		Disinfected (3)	462758
	506066			474187
Average	509088		Average	468472.5
Check	23475		Check	23475
	23359			23359
Average	23417		Average	23417
Incubated	232448		Incubated	258425
Disinfected (1)	222158		Disinfected (1)	298565
Average	227303		Average	278495
Incubated	218026		Incubated	253286
Disinfected (2)	223065		Disinfected (2)	255384
Average	220545.5		Average	254335
Incubated	240968		Incubated	147677
Disinfected (3)	231833		Disinfected (3)	159758
Average	220545.5		Average	254335

ATP Tests (RLUs)				
Humic Acid				
2 mg/L			20 mg/L	
Check	22046		Check	22046
	24567			24567
Average	23306.5		Average	23306.5
Raw	332115		Raw	263836
	389281			319044
Average	360698		Average	291440
Disinfected (1)	665989		Disinfected (1)	693734
	644516			694463
Average	655252.5		Average	694098.5
Disinfected (2)	695239		Disinfected (2)	669061
	701542			680000
Average	698390.5		Average	674530.5
Disinfected (3)	662828		Disinfected (3)	670011
	686904			684405
Average	674866		Average	677208
Check	14630		Check	14630
	19459			19459
Average	17044.5		Average	17044.5
Incubated	244635		Incubated	224448
Disinfected (1)	244466		Disinfected (1)	241404
Average	244550.5		Average	232926
Incubated	251476		Incubated	492040
Disinfected (2)	266456		Disinfected (2)	530073
Average	258966		Average	511056.5
Incubated	215715		Incubated	594579
Disinfected (3)	236056		Disinfected (3)	747991
Average	225885.5		Average	671285

ATP Tests (RLUs)				
Kaolin Clay				
100 NTU			1000 NTU	
Check	24100		Check	24100
	21975			21975
Average	23037.5		Average	23037.5
Raw	419954		Raw	459648
	448398			477567
Average	434176		Average	468607.5
Disinfected (1)	516895		Disinfected (1)	589128
	576278			604675
Average	546586.5		Average	596901.5
Disinfected (2)	561106		Disinfected (2)	640268
	608518			613342
Average	584812		Average	626805
Disinfected (3)	600310		Disinfected (3)	737076
	573203			694320
Average	586756.5		Average	715698
Check	15414		Check	15414
	17308			17308
Average	16361		Average	16361
Incubated	231546		Incubated	204522
Disinfected (1)	248385		Disinfected (1)	205143
Average	239965.5		Average	204832.5
Incubated	234224		Incubated	271304
Disinfected (2)	246616		Disinfected (2)	325508
Average	240420		Average	298406
Incubated	217860		Incubated	254866
Disinfected (3)	213087		Disinfected (3)	222762
Average	215473.5		Average	238814

Appendix E – Raw Parameter Study Data (HPC Tests)

CFU Tests			
Tryptophan			
2 mg/L		20 mg/L	
Before UV			
10^0	TNTC	10^0	TNTC
	TNTC		TNTC
10^-1	TNTC	10^-1	TNTC
	TNTC		TNTC
10^2	TNTC	10^2	TNTC
	TNTC		TNTC
10^3	TNTC	10^3	TNTC
	TNTC		TNTC
After UV (1)			
10^0	TNTC	10^0	50
	TNTC		133
10^-1	21	10^-1	6
	13		6
10^2	0	10^2	1
	1		1
10^3	1	10^3	0
	0		0
After UV (2)			
10^0		10^0	134
10^-1	17	10^-1	16
	10		12
10^2	1	10^2	1
	5		0
10^3	1	10^3	0
	0		0
After UV (3)			
10^0	37	10^0	69
	TNTC		51
10^-1	7	10^-1	5
	6		3
10^2	0	10^2	1
	1		0
10^3	0	10^3	0
	0		0

CFU Tests			
Humic Acid			
2 mg/L		20 mg/L	
Before UV			
10^0	TNTC	10^0	TNTC
	TNTC		TNTC
10^-1	TNTC	10^-1	TNTC
	TNTC		TNTC
10^2	TNTC	10^2	TNTC
	TNTC		TNTC
10^3	TNTC	10^3	TNTC
	TNTC		TNTC
After UV (1)			
10^0	45	10^0	70
	155		66
10^-1	5	10^-1	23
	3		
10^2	0	10^2	3
	0		2
10^3	0	10^3	2
	0		4
After UV (2)			
10^0	103	10^0	TNTC
	61		TNTC
10^-1	31	10^-1	32
	44		25
10^2	3	10^2	6
	7		8
10^3	3	10^3	0
			1
After UV (3)			
10^0	TNTC	10^0	
	TNTC		
10^-1	31	10^-1	44
	40		30
10^2	4	10^2	4
	3		2
10^3	0	10^3	0
	1		1

CFU Tests			
Kaolin Clay			
100 NTU		1000 NTU	
Before UV			
10^0	TNTC	10^0	TNTC
	TNTC		TNTC
10^-1	TNTC	10^-1	TNTC
	TNTC		TNTC
10^2	TNTC	10^2	TNTC
	TNTC		TNTC
10^3	TNTC	10^3	TNTC
	TNTC		TNTC
After UV (1)			
10^0	50	10^0	102
	75		90
10^-1	6	10^-1	10
	4		13
10^2	1	10^2	0
	2		1
10^3	0	10^3	0
	0		0
After UV (2)			
10^0	50	10^0	TNTC
	51		TNTC
10^-1	6	10^-1	25
	5		39
10^2	1	10^2	0
	0		6
10^3	0	10^3	1
	0		0
After UV (3)			
10^0	145	10^0	TNTC
	57		TNTC
10^-1	10	10^-1	26
	12		34
10^2	0	10^2	2
	0		3
10^3	0	10^3	0
	0		2

Appendix F – Superfluous Extended Incubation Data

			Relative Light Units (RLU)			
Sampling Date	Plant	Stage	4 hr	2 days	7 days	14 days
March 23 2015	Calibration (RLU)		19361	24805	16038	
			18984	23296	13357	
	HC	Before UV	1272	Over Scale	223845	
			1427	Over Scale	250913	
		After UV	1692	Over Scale	304797	
			1578	Over Scale	327174	
	HFX	Before UV	6513	Over Scale	235294	
			5012	Over Scale	252038	
		After UV	1195	Over Scale	166766	
			1060	Over Scale	200562	
	DRT	Before UV	11843	Over Scale	264245	
			14780	Over Scale	298240	
		After UV	2351	Over Scale	343108	
			2060	Over Scale	389717	
	Volume Filtered (mL)		10	10	1	
March 25 2015	Calibration (RLU)		24805			19226
			23396			20030
	HC	Before UV	4882			436911
			5782			412686
		After UV	2077			282106
			2377			298532
	HFX	Before UV	6239			173193
			6956			164057
		After UV	1447			246212
			1639			245217
	DRT	Before UV	16356			202413
			17037			223758
		After UV	1948			396388
			2143			450708
	Volume Filtered (mL)		10			1
April 9 2015	Calibration (RLU)		21020	19941	28623	
			23032	19035	29558	
	HC	Before UV	672	859107	292567	
			571	over	324864	
		After UV	1596	329735	402852	
			1386	326975	486616	
	HFX	Before UV	2076	693101	238139	
			2439	728074	224925	
		After UV	1136	619200	518918	
			1212	568946	455942	
	DRT	Before UV	14840	over	592041	
			15594	over	581403	
		After UV	2371	799360	363085	
			2269	913675	339461	
	Volume Filtered (mL)		10	1	1	