UV LED TECHNOLOGY FOR DRINKING WATER POU APPLICATIONS AND BIOFILM DISINFECTION

by

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DEDICATION PAGE

To my grandmother, who raised me. You taught me the basics in life, from cooking and multiplications to how to ride a bike. You taught me that I needed to be responsible and do my homework. You sat by me every afternoon, helping me with my school assignments, until the day you stop understanding them.

You passed away while I was in my trip to the Arctic. I like to think that you knew how far away I was. You always took care of me. I owe you what I am. My successes are also yours. You will always live in my heart.

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ABSTRACT

UV LEDs are a promising technology for the water sector because of their small size and low energy consumption, which makes them ideal for POU applications. UV LED POU device testing is required to verify their effectiveness in water disinfection.

The objective of this research was to verify the capability of two commercial UV LED POU reactors, as well as UV LEDs in a collimated beam configuration for biofilm inhibition. The UV LED POU reactors were tested using *E. coli* as a target microorganism, paired with different UVT, turbidity and flow-rate levels. Results showed that UVT and flow rate had a significant impact on the reactor's efficiency. UV LEDs at 265nm were effective at inactivating biofilm-bound *P. aeruginosa* in coupons. Furthermore, the combination of UV LED and wiping had synergistic effects. This could indicate advantages in incorporating UV LEDs into existing biofilm mitigation strategies.

LIST OF ABBREVIATIONS AND SYMBOLS USED

±	Plus or minus symbol
°C	Degree Celsius
µl	Micro liters
AlGaN	Aluminium gallium nitride
ANOVA	Analysis of variance
ATCC	American type culture collection
ATP	Adenosine triphosphate
BSC	Biosafety cabinet
cATP	Cellular adenosine triphosphate
CDC	Centre of Disease Control
CFU	Colony-forming unit
CFU/cm^2	Colony-forming unit per centimeter square
CFU/ml	Colony-forming unit per millilitre
Cl_2	Chlorine
CSTR	Continuous stirred tank reactor
DBPs	Disinfection by-products
DNA	Deoxyribonucleic acid
DWDS	Drinking water distribution systems
E. coli	*Escherichia coli*
E. coli K 12	*Escherichia coli* strain K 12 (ATCC MG1655)
E. coli O157:H7	*Escherichia coli* pathogenic strain
EPA	Environmental protection agency
EPS	Extra polymeric substances
GBP (£)	Pound sterling
GCDWQ	Guidelines for Canadian Drinking Water Quality
h	Hour
HPC	Heterotrophic plate count
L	Liter
LPM	Liters per minute
log	Logarithm
LPUV	Low pressure ultraviolet lamp
mg/L	Milligrams per liter
min	Minutes
mJ/cm^2	Millijoules per centimeter square
ml	Millilitres
mm	Millimetres
MPUV	Medium pressure ultraviolet lamp
mW	Milliwatts

nm	Nanometers
NOM	Natural organic matter
NSF/ANSI	National Sanitation Foundation/ American National Standards Institute
NTU	Nephelometric turbidity unit
OD 600	Optical density at 600 nm
P. aeruginosa	*Pseudomonas aeruginosa*
PBS	Phosphate buffer solution
pg cATP/ml	Picograms of cellular adenosine triphosphate per millilitre
pH	Potential of hydrogen
POE	Point of entry
POU	Point of use
PUV	Pulsed ultraviolet
QGA	Quench-gone Aqueous
RNA	Ribonucleic acid
RPM	Revolutions per minute
SDWS	Small drinking water systems
SRHA	Suwannee River humic acid
TOC	Total organic carbon
TSA	Tryptic soy agar
TSB	Tryptic soy broth
USD ($)	United States dollar
UV	Ultraviolet
UV LED	Ultraviolet light emitting diode
UVR	Ultraviolet radiation
VBNC	Viable but nonculturable
WHO	World Health Organization
α	Alpha, significance level

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CHAPTER 1 INTRODUCTION

1.1 UV TECHNOLOGY IN THE WATER INDUSTRY

Ultraviolet (UV) disinfection has been an accepted technology for the disinfection of water and wastewater for many years. UV radiation is capable to inactivate viruses, bacteria, and protozoan oocysts without chemical addition, and without changing significantly water quality. Furthermore, UV radiation does not add odour or taste to the water. With the growing interest of UV technology as disinfection technology, more sources of UV radiation have been investigated.

Although, there are some commercially available UV reactors for point of use (POU) applications, UV reactors remain not as commonly applied as POU technologies due to UV lamps disadvantages, such as fragility, mercury content and high energy consumption. UV light emitting diodes (UV LED) technology is a novel source of UV radiation that has gained interest within the water research community due to its advantages over conventional UV low-pressure (LPUV) or UV medium pressure (MPUV). Some of the advantages of UV LEDs' include their smaller footprint, lower energy requirements, customizable wavelengths, and the absence of mercury. All these aforementioned advantages make UV LEDs applicable for a wider range of applications than LPUV or MPUV.

One of the applications that is gaining popularity is the use of UV LEDs for point of use (POU) application (Chatterley & Linden, 2010, 2009; Lui et al., 2014; Lui., 2016).UV LEDs have characteristics that make them an excellent fit for POU applications, such as being small in size, low energy consumption, easy operation and instant turn on/off.

UV LED disinfection has been proven to work in several studies (Chen et al., 2017; Rattanakul & Oguma, 2018; Song et al., 2016), however, the majority of these studies have been performed in laboratory conditions. Therefore, more research on the application of UV LED for POU drinking water treatment is required.

1.2 Need for Water Disinfection

According to the World Health Organization (WHO), in 2015 around 29% of the global population did not have access to safe, readily available drinking water (World Health Organization, 2017). This reality is especially common in remote rural areas, where the delivery of safe water becomes more complex. Rural communities usually do not have a central drinking water treatment plant, and thus, they have to rely on different strategies, such as the water treatment at the point of use. There are different POU water treatment technologies, such as boiling, chlorine and solar water disinfection (SODIS); however, each technology has its weaknesses and thus, they cannot alleviate the need of water treatment in all the cases.

Arctic communities (communities above a latitude of 60º) are a place where POU technology could be applied. Arctic communities usually do not have centralized drinking water treatment. Instead, they take water from a reservoir and pump it into a truck, where the water is chlorinated and then delivered to the households within the community (Daley et al., 2014). In the households, water sits in a tank until its consumption where is at risk of recontamination due to stagnation (Ashbolt, 2004; Ercumen, Gruber, & Colford, 2014). Moreover, during the delivery and storage of the potable water, there are several points were water can be contaminated with bacteria, viruses and protozoa.

1.3 Opportunities for UV LEDs in Water Industry

A better understanding leading to successful application of UV LEDs as POU technology could help remote communities to have a reliable source of microbiologically safe readily water. The knowledge gained in this study could be translated into other communities in the Arctic or even in other rural communities in the world.

On the other hand, the advantages of UV LEDs can be used in other applications different from the drinking water industry such as biofilm control and disinfection field. Biofilms can grow basically in any surface that is in contact with non-sterile water (Flemming et al., 2011). The advantages that UV LEDs posses over the traditional mercury lamps, open a great window of opportunity for the application of UV LEDs in surface disinfection.

There are some studies that have investigated the use of UV radiation for biofilm disinfection (Bak et al., 2010; Garvey et al., 2015; Li et al., 2010), however, these are hardly comparable within each other, due to the different methods being used to measure UV light and the UV light source used. Nonetheless, all the mentioned studies have concluded that UV light was effective to inactivate to a certain extent the bacteria within the biofilms. In addition some studies (El-Azizi & Khardori, 2016; Murphy et al., 2008) have found that by pairing UV light with other disinfection techniques, such as chemical disinfection, biofilm disinfection achieved significantly increased, making the technologies combined more efficient than any of them alone.

1.4 RESEARCH OBJECTIVES

The overall goal of this thesis is to understand the suitability of UV LED technology for water industry as a POU device for treating drinking water, as well as a technology for treating biofilm, which may also have long-term POU applications.

The first objective of this research project was the evaluation and characterization of UV LEDs reactors intended for POU drinking water applications that are currently on the market. The UV LED POU reactors manufacturer's claims were tested, as well as challenged with different water quality parameters on laboratory scale to evaluate their effectiveness and robustness. In addition, one UV LED POU reactor was installed and monitored in an Arctic community.

The second objective of this research project is the application of UV LED technology for biofilm disinfection. To enhance biofilm disinfection, this research will also explore synergistic effects between UV LED irradiation and other disinfection techniques on biofilm-bound microorganisms. The specific objectives to accomplish these two goals are explained in the objectives section of Chapter 4 and Chapter 5.

1.5 THESIS ORGANIZATION

This dissertation is organized in six chapters as follows:

- Chapter 1 provides a general introduction to the topics that are discussed in this dissertation and outlines the major goals of the research project.
- Chapter 2 presents some background information related to the topics presented in this dissertation, as well as relevant regulations currently available for this work.

This chapter also gives a state-of-the-art on recent papers that are relevant to the presented topics in order better explain the rationale behind this research project.

- Chapter 3 explains generally materials and methods used for the experimentation performed. More specific methodology is found in Chapter 4 and Chapter 5.
- Chapter 4 explains the experimental design in the evaluation of commercially available UV LED for drinking water POU application. It also gives detailed information about testing, results, discussion and conclusion.
- Chapter 5 presents the experimentation of the UV LED applied for biofilm disinfection. In this chapter is presented a detailed methodology concerning the biofilm experimentation as well as detailed results, discussion and conclusions.
- Chapter 6 gives a conclusion summary, as well as future work.

CHAPTER 2 BACKGROUND INFORMATION

2.1 ULTRAVIOLET (UV) DISINFECTION

Ultraviolet (UV) light is defined as the part of the electromagnetic spectrum with wavelengths ranging from 200 to 400 nm. It can be divided into UV-A, UV-B and UV-C (Figure 2-1). The UV-C region is called the germicidal range because this UV radiation is absorbed by the DNA and RNA of microorganisms. When the structure of the DNA changes due to the absorption of the UV light photons, the microorganisms lose their ability to replicate even though they are still "alive" (metabolically active). The amount of UV light applied to the microorganisms during UV treatment is called UV dose or fluence and is usually measured in mJ/cm^2.

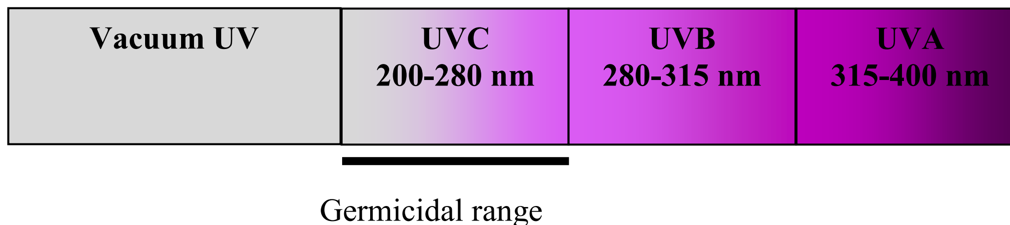


Figure 2-1 Ultraviolet (UV) spectrum ranges.

UV disinfection is a physical disinfection process. No chemicals are added to the water and there is no residual effect in the water after treatment. Furthermore, quality parameters of the treated water do not have a significant change after being treated with UV radiation.

The main sources for artificially creating UV light are low pressure and medium pressure mercury lamps. These can be described as gas discharge lamps that usually contain two electrodes, one at each end of a gas containing tube (most commonly mercury, but xenon can also be found).

UV disinfection, as with any other technology, has many advantages and disadvantages. Some of the advantages of UV disinfection are: its effectiveness against *Cryptosporidium* and *Giardia*, in which the traditional chlorine disinfection is almost not effective (Hijnen et al., 2006), its low impact on water quality parameters, such as turbidity, pH and TOC (Bolton, 2008b), the impossibility of overdosing UV light in the water, and virtually no generation of DBPs and other chemical residues (Spiliotopoulou et al ., 2015). On the other hand, some of the disadvantages are: the high energy consumption and equipment fragility, the mercury inside the lamps, which convert them in hazardous waste when its time of disposal. Moreover, UV disinfection does not have a residual effect on the treated water, and thus the addition of chlorine becomes necessary for further distribution or storage.

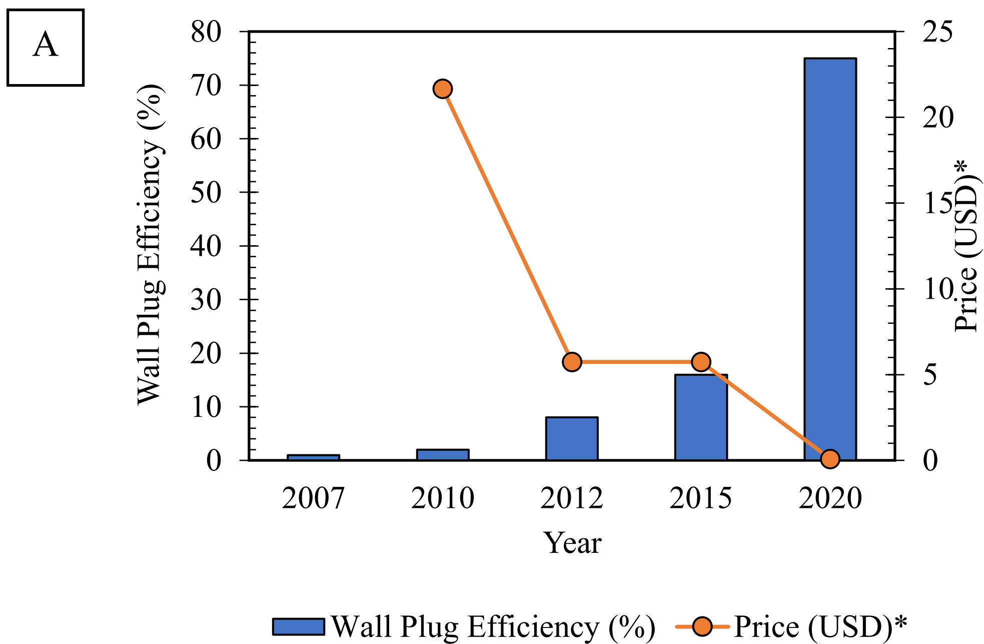
2.1.1 Ultraviolet Light Emitting Diodes (UV LEDs)

When UV technology gained popularity in a wide range of fields, the limitations of the use of mercury-based lamps started raising concerns. The fact that mercury is a toxic compound and a hazardous waste was one of the factors that led the search for new technologies that could replace the use and further disposal of mercury in the environment. The most popular alternative is the use of ultraviolet light emitting diodes (UV LED), which consist of an AlGaN based diode that emits UV light when activated (Tamulaitis, 2011).

With the advance and evolution of the semiconductors industry, UV disinfection using UV LEDs as a source of UV light has gained interest due to its advantages over the traditional mercury lamps. Some of the advantages of UV LEDs over mercury lamps are: absence of mercury, no warm-up time, less power required in operation, wide range of wavelengths,

smaller size as well as the ability to immediately turn on and off, which makes them ideal from the design standpoint.

Despite all the advantages that UV LEDs present over mercury lamps, this novel technology still has many areas of improvement, such as low wall plug efficiency, relatively low power output and high costs. However, according to Ibrahim et al, (2014) UV LEDs can reach a 75% wall plug efficiency and their cost could drop to 0.089 USD by 2020.



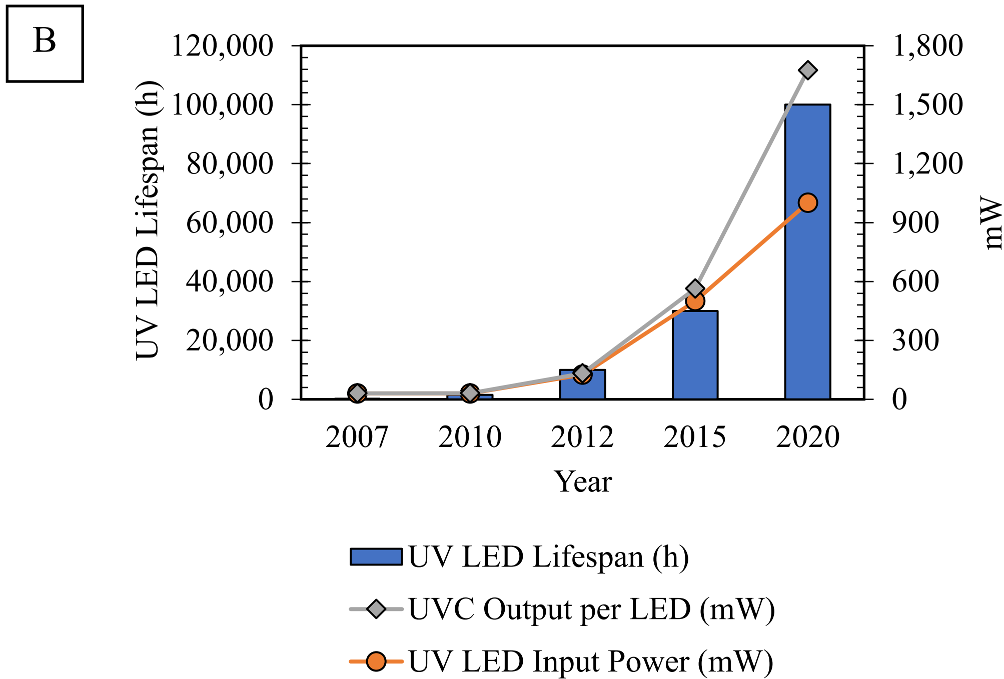


*Figure 2-2. Key parameters in the development of UV LEDs from 2007 and projected to 2020. A. Wall plug efficiency (%) and price (USD). B UV LED lifespan, UV input and output (mW). *Prices converted from GBP (£) to USD ($) at currency exchange in November 2018. Data adapted from Ibrahim et al., (2014).*

With the future improvement of the semiconductor industry, UV LEDs will highly improve their efficiency and the design's possibility will be greatly benefited.

2.1.2 Regulations for UV Disinfection

UV disinfection is primarily used in treatment plants, thus, the regulation regarding UV disinfection is focused on this application. The USEPA has the Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule, which is a compilation of recommendations for the use of UV disinfection in water treatment plants using mercury-based lamps (United States Environmental Protection

Agency, 2006). This document includes procedures for UV dose validation, operational parameters, reactor configuration, water quality parameters to be considered when applying UV technology and design considerations for UV facilities.

The Guideline Technical Document for enteric viruses from the Guidelines for Canadian Drinking Water Quality (GCDWQ) (Health Canada, 2011) mentions that the most common dose applied in water supply systems in Canada is 40 mJ/cm^2 and is often applied in combination with chlorine disinfection or other physical removal barriers. The dose of 40 mJ/cm^2 is often used because at this dose the majority (i.e., 4-log removal) of enteric viruses would be inactivated. These guidelines also mention that in the case of drinking water sources that are less vulnerable to fecal contamination, a different ideal UV dose should be designated by a responsible authority. In the case that the drinking water source is prone to be contaminated, a higher UV dose or a multi-disinfectant strategy should be considered.

Furthermore, there is NSF/ANSI 55 certification: UV microbiological water treatment systems. This certification helps the manufacturers of UV POU and UV POE systems to verify that their products, in fact, can disinfect contaminated water. This certification has two classes, class A and class B. Class A systems are designed to disinfect contaminated water to a safe level. Class A should provide a fluence of 40 mJ/cm^2 and it may claim to be able to disinfect water that has been contaminated with pathogens, such as *Cryptosporidium* and *Giardia*. Class B systems may claim to reduce the normal microbial load present in water. These systems are designed as a supplemental treatment of water that has been declared under acceptable microbial parameters from a local health agency. Class B systems should deliver a fluence of 16 mJ/cm^2. Even though the NSF/ANSI is a

certification, not a regulation, this mark is well recognized by customers and health officials as a symbol of product quality (NSF International, 2016).

To the best of the author's knowledge, there is no specific regulation that talks about UV LEDs. The reason for this could be that the novelty of UV LED technology and that this technology is not being currently applied in great scale. However, with the development of the semiconductor technology and the improvement of UV LEDs, it is expected to see this technology more commonly applied within the near future. In terms of regulation, even though the technology is not the same and different design parameters need to be considered for the effective application of UV LEDs, such as reactor design and wavelength combination, the treatment objectives presented in current regulation should be kept the same.

2.2 Parameters Affecting UV Disinfection During Water Treatment

UV disinfection efficiency is highly affected by water quality parameters, being ultraviolet transmittance (UVT) and turbidity the most important ones. According to Qualls et al., (1983), suspended particles in unfiltered water can produce adverse outcomes on the effectiveness of UV light for microbial disinfection because they scatter, absorb, and block UV light. The more UV light that is absorbed by suspended particles, the less UV light that will hit the microorganisms in the water. Additionally, microorganisms can be shielded behind the particles and thus UV cannot penetrate the cell to damage and/or disrupt DNA. Furthermore, microorganisms can aggregate to form bigger particles that could possibly protect pathogens inside these aggregates. In the following section, it would

be described how UV disinfection efficiency is affected by UVT and turbidity and how these parameters need to be considered for better optimization of UV disinfection.

2.2.1 UV Absorbance and Transmittance

UVT is the measurement of the amount of UV light that passes through a water sample. This parameter is important because the lower the UVT, the less UV light can pass through the water, and thus, more UV light sources are necessary to achieve the desired dose.

Absorption is the ability of a particle or substance to absorb light and is calculated using the Beer-Lambert Law. The decrease of an incident light that passes through a water sample is called Absorbance (A). The absorbance value is unitless and it can be calculated as shown in Equation 2-1:

$$A = \log \frac{I_0}{I}$$

Equation 2-1

Where:

A= absorbance at a specified path length and wavelength

I_0 = intensity of light incident on the sample (mW/cm^2)

I = intensity of light transmitted through the sample (mW/cm^2)

UVT is expressed in percentage (%) and is usually measured in a 1-cm cell at 254nm. UVT is described in Equation 2-2 (Bolton, 2008a):

$$UVT = 100 \times \frac{E^t}{E^0} = 100 \times 10^{A_{254}}$$

Equation 2-2

Where:

E^0 = irradiances incident on the cell

E^t = irradiances incident transmitted through the cell

A_{254} = absorption coefficient at 254 nm at 1-cm path length

UVT is normally measured at 254 nm because at this wavelength low-pressure mercury lamps emit light. However, UVT scans are also important when the UV light source is a polychromatic light (medium pressure mercury lamp) because absorbance in other wavelengths can affect the UV disinfection performance. Furthermore, when the absorption coefficient increases, UVT decreases. The absorption coefficient increases when there are absorbing components in the water, with the principal one being total organic carbon (TOC). TOC consists generally of humic acid, alginic acid and possibly, phenols.

The effect of particles absorption, light scattering and UVT have been widely studied in order to know the real effect of this in UV disinfection. In the Handbook of Ultraviolet Disinfection (Bolton, 2008) suggests that as a general rule, when TOC increases, UVT decreases. However, other authors (Cantwell & Hofmann, 2011) have found that in surface water treated with UV light, there was no correlation between water quality parameters,

such as total organic carbon, total suspended solids, turbidity, or UV absorbance (254). Moreover, in the Christensen & Linden, (2003) study, the authors concluded that the impact that particles have on UV absorbance measurement is accounted for properly in the UV design, the effect on delivery of UV dose appears negligible for turbidity up to 10 NTU.

2.2.2 Turbidity

According to the Standard Methods of Examination of Water and Wastewater (2011) turbidity is caused by suspended and colloidal matter in water, such as silt, clay, finely organic and inorganic matter, plankton and microorganisms. The turbidity measurement is an expression of the light scattered and absorbed, rather than transmitted caused by particles in a sample of water. Furthermore, it is difficult to correlate turbidity with the number of particles in a water sample because of the shape, size and refractive-index of the particles.

Turbidity is usually measured in nephelometric turbidity units (NTU) using a nephelometer, which consists of a light source to illuminate the sample, indicating the intensity of the light scattered at 90° to the path incident light. The instrument used to measure turbidity should have a sensitivity of 0.02 NTU or less in water samples lower than 1 NTU. The sample cells used to measure turbidity should be a clear, clean, colorless cell, without scratches. Samples should be inverted a few times before portioning an aliquot to the cell. A blank using extra pure water should be taken and the value obtained should be subtracted from the measured turbidity value of the water sample.

The exact effect of turbidity in water when it is being treated with UV light is not well understood, and thus, it needs to be studied. Bolton (2008) suggests that in water with turbidity measurements below than 1 NTU, the effects on UV reactor performance can be ignored. In contrast, Cantwell & Hofmann, (2011) found that in some cases, where unfiltered surface water in small systems is being treated with UV light, turbidity values below 2 NTU interfered with UV disinfection mechanisms. Furthermore, Cantwell & Hofmann, (2008) found that particles naturally occurring in surface water as small as 11µm are able to protect indigenous coliform bacteria from UV radiation at 254 nm in doses up to 40 mJ/cm^2. The authors observed this phenomenon at a turbidity as low as 0.8 NTU. Finally, the study concluded that turbidity had a partial effect on the UV treatment.

Every source water is different; therefore, a source water characterization and monitoring are required when UV systems are being used. In addition, certain events, such as storms, could induce higher turbidity levels and lower UVT levels. For this reason, some jurisdictions require that small systems using UV systems to disinfect surface water need to use an upstream filter before the UV light treatment (Agriculture and Agri-Food Canada, 2008; Nova Scotia Environment and Labour, 2007; United States Environmental Protection Agency, 2006)

2.3 Microbial Testing Tools

Enteric diseases are considered the main public health concern related to microbial parameters in drinking water. The use of different microbial testing and monitoring tools to identify any microbial risk in water is imperative to keep the population safe from waterborne diseases.

2.3.1 Indicator Organisms

Due to the impracticality of looking and counting all the microbes that can be present in drinking water, microbial safety is evaluated through the detection of indicators of faecal contamination. A microorganism is considered a microbial indicator of faecal pollution when it is an organism that is naturally occurring in human or animal faeces. When these microorganisms are detected, it can indicate possible faecal contamination in a water body or distribution system and, potentially, the presence of enteric pathogens. A microorganism needs to meet the following criteria to be considered as an indictor microorganism (World Health Organization, 2003):

- The indicator organism should not be present in uncontaminated water and it should be present when the source of pathogenic microorganisms of concern are present. It should respond to environmental changes in a similar manner than the pathogen of concern.
- The indicator organism should be present in higher amounts than the pathogenic organisms.
- The indicator organism should not be a pathogenic organism itself, in order to minimize the analyst's health risks.
- The indicator organism should not be difficult to enumerate, identify and isolate.
- The test to identify the indicator organism should be economically reasonable in order to analyze of a large number of samples.

There is no perfect microbial indicator; even when an indicator microorganism meets all the criteria mentioned above, it does not ensure that its presence/absence would indicate the presence/absence of a pathogen. One of the major setbacks in the use of microbial indicators is the time that it takes to culture these microorganisms. Some of the microbial tests could take up to 48 hours to culture (Rice, Baird, Eaton, & Clesceri, 2012), which could lead to the risk of consumption of contaminated water.

2.3.1.1 Escherichia coli

E. coli is considered a model microorganism for a great variety of experiments because of its relatively easy culture, low nutritional requirements and rapid growth. Not all the *E. coli* strains are pathogenic, and usually, experimentation using *E. coli* as target microorganism uses one of the non-pathogenic strains (Verhille, 2013).

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium that is commonly found in the lower intestine of warm-blooded organisms and it is expelled to the environment within faecal matter. *E. coli* ideally grows at 37°C, but in laboratory it could grow up to 49°C (Verhille, 2013). *E. coli* is well referenced the indicator for the occurrence of recent faecal contamination in drinking water systems because it meets the criteria described previously. Only certain strains of *E. coli* can cause disease and only under certain conditions (i.e., *E. coli* O157:H7). When a water sample is tested for *E. coli*, encompass both the abundant non-pathogenic strains of *E. coli* (Leclerc, Mossel, Edberg, & Struijk, 2001) and pathogenic strains.

2.3.2 Heterotrophic Plate Count (HPC)

According to the Standard Methods for the Examination of Water and Wastewater (Rice, Baird, Eaton, & Clesceri, 2012), HPC is a procedure that estimates the amount of live and culturable heterotrophic bacteria in water. This technique can be used to measure changes during water treatment and distribution. In HPC, bacteria are quantified as colony-forming units (CFU), that can appear as single cells, clusters, pairs or chains. There are four different methods for bacterial quantification: the pour plate method, the spread plate method, membrane filter method and enzyme substrate method for heterotrophic bacteria. These aforementioned methods need to be chosen according to the specific needs and characteristics of the study. The type of agar, sample volume, dilutions, incubation time and temperature would also be selected according to the specific needs of the study. For the pour and spread plate methods, resulting colonies need to be counted after the indicated incubation time. The Standard Methods for the Examination of Water and Wastewater (Rice, Baird, Eaton, & Clesceri, 2012) suggest only taking into account plates with colonies between 30 and 300. Bacterial count per millilitre should be computed using the following equation:

$$CFU/mL = \frac{colonies\ counted}{actual\ volume\ of\ sample\ plated(ml) \times dilution}$$

Equation 2-3. Bacterial count per millilitre. Standard Methods for the Examination of Water and Wastewater section 9215-8. (Rice, Baird, Eaton, & Clesceri, 2012).

HPC serves as an indication of the load of general aerobic bacteria in a water sample. An increase in bacterial counts in finished water could indicate a problem with the water treatment or a change in the quality of the source water. When there is a significant increase

of bacterial counts in the distribution systems it could indicate that regrowth may be occurring (Verhille, 2013). Some disadvantages of HPC are the incubation time necessary, the limited bacterial population that can grow in laboratory conditions (0.01% to 1%) (Watkins & Jian, 1997) and the viable-but-non-culture state (VBNC) (Ayrapetyan & Oliver, 2016; Xu et al., 1982).

2.3.3 Adenosine Triphosphate (ATP) Assays

Adenosine triphosphate (ATP) is an energy molecule present in all living cells. The ATP molecule is a complex molecule that serves as the primary energy source in cellular metabolism. ATP is released from cells when they die, but this molecule is rapidly absorbed by other organisms or degrades, thus, the amount ATP is considered as a measure of living organisms within a system (Tifft & Spiegel, 1976).

Intracellular ATP released from the cells is measured using bioluminescence techniques. Released ATP reacts with luciferin (D-LH2) in the presence of the enzyme luciferase, magnesium ions and oxygen. This reaction results in a determinate amount of light per each ATP molecule present, therefore, light production rate and total light output can be correlated with ATP concentration. Furthermore, the light output intensity after the reaction needs to be measured rapidly because the reaction peaks within a few seconds and then decreases. An ATP photometer is usually used to measure the peak light output (Neethling, Johnson, & Jenkins, 1985).

ATP assays are considered as a rapid and reliable indicator of disinfection techniques, faster than culture-based methods (Neethling et al., 1985; Tifft and Spiegel 1976) and

some studies have shown a linear relation with HPC methods (Deininger & Lee, 2001; Delahaye, Welté, Levi, Leblon, & Montiel, 2003). However, other studies have found substantial differences between HPC results and ATP assay results (Kim et al. 2011; Neethling et al., 1985). It can be hard to compare HPC results with ATP results because of the inner difference between them. ATP measures the amount of ATP molecules in a water sample, while HPC measures the ability of microorganisms to reproduce. Microorganisms that are alive, but unable to reproduce (i.e. VBNC), will be counted for ATP but not for HPC.

Moreover, some authors suggest that ATP assays have monitoring potential in drinking water quality, where an increase of ATP in the same location and sampling conditions could indicate an increase of bacterial activity in real time (Keasler et al., 2013; Vang, Corfitzen, Smith, & Albrechtsen, 2014).

As mentioned before, ATP assays can be used for the rapid detection of bacterial activity in a sample, or to monitor the efficacy of a disinfection technique. In disinfection techniques where the microorganisms are removed from the water (i.e. filtration, coagulation/flocculation) or when the microorganisms are destroyed (i.e. chlorination, ozone) it is easy to correlate the decrease of ATP with the effectivity of the treatment. However, in UV disinfection, microorganisms are neither removed from the water, nor destroyed immediately after the UV dose. As previously mentioned, UV disinfection affects the DNA structure of the microorganisms, preventing them from replicating, but it does not remove them. Therefore, the amount of ATP present in a water sample that has been treated with UV radiation would not have a significant change before and after treatment. It has been shown in literature that ATP quantification will not show significant

difference when performed immediately before and after UV treatment (Linklater & Örmeci, 2014), but a more recent study conducted by Rauch et al., (2018) found that incubating the water sample that has been treated with UV radiation can make ATP assays suitable to quantify the effect of UV disinfection.

2.4 Point Of Use Technology (POU)

2.4.1 Drinking Water Situation Worldwide, Where POU Can Be Applied?

Water safety is essential to human development and well-being. One of the most effective means to promote health and reduce poverty levels worldwide is to provide access to safe water. The United Nations General Assembly in 2010 established the human right to water and sanitation. Everyone has the right to adequate, continuous, safe, reachable, and affordable water for personal and domestic use (United Nations, 2010). Furthermore, according to World Health Organization (2017) there is a direct relation between contaminated water and poor sanitation to the transmission of diseases such as hepatitis A, diarrhoea, dysentery, cholera, typhoid, and polio. In addition, there are approximately 502,000 diarrhoeal deaths each year due to contaminated drinking water. The absence or inappropriate management of water and sanitation services expose people to preventable health risks. This is especially important in health care facilities, where patients and staff have an additional risk of infection and disease if there is not enough water, sanitation, and hygiene services.

The WHO estimates that 15% of patients worldwide acquire an infection during a hospital stay and this number is even greater in developing countries. At the same time, the WHO has statistics that indicate that in 2017, 29% (2.1 billion) of the world's population do not use safely managed drinking water services, that is one readily available on premises and free from contamination. Furthermore, 159 million people still collect water directly from surface water sources (World Health Organization, 2017).

In situations where water is contaminated with pathogens, water intended for drinking needs to be treated before consumption. These situations raise a concern about the development and implementation of POU technologies. POU technologies are intended to prevent and reduce the number of diseases acquired from the lack of clean water. Boiling water before drinking is one of the most common strategies to disinfect water, although it has a high energy requirement and does not have a residual effect. Chemical disinfectants such as chlorine can be a suitable option, but the correct dosage can be compromised if the customer does not have the proper training to use it appropriately. Besides, chemical disinfectants can be expensive and the transportation to rural communities with difficult access can be challenging.

Taking into consideration that every POU technology has advantages and disadvantages, more approaches need to be researched and applied, in order to reduce the number of people that do not have access to readily safe water.

2.4.2 Point of Use Technology for Small Systems

Small drinking water systems (SDWS) are systems that serve less than 10,000 people. In most cases SWDS face the impossibility to have a centralized drinking water treatment plant, and thus, they need to rely on different strategies to deliver water to their costumers

that meet the drinking water guidelines. The implementation of Point of use (POU) or point of entry (POE) technologies is one way to facilitate smalls systems to meet the requirements for drinking water. POU and POE systems rely on the same treatment technologies that are usually used in centralized water treatment plants but designed to treat just a portion of the water. According to the Environmental Protection Agency, (2006), the term POU refers to systems or devices that can be installed in an individual source line ahead of some or all taps dedicated to dispensing water for direct consumption, such as drinking or cooking. POU systems can have several installation types, such as plumbed-in units, or faucet-attached units. Some of these systems typically have a separate tap for untreated water, so untreated water can be used for washing or cleaning and the treated water remains separate for consumption.

2.4.3 Point of Use Regulations

Water technologies such as adsorptive media, granular activated carbon (GAC), ion exchange (IX), and reverse osmosis (RO) are considered as POU and POE treatment technologies by Environmental Protection Agency (2006). These technologies are used for their ability to remove contaminants such as metals (i.e. arsenic, copper, lead), nitrates and pesticides. Although some technologies intended for POU have the ability to remove microbial contaminants, the EPA advises against using them to meet compliance with microbial contaminants. Furthermore, the Environmental Protection Agency (2006) advises that small systems need to have operation and maintenance procedures in case they are using POU or POE devices.

Canada does not have a specific regulation for POU/POE technology, however, the government of Nova Scotia talks about the use of POU or POE technology in case well water is being used (Nova Scotia Environment, 2009). Nova Scotia Environment does not recommend specific brands of drinking water devices, but it recommends the use of technology that is NSF certificated.

The NSF International certifies products intended for POU and POE applications, however, the NSF standard is technology-specific. Current technologies for water treatment covered for NSF standards include UV, reverse osmosis, ceramic filters, adsorptive medias, ion exchange among others.

2.4.4 Ultraviolet Technology for POU

The small size and wide range of wavelengths of UV LEDs make them suitable to be applied as POU. In POU technologies a residual effect is not necessary, because POU is intended for immediate consumption of the treated water, without further storage or distribution.

The use of UV light for POU in small communities for water disinfection has gained interest since the beginning of the century (Chatterley & Linden, 2010). The use of the traditional mercury lamp as a source of UV light comes with a number of sustainability issues, such as the fragility and high energy consumption, making small communities face difficulties to dispose the bulbs containing toxic mercury. The development of new UV light sources has helped to subside the disadvantages presented in UV lamps. Therefore, the interest of applying UV light as POU technology has increased.

UV LEDs in contrast with mercury lamps, do not contain mercury, which makes them a better option in terms of disposal. Also, they are smaller in size and robust, which makes them more ideal in terms of transportation. Furthermore, they have a lower energy consumption and warm-up time is not required, which allows applications where energy is limited, and the system can be turned on and off whenever is necessary.

The application of UV LEDs in flow-through reactors for POU drinking water treatments bring the same advantages of the UV technology (treatment-wise), without some of the major disadvantages of UV low and medium pressure lamps, allowing to disinfect water almost immediately. These systems can become an aid in a large number of communities where safe water is not readily available.

2.4.5 The Use of UV LEDs for POU

Despite the advantages of UV LEDs offer to POU technology over the use of mercury lamps, there are still some concerns about this technology. UV disinfection does not have a residual disinfection effect on the water, which narrows the market of UV disinfection technology to systems where the water will be ideally immediately consumed, without further storage rather than for distribution. Additionally, UV disinfection effectiveness is dependent on the initial water quality being treated and the reactor design, thus, these parameters need to be considered for UV LEDs as POU applications (Bolton & Cotton, 2008) (Cantwell & Hofmann, 2011).

Furthermore, UV LEDs efficiency are still a concern for the application of UV LED POU systems, with further technology improvement, increase efficiency and decrease price, the

potential application of UV LED as a POU technology would increase (Green et al., 2018; Ibrahim et al., 2014; Lui et al., 2014, 2016)

2.5 BIOFILM

Biofilm can be defined as a group of cells enclosed in extra polymeric substances (EPS), that they produce, growing on a surface (Lewis, 2001). The extra polymeric substances are primarily composed of polysaccharides, proteins, DNA and lipids, however, noncellular materials, such as silt or clay particles, corrosion particles and mineral crystals are also commonly found enclosed within the biofilm. The noncellular material that can be found in a biofilm matrix is often dependent on the surface where the biofilm has developed. Biofilms can form on a wide range of surfaces, such as living tissue, industrial and potable water piping, natural aquatic systems and medical devices (Flemming et al., 2011). Biofilms can be very diverse: there are single and multi species biofilm, depending on the surface, substrate and other conditions.

The biofilm development process depends on various factors, but some general development steps have been recognized such as the initial surface attachment of cells, cells aggregation, growth, maturation into an established biofilm, and the passive detachment of biofilm material (cells, noncellular material, toxins) into the surrounding environment (Hall-Stoodley, Costerton, & Stoodley, 2004). The enclosed biofilm provides a well protected ecosystem, allowing survival of microorganisms under unfavourable environmental conditions and offering tolerance to antimicrobial compounds (Lewis, 2001). Microorganisms enclosed in a biofilm matrix differ from their planktonic (free-

floating) counterparts. The microorganisms in biofilms have the ability to exhibit and pass a distinct phenotype with respect to gene transcription and growth rate (Donlan, 2002).

2.5.1 Opportunistic Pathogens and Health Considerations

According to Lewis, (2001), biofilms are responsible for more than 60% of human infections. These include device-related and non-device related infections. Common infections, such as urinary track infections, common dental plaque formation, and gingivitis are familiar examples of non-device related infections that are caused by biofilm formations. Furthermore, device-related infections include catheter infections, infected implants and prosthetics, ventricular assisted devices, contact lenses, central venous catheters, among others (Jamal et al., 2018). In summary, biofilm formation on indwelling medical devices has a significant influence in nosocomial acquired infections. Consequently, biofilm prevention and control are a major public health concern.

Other sources of biofilm related infections are drinking water systems, which can become temporary or permanent environments for health-related microorganisms. It is known that biofilms will form in any surface that is in contact with non-sterile water (Wingender & Flemming, 2011). Furthermore, pathogens present in water (often times in below detection limit) can attach to biofilms, and these can act as pathogen's reservoirs. The later detachment of biofilm particles can become a source of water contamination.

According to Flemming (2002), around 95% of the overall bacterial biomass found in drinking water distribution systems (DWDS) is in biofilm-bound form, attached to premise plumbing, and not dissolved in the water, which is often the portion sampled. Moreover,

low culturability is common of biofilm naturally occurring in drinking water, especially in old biofilm. Drinking water suppliers often look for water with a reduced amount of nutrients, thus, less amount of biofilm can be formed. However, the naturally occurring microflora usually found in biofilms consist in microorganisms that are not a threat to human health. The major concern about biofilm formation in DWDS is that they can harbour pathogenic microorganisms (i.e. *Legionella s.p*, *P. aeruginosa*), and prevent disinfectants to act on them.

Wingender & Flemming (2011) describe two categories of relevant microorganisms found in DWDS biofilms. First, there are the pathogenic microorganisms which have been associated with water-related illness and outbreaks, and secondly, microorganisms which are primarily used as indicators in water analysis.

The first category, the pathogenic organisms, can be divided in two sub-categories. The first one consists in enteric pathogens, usually faecally originated, which can cause disease to humans independently of their health status, while the second sub category consists in opportunistic pathogens, which cause disease in vulnerable populations (children, elderly, or individuals with a compromised immune system). Some of the opportunistic pathogens that can be found in DWDS include *Campylobacter spp*., *H. pylori*, *Legionella spp*., Cryptosporidium spp., enterohaemorrhagic *E. coli* O157:H7, Faecal streptococci and *P. aeruginosa*.

2.5.1.1 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a non-fermenting, Gram negative bacteria. *P. aeruginosa* is an opportunistic pathogen, inherently resistant to many antibiotics and can cause infections in eyes, skin, ears, urinary track and respiratory track, which can lead to sepsis (Smith &

Iglewski, 2003). *P. aeruginosa* is a common cause of nosocomial diseases. Some of the most common environmental reservoir of *P. aeruginosa* in hospitals include: taps, shower heads, respiratory therapy equipment, flower vases, water fountains, among others (Kerr & Snelling, 2009). *P. aeruginosa* is an organism commonly found in biofilm and has become one of the most studied model organisms in the biofilm research field (McDougald et al., 2008).

2.5.2 Biofilm Control and Biofilm Disinfection Techniques

In the biofilm control field, there is a wide range of techniques that can be applied. Ideally, biofilm formation prevention would be the best practice to prevent any biofilm-related risk. However, there is no technology that could totally guarantee the prevention of unwanted biofilms formation without side effects. The main strategy for biofilm control is the regularly cleaning and disinfection of surfaces of interest, to prevent bacteria to attach firmly to the surfaces (Midelet & Carpentier, 2004). Other biofilm prevention strategies are biofilm detectors (Pereira et al., 2008) and the use of materials that can prevent biofilm formation (Rogers et al., 1994).

Surface cleaning and disinfection remain as the most common practices for biofilm control. It is known that disinfectants have a poor penetration in biofilms, therefore, the cleaning procedure before the disinfection is the most important to improve sanitation effectiveness (Simões et al., 2006). According to Simões et al. (2006), an effective biofilm cleaning procedure must be able to dissolve the EPS material so the chemical disinfectants can penetrate to the cells within the biofilm matrix. Due to the possibility of biofilm

regrowth, a strict cleaning and disinfecting regime must be followed. However, these procedures tend to be expensive and involve the interruption on surfaces usage.

The disinfectant needs to be chosen according to the type of biofilm and the surface that needs to be disinfected. It is important to take in to account that most of the disinfection studies using disinfectants are performed using planktonic (free-floating) cells, however, studies have shown (Bridier., 2011; Høiby., 2010; J.A. Otter., 2015) that biofilm have higher resistance to antimicrobials than their planktonic counterparts. Biofilm-bound bacteria high resistance to disinfectants is a multifactorial process, where different mechanisms (i.e. the phenotypic adaptations of biofilm cells, gene transfers and mutations, pathogens being protected in multispecies biofilm) play a role (Bridier et al., 2011). The risk of infections caused by biofilms and the increasing bacterial and biofilm resistance to antimicrobials increases the need to new and more efficient biofilm control strategies.

2.5.2.1 Ultraviolet disinfection on biofilm

Since biofilms have gained resistance to antimicrobials, more biofilm control strategies have started to be investigated. UV radiation as a part of disinfection regimes for biofilm mitigation has started to gain popularity (El-Azizi & Khardori, 2016; Friedman, Harif, Herzberg, & Mamane, 2016; Garvey et al., 2015; Markvart., 2017). However, the amount of UV radiation necessary to penetrate in a biofilm matrix remains unclear. Despite several studies have used UV radiation for biofilm disinfection in the past (Bak., et al. 2010; Garvey et al. 2015; Li et al. 2010), a UV radiation effectiveness comparison between them is hard to achieve due to their differences in UV light sources.

Bak et al. (2010) found that UV C LEDs were effectively used to disinfect *P. aeruginosa* biofilm contaminated catheters. The authors found a significant impact between the lumen tube material and the disinfection achieved. Garvey et al. (2015) used pulsed UV (PUV) light to achieve significant rates of inactivation of *P. aeruginosa* and *Staphylococcus aureus* in biofilm form grown in a CDC reactor. The authors suggest that their findings could provide a method for POU inactivation of biofilm, but more research needs to be conducted in the subject. Furthermore, Li et al. (2010) investigated the effect of pulsing UVA-LEDs at 365 nm on *Candida albicans* or *Escherichia coli* biofilm. This study found that 5 min pulsed UVA LED irradiation at 100 Hz was the most efficient for the inactivation of both *C. albicans* and *E. coli* biofilms. Furthermore, Gora et al, (2019) summarized UV inactivation studies on biofilm and found that the results differed significantly, in terms of inactivation even at the same microorganism. A reason for this could be because of the difference in experimental design and UV apparatus used.

Despite all the aforementioned studies concluding that UV radiation had a significant effect on biofilm disinfection, the most effective UV doses and wavelengths for biofilm disinfection have not been determined, due to the different approaches that these studies have used to report their results.

2.5.2.2 Efficacy of UV light in combination with other disinfection techniques

Some studies have investigated at the effects of UV radiation when is combined with other disinfection techniques. Koivunen & Heinonen-Tanski (2005) applied a combination of peracetic acid (PAA) and UV disinfection, finding that this combination increased disinfection efficiency and showed synergistic benefits, being the highest synergy values

reaching 2 log units for enteric bacteria. In a similar way, Murphy et al., (2008) combined monochloramine, chlorine and chlorine dioxide alone or in combination with UV radiation to study the effects on *E. coli* growth and persistence in planktonic and biofilm-bound form. Even though Murphy et al., (2008) applied the UV radiation and chemicals at the inlet of the systems, and not directly on the biofilms, synergistic effects were found on biofilm formation as well. This study found that in overall, the combination of UV radiation with chlorine-based disinfectants achieved higher log reduction in both *E. coli* cultures and reduced biofilm formation than the chlorine-based treatments alone. Similarly, El-Azizi & Khardori (2016) paired UV C light with antistaphylococcal antibiotics to disinfect catheter biofilms of antibiotic-resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*. This study established that the use of the antibiotics on the antibiotic-resistant strains or the UV radiation alone did not have a significant impact in the disinfection of the biofilms. However, the antibiotics treatment of the biofilms after the UVC light significantly reduced the number of viable cells within the biofilms.

To summarize, the combination of UV radiation with other disinfection technologies could ultimately improve biofilm control strategies.

CHAPTER 3 MATERIALS AND METHODS

The overall materials and methods applied in this project are outlined below. The specific methodologies used during specific phases of this research project are explained in each chapter.

3.1 Experimental Set-up for UV LED POU Reactors Testing

Experiments were conducted at Dalhousie University at the microbiology lab in the Clean Water Laboratory. For the test of UV LED POU reactors, two different models of UV LED POU reactors available at the time were chosen. To test the efficiency of these reactors, Phosphate Buffer Solution (PBS) prepared with deionized water was spiked with *E. coli* and passed through the reactors. *E. coli* K-12 was used as a target microorganism because is a non-pathogenic strain of *E. coli*. *E. coli* is commonly used as a model microorganism for different types of studies, such as water disinfection studies, and it is the most thoroughly studied prokaryotic microorganism due to its rapid growth and simple nutritional requirements.

The *E. coli* spiked solution was pumped in to the UV LED reactor using a peristaltic pump (Cole-Parmer Canada Inc., Anjou, QC, Canada) and opaque tubing (Masterflex Precision Tubing, Cole-Parmer Canada Inc., Anjou, QC, Canada) at different sizes, to achieve different flow rates used in the described tests (see CHAPTER 4 of this dissertation).

The peristaltic pump used was calibrated and set to the intended flow rate every time before the experiment was conducted, to minimized error introduced by the pump affecting the actual flow rate at which the reactor was operating.

3.1.1 Reactor Cleaning Procedure

Before each experiment, the whole system needed to be carefully cleaned. Due to the impossibility of autoclaving the reactors or conducting the experiments inside the biosafety cabinet (BSC), a chemical cleaning method was chosen. A solution of 70% ethanol was circulated for about 30 minutes through the reactors and tubing.

Afterwards, the UV LED POU reactors were flushed with 4L of deionized sterile water to ensure no residual ethanol. Furthermore, the reactors were flushed with 1 L of sterile phosphate buffer solution (PBS), and samples of each reactors, as well as blank line were taken (See Figure 3-1). Standard plate count and ATP tests were performed on the samples, in the same manner as the treated samples.

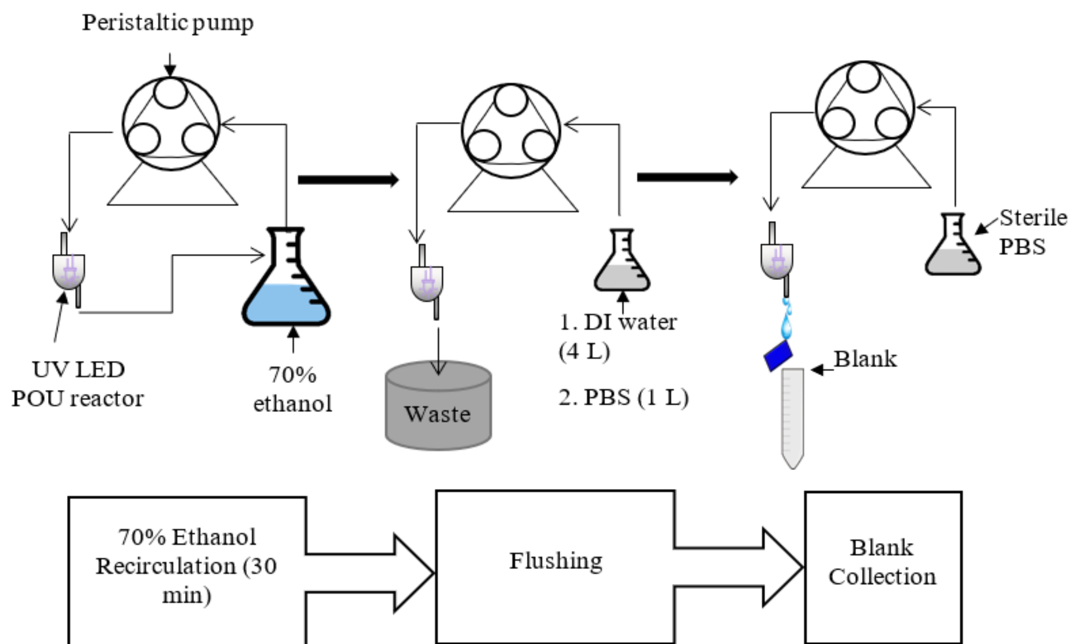


Figure 3-1 UV LED POU reactor cleaning procedure

The cleaning process was considered successful if blanks samples resulted in > 30 CFU/mL and 15 pg cATP/mL. This level was chosen because were the highest bacterial levels found in the system after the described cleaning procedure was performed.

3.1.2 Sample Treatment and Collection

After cleaning, water solutions spiked with *E. coli* were pumped through the reactor, ensuring that all samples taken passed uniformly through the reactor and they were equally impacted by light, without any extra contact time inside the reactor's chamber. After each experiment, samples were taken and kept in amber bottles to avoid light exposure and possible bacterial reactivation. These experiments were not conducted under red light or dark conditions, because according to the author's criteria, this would not represent what would happen in usual household conditions where these reactors are supposed to be applied.

Reactor testing was conducted in two timelines, the first reactor (Reactor A) was tested during the summer of 2017 and the second reactor (Reactor B) was tested during the summer of 2018. The system was set up as described in Figure 3-2.

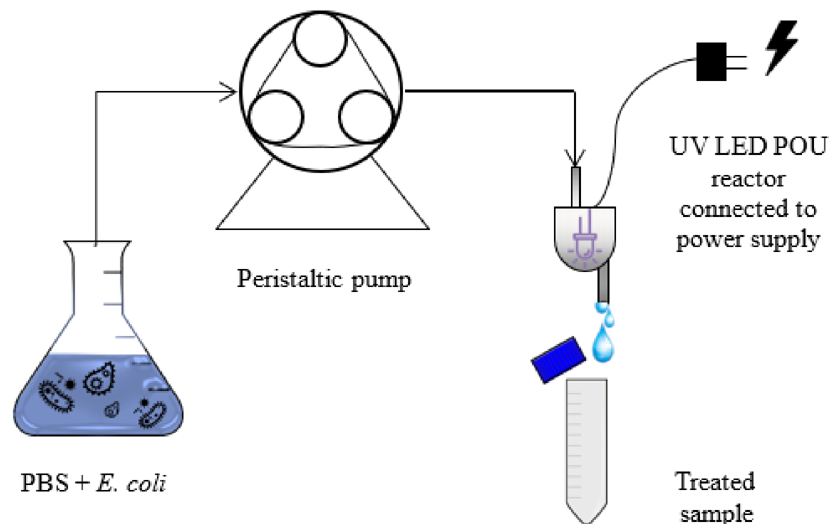


Figure 3-2 Diagram of UV LED reactor set up and sample collection

3.2 *E. coli* Preparation and Inoculation Procedure

For the experiments conducted in this study, *E. coli K-12* was chosen because is widely use and because is a non-pathogenic strain.

E. coli K-12 (ATCC #47076 strain MG1655) was obtained from the Centre for Research in Environmental Microbiology at the University of Ottawa. *E. coli* stock was kept frozen in vials (50% culture in TSB and 50% glycerol) at -80°C until the time of the experiment.

E. coli was grown in tryptic soy broth (TSB). TSB was prepared according to package instructions and autoclaved for sterilization. TSB was cooled down until room temperature before use. In every experiment, one vial of *E. coli* was taken out from the freezer and thawed inside the BSC. After thawing, 125mL of sterile TSB was inoculated with 250 µL inoculum and grown overnight (12-16 hours) at 37°C and shaken at 175 RPM. After the overnight growth, 50 mL of sterile TSB was inoculated with a 50 µl inoculum from the overnight culture, which was grown until mid-exponential phase. The *E. coli* was grown until mid-exponential phase to ensure that the bacteria was metabolically active

In order to determine when *E. coli* reached mid-exponential phase, a growth curve based on the optical density at 600 nm (OD 600) was created from this procedure (see Figure 3-3). To measure cell optical density (OD 600), a 700 mL inoculum was taken from the culture and placed in a 10 mm quartz cell. Afterwards, light absorption at 600 nm was measured using a Hach DR 500.

When the *E. coli* was at mid-exponential phase, an extra aliquot was taken, diluted and plated in triplicate in a TSA and incubated for 16 hours. After incubation, colonies were

counted. This plating procedure was performed to correlate CFU/mL with optical density at 600 nm (OD 600) and to make an estimate of cell concentration (see Figure 3-4).

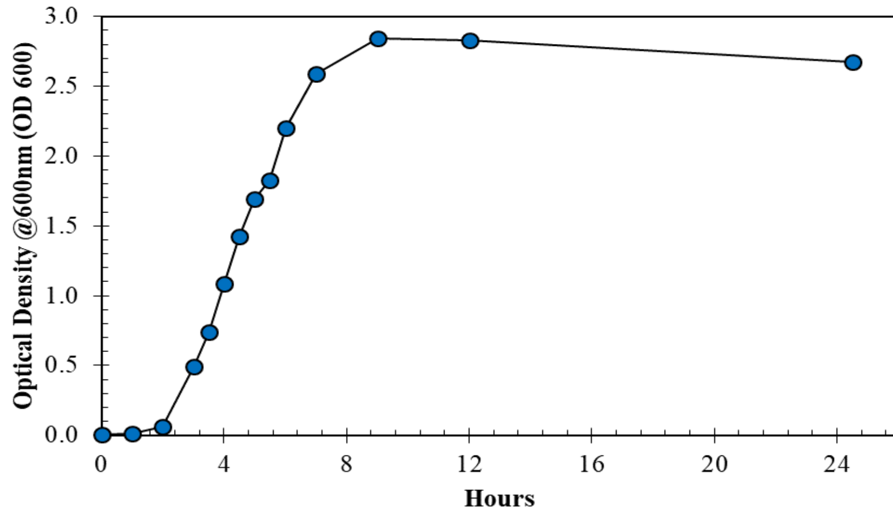


Figure 3-3 Time and OD 600 values

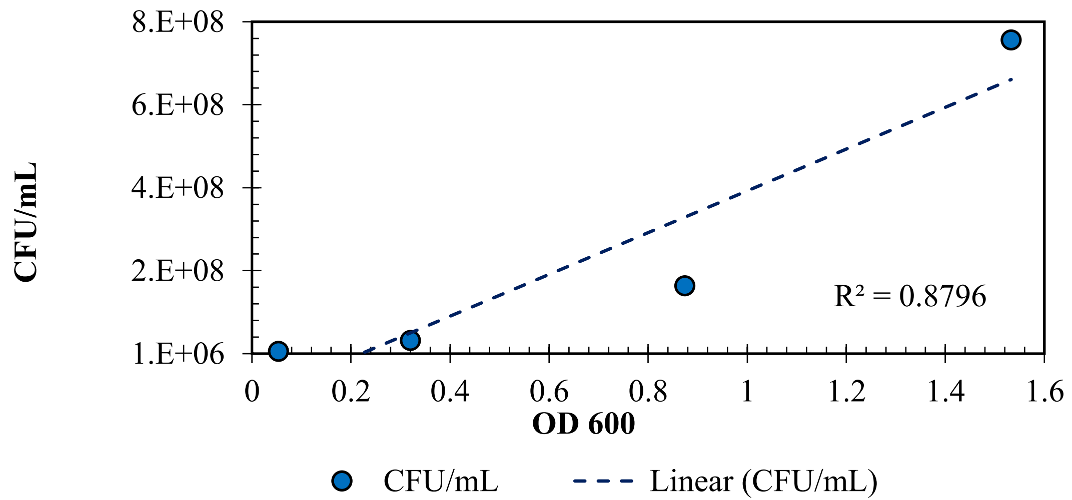


Figure 3-4 Correlation between OD 600 and CFU/mL.

After approximately 4 hours of subculture, OD 600 was measured and a value around 1.0 was expected, which would mean that the *E. coli K-12* culture was in the middle of the logarithmic growth phase and according to the line equation, the concentration would be around 4 x 10^8 CFU/mL. After 4-h of growth and confirmation that OD 600 was around 1.0, bacterial culture was transferred to a sterile Falcon™ 50 mL conical centrifuge tube. The tube with the culture was then centrifuged for 15 minutes at 3.6 x1000 RPM to form a cell pellet. The supernatant was decanted, and the tube was refilled to its previous volume with PBS. Subsequently, the tube was vortexed to resuspend the pellet. After resuspension, the tube was centrifuged, decanted and vortexed two more times, to wash the cells from the previous TSB. In total, cells were washed twice. This final solution is the concentrated work solution which had the same concentration stated before (4 x 10^8 CFU/mL). This concentrated *E. coli* solution was further diluted to the desired concentration.

All the sample handling and preparation was done under sterile conditions, inside the BSC.

3.3 Bacterial Quantification Methods

3.3.1 Standard Plate Count

To quantify the effect of UV treatments, standard bacterial plate count and ATP analysis were performed. Before plating, a set of serial dilutions was done, depending on the initial concentration used at the time and the expected concentration after treatment. For the serial dilutions, a 1 mL aliquot was taken and transferred to a 9 mL sterile PBS tube using a sterile pipette tip and gently vortexed. This was counted as the first dilution or 10^{-1}. Subsequently, a 1 mL aliquot from the 10^{-1} tube was taken and added to a new 9mL sterile

PBS tube, which was gently vortexed to create the second dilution or 10^{-2}. This procedure was repeated subsequently using a sterile pipette tip every dilution until the desired dilution was reached. Every sample was diluted in triplicate.

Tryptic soy agar (TSA) is a growth media for culturing bacteria. TSA is a non-selective general use media and it was chosen to be used to culture *E. coli* because of its efficiency and simplicity.

TSA was prepared from manufacturer's instructions, autoclaved to be sterilized and poured into sterile Petri dishes inside the BSC. Plates were left half uncovered until agar was dehydrated and solid and all the condensation on the top lid of the plate was evaporated. After agar was solidified, plates were covered with their lids, turned upside down and placed inside the incubator at 37°C overnight. After overnight incubation, plates were examined for possible colonies, in case the agar preparation or pouring was not done with the correct technique. Plates where colonies grew were discarded. Overnight incubation also served to dry the agar even more, so it could easily absorb the aliquot further plated.

After the serial dilution for each treated sample and untreated control was performed, a known aliquot was taken using a new sterile pipette tip, deposited on a TSA plate and spread evenly in the agar surface using a sterile glass rod. Usually, 0.1 mL was the volume aliquoted, but this changed to bigger volumes (up to 0.5 mL) in the cases that it was necessary to capture lower bacterial concentrations. Plates were saved upside down in a dark incubator and incubated for at least 16 hours, up to a maximum of 24 hours, at 37°C ± 0.5 °C.

When adding an inoculum higher than 0.1 mL, plates were left to sit, until all the liquid was absorbed by the agar. After the incubation time, plates were taken from the incubator and all the visible colonies were counted. Only the plates with colonies between 30 and 300 were taken into consideration.

All the dilutions and plating were performed using sterile material and under sterile conditions inside the BSC.

3.3.2 Recovery ATP Method

ATP analysis was also conducted to determine the efficacy of the UV LED treatment. The LuminUltra® ATP QGA kit (LuminUltra, 2017) was used with a few modifications. An incubation step was added to the regular kit instructions, to let the damaged cells try to reproduce and quantify the real effect of UV light. This method is referred in this dissertation as the recovery ATP method and more details can be found in the study by Rauch et al., (2018).

After each UV treatment, samples were collected, and an aliquot was taken and added to a nutrient broth made from a quarter of strength TSB and 0.1% v/v glycerol solution in a ratio of 1:10. The resulting solution was considered as the new sample to be analyzed and it was left to incubation for 4 hours at 37°C. After the incubation time passed, samples were taken from the incubator and processed according to the manufacturer's instructions (LuminUltra, 2017).

3.4 Bacterial Log Inactivation Calculation

Log inactivation was calculated to quantify the efficiency of the disinfection treatments presented in this dissertation.

Log inactivation was calculated using Chick's law as follows:

$$Log\ inactivation = \log_{10} N_0 - \log_{10} N$$

Where:

N_0: Control (untreated) concentration

N: Treated concentration

Equation 3-1 Equation used to calculate bacterial log inactivation

The log inactivation values for each experiment were calculated using the control from each batch.

3.5 Biofilm Growth

P. aeruginosa PA01 was chosen as a target microorganism for all the experiments with biofilm-bound bacteria in this study. *P. aeruginosa* was chosen because is a well-studied microorganism and is capable of growing biofilm in a wide range of conditions (Díaz De Rienzo et al, 2003). *P. aeruginosa* was grown in polycarbonate coupons, using a CDC reactor (BioSurface Technologies Corp, n.d.) following the USEPA's methodology (US Environmental Protection Agency Office of Pesticide Programs, 2013). In detail, one millilitre of pure laboratory grade of *P. aeruginosa* was inoculated in the CDC reactor,

with 500 mL of solution in a concentration of 300mg/L TSB. The reactor was maintained in batch mode rotating at 250 RPM for 24 ± 1 hours. Following the batch mode period, the system was switched to continuous stirred-tank reactor (CSTR) mode. A solution of 100 mg/L TSB (approximately 16L) was continuously passed through the reactor at a 10mL/min during 24 ± 1 hours. The temperature of the reactor was maintained at room temperature (23 ± 1 °C) for all stages.

3.6 Biofilm Collection And Treatment

After 24 ± 1 CSTR period, coupons were carefully removed from the CDC reactor inside the BSC and placed in 47 mm sterile Petri dishes. After all the coupons were removed from the CDC reactor, they were subjected to UV LED treatment at different fluences and to wiping treatments. All coupons treated with UV radiation were kept under red light to avoid *P. aeruginosa*'s reactivation mechanisms, which would skew the results. After the coupons were treated, biofilm was carefully removed from the coupon (see Figure 3-5), using sterile swabs and resuspended in 50mL of PBS (Gagnon & Slawson, 1999). Serial dilutions of the resuspended biofilm were performed followed by plating in TSA plates, in the same manner as E. *coli* solutions mentioned before. All the serial dilutions and plating were aseptically performed under red light to avoid reactivation mechanisms.

Plates were incubated upside down in a dark incubator at 37°C ± 0.5 for 16 to 20 hours. After the incubation time, colonies that had grown on the plates were counted; only plates with colonies between 30 and 300 were considered.

As well as standard plate count, ATP tests were performed to quantify the treatment effect on the biofilm. On biofilm treated with UV light, the same ATP regrowth method mentioned before was applied. An aliquot of the treated and resuspended biofilm was taken

and placed in a special solution in a ratio of 1:10 and incubated for 4 hours, allowing *P. aeruginosa* to reactivate. After the incubation time, ATP was analyzed with LuminUltra® ATP QGA kit according to manufacturer's instructions.

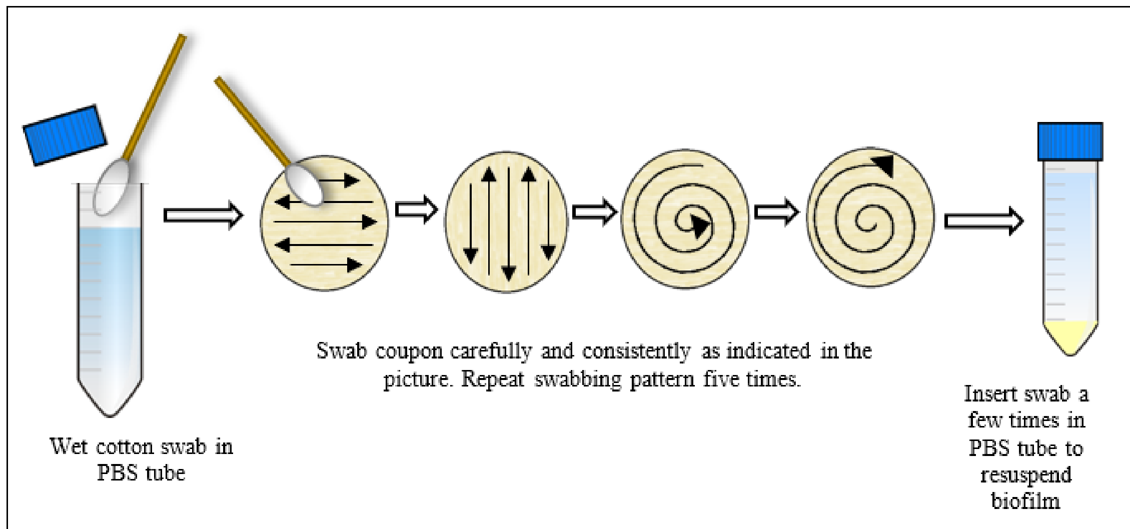


Figure 3-5 Swabbing procedure used to remove biofilm from coupons.

CHAPTER 4 UV LED POINT OF USE REACTORS FOR DRINKING WATER TREATMENT

4.1 INTRODUCTION

The use of UV LED for the inactivation of microorganisms has gained a lot of interest in recent years in different fields, including the drinking water industry. The characteristics of UV LED technology, such as smaller size, no warm-up time and a great range of wavelengths, compared with conventional mercury lamps, make them more suitable for a large range of applications. These characteristics make UV LED technology an excellent fit for point of use (POU) drinking water treatment.

The use of household POU treatment systems can be seen as an alternative to large-scale, centralized and, usually expensive, methods for drinking water disinfection or as an addition to full scale treatment. POU technology is primarily (but non-exclusively) intended for the use in remote and rural areas (Mintz et al , 2001) and for consumption of the water after treatment, without further storage.

The efficiency of UV LED technology has been shown in several studies (Chatterley & Linden, 2010; Green et al., 2018; Kim et al, 2017; Rattanakul & Oguma, 2018) but these experiments were mainly conducted using collimated beam systems; consequently, research in UV LED flow through systems applied as a POU remains limited.

Despite the advantages of UV LED compared to traditional mercury lamps, the specific characterization of UV LEDs, their operational requirements, the measurement of their efficiency and performance is still not standardized for water treatment operations.

Even though research in the POU industry using UV LED reactors is still limited, UV LED POU reactors are currently available on the market. Therefore, more testing of UV LED reactors intended for POU applications that are commercially available on the market is necessary, especially if they will be used under challenging drinking water quality conditions, such as high turbidity levels and low ultraviolet transmittance (UVT).

4.1.1 Objectives

The objectives of this chapter were to:

1. Create dose-response curves using the UV LED reactors under challenging conditions.
2. Analyze different quantification techniques applied to the evaluation of UV LED POU technology.
3. Conduct a field scale evaluation of UV LED POU technology in an Arctic community.

4.2 UV LED POU Devices Tested

Two commercially available UV LED devices intended for POU drinking water treatment were used in this study. Both models were low-priced, used solely UV LED for disinfection, operated in the UV-C range, and were commercially available at the time of purchase. Both reactors operate in a flow-through mode, which means the water is exposed to light only when it is passing through the device without any extra contact time from

water sitting inside the reactor's chamber. For clarity, in this document the two UV LED POU reactors will be referred as reactor A and reactor B. However, the model and reactor specifications provided in Table 4-1.

Table 4-1 Specifications of UV LED POU reactors for drinking water treatment tested.

	Reactor A: H-WSE06 from H-CEN®	**Reactor B: PearlAqua from AquiSense®**
Maximum operational capacity	1 LPM	5.3 LPM
Sterilization rate	99.6% (2.5 log inactivation)	99.99% (4 log inactivation)
Wavelength	275 nm	280 nm

Reactor's B manufacturer suggests that the drinking water intended for treatment should have an ultraviolet transmittance (UVT) above 90%. Reactor A's manufacturer does not mention any drinking water quality specification.

4.3 Laboratory Scale Testing of UV LED Reactors Intended for POU Drinking Water Treatment

Reactors were connected to the pump in order to pass the bacterial solution through them (see Figure 3-2). This set up was indented to represent a typical installation of an UV LED POU reactors connected to a tap. Details of the reactor set up are explained in Section 3.1 of this dissertation. All experiments were conducted using aseptic techniques. Samples were taken in sterile amber bottles, ensuring that all the samples collected were passed through the reactor.

4.4 Reactor A Characterization and Testing

Reactor A characterization was interesting to test due its simple operation and low price. Reactor A has a simple operation procedure, which consists of plugging in the reactor to the power source and immediately the LEDs inside will turn on and start treating the water that is being passed through.

Samples that were passed through the reactor at each condition were taken and the efficiency of the treatment was calculated and expressed as log inactivation.

Four devices of the same model (Reactor A) were connected for testing, finding no significant difference between them, in terms of operation efficiency.

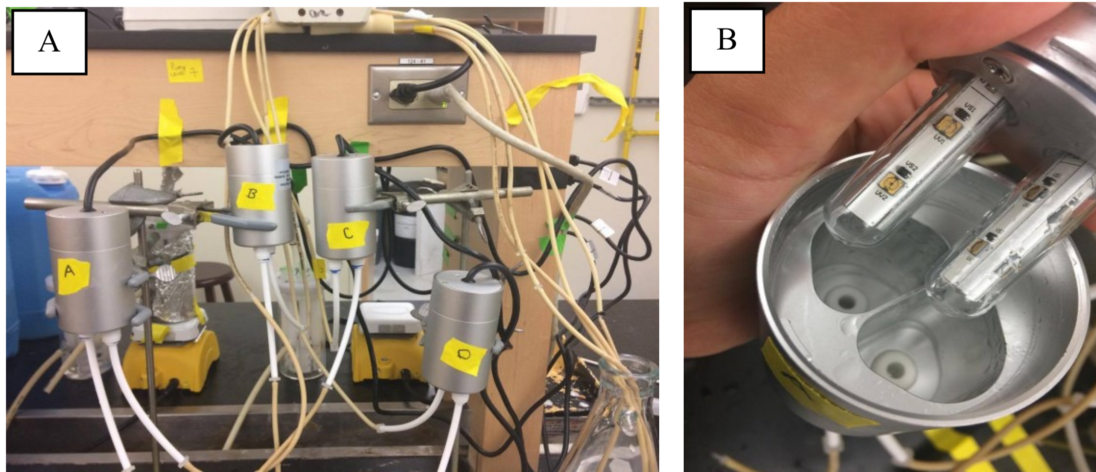


Figure 4-1(A) Reactor A units connected to peristaltic pump (B) Interior of reactor A

Reactor A were connected to a peristaltic pump (Cole-Parmer Canada Inc., Anjou, QC, Canada) and opaque tubing (Masterflex Precision Tubing, Cole-Parmer Canada Inc., Anjou, QC, Canada) at different sizes, to achieve different flow rates used in the described

tests. Flow rates were measured before each experiment to make sure that the pump was appropriately calibrated.

4.4.1 The Impact of Bacterial Concentration on Log Inactivation

Two different *E. coli* concentrations were tested to explore the impact of high and low bacterial concentration.

The high bacterial concentration was $1.16 \times 10^6 \pm 1.14 \times 10^5$ CFU/mL and the low bacterial concentration was $1.14 \times 10^3 \pm 8.1 \times 10^1$ CFU/mL. *E. coli* was grown using TSB and cells were washed in PBS afterwards, as mentioned in Section 3.2. The bacterial solution in PBS had a UVT of 99.05% ± 0.78% and a turbidity of 0.15 NTU ± 0.01 NTU. Artificial water matrix created did not disturb the operation of the reactor, nor affect the light transmittance in any significant manner, therefore, Reactor A operated in the best-case-scenario conditions.

4.4.2 The Impact of Flow Rate in Log Inactivation.

Three different flow rates, 0.06 LPM, 0.11 LPM and 1.0 LPM were evaluated. The highest flow rate (1.0 LPM) was chosen because the manufacturer's manual states that the units could achieve 99.96 % inactivation at 1.0 LPM. The lower flow rates were chosen to show how disinfection will change when changing the flow rate. The flow rate is directly proportional with water residence time inside the reactors, and, thus, the UV dose applied on to the water passing through.

Table 4-2 Experimental design used to test reactor A

Run	Flow Rate (LPM)	Initial Bacterial Concentration[1]
1	0.06	Low
2	0.11	Low
3	1.00	Low
4	0.06	High
5	0.11	High
6	1.00	High

[1] Low concentration represents 1.5 x10^3 CFU/ml and High concentration represents 1.5 x10^6 CFU/mL.

4.4.3 Reactor A Testing Results

The results from the experimentation described above are presented in Figure 4-2

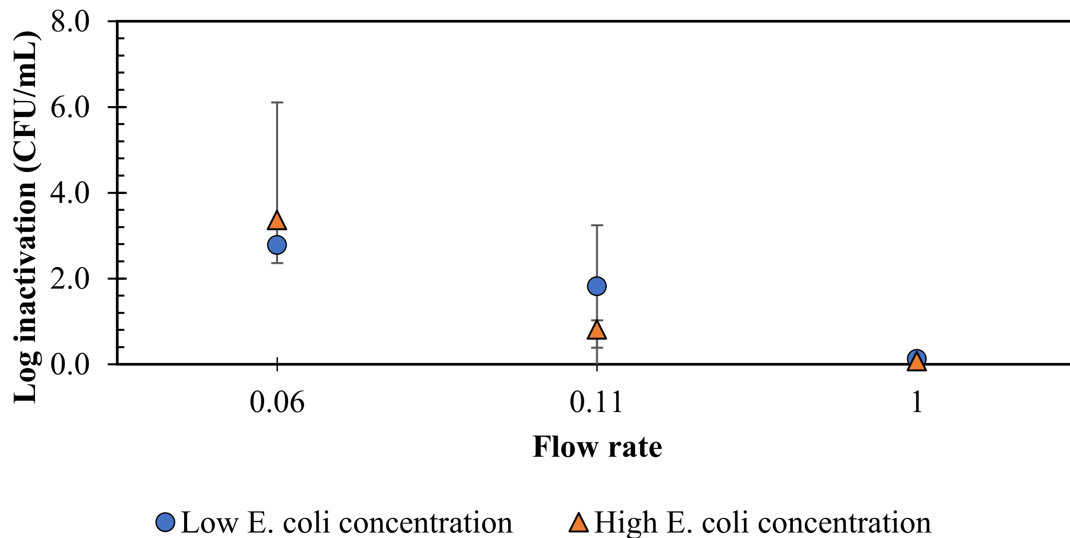


Figure 4-2 Log inactivation values of E. coli for three flow rates using reactor A. Log inactivation values were obtained using the standard plate count method. Each point represents the average of the two units. Error bars represent standard deviation, n=6

Under the conditions studied, the 2.5 log inactivation claimed by the manufacturer was not achieved at 1 LPM at either high or low bacterial concentration (Figure 4-2). One-way ANOVA was conducted between the control (untreated) sample and the treated sample at 1 LPM at the low bacterial concentration to determine if there was any significant difference between the untreated and treated sample. The resulting p-value was 0.001, rejecting the null hypothesis that all means are equal (at $\alpha=0.05$) and indicating that there was a significant difference between UV treated and non-treated samples. Similarly, a second one-way ANOVA was conducted between the control (untreated) sample and the treated sample at 1 LPM at the high bacterial concentration. The p-value, was 0.297, indicating that there was no significant difference between the untreated and treated sample at the high bacterial concentration.

To investigate which factor had a greater effect in the log inactivation, a two-way ANOVA was conducted between the initial bacterial concentration (high and low) and the flow rate (0.06, 0.11 and 1 LPM). The resulting p-values indicate that the initial bacterial concentration factor (high and low) did not have a significant impact in the log inactivation (p-value = 0.721), however, the flow rate had a significant impact in the log inactivation response (p-value = 0.000).

In summary, there is no statistically significant effect at high or low initial bacterial concentration, but there is a significant effect in the flow rates tested.

These results contradict the manufacturer's statement that the reactor can be efficient at 1 LPM. On the other hand, a log inactivation of 3.3 was achieved when the reactor was operated at 0.06 LPM and using the high bacterial concentration. This result was similar at the same flow rate but at low bacterial concentration, achieving 2.7 log inactivation.

These are positive results confirming that the unit is able to inactivate microorganisms, in this case, *E. coli*, in water, but only at a very low flow rate, lower than the medium flow rate at a tap which is 5 LPM (Health Canada, 2017b). For this reason, this unit would not be suitable in a real-life scenario where a POU disinfection is needed.

As shown above, Reactor A did not meet the manufacturer's specifications of 99.6% efficiency at 1 LPM. No further experimentation was conducted using Reactor A. It was concluded that any additional challenge in its operational parameters (higher flow rate) or any disturbance in the initial water quality (higher turbidity or lower % UVT) would have a significant impact in its performance.

4.5 Reactor B Characterization and Testing

A second UV LED POU reactor was also tested (Reactor B). Set-up was kept the same as reactor A, with the exception that only two units were used for the testing. The two reactor B units were connected to a peristaltic pump (Cole-Parmer Canada Inc., Anjou, QC, Canada) using opaque tubing (Masterflex Precision Tubing, Cole-Parmer Canada Inc., Anjou, QC, Canada) at different sizes, to achieve different flow rates used in the described tests. Flow rates were measured before each experiment to make sure that the pump was properly calibrated.

Two units were used for each test. Since both units would have similar efficiencies, samples taken under the same conditions were considered replicates. Treated samples were taken in duplicates.

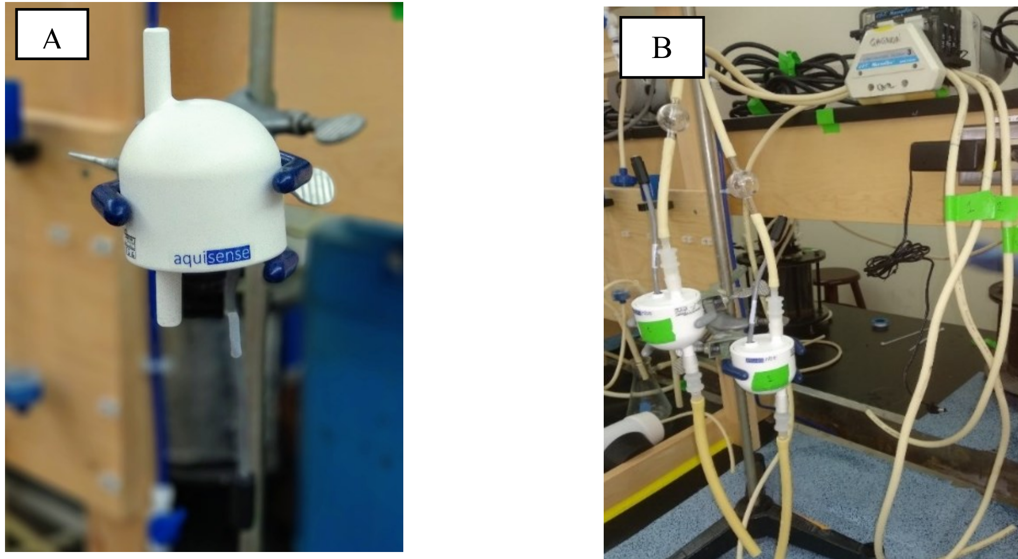


Figure 4-3 (A) Reactor B picture. (B) Two Reactor B units connected to peristaltic pump

4.5.1 Impacts of Bacterial Concentration and Flow Rate in Reactor's B Efficiency

In this set of experiments, all the solutions created had an approximate *E. coli* concentration of 3.0 x 10^6 CFU/mL ± 3.9 x 10^5. This bacterial concentration was chosen to represent an exaggerated scenario of bacterial contamination. Therefore, it is possible to observe the UV LED reactor performance and extrapolate its efficiency.

E. coli culture was grown as explained in Section 3.2 of this dissertation. Experiments were conducted using three different flow rates, 0.25 LPM, 0.75 LPM and 1.5 LPM ± 0.02. Flow rates were chosen accordingly to the peristaltic pump's capacity.

UV disinfection is dependent on water quality parameters, such as turbidity and UVT, and reactor characteristics, such as intensity (Jenny, Simmons, Shatalov, & Ducoste, 2014). When UV efficiency is paired with flow rate, it corresponds directly to contact time and, thus, UV dose. The simplest reactor parameter to control is flow rate because it would be

the only one that a household customer would have the opportunity to modify since intensity is set within the reactor design.

4.5.2 Impacts of Turbidity and UVT in *E. coli* Inactivation on Reactor's B efficiency

Drinking water quality parameters such as UVT and turbidity play an important role in the evaluation of UV disinfection. Suspended particles in water can cause light to scatter, to be absorbed or act as a shield and block light affecting the quantity of UV light hitting the water and thus, the extent of bacterial inactivation (Qualls et al., 1983). Turbidity and Ultraviolet Transmittance (UVT) are two parameters that indicate the amount of light passing through a water sample, hence, they were chosen to be the characteristics to be altered in the artificial water matrices to test the efficiency of UV LED reactors.

4.5.2.1 Turbidity

Turbidity can be expressed as the optical property that causes light to be absorbed and scattered instead of being transmitted without change in direction through the sample. Turbidity is caused by the amount of colloidal and suspended matter, such as silt, clay, fine organic and inorganic matter and microorganisms (Standard Methods for the Examination of Water and Wastewater, 2011). High turbidity can present a risk in drinking water, because bacteria and viruses can attach to the particles, and, thus, prevent UV light from penetrating and inactivating them.

To simulate different water conditions, artificial water matrices were created. To simulate different turbidity levels a PBS solution was spiked with different concentrations of laboratory grade kaolin clay to increase turbidity. Using PBS solution ensured that when spiking *E. coli K-12*, it will survive during the experiment time. Turbidity levels were

measured in the control solutions and the treated samples, to confirm that turbidity did not change significantly among the sampling process. Turbidity was measured using a Hach 2100Q Portable turbidimeter (Cat. No. 2100Q01).

Three levels of turbidity were chosen and compared: 0.1, 1.0 and 10 NTU. 0.1 NTU was the turbidity of pure PBS, without the addition of kaolin clay, representing the best-case scenario and the lowest turbidity achievable in this experiment. The turbidity of 1 NTU was chosen since the Health Canada recommends maintaining a level below 1.0 NTU (Health Canada, 2017a). The highest level (10 NTU) represents 10 times the maximum recommendable value. Thus, a real-life drinking water with a turbidity level as high as 10 NTU would be considered above regulation and would need treatment before being suitable for consumption. This high-level turbidity could be present in different situations such as recontamination in the distribution system. In Arctic communities, where surface water is often drawn from lakes or reservoirs and chlorinated without a filtration step, it is common to find turbidity levels above what is recommended in the Health Canada Regulations (Medeiros, Wood, Wesche, Bakaic, & Peters, 2017).

Table 4-3 Description of the solutions created to test the efficiency of reactor B at different turbidity levels.

Turbidity (NTU)	**Turbidity Value Measured (NTU)**	**Corresponding UVT Value**	**Kaolin Clay Concentration (mg/l)**
0.1 NTU	0.15 ± 0.01	98.5 ± 0.01	0.00
1 NTU	0.98 ± 0.31	93.6 ± 0.01	0.65
10 NTU	9.88 ± 0.66	90.77 ± 1.39	1.65

4.5.2.2 UV Transmittance (UVT)

UV transmittance (UVT) is a measurement of the amount of UV light (usually at 254 nm) that passes through a water sample, compared to the amount of UV light that passes through a pure water sample. This measurement is presented as percent UVT. UVT can be affected due to organics, colloidal particles, or other solids in the water that can scatter UV light through the water. UV inactivation treatment is less effective when UVT is low because less UV light reaches the targeted microorganisms due to interference by UV absorbing water matrix components (Bolton & Cotton, 2008). In this study, UVT was measured using a Hach DR 500 in the untreated samples, as well as the treated.

Three levels of UVT were chosen: 50 % UVT, 75 % UVT and 99 % UVT to represent different water quality scenarios. A PBS solution was spiked with different concentrations of Suwannee River humic acid (SRHA) until the desired UVT was reached. Detailed UVT values and SWHA concentration are shown in Table 4-4. The solution used as the highest UVT (99%) was solely PBS solution with same *E. coli* concentration and did not contain any SRHA. The lower UVT values were chosen to simulate scenarios where water would have a high level of organics and would represent challenging conditions for UV treatment to operate, taking into consideration that UVT above 90% is considered ideal for UV operation.

Table 4-4 Description of the different solutions created to test the efficiency of reactor B at different UVT levels.

Intended value	**Measured UVT in solution.**	**Concentration of SRHA (mg/l) added**	**Turbidity value (NTU)**	**TOC value (mg/l)**
99 %	99.05 ± 0.78	0.00	0.15 ± 0.01	N/A
75 %	72.75 ± 2.70	5.77	0.27 ± 0.01	2.23
50 %	48.32 ± 3.22	13.27	1.38 ± 0.18	5.13

4.5.2.3 Sample Analysis

After collection, samples were diluted and plated immediately accordingly to standard plate count procedures, explained in detail on Section 3.3.1 of this dissertation. When immediate dilution and plating were not possible, samples were kept in a 4°C fridge, for no more than 4 hours. For ATP analysis, the recovery ATP method was used. This method has been explained to detail in Section 3.3.2 of this dissertation.

Reactor B Laboratory Testing Results

4.5.2.4 UVT Impacts in Log Inactivation Using the Standard Plate Count Method

A one -way ANOVA between the UV unit 1 and UV unit 2 was conducted to find if the two units were comparable under the same conditions. There was no significant difference between units at the $p<0.05$ level ($p=0.625$), therefore, the log inactivation values from the two units under the same conditions were taken as independent replicates.

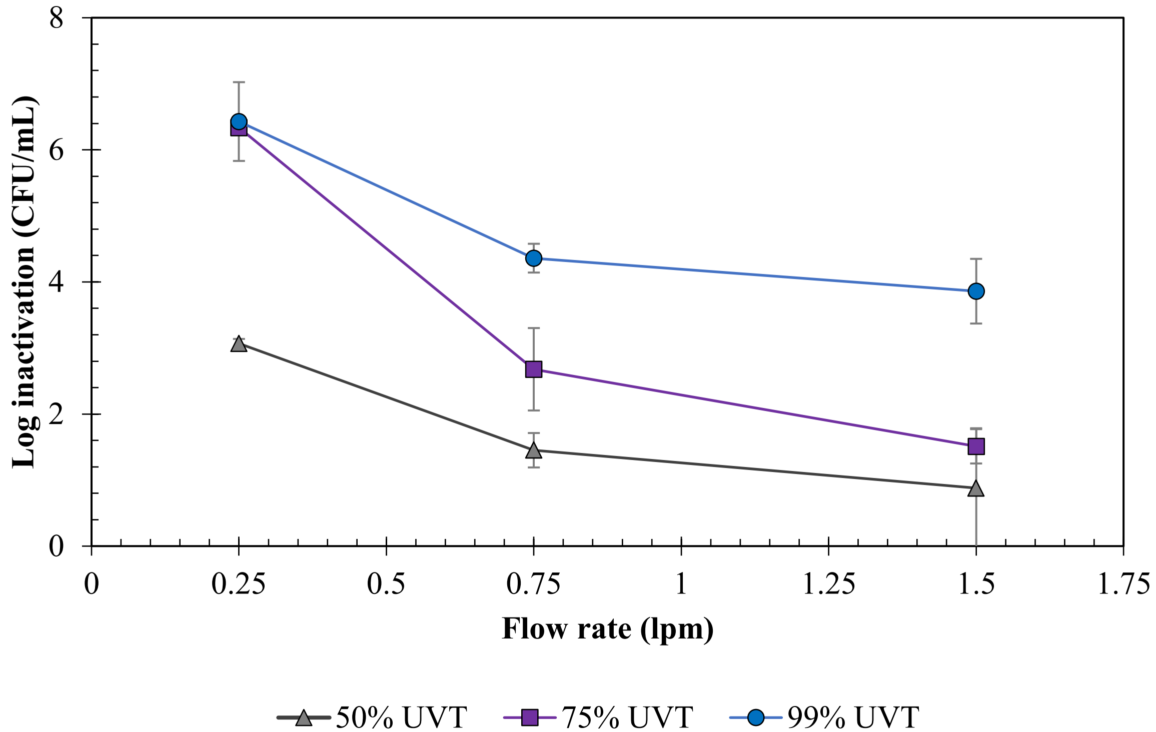


Figure 4-4 Log inactivation values achieved by reactor B using standard plate count, at different UVT levels. Error bars represent standard deviation, n=4

At the higher flow rates studied log inactivation decreased by at least one order of magnitude (Figure 4-4). This was an expected behaviour, because when flow rate increases, the time that the water is in the disinfection chamber inside the reactor is reduced, and thus, the contact time between the water sample and the UV light is lower.

At 99% UVT, which represents the best-case-scenario, log inactivation decreased from 6.4 (at 0.25 LPM) to 3.8 (at 1.5 LPM). This is equivalent to a reduction of 40.6 % of inactivation when the flow rate is increased. Log inactivation of 6.4 is the maximum log inactivation achievable in this experiment.

On the other hand, at 50% UVT, representing the worst-case-scenario and a poor water quality, the highest log inactivation was 3 log and it was achieved at 0.25 LPM. Log inactivation dropped to 0.8 log, which represents a 71.3 % reduction in bacterial inactivation. A two-way ANOVA was performed to find if the flow rate and UVT had significant impacts on the log inactivation results. Both factors had a significant impact on log inactivation, both factors resulted in p-value = 0.000.

The effect of particles in UV disinfection has been studied since UV disinfection was incorporated more commonly in wastewater treatment (Qualls et al., 1983). A more recent study conducted by Cantwell & Hofmann (2008) found that particles as small as 11 μm are able to offer protection from UV light to coliform bacteria up to a dose of 40 mJ/cm^2. This phenomenon was also observed in turbidity levels as low as 0.8 NTU, which is below the Canadian guidelines for drinking water quality (Dunn, Bakker, & Harris, 2014; Health Canada, 2017a).

The majority of the studies exploring the effect of particles in UV disinfection have been conducted with wastewater samples and using standard sources of UV light (medium or low-pressure mercury lamps). They illustrated the potential effects of colloidal matter have in UV disinfection for drinking water treatment but further research on the effect of UVT in UV LED rectors intended for POU drinking water treatment is required.

In summary, flow rate and UVT had a significant impact on the performance of reactor B. Bacterial log inactivation dropped significantly when flow rate increased at the three levels of UVT (50,75 and 99%), whereas at 50% UVT, only 3 log inactivation was achieved even at the lowest flow rate tested (0.25 LPM).

In addition, the increase of UV absorbing substances (i.e. humic acids), which leads to a decrease in UVT, has a direct impact to the amount of light impacting the *E. coli* that are suspended in the water sample. UVT is a critical water quality parameter that needs to be considered in the evaluation of a UV LED POU system because it has a direct impact on its performance.

4.5.2.5 UVT Impacts in Log Inactivation Using the Recovery ATP Method

Besides standard plate count, recovery ATP method was used to measure the efficiency of reactor B. Results from this test are presented in Figure 4-5.

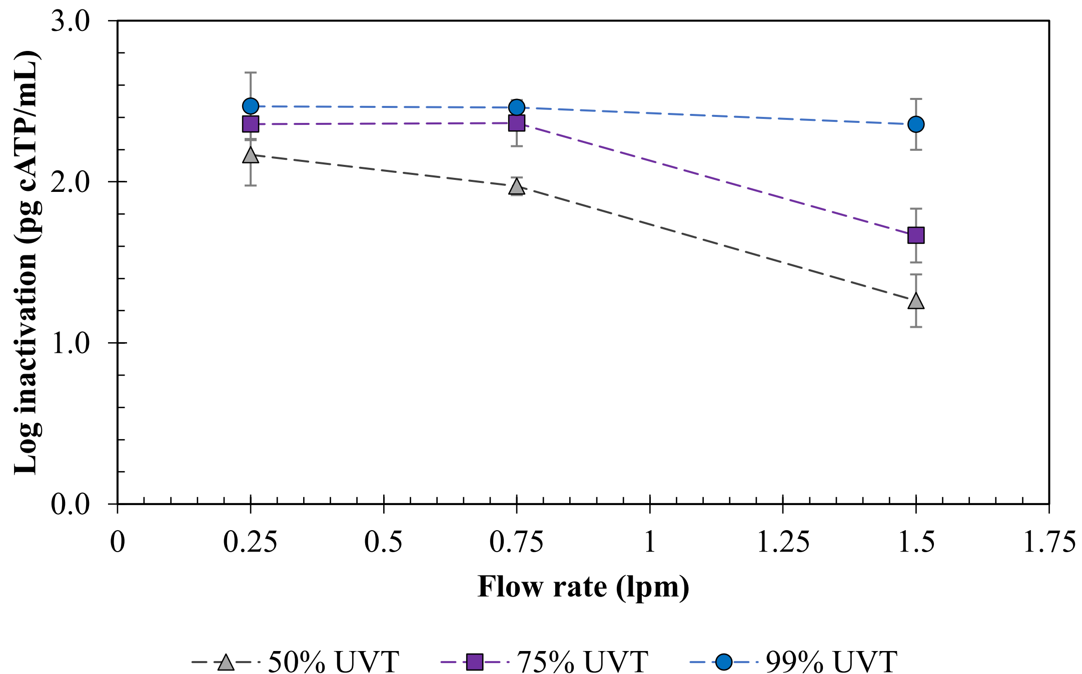


Figure 4-5 Log inactivation values achieved by reactor B using the recovery ATP method, at different UVT levels. Error bars represent standard deviation, n=4

The log inactivation calculated from the recovery ATP results followed the same trend as the standard plate count method: Log inactivation decreased when flow rate increased and when UVT decreased.

A two-way ANOVA was conducted to determine whether the changes in flow rate and UVT had a significant impact in the log inactivation response. The results indicate that both flow rate and UVT (p-value = 0.000) had a significant impact in the log inactivation. The unit number (1 or 2) did not have a significant impact (p-value = 0.949) nor the sample number (p-value = 0.824).

At 0.25 LPM, log inactivation values were not significantly different at the three levels of UVT (p-value = 0.093). This phenomenon could be because the recovery ATP method might not able to quantify changes higher than 2.5 log.

At 50% UV, representing the poorest water quality in this set of experiments, log inactivation decreased from 2.17 log (at 0.25 LPM) to 1.26 log (at 1.5 LPM), which means a decrease of 41.9% of bacterial inactivation.

Finally, at 75% UVT, log inactivation decreased from 2.3 log (at 0.25 LPM) to 1.6 log (at 1.5 LPM). This represents a 29.6% decreased in bacterial inactivation.

In summary, flow rate and UVT conditions have an overall significant effect in the bacterial log inactivation response. However, at 99% UVT specifically, flow rate did not have a significant impact. Moreover, at 75% UVT and 50% UVT, bacterial log inactivation dropped significantly when the flow rate was increased.

4.5.2.6 Turbidity Impact in Log Inactivation Using the Standard Plate Count Method

As mention before, it was hypothesized that turbidity would have an impact on the performance of UV LED disinfection process. Figure 4-6 presents the log inactivation results of different turbidity levels in reactor B.

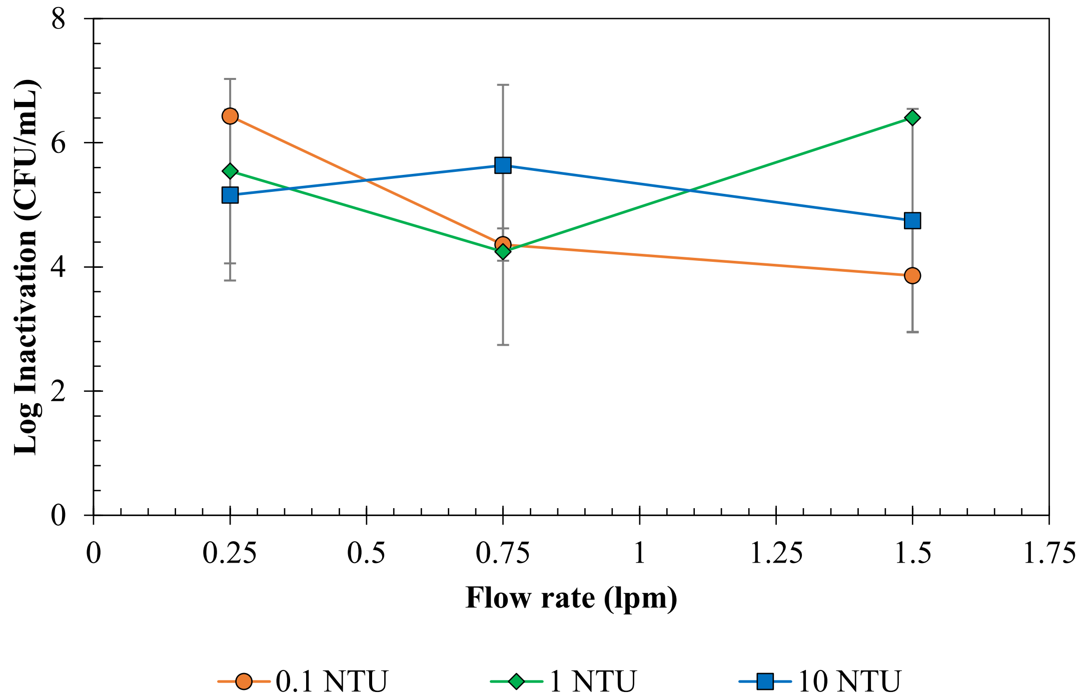


Figure 4-6 Log inactivation values achieved by reactor B at different turbidity levels using standard plate count. Error bars represent standard deviation, n=3.

Figure 4-6 does not show a clear trend on how the changes in turbidity affect bacterial inactivation. The error bars presented in Figure 4-6 overlap with each other in almost all points. This could indicate there is no significant difference between the turbidity levels. To further analyze these results, a two-way ANOVA was conducted to find which factor had a significant effect in log inactivation. There was no significant effect found due to the change of turbidity (p-value = 0.26) but the change of flow rate did result a significant difference (p-value = 0.01).

This outcome contradicts a study by Cantwell & Hofmann (2011) where it was found that small particles can interfere with the penetration of UV light in water samples, even at turbidity levels below 2 NTU. The study by Cantwell & Hofmann (2011) also mentions that factors such as particle size, composition, shape, and porosity also have an effect on

how the light will be absorbed by the water sample and thus, how effective the UV disinfection will result.

There are some potential reasons as to why turbidity did not have a significant effect on the UV LED disinfection performance in this study. It is possible that error within the data affected the log inactivation response and its relation to the turbidity. The error observed in this experimentation could be due to the nature of particles being used to increase the turbidity. As mentioned before, kaolin clay was the compound used to increase turbidity in the solution. Kaolin clay might be blocking or shielding UV light from reaching the microorganisms in the water sample. Moreover, a study conducted by Cantwell & Hofmann (2011) found that a variety of particles contained in surface water could have an effect on how the light passes trough a water sample, even at similar turbidity levels.

4.5.2.7 Turbidity Impact in Log Inactivation Using the Recovery ATP Method

Besides standard plate count, recovery ATP method was used to measure the efficiency of reactor B in bacterial log inactivation at different turbidity levels. Results from these tests are presented in Figure 4-7.

One-way ANOVA was conducted to compare the effect of turbidity on the log inactivation of *E. coli* using the ATP recovery method. There was a significant effect on the log inactivation of *E. coli* at a $p>0.05$ level (p-value = 0.000).

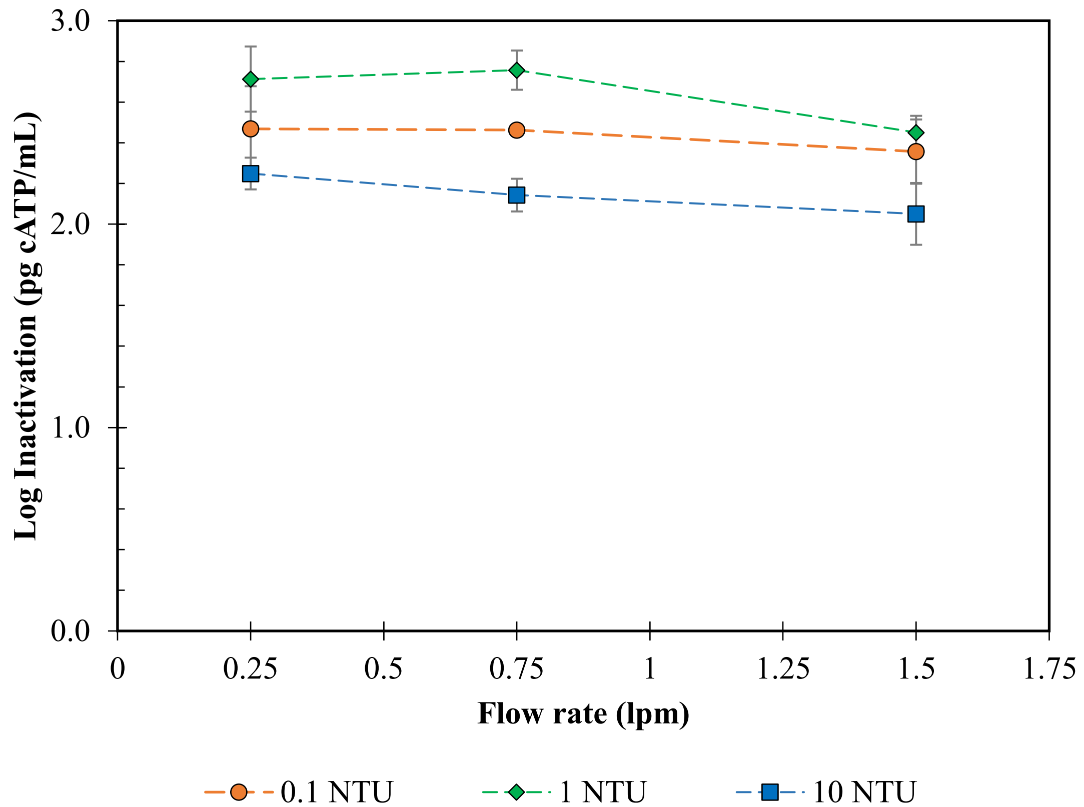


Figure 4-7 Log inactivation values achieved by reactor B at different turbidity levels, using recovery ATP method. Error bars represent standard deviation, n=4.

Because statistically significant results were found, Tukey HSD post hoc test was conducted. Results indicate that the three levels of turbidity are significantly different, and thus, the three levels had a significant effect on the log inactivation of *E. coli.*

These results contradict the results of standard plate count stated before, where no significant effect was found with the increase of turbidity values. The reason for this could be because the amount of error within the standard plate count makes it difficult to determine if there is a significant effect of or not in the log inactivation achieved with turbidity values between 0.1 and 10 NTU. (see Appendix B). Under these circumstances,

it cannot be concluded whether or not there is a significant effect in log inactivation with the change of turbidity values in this study.

4.6 Application of UV LED for POU Treatment. An Exploratory Study in an Arctic Community

4.6.1 Drinking Water Situation in the Canadian Arctic

Arctic communities usually do not rely in centralized water treatment plans for drinking water treatment and distribution. These communities extract water from a reservoir and chlorinate it. Afterwards, chlorinated water is delivered by truck to the community. Trucked water is stored in tanks until the moment of consumption (see figure 4-8). This is a common scenario in the majority of Arctic communities.

The challenging drinking water conditions that are commonly present in these communities represented an opportunity to test UV LEDs as a POU technology.

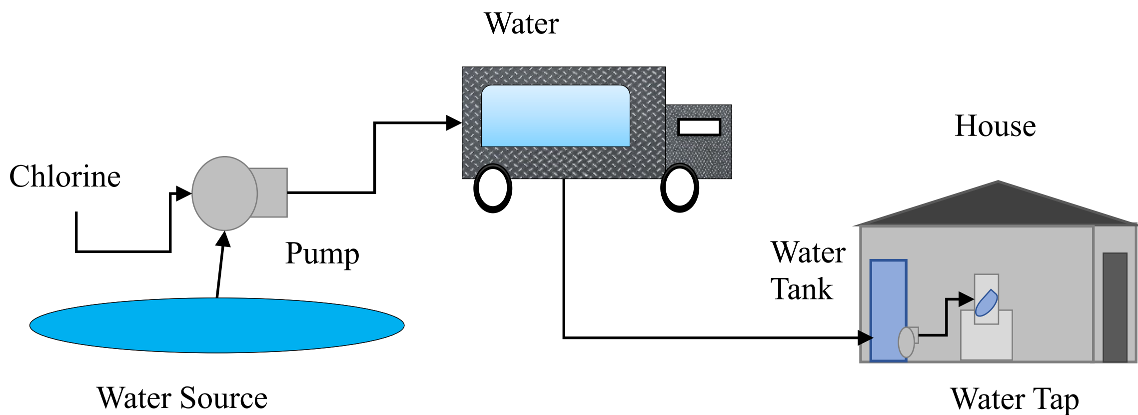


Figure 4-8 Common drinking water treatment and distribution system in Arctic communities

4.6.2 Reactor B Set-Up in an Arctic community

A POU UV LED (referred as reactor B) apparatus was installed on the sink tap in the laboratory inside a government building, which currently houses a research laboratory and offices for family and health services professionals. This location was chosen because it was a building where the access was not compromised, and it was considered to be representative of an average household. Reactor B was chosen for this study because it demonstrated a higher efficiency on laboratory testing than reactor A.

Two reactor B units were installed in parallel in a sink as shown in Figure 4-9. The units were installed in parallel with the help of a manifold built for this purpose. The manifold has three ports and a UV LED apparatus was collocated in each side, leaving the central port for control (untreated) sampling. After the first week, one of the UV LED units was removed from the system, and sampling was continued with just one unit.

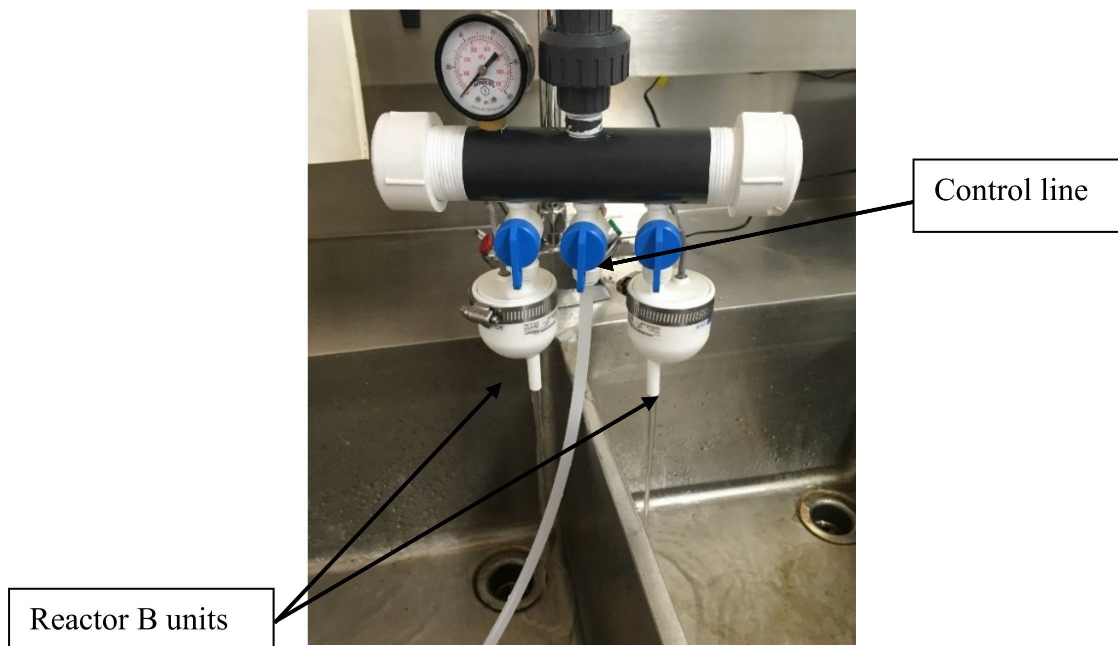


Figure 4-9 Two reactor B units installed in parallel using a manifold. The central line was used as control (untreated) sampling.

Collected samples were analyzed for ATP using the recovery ATP method, (Rauch et al., 2018), which has been explained in the materials and methods section of this dissertation.

4.6.3 Sampling Process

Sampling was performed according to standard methodology (Rice, Baird, Eaton, & Clesceri, 2012) and after sample collection, turbidity, free chlorine, total chlorine, ATP and UVT were measured. All samples were analyzed in the field, except UVT. All the sampling was performed at the same Arctic community, over a period of 10 weeks from May to July 2018.

Turbidity in the water samples was measured using a Hach 2100Q Portable turbidimeter (Cat. No. 2100Q01). Total and Free chlorine was measured using a Hach Pocket Colorimeter II ™ (Cat. No. 58700-00) and the Hach Permachem® Reagents for total and free chlorine. For UVT measurements, samples were sent to the Centre of Water Resources Studies at Dalhousie University and analyzed there using the Hach DR 500.

To monitor microbial activity in the water, ATP analysis was performed. The ATP QGA kit ® by LuminUltra was chosen for its simple operation. Samples for microbial analyses were collected according to standard methods (Rice, Baird, Eaton, & Clesceri, 2012) and ATP analysis was performed according to manufacturers instructions.

4.6.4 Application of Reactor B in the Field: Results

4.6.4.1 Water Quality in the Arctic Community

Table 4-5 presents a summary of water quality parameters found in the Arctic community

Table 4-5 Average water quality measurements in the Arctic community and average flow rate at which reactor B was operated on the field.

	Free Cl_2 (mg/L) – Outlet	**Turbidity (NTU) – Outlet**	**UVT (%) – Outlet**	**Flow Rate (LPM) – Outlet**
Mean ± StdDev	0.1 ± 0.08	3.0 ± 0.7	71 ± 0.07	1.7 ± 0.3

As it can be seen on Table 4-5, turbidity levels measured during the sampling period were around 3 NTU and UVT (%) were around 71%. Reactor B was operated at 1.7 lpm. UVT values in the Arctic community as well as turbidity, were similar to the UVT values at which Reactor B was tested on the laboratory.

4.6.4.2 Reactor B Performance in the Arctic community

Figure 4-10 shows the performance reactor B during the 10 weeks sampled in the Arctic community.

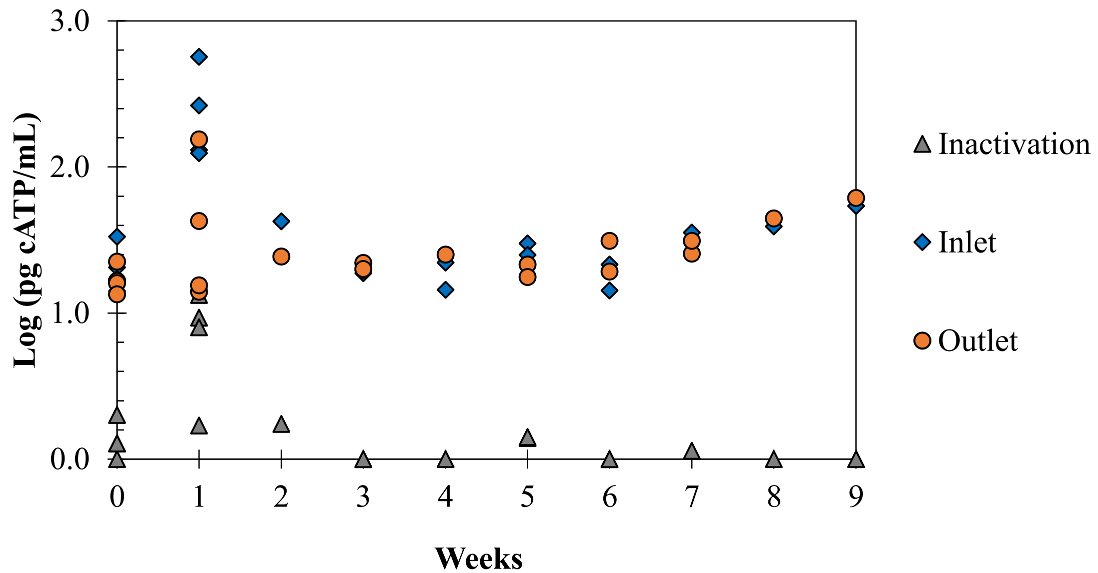


Figure 4-10 Reactor B tested in the Arctic community. The x-axis shows the weeks when the reactor was sampled (May to July 2018). Y-axis shows pg cATP/mL in log scale.

The average cATP concentration during the sampling period at the inlet (before treatment) was 74 pg cATP/mL, which according to the kit manufacturers instructions (LuminUltra, 2017), is higher than what is considered suitable for potable water (<1.0 pg cATP/mL).

Bacterial inactivation was not consistently achieved during the ten weeks were reactor B was sampled (Figure 4-10). In the majority of the samples, the cATP concentration on the outlet (treated) sample was lower than the cATP concentration on the inlet (untreated). As it can be seen, in week 1 there is an event of high ATP and only in this week 1 log inactivation was achieved.

In week 1, a 1 log inactivation can be observed, and it could be due that the cATP levels at the inlet (untreated) were higher than in the following weeks.

There was no clear inactivation achieved during the rest of the sampling events but neither any high ATP level event. One hypothesis that could explain this phenomenon is that the recovery ATP method might be not sensitive enough to quantify any significant difference between inlet (untreated) and outlet (treated) samples in this low cATP scenario. However, this hypothesis was not proven, and the recovery ATP method's methodology does not mention a minimum cATP level required in the analyzed sample. Moreover, it was not possible to perform an additional bacterial quantification method in the field.

A second hypothesis is that the UV LED unit became fouled after week 2, which could lead to lower light intensity being delivered into the water. However, this hypothesis was not tested.

4.6.4.3 Comparison Between Laboratory Testing and testing on the field

Table 4-6 shows the main differences between the laboratory testing and the testing on the community on the field, in terms of operation and water quality.

Table 4-6 Comparison of water quality and flow rate between laboratory and in the field testing

	Turbidity (NTU)	**UVT (%)**	**Flow Rate (LPM)**	**Bacterial Concentration (pg cATP/mL)**
Arctic community	3.0 ± 0.7	71 ± 0.07	1.7 ± 0.3	74 ± 127.7
Laboratory	0.27 ± 0.01	72.75 ± 2.70	1.5 ± 0.1	101,255 ± 29,113

As it can be seen on Table 4-6, UVT and flow rate values were very similar, and the main difference between the two testing is the initial bacterial concentration. Turbidity was not considered because, on the laboratory testing, there was not significant difference between the log inactivation achieved at different turbidity levels, from 0.1 to 10 NTU.

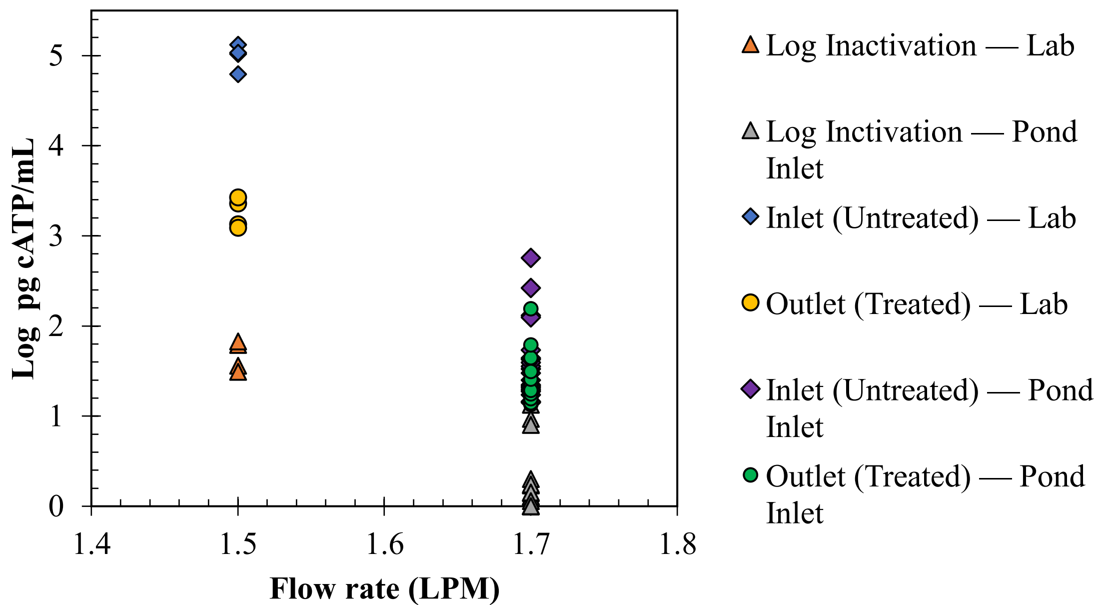


Figure 4-11 Reactor B comparison between laboratory testing and testing in the field. cATP concentrations are expressed in log scale.

As Figure 4-11 shows, the average initial cATP concentration used in the laboratory testing was 101,255 pg cATP/mL (5 log). After UV treatment, cATP concentration decreased to 1,883 pg cATP/mL (3.2 log), achieving 1.6 log inactivation ± 0.1.

In comparison, the average initial cATP concentration measured in the Arctic community at the UV LED reactor's inlet was 74 pg cATP/mL (1.6 log). cATP concentration decreased to 32 pg cATP/mL (1.4 log), achieving 0.2 log ± 0.3 inactivation in average.

4.7 CONCLUSIONS

4.7.1 Lab Scale Conclusions

Reactor A did not meet the claims of being able to achieve a 2.5 log inactivation at 1 LPM. For this reason, experiments using this reactor ceased. Reactor A achieved 2.5 log inactivation at flow rates of 0.06 LPM. Moreover, log inactivation declined by 70% when the flow rate increased from 0.06 LPM to 0.11 LPM. Reactor B demonstrated a better performance than reactor A, being able to achieve up to 4.2 log under the same conditions (1 LPM, 99% UVT). Reactor B achieved up to 6.4 log inactivation at 0.25 LPM and 99-75% UVT. However, log inactivation decreased to 3.8 log (40.6%) when flow rate increased to 1.5 LPM. It was not possible to test reactor B at its maximum operational capacity (5.3 LPM), but it could be anticipated that reactor B might not be able to meet its claims. Nonetheless, reactor B performed better than reactor A and resulted in a better fit for field applications.

Both reactors indicated, when the flow rate increased, their efficiency decreased, which was an expected phenomenon.

Even though none of the two reactors tested were capable to meet the manufacturer's claims, UV LED technology is be promising for POU disinfection applications. Parameters such as the water quality and the operational flow rate play a key role in reactors operation. UV LED reactors intended for POU applications should include a flow rate controller that limits the user from operating the units at higher flow rates than the flow rates intended.

4.7.2 Conclusions for UV LED POU Unit in the Field

Results from reactor B tested in the field differed from laboratory results. The log inactivation achieved by reactor B in the field was lower than log inactivation achieved on laboratory testing. One hypothesis that could explain this is that the cATP levels measured in the field were not consistent during the study period and lower than cATP levels used during the laboratory testing. Without enough cATP at the inlet of reactor B, it could not be possible to measure bacterial inactivation. However, this hypothesis was not proven, due to the impossibility of performing an additional bacterial quantification method in the field. In addition, the recovery ATP method's methodology does not mention a minimum cATP level required.

Another important factor for reactor's B performance was the % UVT in the water samples. With a UVT being 75% on average, there is a risk that the UV LED reactor could not operate at its best conditions. In overall, the DWDS as well as the drinking water quality in the Arctic community, creates a window of opportunity for the application of POU technologies. However, in this study, the efficiency of this particular UV LED apparatus cannot be finally proven, nevertheless, there is sufficient data for further experimentation in direction of improving drinking water quality in this community.

CHAPTER 5 UV LED TECHNOLOGY FOR BIOFILM DISINFECTION

5.1 INTRODUCTION

Microorganisms in the environment are much different from their laboratory counterparts, because given the opportunity, the majority of them would form biofilms or attach to a biofilm matrix. Biofilms can be defined as the attachment of planktonic cells onto a surface, which form an enclosed matrix of extra polymeric substances (EPS) that they produce (Donlan, 2002). Depending on the environment where the biofilms attach, non-cellular materials such as blood components, corrosion particles, mineral crystals, clay or silt particles can be found. Biofilm can develop on any surface that is in contact with non-sterile water, such as medical devices, water system piping, natural aquatic systems and even living tissue (Flemming et al., 2011).

The presence of biofilms on different surfaces represents a health risk. It is estimated that about 65% of all bacterial infections are associated with bacterial biofilms (Lewis, 2001). Biofilms can act as a reservoir for opportunistic human pathogens such as *Pseudomonas aeruginosa*, which can develop resistance to antibiotics, disinfectants, and antimicrobials (Kerr & Snelling, 2009). There are numerous disinfection techniques for biofilm control, ranging from preventing biofilm from attaching to surfaces, to the disinfection of formed biofilms using chemical disinfectants such as chlorine (Beloin et al., 2014).

Biofilm formation is a problematic issue in a wide range of industries, and the drinking water industry is no exception. During the drinking water treatment process, biofilm can form on membrane filtration systems, causing a significant hindrance to membrane filtration systems. Furthermore, when biofilms grow in the distribution systems, it can

jeopardize the quality of the water being delivered to the customer at the tap. To prevent this, drinking water utilities routinely use chlorine to inhibit microbial regrowth in their distribution systems, but this creates the risk the forming disinfection by-products (DBPs) when chlorine reacts with residual natural organic matter (NOM).

UV light has been emerging as a new strategy to prevent and manage biofilm formation. There are studies that have used UV mercury lamp for biofilm disinfection (Pozos et al., 2004), but more recent studies are focusing on the use of UV LEDs (Li et al., 2010), for all the advantages that UV LED have compared to traditional mercury lamps (smaller size, instant on/off and variety of wavelengths).

The energy required for disinfection remains dependent on the surface where the biofilm is growing and the microorganisms that are present in the biofilm matrix. UV LED technology could be applied not only to control biological load in the water but also for surface disinfection in drinking water systems. The use of UV LEDs adds a significant number of possibilities to biofilm control, but more research and development is required.

5.1.1 Objectives

The objectives of this set of experiments were to:

1. Create a UV LED dose-response curve creation for the biofilm-bound of *P. aeruginosa.*
2. Analyze different bacterial quantification techniques applied to biofilm disinfection using UV LED irradiation.
3. Explore synergistic effects in common wiping techniques paired with UV LED disinfection.

5.2 UV LED Apparatus Set-Up

A UV LED collimated beam apparatus from AquiSense® technologies (Picture 1-1) was used for all the UV experiments. The apparatus consists of a central unit with three UV LEDs, a ventilation system and a collimator, which directs the light from the UV LEDs to the sample. This device can be set to operate at 255 nm, 265 nm or 285 nm.

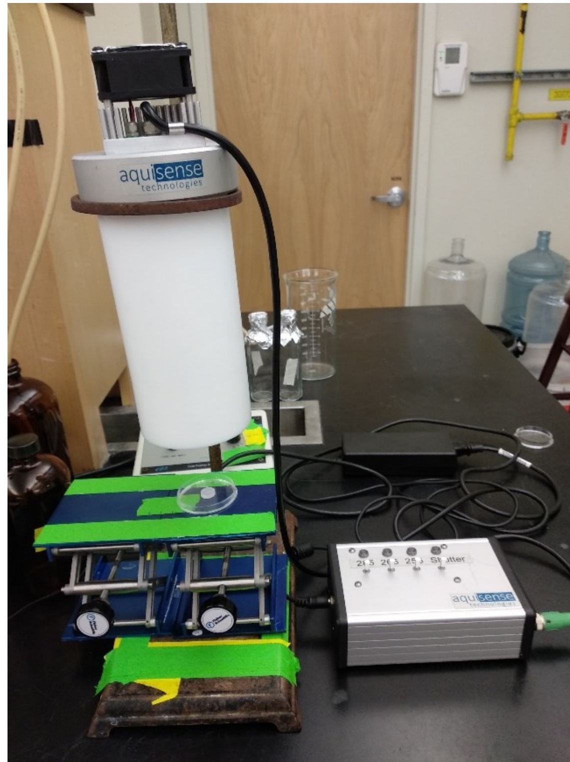


Figure 5-1 Collimated beam used for UV LED biofilm disinfection. (AquiSense technologies®)

5.3 CHARACTERIZATION AND DOSE CALCULATION

Irradiance measurements were taken according to Gora, Rauch & Ontiveros (2018) using a USB4000 spectrometer (Ocean Optics Inc., Fl, USA) to characterize the beam of light. Measurements every 1 cm of the collimator's area were taken at 1.5 cm from the edge of the collimator to the surface of the coupon. The coupons were placed at this distance in all the following experiments. After taking measurements, the average irradiance was calculated.

Exposure time was calculated according to Bolton & Linden (2003) as follows:

Equation 5-1 UV dose calculation

$$Time = \frac{Fluence}{Average\ irradiance}$$

Where:

Fluence is in mJ/cm^2

Average irradiance is in mW/cm^2

Time in seconds.

Bolton & Linden (2003) mention that microbial suspensions must be stirred during UV irradiation, but in this case, the treatment target was not in a suspension, but in a biofilm form. For this reason, after coupons were placed in a sterile petri dish, the dish was placed on a rotator device that continually rotates the petri dish with the coupons at 5 RPM. This rotation ensures a homogenous light exposure on the coupon surface.

5.4 *P. AERUGINOSA* CULTURE

5.4.1 Bacterial Strain

Pseudomonas aeruginosa is a ubiquitous, environmentally important microbe that is frequently associated with disease in humans, where it acts as an opportunistic pathogen with the potential to cause infections especially in immunocompromised population. *P. aeruginosa* can be found in water systems and is especially a high risk in hospitals and other health centres. The capacity of *P. aeruginosa* to cause disease is improved by both inherent and developed resistance to many antimicrobials and disinfectants, virulence factors and ability to adapt to a wide range of environments (Kerr & Snelling, 2009).

P. aeruginosa PA01 was chosen as a target microorganism in this study due to its ability to quickly form biofilms. There a several studies that have used *P. aeruginosa* for biofilm studies, and thus, methodologies for its formation are well studied (Bak et al., 2010; Bridier et al., 2011; Teitzel & Parsek, 2003).

5.4.2 Biofilm Growth

As explained in Section 3.5, *P. aeruginosa* was grown according to the USEPA's methodology (US Environmental Protection Agency Office of Pesticide Programs, 2013). When the biofilm was ready, coupons were carefully extracted from the CDC reactor and placed in a 45 mm sterile petri dish. Afterward, coupons were immediately exposed to UV LED treatment for times ranging 41 to 207 seconds which corresponded to UV doses from 4 to 20 mJ/cm^2.

Immediately after each treatment, the biofilm was carefully swabbed from the coupons with sterile cotton swabs and resuspended. The bacterial enumeration in the resuspended biofilm was done using standard plate count, and cellular ATP was quantified using the recovery ATP method (Rauch et al., 2018).

5.4.3 Initial Bacterial Concentration

For the control (untreated condition) the total bacterial concentration after biofilm extraction and resuspension was recorded. The average bacterial concentration using standard plate count for the controls was 3.94E+07 ± 1.68E+07 CFU/cm^2 .

A one-way ANOVA at 95% level confidence was performed with all the control data to see if all the controls have a statistically significant equal mean. The resulting P-value was 0.000 which rejects the null hypothesis that states that all means are statistically equal. Even though the same methodology was followed to grow *P. aeruginosa* biofilm in the coupon, the results are not significantly the same. In this case, this difference was not considered significant because log inactivation data was normalized with each of its control.

A second one-way ANOVA was performed with all the control data from the ATP, and the resultant p-value was 0.088. This value indicates that the null hypothesis that all means are equal is accepted. This difference between the results from the ANOVA tests could be because the ATP test has a lower range of detection than the standard plate count. For the analysis presented in this study, all the log inactivation data was normalized with its each control batch.

5.4.4 Log Inactivation Calculation

After biofilm treatment and resuspension, bacterial enumeration was performed using standard plate count and cellular ATP quantification was performed using the biomass recovery ATP method. Log inactivation was calculated according to as explained in Section 3.4 using Equation 3-1.

5.5 BIOFILM TREATMENT

5.5.1 UV LED Treatment Procedure

A 265 nm wavelength was chosen for the treatment of *P. aeruginosa* biofilm. According to Rattanakul & Oguma (2018), 265 nm is more effective for the inactivation of *P. aeruginosa* compared to other wavelengths. Although Rattanakul & Oguma (2018) used planktonic (free-floating) cells, it was still used as a reference assuming that biofilm-bound bacteria would respond better to 265 nm as well because it was assumed that the action spectra of the *P. aeruginosa* DNA will similar in planktonic and biofilm form.

Taking into consideration that Rattanakul & Oguma's (2018) study shows that a fluence of approximately 5 mJ/cm^2 achieved a 3 log inactivation, fluences from 4 mJ/cm^2 to 20 mJ/cm^2 were chosen. Higher doses than the ones shown in the aforementioned study were used to create a new response curve because it was hypothesized that higher doses would be required to achieve the same log inactivation in biofilm as in planktonic cells.

Table 5-1 shows are the UV doses used and their corresponding times:

Table 5-1 Time correspondent to each UV fluence used.

Fluence (mJ/cm^2)	Time (seconds)
4	41.22
8	82.44
12	123.67
16	164.89
20	206.11

All the UV experimentation was performed in triplicate and under red-light light conditions. Treated and resuspended biofilm samples were kept under red light conditions until the moment of microbial analysis to avoid photoreactivation (Rattanakul & Oguma, 2018).

5.5.2 Wiping Procedure

Wiping with commercial disinfectant wipes was also investigated to treat biofilm. Wiping is a conventional method to disinfect common surfaces. It can be used to remove biofilm from surfaces or to prevent its formation. Despite wiping's simplicity, this method can be troublesome and time-consuming, and there is a risk of insufficient contact time between the solution in the wipes and the surface. For this study, CaviWipes ®, a commercial brand of cleaning wipes, was tested. These wipes are intended for the disinfection of common surfaces. These wipes contain 17.2% isopropanol and 0.28% of benzethonium chloride. According to manufacturer suggestions, three minutes of wiping is recommended. However, in real life applications, the recommended treatment level is unlikely to be achieved. Wiping a surface for three minutes in real life practice is a time-consuming and laborious task.

In this study, coupons were carefully wiped with the commercial wipes at different times to find a response disinfection curve for the wiping procedure. The times tested were: 3 minutes, 1 minute, 30 seconds, 15 seconds and a single pass. Biofilm was extracted right after each wiping treatment, avoiding extra contact time for the disinfectant in the biofilm. Biofilm extraction and resuspension were performed in the same manner as described above. Disinfection efficiency was analyzed with standard plate count, and ATP quantification was performed with the ATP QGA kit (LuminUltra®), according to the manufacturer's instructions.

5.5.3 Wiping with Subsequent UV LED Exposure

Different combinations between wiping times and UV light doses were evaluated for the purpose of trying to find an alternative method to disinfect biofilm. Fluences of 8 mJ/cm^2 and 12 mJ/cm^2 were paired with 15 sec and a single pass of wiping to explore if the two treatment approaches had a synergistic effect.

First, coupons were wiped and immediately afterward, coupons were immediately placed under the UV LED collimator, and treatment was performed as stated above. To make sure that the disinfection that was being observed was due to the treatments, any extra contact time between or after treatments was avoided.

5.5.3.1 Synergy Calculation

The synergy between wiping and UV LED treatment was calculated according to Koivunen & Heinonen-Tanski (2005) and is explained in Equation 5-2.

Synergy (log units) = log reduction by combined chemical/UV disinfection – (log reduction UV disinfection + log reduction chemical disinfection)

Equation 5-2 Synergy equation

Chemical disinfection, in this case, refers to the disinfection obtained after the wiping procedure.

According to Koivunen & Heinonen-Tanski (2005), a synergistic benefit in combined disinfection treatment is observed when the synergy value is positive, while a negative value represents an adverse effect. If the resultant value is zero, it means that the efficiency of combined treatment is the same as the sum of the two individual treatments.

5.6 RESULTS

5.6.1 UV LED Treatment Results

Figure 5-2 shows the log inactivation results for the UV treatment. Results from the standard plate count and recovery ATP are presented on the same graph. The results for recovery ATP are, for almost all the points, slightly below those for standard plate count. It is interesting that recovery ATP method works to estimate the log inactivation of biofilm-bound after UV disinfection.

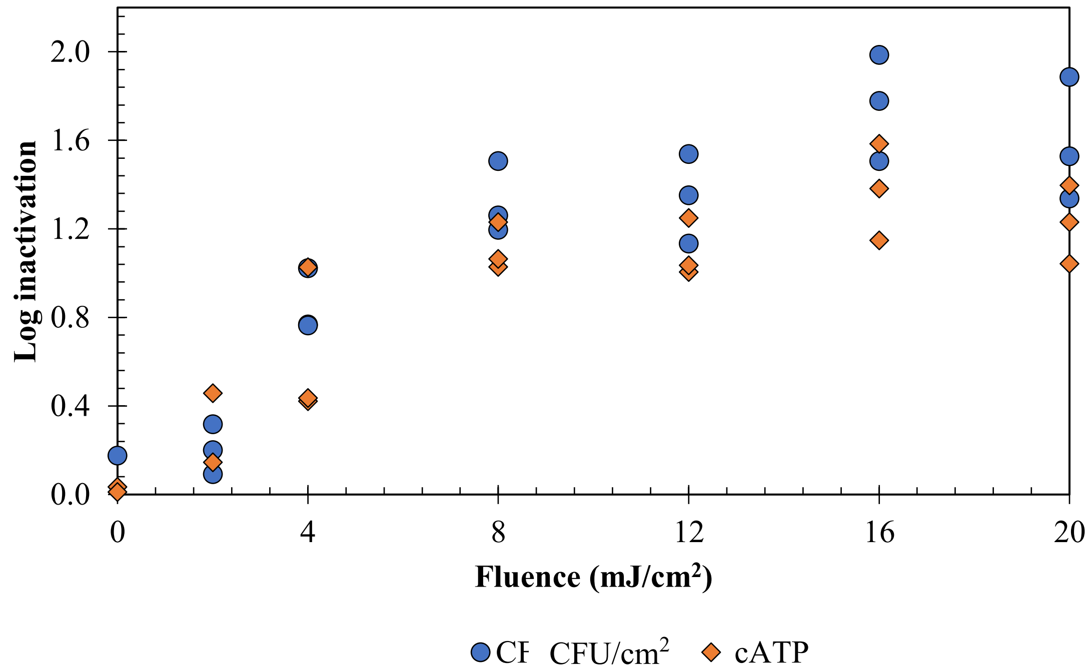


Figure 5-2 Inactivation profile of P. aeruginosa biofilm with UV LED at 265 nm. CFU/cm² was quantified using standard plate count, and cATP was quantified using recovery ATP.

Log inactivation of 0.2 was achieved at a UV dose of 2 mJ/cm², and it increased to 1.3 at a UV dose of 8 mJ/cm². The log inactivation remained constant up to the UV dose 20 mJ/cm².

A one-way ANOVA was conducted to compare the effect of UV dose on the log inactivation of *P. aeruginosa* bacterial-bound biofilm when using standard plate count. There was a significant effect on the log inactivation of *P. aeruginosa* biofilm at a $p>0.05$ level for the treatments $p= 0.000$).

Because statistically significant results were found, a post hoc test was conducted. Tukey's HSD test was chosen because it is designed to compare each of the conditions to every other condition. Results indicate that there is no significant difference between the control

(UV dose= 0) and the UV dose of 2 mJ/cm^2 (adjusted p-value= 0.881), but there was a significant difference between the control (UV dose=0) and the rest of the UV doses. Furthermore, all the comparisons between doses of 8 mJ/cm^2 and 20 mJ/cm^2 were not significantly different (See Appendix B)

Garvey et al., (2015) showed that pulsed ultraviolet light (PUV) was effective in the inactivation of *P. aeruginosa* biofilm grown in a CDC reactor. It is difficult to compare the results with this study, because it has been shown that PUV is more effective against that steady UV light and because this study used a xenon-filled flashlamp at wavelengths between 200 and 300 nm, opposite to this study where it was used UV LED at 265nm.

Additionally, Bak et al. (2010) used UV LEDs for the disinfection and inhibition of bacterial biofilms in long-term catheters. The exposure of catheters to265 nm UV LEDs to 78 mJ/cm^2 achieved 99.99% of germicidal rate. The authors concluded that there is potential for UVC LED light sources to be used for biofilm disinfection in thin biofilms.

5.6.2 Relationship Between Recovery ATP Quantification and Standard Plate Count Results.

The log inactivation results after UV LED treatment, up to 20 mJ/cm^2, when using the recovery ATP method was compared with the standard plate count.

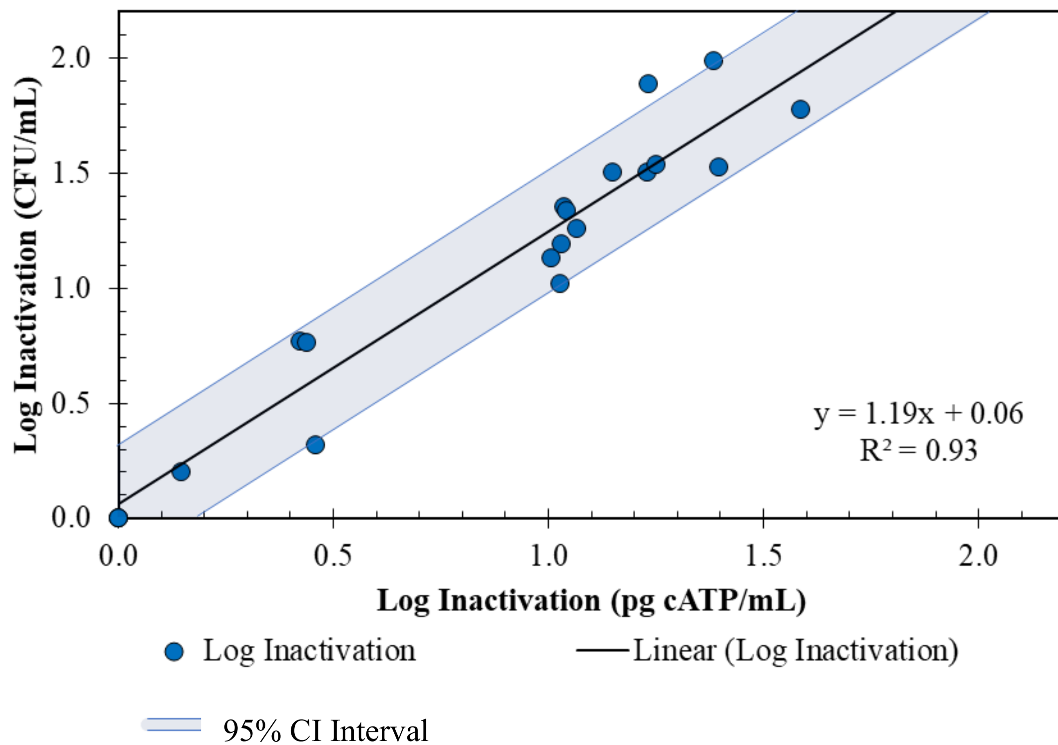


Figure 5-3 Relation between log inactivation cATP and log inactivation of CFU/cm^2. The blue shadow represents the 95% confidence interval (95% CI 0. 1792, 0. 3812, p-value= 0.000).

The resultant coefficient of determination, R^2 is 0.93, which is a relatively good fit to the linear model. This result can be interpreted that the log inactivation obtained from the standard plate count and the log inactivation obtained from the recovery ATP method have

a high correlation. Furthermore, standard plate counts log inactivation results are approximately 1.19 times higher than recovery ATP log inactivation results (95% CI 0. 1792, 0. 3812, p-value= 0.000). Confidence intervals were calculated using t-test, α=0.05.

Given these points, the recovery ATP is a suitable method to measure the log inactivation in biofilm-bound *P. aeruginosa* after UV LED exposure at 265 nm at least for doses below 20 mJ/cm^2, however, it could underestimate the amount of disinfection achieved at higher UV doses.

5.6.3 Wiping Results

As mentioned before, wiping experiments were also investigated to find its effect in biofilm disinfection. A response curve of wiping times and log inactivation was created, and it is presented in Figure 5-4.

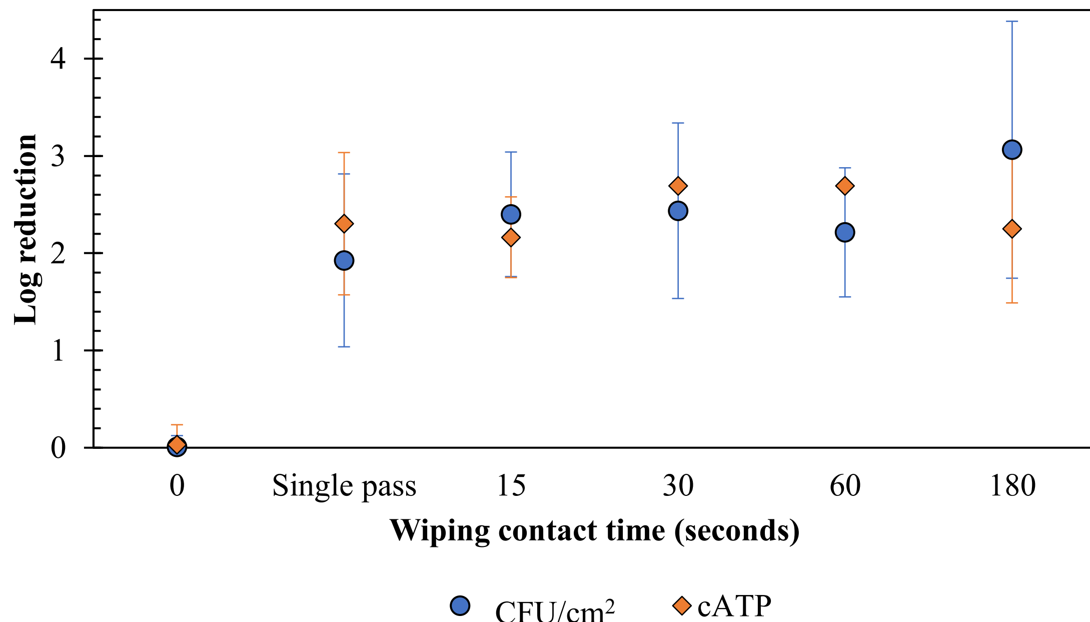


Figure 5-4 Log inactivation of P. aeruginosa biofilm at different wiping contact times using CaviWipes^R. Standard plate count was used to obtain the CFU/cm^2 values, and ATP QGA test was used to the cATP quantification. Error bars represent standard deviation, n=3

It is interesting to find that a single pass wiping was sufficient to achieve 1.9 log inactivation (based on standard plate count), and with the increase of wiping time to three minutes, as recommended for the manufacturer, log inactivation increased up to 3 log. In the times in between that were studied, approximated 2.5 log was achieved in all wiping times.

A one-way ANOVA at 95% level confidence was performed with the responses (log inactivation) of the wiping treatment to find if there is a statistically significant difference among the treatments (wiping times). The resulting P-value was 0.621 which is above the 95% confidence level (P-value= 0.05) and, therefore, there is no statistically significant difference between the treatments. Additionally, a Tukey's test was used to determine if the responses from each treatment (wiping time) were statistically significantly different from the control and the results show that the treatment of 180 seconds is the only group that was statistically significantly different from the control group. On the other hand, Fisher's test showed that all treatments (wiping times) were statistically significantly different from the control group.

There are some studies that look into different combinations of disinfection techniques to inactivate biofilms (Díaz De Rienzo et al., 2016; El-Azizi & Khardori, 2016), but to the author's best knowledge, there are not any studies that pairs wiping techniques using commercial brand of wipes for disinfection with UV treatments. This technique can be applied to several fields, such as the food industry, and with further research, this technique could be adapted to the water industry such as for membrane cleaning.

The ATP quantification results followed the same trend as the standard plate count results, although, when compared these two quantification methods, an R^2 of 0.73 was found, which is not as high as in Figure 5-3. This difference could be due to the inherent variability of the wiping procedure compared to the UV treatment. Nonetheless, cATP quantification can also quantify log inactivation of biofilm after wiping treatment.

5.6.4 Wiping and UV LED Exposure: Synergistic Effects

The synergistic effects between wiping and UV LED disinfection were also explored. The log inactivation results achieved when both methods were combined are summarized in Figure 5-5.

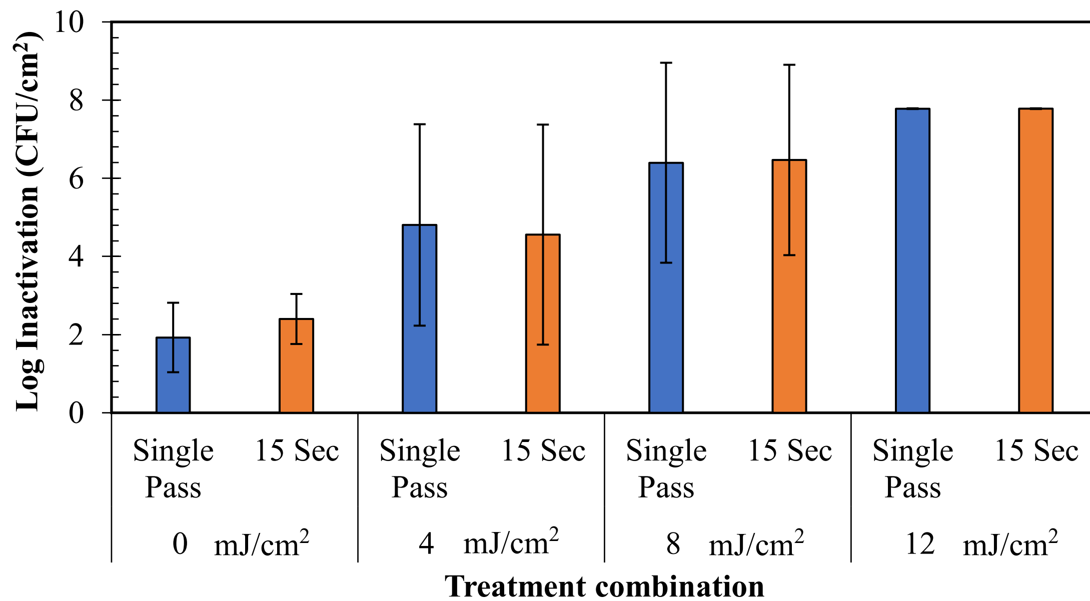


Figure 5-5 Combination between wiping and UV treatment. Wiping contact time tested were single pass and 15 seconds and UV doses ranged from 0 to 12 mJ/cm². Error bars indicate standard deviation, n=3.

The log inactivation achieved with 15 seconds wiping time and 0 mJ/cm^2 was slightly higher than with single pass and 0 mJ/ cm^2 dose being 2.4 and 1.9, respectively. This result was expected, because the more wiping time, the higher contact time between the wipe and the biofilm in the coupon. Furthermore, when the single pass wiping treatments and 15 seconds wiping time were paired with UV dose of 4 mJ/cm^2, a log inactivation of 4.8 and 4.5 were achieved, respectively. In this case, taking into account the large standard deviation value observed (±2 log) the difference between the two wiping times was not significant different. A similar result was observed in the combination of single pass/ 15 seconds wiping treatment paired with 8 mJ/ cm^2, where the log inactivation achieved was 6.4 log in both cases (±2 log). Finally, in the combination of single pass/ 15 seconds wiping treatment paired with 12 mJ/ cm^2, a log inactivation of 7.7 (±0 log) was achieved in both treatments. This outcome was because all the plate counts resulted below the detection limit (<30 CFU/ml) at this treatment level.

The log inactivation results after single pass wiping time paired with UV doses from 0 to 12 mJ/cm^2 were compared with the log inactivation results after 15 seconds wiping times paired with UV doses from 0 to 12 mJ/cm^2.

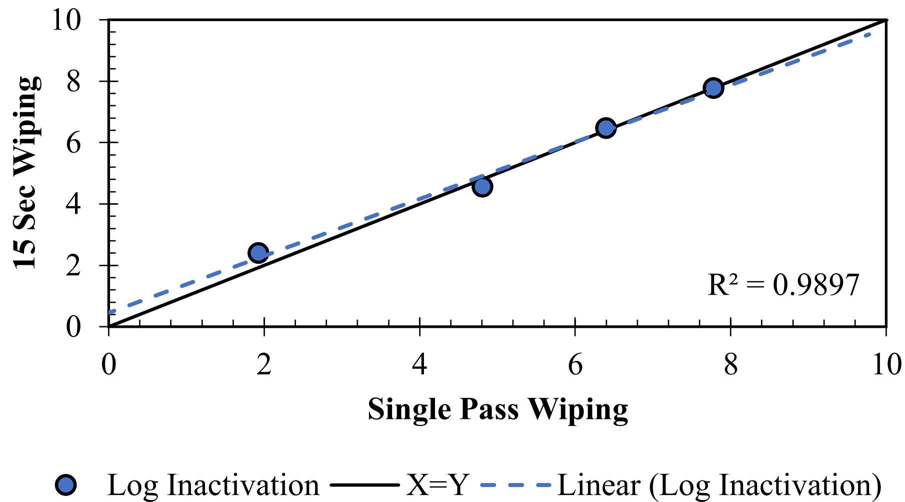


Figure 5-6 Relation between log inactivation with 15 seconds wiping treatment and single pass wiping, combined with UV doses from 0 to 12 mJ/cm^2.

The resultant coefficient of determination, R^2 is 0.98, which is good fit to the linear model. This result can be interpreted that the log inactivation obtained from single pass wiping time and the log inactivation obtained from the 15 seconds wiping time have a high correlation. Furthermore, the majority of the data points falling within the X=Y line indicates that there is not enough evidence in the data to determine that both treatments are significantly different. Additionally, a paired t-test confirmed that both treatment combinations are not significantly different (P-value = 0.654).

The combination treatments synergistic effects were calculated using Equation 5-2 to find if the effects were positive or negative. The results are presented in Table 5-2.

Table 5-2 Synergistic effects of wiping and UV treatment.

Treatment		Synergy Calculated Using Standard Plate Count Values (Log Units)
Wiping Time	**UV Dose (mJ/cm^2)**	
Single Pass	0	0.00
	4	2.03
	8	3.15
	12	4.51
15 Sec	0	0.00
	4	1.31
	8	2.75
	12	4.04

Positive synergistic effects were observed using standard plate count for the combination of wiping and UV treatment. These results can indicate that the combination of wiping and UV treatment can be used to achieve a higher inactivation effect in *P. aeruginosa* biofilm that wiping or UV treatment alone. One hypothesis that could explain this phenomenon is that the wiping treatment, which is a combination of physical and chemical treatment, disrupted the thing layer of biofilm on the coupon and left the bacteria in it exposed without the EPS protection. As a result, the UV dose applied to the coupon afterwards had more impact in the already weakened biofilm. However, this hypothesis was not tested. These findings are consistent with Koivunen & Heinonen-Tansk (2005), were the combination of peracetic acid (chemical disinfection) and UV disinfection increased disinfection efficiency and showed synergistic benefits, with synergy values reaching up to 2 log units for enteric bacteria. However, this study used planktonic cultures instead of biofilm-bound bacteria, and the UV light source was a low-pressure mercury arc lamp.

A study published by El-Azizi & Khardori (2016) assessed the combination approach between UVC light at 254nm and antibiotics for antibiotic-resistant strains commonly fund inside inserted medical devices (i.e., catheters). El-Azizi & Khardori (2016) found that when biofilms were exposed to the antibiotics or UVC light alone, both were not effectively inactivating the bacteria, but when biofilms were treated with UVC light then with antibiotics, the number of viable cells was significantly reduced.

Furthermore, Zyara et al., (2016) investigated the effect of the combination of chlorine/ultraviolet disinfection in seventeen coliphages from treated municipal wastewater. Zyara et al. (2016) concluded that the combination of total chlorine of 0.05–0.25 mg/L and UV irradiation (14–22 mW/cm^2) was more effective than chlorine alone, and 3 to 5 Log reductions were achieved for the chlorine-resistant strains.

Synergistic effects between disinfection treatments for biofilm inactivation need to be further explored, but there is good evidence that some treatment combinations result in higher biofilm inactivation than their counterparts alone.

5.7 Conclusions for Biofilm Study

UV LED technology at 265nm achieved up to 1.8 log inactivation of biofilm-bound *P. aeruginosa*. A UV dose of 8 mJ/cm^2 achieved 1.4 log inactivation which is lower than what studies performed on planktonic *P. aeruginosa* achieved, where a dose of 5 mJ/cm^2 achieved 3-log. A lower log inactivation in biofilm-bound bacteria than in planktonic bacteria even applying the same UV dose was expected and it is consistent to the existing literature in the field (Rattanakul & Oguma, 2018).

The recovery ATP method was suitable for the quantification of biofilm after UV treatment, and it had a linear relation with the standard plate count ($R^2 > 0.9$).

Synergistic effects were found when pairing common wiping disinfection techniques, and UV LED disinfection. These two disinfection techniques successfully achieved higher inactivation than either of them alone. Further investigation is required for the use of UV LED technology in biofilm disinfection, but this study found enough evidence that UV LED are successfully able to inactivate bacteria in biofilms. With the advance of the UV LED industry, more methodologies can be developed and applied in the biofilm control field.

CHAPTER 6 CONCLUSIONS AND RECOMENDATIONS

6.1 Conclusions

UV LEDs were tested for POU applications at lab scale under various water quality conditions using *E. coli* as a target microorganism and on the field in an Arctic community. UV LEDs were also evaluated at bench-scale for the mitigation of *P. aeruginosa* biofilm, alone and paired with common wiping techniques.

The impacts of flow rate, UVT, and turbidity were evaluated at the laboratory scale to establish the practical treatment limitations of the UV LED POU units. UV LED reactors intended for POU drinking water successfully inactivated *E. coli* in purified water, achieving up to 6-log inactivation at 0.25 LPM and 99% to 75% UVT. However, at 1.5 LPM the log inactivation dropped to 0.8 log, representing a 71% reduction in bacterial inactivation. The final analysis showed that UVT in water as well as the flow rate at which the UV LED POU reactor was being operated had a great impact on the reactor performance. However, the impact on the *E. coli* log inactivation when turbidity was increased from 0.1 NTU to 10 NTU was unclear. A modified ATP biomass regrowth method successfully quantified the bacterial inactivation after UV treatment at the lab scale testing, but in a lower range than standard plate counts.

The UV LED POU reactor tested in lab scale conditions did not perform at the same efficiency in field conditions. The UV LED POU reactor was able to achieve 1-log inactivation in one sampling event, during the whole sampling period (10 weeks). This discrepancy might have happened due to a combination of different reasons, including but not limited to the method used to quantify bacterial inactivation and/or reactor damage.

On the other hand, UV LEDs at 265 nm were also able to inactivate biofilm-bound *P. aeruginosa* achieving a maximum of up to 1.8 log inactivation at 16 mJ/cm^2. These inactivation results were lower than what studies using planktonic *P. aeruginosa* showed using UV LEDs at the same wavelength (Rattanakul & Oguma, 2018). Additionally, the biomass recovery ATP method had a linear relationship with the standard plate counts ($R^2 > 0.9$), at least at doses below 20 mJ/cm^2. Furthermore, this study found that the combination UV LED radiation at 265 nm with common wiping disinfection techniques increased disinfection efficiency on biofilm-bound *P. aeruginosa* and showed synergistic benefits, of up to 4.5 log. This high synergistic effect was observed when single pass wiping was combined with of 12 mJ/cm^2 dose of UV LED radiation.

6.2 Recommendations for Future Study

Despite the limited research on the use of UV LED POU for drinking water applications, such reactors are currently available on the market. Therefore, more testing of UV LED reactors intended for POU applications that are commercially available on the market is necessary, especially if they will be used under challenging drinking water quality conditions.

This work could serve as ground work for the future testing of more available UV LED technology intended for POU applications. Similarly, the experience gained during the testing on the Arctic could serve to for future testing of POU UV LED reactors in remote and rural areas. More field experiments are necessary to determine the best way to apply UV LED POU reactors in challenging conditions. Furthermore, where feasible, a different bacterial quantification method should be considered to quantify reactor performance. In

addition, UV LED POU testing should be accompanied with biodosimetry tests, in order to accurately quantify the real UV dose being applied at the treated water.

The successful inactivation of biofilm-bound *P. aeruginosa* using UV LED technology results gained in this research, as well as the synergistic effects found between the UV LEDs and wiping techniques could be of a great interest in different fields, such as surface disinfection industry. These results could greatly improve surface disinfection methods. Therefore, these results could provide great insights for contamination-related risk reduction in target areas, including food preparation and handling surfaces, hospitals, health clinics, and dental environments. However, more testing pairing different disinfectants (i.e. chlorine, H_2O_2 and ethanol) with different UV light doses is required to optimize and further include this knowledge in common disinfection practices. It would also be of interest to investigate the effect of combinations of different wavelengths on biofilm-bound bacterial inactivation. In addition, it would be interesting to investigate the mechanism behind the synergistic effect found between wiping and UV treatment. Scanning electron microscopy (SEM) could be applied to understand the damage that wiping might be causing on the upper layer of the biofilm, if any.

Given these points, UV LEDs are a promising technology for drinking water treatment and for biofilm disinfection. For this reason, more research investigating the use of UV LEDs alongside pairing combinations with other disinfection techniques needs to be done for the further understanding of its applications and limitations.

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APPENDIX A. COPYRIGHT RELEASE FORM

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Title: Inactivation of biofilm-bound Pseudomonas aeruginosa bacteria using UVC light emitting diodes (UVC LEDs)

Author: Stephanie L. Gora,Kyle D. Rauch,C. Carolina Ontiveros,Amina K. Stoddart,Graham A. Gagnon

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APPENDIX B. ADITTIONAL DATA

Raw Data from Section 4.4

Reactor A Testing

Flow Rate (Lpm)	Replicate	Raw Plate Count (CFU/mL)	Bacterial Concentration Level	Reactor	Log Inactivation
1.00	1	1,620	Untreated Low	--	-0.03
1.00	2	1,320	Untreated Low	--	0.06
1.00	3	1,560	Untreated Low	--	-0.02
1.00	1	1,140	Low	A	0.12
1.00	2	1,130	Low	A	0.12
1.00	3	1,050	Low	A	0.15
1.00	1	1,200	Low	B	0.10
1.00	2	1,160	Low	B	0.11
1.00	3	1,080	Low	B	0.14
1.00	1	1,250,000	Untreated High	--	0.00
1.00	2	1,450,000	Untreated High	--	-0.06
1.00	3	1,090,000	Untreated High	--	0.06
1.00	1	1,060,000	High	D	0.08
1.00	2	1,290,000	High	D	-0.01
1.00	3	880,000	High	D	0.16
0.11	1	1,370	Untreated Low	--	-0.02
0.11	2	1,260	Untreated Low	--	0.02
0.11	3	1,300	Untreated Low	--	0.00
0.11	1	350	Low	A	0.57
0.11	2	400	Low	A	0.52
0.11	3	470	Low	A	0.45
0.11	1	< 300	Low	D	3.12
0.11	2	< 300	Low	D	3.12
0.11	3	< 300	Low	D	3.12
0.11	1	1,540,000	Untreated High	--	0.02
0.11	2	1,490,000	Untreated High	--	0.03
0.11	3	1,760,000	Untreated High	--	-0.04
0.11	1	311,500	High	B	0.71
0.11	2	269,000	High	B	0.77
0.11	3	91,500	High	B	1.24
0.11	1	360,000	High	C	0.65
0.11	2	280,000	High	C	0.76

0.11	3	300,000	High	C	0.73
0.06	1	620	Untreated Low	--	-0.01
0.06	2	560	Untreated Low	--	0.03
0.06	3	620	Untreated Low	--	-0.01
0.06	1	< 300	Low	A	2.78
0.06	2	< 300	Low	A	2.78
0.06	3	< 300	Low	A	2.78
0.06	1	< 300	Low	D	2.78
0.06	2	< 300	Low	D	2.78
0.06	3	< 300	Low	D	2.78
0.06	1	820,000	Untreated High	--	-0.05
0.06	2	700,000	Untreated High	--	0.02
0.06	3	690,000	Untreated High	--	0.03
0.06	1	114,000	High	B	0.81
0.06	2	93,000	High	B	0.90
0.06	3	105,000	High	B	0.85
0.06	1	< 300	High	C	5.87
0.06	2	< 300	High	C	5.87
0.06	3	< 300	High	C	5.87

Raw Data from Section 4.5. Reactor B Testing

UVT – Standard Plate Count

UVT Value (%)	Flow Rate (LPM)	UV Unit	Sample	Replicate	Log Inactivation (CFU/mL)	CFU/mL
50	0.25	1	1	1	3.72	640
50	0.25	1	1	2	3.68	690
50	0.25	1	1	3	3.80	530
50	0.25	1	2	1	3.49	1,080
50	0.25	1	2	2	3.54	950
50	0.25	1	2	3	3.53	980
50	0.25	2	1	1	2.58	10,100
50	0.25	2	1	2	2.62	9,100
50	0.25	2	1	3	2.69	7,800
50	0.25	2	2	1	2.38	15,900
50	0.25	2	2	2	2.36	16,900
50	0.25	2	2	3	2.43	14,400
50	0.75	1	1	1	1.15	204,000
50	0.75	1	1	2	1.25	163,000

50	0.75	1	1	3	1.23	169,000
50	0.75	1	2	1	1.50	92,000
50	0.75	1	2	2	1.59	74,000
50	0.75	1	2	3	1.90	62,000
50	0.75	2	1	1	1.32	173,000
50	0.75	2	1	2	1.33	171,000
50	0.75	2	1	3	1.34	166,000
50	0.75	2	2	1	1.66	79,000
50	0.75	2	2	2	1.58	96,000
50	0.75	2	2	3	1.56	99,000
50	1.5	1	1	1	0.57	680,000
50	1.5	1	1	2	0.47	850,000
50	1.5	1	1	3	0.63	590,000
50	1.5	1	2	1	0.51	780,000
50	1.5	1	2	2	0.57	680,000
50	1.5	1	2	3	0.60	630,000
50	1.5	2	1	1	1.75	44,000
50	1.5	2	1	2	1.63	59,000
50	1.5	2	1	3	1.65	56,000
50	1.5	2	2	1	0.77	420,000
50	1.5	2	2	2	0.73	470,000
50	1.5	2	2	3	0.69	510,000
75	0.25	1	1	1	6.34	0
75	0.25	1	1	2	6.34	0
75	0.25	1	1	3	6.34	0
75	0.25	1	2	1	6.34	0
75	0.25	1	2	2	6.34	0
75	0.25	1	2	3	6.34	0
75	0.25	2	1	1	6.34	0
75	0.25	2	1	2	6.34	0
75	0.25	2	1	3	6.34	0
75	0.25	2	2	1	6.34	0
75	0.25	2	2	2	6.34	0
75	0.25	2	2	3	6.34	0
75	0.75	1	1	1	3.18	2,440
75	0.75	1	1	2	3.16	2,610
75	0.75	1	1	3	3.21	2,310
75	0.75	1	2	1	3.14	2,140
75	0.75	1	2	2	3.12	2,850
75	0.75	1	2	3	3.27	2,010
75	0.75	2	1	1	2.84	5,400
75	0.75	2	1	2	2.65	8,400
75	0.75	2	1	3	2.35	16,700

75	0.75	2	2	1	1.75	66,000
75	0.75	2	2	2	1.73	69,000
75	0.75	2	2	3	1.74	68,000
75	1.5	1	1	1	1.20	157,000
75	1.5	1	1	2	1.19	160,000
75	1.5	1	1	3	1.25	139,000
75	1.5	1	2	1	1.33	116,000
75	1.5	1	2	2	1.36	110,000
75	1.5	1	2	3	1.28	132,000
75	1.5	2	1	1	1.77	42,000
75	1.5	2	1	2	1.68	52,000
75	1.5	2	1	3	1.83	37,000
75	1.5	2	2	1	1.78	41,000
75	1.5	2	2	2	1.67	53,000
75	1.5	2	2	3	1.77	42,000
99	0.25	1	1	1	6.49	2
99	0.25	1	1	2	6.49	0
99	0.25	1	1	3	6.49	0
99	0.25	1	2	1	6.49	2
99	0.25	1	2	2	6.49	2
99	0.25	1	2	3	6.49	0
99	0.25	2	1	1	6.36	0
99	0.25	2	1	2	6.36	0
99	0.25	2	1	3	6.36	0
99	0.25	2	2	1	6.36	0
99	0.25	2	2	2	6.36	0
99	0.25	2	2	3	6.36	0
99	0.75	1	1	1	4.05	276
99	0.75	1	1	2	4.01	266
99	0.75	1	1	3	4.04	212
99	0.75	1	2	1	4.73	58
99	0.75	1	2	2	4.75	56
99	0.75	1	2	3	4.64	72
99	0.75	2	1	1	4.19	200
99	0.75	2	1	2	4.27	168
99	0.75	2	1	3	4.33	146
99	0.75	2	2	1	4.49	100
99	0.75	2	2	2	4.39	128
99	0.75	2	2	3	4.43	116
99	1.5	1	1	1	3.39	1,270
99	1.5	1	1	2	3.30	1,560
99	1.5	1	1	3	3.36	1,370
99	1.5	1	2	1	4.91	38

99	1.5	1	2	2	4.89	40
99	1.5	1	2	3	5.05	28
99	1.5	2	1	1	2.83	4,600
99	1.5	2	1	2	2.72	6,000
99	1.5	2	1	3	2.68	6,600
99	1.5	2	2	1	4.50	98
99	1.5	2	2	2	4.33	146
99	1.5	2	2	3	4.35	138

UVT – Recovery ATP Method

Flow Rate (LPM)	UV Unit	% UVT	Sample	Log Inactivation (pg cATP/mL)	pg cATP/mL
0.25	1	50	1	2.23	1,160.60
0.25	1	50	2	2.38	806.10
0.25	2	50	1	2.13	1,435.09
0.25	2	50	2	1.93	2,306.06
0.25	1	75	1	2.42	743.26
0.25	1	75	2	2.31	950.09
0.25	2	75	1	2.45	692.97
0.25	2	75	2	2.25	1,093.64
0.25	1	99	1	2.49	570.71
0.25	1	99	2	2.50	561.79
0.25	2	99	1	2.19	1,163.07
0.25	2	99	2	2.69	362.33
0.75	1	50	1	2.01	3,711.70
0.75	1	50	2	2.02	3,636.34
0.75	2	50	1	1.96	4,203.34
0.75	2	50	2	1.90	4,773.30
0.75	1	75	1	2.50	1,201.45
0.75	1	75	2	2.47	1,275.19
0.75	2	75	1	2.25	2,129.33
0.75	2	75	2	2.23	2,241.82
0.75	1	99	1	2.45	639.04
0.75	1	99	2	2.44	640.18
0.75	2	99	1	2.49	581.82
0.75	2	99	2	2.47	602.18
1.5	1	50	1	1.19	7,695.59
1.5	1	50	2	1.06	10,337.68

1.5	2	50	1	1.40	4,803.79
1.5	2	50	2	1.40	4,821.42
1.5	1	75	1	1.56	2,288.79
1.5	1	75	2	1.49	2,670.29
1.5	2	75	1	1.79	1,342.68
1.5	2	75	2	1.83	1,231.32
1.5	1	99	1	2.13	1,336.26
1.5	1	99	2	2.45	633.12
1.5	2	99	1	2.39	731.13
1.5	2	99	2	2.46	611.62

Turbidity – Standard Plate Count

Flow Rate (LPM)	Turbidity (NTU)	UV Unit	Sample	Replicate	Log Inactivation (CFU/mL)	Log Inactivation (CFU/mL)
0.25	1	1	1	1	6.36	1
0.25	1	1	1	2	6.36	1
0.25	1	1	1	3	6.36	1
0.25	1	1	2	1	6.36	1
0.25	1	1	2	2	6.36	1
0.25	1	1	2	3	6.36	1
0.25	1	2	1	1	3.17	1,550
0.25	1	2	1	2	2.98	2,400
0.25	1	2	1	3	3.08	1,900
0.25	1	2	2	1	6.36	1
0.25	1	2	2	2	6.36	1
0.25	1	2	2	3	6.36	1
0.25	10	1	1	1	3.72	560
0.25	10	1	1	2	3.62	710
0.25	10	1	1	3	3.66	640
0.25	10	1	2	1	6.47	1
0.25	10	1	2	2	6.47	1
0.25	10	1	2	3	6.47	1
0.25	10	2	1	1	3.99	300
0.25	10	2	1	2	4.02	280
0.25	10	2	1	3	4.07	250
0.25	10	2	2	1	6.47	1
0.25	10	2	2	2	6.47	1
0.25	10	2	2	3	6.47	1
0.25	0.1	1	1	1	6.49	2

0.25	0.1	1	1	2	6.49	0
0.25	0.1	1	1	3	6.49	0
0.25	0.1	1	2	1	6.49	2
0.25	0.1	1	2	2	6.49	2
0.25	0.1	1	2	3	6.49	0
0.25	0.1	2	1	1	6.36	0
0.25	0.1	2	1	2	6.36	0
0.25	0.1	2	1	3	6.36	0
0.25	0.1	2	2	1	6.36	0
0.25	0.1	2	2	2	6.36	0
0.25	0.1	2	2	3	6.36	0
0.75	1	1	1	1	3.54	1,040
0.75	1	1	1	2	3.58	960
0.75	1	1	1	3	3.60	920
0.75	1	1	2	1	6.56	1
0.75	1	1	2	2	6.56	1
0.75	1	1	2	3	6.56	1
0.75	1	2	1	1	3.14	2,640
0.75	1	2	1	2	3.06	3,140
0.75	1	2	1	3	3.08	3,045
0.75	1	2	2	1	3.50	1,140
0.75	1	2	2	2	3.50	1,140
0.75	10	1	1	1	6.47	1
0.75	10	1	1	2	6.47	1
0.75	10	1	1	3	6.47	1
0.75	10	1	2	1	5.17	20
0.75	10	1	2	2	6.47	1
0.75	10	1	2	3	6.47	1
0.75	10	2	1	1	3.55	830
0.75	10	2	1	2	3.60	730
0.75	10	2	1	3	3.57	790
0.75	10	2	2	1	6.47	1
0.75	10	2	2	2	6.47	1
0.75	10	2	2	3	6.47	1
0.75	0.1	1	1	1	4.05	276
0.75	0.1	1	1	2	4.01	308
0.75	0.1	1	1	3	4.04	286
0.75	0.1	1	2	1	4.73	58
0.75	0.1	1	2	2	4.75	56
0.75	0.1	1	2	3	4.64	72
0.75	0.1	2	1	1	4.19	200
0.75	0.1	2	1	2	4.27	168
0.75	0.1	2	1	3	4.33	146

0.75	0.1	2	2	1	4.49	100
0.75	0.1	2	2	2	4.39	128
0.75	0.1	2	2	3	4.43	116
1.5	1	1	1	1	6.40	1
1.5	1	1	1	2	6.40	1
1.5	1	1	1	3	6.40	1
1.5	1	1	2	1	6.40	1
1.5	1	1	2	2	6.40	1
1.5	1	1	2	3	6.40	1
1.5	1	2	1	1	6.40	1
1.5	1	2	1	2	6.40	1
1.5	1	2	1	3	6.40	1
1.5	1	2	2	1	6.40	1
1.5	1	2	2	2	6.40	1
1.5	1	2	2	3	6.40	1
1.5	10	1	1	1	2.92	3,525
1.5	10	1	1	2	2.96	3,185
1.5	10	1	1	3	2.90	3,700
1.5	10	1	2	1	6.47	1
1.5	10	1	2	2	6.47	1
1.5	10	1	2	3	6.47	1
1.5	10	2	1	1	3.11	2,290
1.5	10	2	1	2	3.14	2,130
1.5	10	2	1	3	3.11	2,260
1.5	10	2	2	1	6.47	1
1.5	10	2	2	2	6.47	1
1.5	10	2	2	3	6.47	1
1.5	0.1	1	1	1	3.39	1,270
1.5	0.1	1	1	2	3.30	1,560
1.5	0.1	1	1	3	3.36	1,370
1.5	0.1	1	2	1	4.91	38
1.5	0.1	1	2	2	4.89	40
1.5	0.1	1	2	3	5.05	28
1.5	0.1	2	1	1	2.83	4,600
1.5	0.1	2	1	2	2.72	6,000
1.5	0.1	2	1	3	2.68	6,600
1.5	0.1	2	2	1	4.50	98
1.5	0.1	2	2	2	4.33	146
1.5	0.1	2	2	3	4.35	138

Turbidity – Recovery ATP Method

Flow Rate (LPM)	Turbidity (NTU)	UV Unit	Sample	Log Inactivation (pg cATP/mL)	pg cATP/mL
0.25	1	1	1	2.89	288.18
0.25	1	1	2	2.78	376.79
0.25	1	2	1	2.51	686.71
0.25	1	2	2	2.67	479.11
0.25	10	1	1	2.21	552.37
0.25	10	1	2	2.35	397.94
0.25	10	2	1	2.17	601.00
0.25	10	2	2	2.27	483.89
0.25	0.1	1	1	2.49	570.71
0.25	0.1	1	2	2.50	561.79
0.25	0.1	2	1	2.19	1,163.07
0.25	0.1	2	2	2.69	362.33
0.75	1	1	1	2.76	608.40
0.75	1	1	2	2.79	570.18
0.75	1	2	1	2.62	836.30
0.75	1	2	2	2.85	494.29
0.75	10	1	1	2.17	596.37
0.75	10	1	2	2.21	553.88
0.75	10	2	1	2.03	841.27
0.75	10	2	2	2.16	610.82
0.75	0.1	1	1	2.45	639.04
0.75	0.1	1	2	2.44	640.18
0.75	0.1	2	1	2.49	581.82
0.75	0.1	2	2	2.47	602.18
1.5	1	1	1	2.40	927.68
1.5	1	1	2	2.47	781.22
1.5	1	2	1	2.37	987.20
1.5	1	2	2	2.55	649.98
1.5	10	1	1	1.84	1,282.44
1.5	10	1	2	2.10	713.16
1.5	10	2	1	2.06	779.80
1.5	10	2	2	2.20	557.01
1.5	0.1	1	1	2.13	1,336.26
1.5	0.1	1	2	2.45	633.12

1.5	0.1	2	1	2.39	731.13
1.5	0.1	2	2	2.46	611.62

Raw Data from Testing on Field

Week	Date	Control (pg cATP/mL)	UV Treated (pg cATP/mL)	Log Inactivation (pg cATP/mL)
0	May 18, 2018	33.33	16.62	0.30
0	May 18, 2018	14.15	22.55	0.00
0	May 21, 2018	20.55	16.04	0.11
0	May 21, 2018	17.18	13.43	0.11
1	May 23, 2018	569.49	42.73	1.12
1	May 23, 2018	263.30	154.79	0.23
1	May 28, 2018	130.96	14.04	0.97
1	May 28, 2018	124.25	15.53	0.90
2	June 4, 2018			
2	June 4, 2018	42.44	24.38	0.24
3	June 13, 2018	18.75	22.02	0.00
3	June 13, 2018	19.65	20.08	0.00
4	June 19, 2018	22.13	25.12	0.00
4	June 19, 2018	14.45		
5	June 25, 2018	30.03	21.52	0.14
5	June 25, 2018	25.03	17.65	0.15
6	July 7, 2018	14.31	19.23	0
6	July 7, 2018	21.46	31.19	0
7	July 13, 2018		25.58	
7	July 13, 2018	35.65	31.31	0.06
8	July 17, 2018	44.18	44.47	0
8	July 17, 2018	39.13		
9	July 23, 2018	54.20	61.34	0

Raw Data from Chapter 5.

UV Kinetics Curve

UV Dose	CFU/cm^2	Log Inactivation (CFU/cm^2)

0	43,130	-0.05
0	36,270	0.18
0	38,175	-0.08
2	133,072	0.20
2	233,286	0.09
2	64,760	0.32
4	70,280	0.77
4	68,098	0.76
4	17,446	1.02
8	17,395	1.20
8	10,938	1.51
8	15,990	1.26
12	18,338	1.13
12	17,119	1.35
12	10,453	1.54
16	4,831	1.78
16	7,702	1.99
16	13,213	1.51
20	7,449	1.53
20	10,924	1.89
20	16,848	1.34

Wiping Times Kinetics Curve

Wiping Time (Sec)	CFU/cm^2	Log Inactivation
Single pass	5,250,000	1.06
Single pass	88,000	2.84
Single pass	790,000	1.88
15	845,000	1.95
15	105,000	2.85
30	34,500	3.34
30	270,000	2.44
30	2,200,000	1.53
60	155,000	2.68
60	1,345,000	1.75
180	1,380,000	1.73
180	62,000	3.08
180	3,150	4.38
0.00	83,500,000	-0.04759
0.00	87,000,000	-0.06542

0.00	54,000,000	0.141701

Linear Fit Standard Plate Count-Recovery ATP Method

CFU/cm^2	cATP
-0.05	-0.04
0.18	0.03
-0.08	0.01
0.20	0.14
0.09	-0.10
0.32	0.46
0.77	0.42
0.76	0.44
1.02	1.03
1.20	1.03
1.51	1.23
1.26	1.07
1.13	1.01
1.35	1.04
1.54	1.25
1.78	1.59
1.99	1.38
1.51	1.15
1.53	1.40
1.89	1.23
1.34	1.04

Raw Data – Synergy

Wiping Time	UV Dose (mJ/cm^2)	CFU/cm^2	Log Inactivation (CFU/cm^2)	pg cATP/cm^2	Log Inactivation (pg cATP/cm^2)
Single pass	4	0	7.78	1.62	2.50
Single pass	4	33,500	3.26	29.14	1.25
Single pass	4	25,000	3.38	1.07	2.69
Single pass	12	0	7.78	-0.46	2.71
Single pass	12	0	7.78	2.85	2.26

Single pass	12	0	7.78	-1.31	2.71
15 sec	4	160,000	2.58	-0.66	2.71
15 sec	4	0	7.78	0.13	3.61
15 sec	4	29,000	3.32	1.92	2.43
15 sec	12	0	7.78	0.70	2.87
15 sec	12	0	7.78	0.20	3.41
15 sec	12	0	7.78	0.57	2.96
Single pass	0	5,250,000	1.06	17.95	1.46
Single pass	0	88,000	2.84	-4.00	2.71
Single pass	0	790,000	1.88	0.94	2.74
None (control)	None (Control)	69,000,000	-0.06	319.85	0.21
None (control)	None (Control)	44,500,000	0.13	745.41	-0.16
None (control)	None (Control)	67,500,000	-0.05	484.37	0.03
Treatment					
Wiping time	UV dose				
Single pass	8	27,000	3.44	13.84	1.68
Single pass	8	0	7.87	9.96	1.82
Single pass	8	1,500	7.87	8.62	1.89
15 sec	8	0	7.87	9.89	1.83
15 sec	8	0	7.87	3.84	2.24
15 sec	8	16,500	3.66	8.21	1.91
None (control)	None (Control)	83,500,000	-0.05	950.62	-0.16
None (control)	None (Control)	87,000,000	-0.07	596.05	0.05
None (control)	None (Control)	54,000,000	0.14	442.59	0.18