University of Alberta

VALIDATION OF FULL-SCALE UV REACTOR PERFORMANCE IN WASTEWATER TREATMENT USING INDIGENOUS MICROORGANISMS

by

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DEDICATION

To Ardeshir for his love, support and endless patience,

and my parents for their sacrifices, prayers and understanding.

ABSTRACT

The objective of this study was to measure dose delivery of a full-scale ultraviolet (UV) light disinfection reactor, treating municipal wastewater using biodosimetry, based on inactivation of indigenous fecal coliform bacteria. The UV reactor was evaluated at four combinations of high and low flow wastewater rate, and 50% and 100% lamp power. The reduction equivalent UV dose (RED) determined using the indigenous fecal coliform bacteria, was consistently lower than the UV dose computed by the reactor control system, and ranged from 6.1 to 11.7 mJ/cm². On average, the reduction equivalent dose (RED) of the UV reactor bacteria was approximately 30% of the dose reported by the UV reactor control system. The ratio of RED to computed dose observed in this testing was consistent with a relatively broad dose distribution. Ideally, the expected performance of the reactor should be expressed not in terms of averaged calculated dose, but rather in terms of the RED of the target pathogen.

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ABBREVIATIONS

| ACRWWC | Alberta Capital Region Wastewater Commission |
|----------|---|
| ACRWWTP | Alberta Capital Region Wastewater Treatment Plant |
| ANOVA | Analysis of variance |
| ATCC | American Type Culture Collection |
| BNR | Biological Nutrient Removal |
| CBOD | Five-day Carbonaceous biochemical oxygen demand |
| CFD | Computational Fluid Dynamics |
| CFU | Colony Forming Unit |
| DNA | Deoxyribonucleic Acid |
| FC | Fecal Coliform |
| Log | Logarithm |
| LT2ESWTR | Long Term 2 Enhanced Surface Water Treatment Rule |
| MPN | Most Probable Number |
| MPSS | Multiple Point Source Summation |
| N/A | Not Applicable |
| NTU | Nephelometric Turbidity Unit |
| PCTE | Polycabonate Track Etched |
| PFU | Plaque Forming Unit |
| RED | Reduction Equivalent Dose (mJ/cm ²) |
| RNA | Ribonucleic Acid |
| SC | Somatic Coliphage |
| STD | Standard Deviation |
| TC | Total Coliform |
| TSB | Trypticase soy broth |
| TSS | Total Suspended Solids |
| US | United States |
| US EPA | United States Environmental Protection Agency |
| UV | Ultraviolet |
| UV | Ultraviolet |
| UVT | Ultraviolet Transmittance |
| UVT | UV Transmittance |
| WWTP | Wastewater Treatment Plant |

| SYMBOLS | 5 |
|---------|---|
|---------|---|

| a | Absorption Coefficient |
|----------------------------|---|
| С | Speed of light |
| E^{o}_{λ} | Incident light |
| E'_{λ} | Transmitted light |
| G(H) | UV survival-ratio function |
| h | Plank's constant |
| Н | UV dose |
| I avg | Depth averaged UV irradiance |
| k | Rate constant |
| 1 | Pathlength |
| L | Distance from UV lamp to the water surface |
| N | Number of surviving microorganisms after exposure to UV |
| N_{0} | Initial number of microorganisms prior to UV exposure |
| $N_{D,\theta}$ | Number of dispersed microorganisms prior to UV exposure |
| $N_{p,0}$ | number of particle-associated microorganisms prior to UV exposure |
| <i>p</i> -value | Probability of making a type I error in a statistical test |
| <i>P(H)</i> | UV dose distribution probability density function |
| Q | Volumetric flow rate |
| t | Exposure time |
| T_{λ} | Transmittance at specific wavelength |
| V | Irradiation volume |
| x, y, z | Orthogonal directions |
| | |

GREEK SYMBOLS

| λ | Wave | elength | |
|---|------|----------|--|
| | | T TT 7 1 | |

- μ Average UV dose
- σ Standard deviation of UV dose distribution

UNITS

| - | Negative |
|-----|----------------|
| % | Percentage |
| °C | Degree Celsius |
| μ | micrometers |
| cm | centimetres |
| hr | hours |
| kg | kilograms |
| L | litres |
| m | meters |
| min | minutes |
| mJ | miliJoules |
| mL | mililiters |
| mm | milimeters |
| nm | nanometer |
| Pa | Pascal |
| S | seconds |
| | |

1 INTRODUCTION

1.1 BACKGROUND

The use of UV light for disinfection of water was pioneered in both the United States and Europe in the early 1900's with the advent of the quartz lamp. The first UV system was placed into the water supply in 1910. The extensive use of UV equipment relied on integrating the latest developments in the areas of lamps, ballasts and sensor technology. Apparently, difficulties faced in those early experiments and advances in production and use of chlorine gas led to discouragement in the use of UV systems in North America. Disinfection of wastewater is considered to be one of the significant acts in the protection of human health and the maintenance of a natural, healthy environment. Inactivation of pathogenic microorganisms in municipal wastewater plants will reduce the release of pathogens into receiving waters, lowering the threat of deadly waterborne disease outbreaks through contamination of drinking water. Chlorination of treated wastewater for disinfection purpose played a key role in public health achievements in the 20th century.

The outstanding improvement in lamps and ballasts technologies in the 1940's, led to the acceptance and broad use of UV systems for disinfection of drinking water in Europe. Findings about production of carcinogens by using chlorine in wastewater treatment processes turned people's attention to more environmentally friendly disinfection technologies. In the late 1970's the US EPA began to discourage the use of chlorine for wastewater disinfection, and began to promote UV disinfection research. The increasing cost of chlorinating agents, the hazards involving in the transport, storage and handling of chlorine and the toxicity of the free chlorine residual itself to fish and aquatic life, are of concern in chlorine treatment of wastewater. The replacement of the imperfect practice of chlorination with the smaller environmental footprint requirements of UV disinfection, have made UV disinfection a more reliable, and popular alternative to chlorination in both drinking water, and wastewater treatment. Substitution of UV light for permanent disinfection eliminated formation of trihalomethanes, reduced the formation of aldehydes and there were no evidence of toxicity from the full scale UV irradiated effluent at target

UV dose of 40 mJ/cm² (Oppenheimer et al. 1997; Jolis et al. 1999; Bukhari et al. 2004). UV light has been widely used for the disinfection of treated wastewater effluents, which are a major source of fecal contamination of aquatic ecosystems. Since 1978, full-scale innovative UV systems have been successfully used to disinfect effluents, which were reused for irrigation and recreation purposes. There was no evidence of appreciable quantities of harmful disinfection by-products. At a peak wavelength of 254 nm, UV is an effective biocide for both secondary and tertiary effluents, and is considered to be safe to the environment (Dizer et al. 1992; Oppenheimer et al. 1997; Jolis et al. 1999; Scott et al. 2005). Over the past two decades, UV radiation has become an established disinfection technology for primary, secondary and filtered tertiary effluents with the number of plants using UV for final disinfection numbering more than two thousand in the United States (Jolis et al. 1999; Whitby and Scheible 2004).

Most of the early UV systems for disinfecting wastewater were adapted from the facilities used for disinfecting drinking water. The problems associated with cleaning systems, inadequate hydraulics and sizing a UV system for specific wastewater applications and difficulties in maintenance drew industry attention. Taking into account the hydraulic and water quality aspects of design for wastewater applications turned those early UV systems into the second-generation systems. Soon advanced open-channel, gravity flow, modular systems dominated the wastewater disinfection market (Whitby and Scheible 2004).

Reuse of wastewater has recently gained considerable attention as an ecological, and economically necessary practice, due to decreasing ground water tables and deterioration of surface water qualities. Therefore, increasing efforts are being invested to evaluate the treatment efficiency of wastewater treatment facilities regarding fecal microorganisms removal. Typical concentrations of total coliforms (TC) and fecal coliforms (FC) in raw sewage are respectively, 10⁷-10⁹ and 10⁶-10⁸ per 100 ml, which represents a major source of TC and FC to rivers and coastal waters. Large numbers of fecal microorganisms are discharged to the environment via treated effluents which have gone through conventional treatment without any specific disinfection (George et al. 2001).

UV disinfection technology is of growing interest in the drinking water industry for the reasons outlined above. In addition, recent studies have demonstrated the capability of UV irradiation to extensively inactivate highly chlorine-resistant protozoan pathogens such as *Cryptosporidium parvum* and *Giardia lamblia* (Bukhari et al. 1999; Shin et al. 2005).

Treatment of municipal wastewater generally requires disinfection to meet regulatory microbial limits. Disinfection must be effective against a wide range of bacteria, viruses, and protozoa to reduce the number of waterborne pathogens to thresholds that lower the risk of public exposure to infective doses of pathogens. UV irradiance as a reliable means of disinfection, has been shown to be effective in inactivation of most waterborne pathogens. However, many researchers have shown that viruses, especially some important human enteric viruses, are more resistant to UV disinfection than are fecal bacteria, and that these viruses and bacteria have very different inactivation kinetics. Therefore, more than 100 enteric viruses that can be transmitted by water will be the limiting organisms in determining reactor designs and UV doses for systems requiring waterborne pathogen inactivation (Tree et al. 1997; Bourrouet et al. 2001; Shin et al, 2005).

Disinfection efficiency of UV systems depends on UV dose, influent microbial concentration, and water transmittance. Delivered UV dose to a target microorganism is a function of exposure time and UV fluence rate. The fluence rate received by microorganisms is mainly influenced by the wastewater UV transmittance and suspended solid content. Fluence rate itself is determined by lamp wattage, and spectral power output of the lamp. Understanding the relationship between the inactivation of microorganisms and the average UV dose is key to microbial risk assessment.

To determine the disinfection efficacy or microbial log reduction of full-scale UV systems, and to assess the dose requirement to obtain a certain microbial log reduction, the inactivation kinetics can be used. However, for using bench scale results to design and evaluate the full-scale UV systems, it is necessary to know the effect of process

conditions on the efficiency of the radiation process. Unlike chemical disinfection processes like chlorination and ozonation, the efficacy of UV disinfection is not affected by conditions like temperature, pH and reactive organic matter. But efficiency of UV disinfection at full-scale could be affected by factors pertaining to the microorganisms: physiological state (pre-culturing, growth phase), strain diversity, repair mechanisms and particle association and by factors pertaining to the dose assessment: dose distribution due to the distribution of the hydraulic retention time, absorbance, reflection and refraction of UV light through the water and changes in lamp intensity due to aging and fouling (Hijnen et al. 2006). The impact of water quality and the optimization of UV reactor design using hydraulic modeling techniques and biodosimetry, will be the focus of future studies.

In the United States, validation of UV reactors used in drinking water systems is required by the US EPA's The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) to make sure that UV reactor is providing the required level of target inactivation. Biodosimetry is the experimental portion of the validation process and includes the following steps: injecting the challenge organism into the water flow, measuring the organism concentration before and after exposure to UV light, and calculating the log inactivation achieved by the unit. The reduction equivalent dose (RED), is then determined by referring microrganism inactivation measured in the reactor, to a calibration curve of the UV inactivation versus dose that is developed in laboratory UV exposure experiments.

Although it is now well developed in the drinking water treatment field, not many studies were published in the literature regarding the validation of a UV reactor used for disinfection of wastewater. The latter is the primary subject of current thesis. This case study intended to examine the performance of a UV reactor designed for treating unfiltered secondary effluent. Alberta Capital Region Wastewater Commission (the study sponsor) was interested in using indigenous fecal coliform bacteria as the challenge microorganism to verify the performance of a newly installed reactor at their recently upgraded wastewater treatment facility in Strathcona County, AB. Fecal coliform were

proposed because they are present in relatively high concentration in most treated municipal wastewaters. This eliminates the need to add a foreign challenge microorganism to the wastewater as is usually done in biodosimetry evaluations in drinking water UV reactors. Indigenous coliphages are another microorganisms that are present in relatively large concentration in treated wastewater. These microorganisms may also potentially be used to determine dose in large wastewater UV reactors.

1.2 PROBLEM STATEMENT

The problem statement of the current study was that it is hard to measure or calculate the actual UV dose delivered to the microorganisms passing through continuous-flow UV systems. Therefore, determining dose delivery in a large-scale municipal wastewater UV reactor is even harder due to non-ideal behaviour of reactors, and the presence of particulate matter that may shelter microorganisms from UV exposure in secondary effluents. The biodosimetry approach is in wide use for validation of drinking water UV reactor performance. Also, addition of challenge microorganisms to large flow UV reactors is impractical and very costly to carry out, especially on installed reactors. In water treatment, validation is required by regulations and is usually carried out at specialized facilities.

1.3 RESEARCH OBJECTIVES

1.3.1 Objectives

The objectives of this research study were to:

 Provide an independent assessment of the disinfection performance of the low pressure, high intensity open-channel UV system which has been installed as part of recent upgrade of the Alberta Capital Region Wastewater Treatment Plant. This UV light system provides final disinfection of the biologically treated secondary effluent. Alberta Capital Region Wastewater Commission (ACRWC) requested an independent measurement of UV dose based on biodosimetry using indigenous fecal coliform. The measured UV dose using biodosimetry was to be compared to the dose computed and reported by the UV reactor monitoring system (provided by the UV reactor vendor) under a variety of operating conditions. A Point Source Summation Method is used to calculate UV dose in the reactor and computed dose relies on the questionable assumption of complete lateral mixing across UV intensity gradients. Biodosimetry is a method for evaluating the performance of UV systems and is well established in the drinking water treatment field, but less developed in municipal wastewater treatment field. Therefore biodosimetric evaluation of a wastewater UV reactor performance using naturally occurring coliform bacteria as an indicator, will set this study apart from existing validation studies. Laboratory cultured organisms are not representative of wild type populations in wastewater effluent, and are considered to be more sensitive to disinfection processes.

- Develop and test an analytical procedure to reduce the impact of particle-associated microorganisms on the accuracy of the biodosimetry results. The concentration of particulate matter in unfiltered secondary effluent is usually much greater than in filtered drinking water. Association between microorganisms and particles present results in tailing of UV-dose inactivation curves. Dose determinations that lie within the tailing region will be more problematic and less accurate.
- Test the effectiveness of existing UV systems in removal of enteric viruses from treated wastewaters using coliphage as a surrogate organism. Coliphages are valuable models or surrogates for enteric viruses in basic genetic research as well as water quality assessment.

1.3.2 Tasks

 Conduct coliphage assay on up stream UV and down stream UV samples from various wastewater treatment plant facilities: Gold Bar WWTP, City of Kelowna WWTP and Winnipeg WWTP (summer 2004)

- Design and perform bench-scale coliphage UV inactivation test to determine the UV dose-response of the coliphage present in the wastewater using a standard collimated beam exposure (summer 2004)
- Develop an analytical method based on membrane filtration to reduce the influence of particles on the reduction equivalent dose determined using biodosimetry, and compare to biodosimetry without membrane filtration
- Determine the performance and the measured dose of the UV reactor and compare it to the calculated dose under a variety of operating conditions
- Develop a model to predict the UV dose required for a given fecal coliform inactivation credit for the particular examined treated wastewater (Alberta Capital Region secondary effluent)

1.4 OUTLINE OF THESIS

This Thesis has been structured into five chapters. Chapter 1 includes general background, the research goals and carried out tasks to achieve these goals. Chapter 2 represents a review of relevant literature. Experimental apparatus, condition specifications and procedures necessary to fulfil current thesis intention are provided in Chapter 3. The experimental results and discussions are presented in Chapters 4. The conclusions derived from synthesising results and discussions are outlined in Chapter 5. The appendices contain raw data and supportive plots.

2 LITERATURE REVIEW

2.1 WASTEWATER DISCHARGE REGULATIONS

Disinfection should be applied when wastewater is to be discharged into receiving waters with special treatment requirements. The amount of coliforms discharged into receiving water has to be controlled. The United States Environmental Protection Agency (USEPA) issued the first municipal wastewater discharge permit in the US to an Illinois municipality, which enforces a limit of 200 fecal coliform bacteria for each 100 millilitres of discharged water (USEPA, July 1973).

Performance standards for wastewater systems imposed by Alberta Environmental Protection, set the best practicable technology standards for municipalities with a population greater than 20,000. These standards require a fecal coliform concentration of less than 200/100 mL based on a geometric mean of daily grab samples collected in a calendar month (Alberta Environmental Protection, December 1997).

The seasonal discharge of treated wastewater to a receiving watercourse must be reviewed on a site-specific basis (Alberta Environmental Protection, December 1997). The site-specific disinfection criteria range from non-detectable FC to less than 200 FC/100mL. UV reactor applications with fecal coliform limits of 25 to 200/100mL typically have design doses ranging from 29 to 60 mJ/cm² (Sakamoto, 2000).

2.2 UV REDUCTION OF MICROORGANISMS

2.2.1 UV Light

In 1666, Isaac Newton first studied visible light, which is only a small portion of electromagnetic radiation, as a spectrum of colours. His experiments revealed the particle characteristic of light. In the mid nineteen hundreds Maxwell proposed the theory of a "sea of space" filled by ether to explain the transmission of light, heat and radio waves. Maxwell's scientific observations led to his proposal that the phenomenon of light

is an electromagnetic phenomenon, just as radio or TV waves are. The demonstration of the Maxwell's electromagnetic theory of light by Heinrich Hertz (1857 – 1894) confirmed that the velocity of light is equal to the velocity of radio waves, and visible light moves freely like Maxwell's waves. A wave is characterized by its wavelength or by its frequency (how many wavelengths pass a fixed point in a given time). In general light photons with wavelengths ranging from 100 to 1000 nanometers (nm) are capable of initiating photochemical reactions. Plank's law of radiation measures the energy carried by light assuming that energy is transmitted in small packets (Equation 1). This relationship considers both wave and particle-like properties of light (Jagger 1967, Bolton 1999). Those individual small packets of energy were latter named photons and considered as particles but their movement follows wave principals. Plank's law is:

$$u = \frac{hc}{\lambda}$$
 Equation (1)

where *u* is the energy of one photon (J), *c* is the light speed $(2.9979 \times 10^8 \text{ m/s})$, λ is wavelength (m) and *h* is the Plank's constant $(6.6261 \times 10^{-34} \text{ J.s})$. The amount of energy carried by a photon is a function of wavelength at fixed temperature, and increases as its wavelength decreases. This explains why UV light, with a wavelength between 100 and 400 nm is the subject of more studies than visible light involving photochemical reactions. Ultraviolet (UV) light has shorter wavelengths than visible light (400 to 700 nm) but longer than soft X rays.

Based on the effect of the ultraviolet part of the spectrum on human health and the environment, the range of UV wavelengths has been subdivided into: UVA (315 to 400 nm), UVB (280-315 nm), UVC (200 to 280 nm) and vacuum UV (100 to 200 nm) (Bolton 1999). UVA is the least harmful which causes sun tanning or skin bronzing. UVB is reaponsible for sunburns which can lead to skin cancer. UVC is known as a "germicidal" light and is capable of cell destruction due to its high energy levels. Unlike the above portions of the UV light range, the vacuum UV range is readily absorbed by water, air and other substances. Thus it can only be transmitted through a vaccum (Bolton 1999) and, therefore, is not very useful in water or wastewater treatment applications. UV light in the UVC range can be employed to impair bacteria and viruses in air and

water, since it is not competely absorbed by the atmosphere or water, but is absorbed by DNA, RNA and proteins which results in cell inactivation.

2.2.2 Mechanisms of UV Inactivation

In most living organisms other than viruses, genetic information is stored in the deoxyribonucleic acid (DNA). Ribonucleic acid (RNA) is the main genetic material in most, but not all, viruses. Unlike the double helical structure of the DNA molecule (reported by Watson and Crick, 1953), RNA is a single-stranded molecule. The four building blocks of DNA are adenine, thymine, cytosine and guanine. These nucleotide bases are made up of nitrogen containing heterocyclic aromatic compounds and the sequence of these four constituent bases in the DNA molecule determines the unique genetic code of an organism. In double-stranded DNA, the nucleotide bases form "base pairs" where adenine on one strand is paired thymine on the other. In terms of their 3-D molecular structure, adenine and thymine are mirror images of each other and bind together by hydrogen bonds. Cytosine with guanine form similar base pairs (Figure 1). In RNA thymine is replaced by another nucleotide base, uracil (Friedberg et al. 1995; Bolton 1999).

The germicidal effects of UV light involve photochemical damage to DNA and RNA within the cells of an organism. Of the four bases, thymine (uracil in RNA) is the most sensitive, and undergoes a unique photochemical reaction by absorption of UV photons. The absorption of a UV photon by adjacent thymine bases, results in a chemical bond formation between the two-thymine molecules, which is called thymine dimer (Figure 2). The formation of thymine dimers alters the structure of the DNA and is primarily responsible for inhibiting replication and transcription of the cell or virus. As few as one hundred thymine dimers in a DNA chain, are enough to inactivate the entire cell (Bolton 1999; Hijnen et al. 2006).



Figure 1: DNA structure



Figure 2: Photochemical dimerization of two thymine bases (Adapted from Bolton 1999)

The nucleic acids (DNA and RNA) in microorganisms are the most important absorbers of the UV light energy in the wavelength range of 240-280 nm. The UV absorbance spectrum of DNA has a maximum around 260 nm. At lower and higher wavelengths the absorbance decreases (Bolton 1999). Since RNA and DNA carry genetic information for reproduction, damage to these substances can effectively prevent the cell or virus from replicating (Wang et al., 1995). A cell that cannot reproduce within a host is not able to cause disease. From this point on, UVC light with a wavelength of between 200 to 280 nm will be referred to simply as "germicidal UV light".

Exactly what makes one microorganism more UV resistant than another is not fully understood. It is assumed that a low thymine content of the genome of an organism might impart greater UV resistance. Double-stranded genomes, such as those of the adenoviruses, may also provide an increased measure of UV resistance. Adenovirus has relatively high resistance to UV compared to other viruses (Meng and Gerba 1996;

Thurston-Enriquez et al. 2003). It is possible that adenovirus resistant may also be related to the ability of the virus to use host cell DNA repair mechanisms (Thompson et al. 2003). According to the literature, viruses are much more resistance to UV radiation than are vegetative bacteria (Bosch et al. 1989; Dizer et al. 1992; Shaban et al. 1997; Lazarova et al. 1998). For example, the high sensitivity of *Escherichia coli* is believed to be due to a very extensive enzyme system that provides a wide spectrum for attack by UV rays. Poliovirus, which is more resistant to UV, lacks this enzyme system (Dizer et al. 1992).

The potential for regrowth and repair after UV exposure has been reported for many microorganisms, particularly bacteria (Bolton 1999; Hancock and Davis 1999; Friedberg 2003). Observed anomalous survival rates when living cells or bacteriophage (bacteria infecting viruses) were exposed to UV radiation led to the discovery of the phenomenon known as photoreactivation. In photoreactivation, a light dependent enzyme reaction is responsible for repairing the DNA damage caused by exposure to UV light (Friedberg 2003). Regrowth of coliform bacteria and *E. coli* in UV disinfected wastewater treatment plant effluents due to photoreactivation, has been reported (Hancock and Davis 1999).

2.2.3 UV Light Sources

Development of UV lamps and establishing the germicidal effect of UV irradiation made them valuable in water treatment industry. UV lamps are categorized based on the method of light production which includes: temperature radiators gas discharge, electroluminescence, and light emitting diodes (LED). Almost all UV lamps used for water treatment are gas discharge lamps. In this type of lamp, a UV photon is emitted when an excited electron of a filler gas drops from a higher excited level to a lower excited or ground level. Neon, argon, krypton, xenon, and mercury gases, all produce UV light by this mechanisms, however mercury has the lowest energy excited state and thus requires the least excitation energy (Bolton 1999; Hijnen et al. 2006). Despite the potential danger of mercury release to the environment, the wide majority of existing UV installations use mercury lamps. Mercury lamps are preferred due to the relatively long lifetime, and the uncertainties surrounding the cost of other types of lamps (Sharpless and Linden 2005).

Mercury gas discharge lamps generate either monochromatic radiation at 253.7 nm, or polychromatic radiation between 230 to 300 nm, depending on the vapour pressure in the lamp. The two types of monochromatic UV lamps used in the water and wastewater treatment industry are: Low Pressure (LP) mercury lamps and Low Pressure High Output (LPHO) amalgam lamps. Eighty five percent of light emitted by an LP lamp, or an LPHO lamp is at the wavelength of 253.7 nm, which is close to the peak absorbance of RNA and DNA (Jagger 1967). The mercury vapour pressure in LP lamps is between 0.1 to 1.0 Pa. The efficiency of LP lamps is greatest when the surface temperature is about 40°C (Bolton 1999). LP lamps have relatively long lifetimes of 8,000 to 12,000 hours, and operate at low power of 40 to 80 W per lamp. Low Pressure High Output (LPHO) lamps are similar to LP lamps but because of using an amalgam as a filler gas, there is one advantage. The amalgam in LPHO lamps is an alloy of mercury with metals such as gold, silver, copper and tin. The current and flow of photons is 3 times greater in LPHO lamps than in LP lamps. Therefore the output power per unit length of LPHO lamps is about (100 to 200 W per lamp) and 3 times higher than that of LP lamps. Thus, about one-third number of LHPO lamps are required for the same UV dose delivery in a treatment reactor.

Broadband UV lamps include Medium-Pressure (MP) mercury lamps and High-Pressure (HP) mercury lamps. Mercury vapour pressure in MP lamps is between 50 to 300 Pa. Medium-pressure (MP) lamps emit polychromatic radiation within the range of 200 to 300 nm, which is make useful for photochemical treatment of water when many chemical contaminants with different UV absorbance spectra are present (Sharpless and Linden 2005). The electrical power efficiency, defined as the radiant power output of the lamp divided by electrical power input, for MP lamps (10 to15%) is lower than that of LP lamps efficiency (35 to 40%). For UV reactor designs using MP lamps, high power (1 to 25 KW per lamp) is needed, but far fewer lamps are required for the same UV dose delivery than UV reactors using LP or LPHO lamps. Maintenance costs tend to be lower

for MP lamps as there are about 10 times fewer lamps to change even though the lifetime of a MP lamp (3000 to 5000 hours) is much shorter than LP or LPHO lamps (8000 to 10,000 h). High Pressure mercury lamps are used only in spectroscopy (Bolton 1999).

2.3 UV INACTIVATION EXPERIMENTAL METHODOLOGY

2.3.1 Collimated Beam Apparatus

Perhaps the most practical experimental arrangement for carrying out laboratory UV exposures of water samples is the collimated beam (or quasi-collimated beam) apparatus (Figure 3). This apparatus consists of a lamp housed in a shuttered box. A Petri dish or some other suitable container containing the water sample is placed some distance below the shutter (Sharpless and Linden 2005). A collimated beam apparatus is typically equipped with a collimation tube, sometimes containing internal baffles, to ensure that the path of most of the photons is perpendicular to the sample surface. Use of leveller helps to place the Perti dish horizontally (Bolton and Linden 2003; Kuo et al. 2003). For samples with low UV transmittance, magnetically stirring the samples is essential to ensure a uniform UV exposure for all microorganisms in the sample. However, creation of a vortex at the liquid surface must be avoided (Bolton and Linden 2003).

In a typical exposure experiment, the lamp is allowed to warm up to a stable temperature and output. For medium pressure lamps, the enclosure must be vented properly throughout the irradiation to keep the temperature constant (Bolton and Linden 2003). Low pressure mercury lamps, which have been used in the majority of studies involving UV inactivation, generate monochromatic light at 253.7 nm but also emit a small amount of radiation at 185 nm. It is of concern since emission of wavelengths below 200nm can cause the formation of ozone in air during irradiation (Bolton and Linden 2003). It seems in literature, the irradiance from wavelengths below 200 nm was assumed to be negligible as it is absorbed by the air gap in the collimated beam arrangement (Sharpless and Linden 2005).

Despite the consistent use of UV intensity and UV irradiance in the literature, the appropriate term for describing the photon flux at a point in UV disinfection reactor is

fluence rate. The fluence rate is defined as the total radiant power passing from all directions through an infinitesimally small sphere of cross-sectional area dA, divided by dA. Irradiance is the total radiant power incident from only upward directions on an infinitesimal element of surface of area dA divided by dA. Irradiance is the appropriate term when a surface is being irradiated by a parallel beam of UV light that is perpendicular to the liquid surface. Therefore for a well-designed collimated beam apparatus, where the beam is perfectly collimated and the flux of electrons is on one direction, fluence rate and irradiance are equivalent (Figure 3). A radiometer is usually used with a collimated beam arrangement to accurately measure the irradiance at the surface of the sample in the Perti dish (Bolton and Linden 2003).



Figure 3: Simple sketch of the collimated beam apparatus, (1) fan, (2) reflector, (3) LP/MP UV lamp, (4) UV transparent window and shutter, (5) collimated tube, (6) Petri dish containing water sample, (7) stir bar, and (8) magnetic platform, after Bolton and Linden (2003)

2.3.2 UV Dose Determination

Radiant exposure or fluence is obtained by multiplying irradiance or fluence rate which is constant in collimated beam arrangement, with exposure time in seconds. The term UV dose is commonly used for fluence in the water and wastewater treatment industry. The term dose is also very common in the UV disinfection literature, and will be used in this thesis. Whereas dose refers to the total absorbed energy in other contexts, fluence is the more technically correct term indicating incident UV energy rather than absorbed UV energy (Bolton and Linden 2003).

In a collimated beam experiment, UV dose (or fluence) delivered to a sample can be calculated as the product of depth averaged irradiance (fluence rate) and exposure time (in seconds) (Equation 2).

UV Dose (Fluence) = Irradiance (Fluence Rate) × time

$$H = I_{ave} \times t$$
 Equation (2)

where:

 $H = UV \text{ dose } (mWs \cdot cm^{-2} \text{ or } mJ \cdot cm^{-2})$ $I_{avg} = \text{Depth averaged } UV \text{ irradiance } (mW \cdot cm^{-2})$ t = Exposure time

The radiometer measures only the incident irradiance at the surface of the liquid and at the centre point of the beam, I_0 . Determination of several correction factors is critical in the computation of depth-averaged irradiance, I_{avg} in a liquid sample. Accurate and reproducible results are expected by properly following the procedure.

The sensitivity of the radiometer sensor of the collimated beam is dependent on wavelength. For polychromatic radiation, therefore, a sensor factor must be applied. No correction is usually necessary for monochromatic (254 nm) light produced from a LP or LPHO lamp because the sensor response is usually calibrated for this wavelength (Bolton

and Linden 2003). Reflection occurs when light passes from one medium to another where the refractive indices of the two media are different. The reflected fraction from an air-water interface derived from the Frensel Law is equal to 2.5%. Therefore even for a quasi-collimated beam, only 97.5% of incident beam enters the water (Bolton and Linden 2003). The irradiance reading at the centre of Petri dish, I_0 , must also be corrected for radial variation of irradiance over the surface area of the liquid that occurs because the collimation of the beam is not perfect. This radial variation is taken into account by multiplying I_0 by the so-called "Petri" factor which is defined as the ratio of the average incident irradiance over the surface area of the liquid divided by the irradiance at the centre of Petri dish. The average incident irradiance is estimated by making incremental radiometer measurements from the centre across horizontal and vertical directions. This is possible because the radiometer sensor is usually much smaller than the light beam itself. A Petri factor greater than 90% is the indication of a well designed collimated apparatus (Bolton and Linden 2003).

The Beer-Lambert Law describes the attenuation of light by absorbing compounds as it travels through the solution. Transmittance of light of wavelength of λ , T_{λ} , can be defined as follows:

$$T_{\lambda} = \frac{E_{\lambda}^{t}}{E_{\lambda}^{0}}$$
 Equation (3)

where E'_{λ} and E^{0}_{λ} are the transmitted and incident light irradiance at a fixed wavelength of λ (Bolton 1999). The transmittance and absorbance of the liquid are related by the following expression (Equation 4):

$$T_{\lambda} = 10^{-a_{\lambda}\ell}$$
 Equation (4)

where a_{λ} is the absorption coefficient at wavelength of λ and *l* represents the pathlength (Bolton 1999). Since the radiometer measures only the incident irradiance at the surface of water sample, a water factor correction must be applied. The integrated form of the Beer-Lambert law over the sample depth gives the water factor which accounts for the attenuation of UV light in water samples (Equation 5) (Bolton and Linden 2003).

Water Factor =
$$\frac{E_{\lambda}^{t}}{E_{\lambda}^{0}} = \frac{\int_{0}^{1} 10^{-ax} dx}{\int_{0}^{l} dx} = \frac{1 - 10^{-al}}{al \times \ln(10)}$$
 Equation (5)

where *a* is absorption coefficient $(\text{cm}^{-1})(=\text{absorption for a pathlength of 1 cm}) and$ *l*represents the pathlength (cm) which is the liquid depth in the case of a collimated beam experiment (Figure 3). If the beam is not perfectly collimated it will diverge as it travels through the liquid sample. The irradiance will decrease as the inverse square of the distance*L* $from the UV lamp to the surface of the water sample. The divergence factor can be obtained by integration of <math>L^2/(L+x)^2$ from the liquid surface to bottom of the Petri dish with pathlength of *l*. The result is (Bolton and Linden 2003):

Divergence Factor =
$$\frac{L}{L+l}$$
 Equation (6)

Finally, the depth averaged germicidal UV irradiance $(I_{avg}, mW/cm^2)$ for low pressure UV lamps can be computed by:

 $I_{avg} = I_0 \times \text{Petri Factor} \times \text{Reflection Factor} \times \text{Water Factor} \times \text{Divergence Factor}$ Equation (7)

Morowtiz (1950) first derived Equation (7) and used it to quantify the effective UV dose for inactivating microorganisms in a sample (Bolton and Linden 2003; Sharpless and Linden 2005).

2.3.3 UV Dose-Inactivation Response of Microorganisms

In UV inactivation studies, determination of the UV dose-inactivation response for a microorganism of interest in the water matrix is a primary task (Bolton and Linden, 2003). Dose-response data generated in collimated beam tests are generally used to determine the required UV dose delivered in full-scale UV systems to achieve a certain desired level of microorganisms inactivation. In addition, the collimated beam tests can

be employed to calibrate the dose calculation models used in full-scale UV reactors and, eventually be used to reasonably validate dose outputs of these models (Kuo J. et al. 2003). In principle, the UV dose delivered to a sample can be determined accurately using a collimated beam apparatus in the laboratory. The apparatus is very useful as it allows the researcher to control the dose fairly precisely and accurately (Emerick et al. 2000). First, all the correction factors for the sample are measured. Then, the exposure time required to yield a target dose is calculated. The delivered UV dose can be adjusted by changing exposure time. Also the survival ratio can be determined by enumerating target microorganisms before and after UV exposure of a specific dose. The survival ratio is defined as (N/N_0) , where N_0 = concentration of viable microorganisms before exposure and N = concentration of viable microorganisms after exposure. Inactivation is usually defined as the negative logarithm of the survival ratio, or $-\log (N/N_0)$. Therefore the relationship between applied UV dose and microorganism inactivation can be estimated in the laboratory. UV dose-inactivation relationships are usually presented as plots of the microorganism survival ratio, on a log scale, versus germicidal average UV dose.

2.4 **KINETICS OF UV INACTIVATION**

Inactivation is defined as the reduction of the initial concentration of microorganisms due to the exposure to a concentration of disinfectant during a specific contact time. When the concentration of disinfection is constant, the inactivation kinetics for chemical disinfectants is often described using the first-order disinfection model of Chick (1908) and Watson (1908). A similar model can be applied to UV disinfection. UV dose-inactivation data generated from several laboratory studies in collimated beam tests have determined that the UV inactivation of microorganisms tends to follow first-order kinetics (Hijnen et al. 2006). According to the first-order model, the linear relationship between log inactivation and the UV dose or fluence is described by:

$$N = N_0 e^{-kH}$$
 Equation (8)

where:

 $H = \text{dose} (\text{mJ/cm}^2)$

k = first-order inactivation rate constant (s⁻¹)

The UV resistance of a microorganism is described by the inactivation rate constant, k. At least for certain dose ranges, most of the inactivation data can be adequately described with the first-order disinfection model. Several studies indicated that the inactivation of tested microorganisms by UV irradiation followed dose dependent first-order kinetics as described by Equation (8) (Qualls and Johnson 1983; Kamiko and Ohgaki 1989; Abbaszadegan et al. 1996; Sommer et al. 1997; Tree et al. 1997; Jolis et al. 2001; Bourrouet, 2001; Lazarova and Savoye 2004).

Other types of UV inactivation relationships that are observed in practice are shown in Figure 4. For some microorganims, such as *Bacillus subtilis* spores, limited inactivation is observed until a threshold dose is exceeded (Curve B in Figure 4). This results in a shoulder in the inactivation curve at low UV dose. Poor mixing and multiple hit processes have been proposed to explain the presence of shoulder effects. The multiple hit theory, suggests that a single target must be hit a number of times before it is inactivated (Hiatt 1964).

Another deviation from first-order kinetics is a significant decrease in the rate of inactivation at high inactivation levels (Curve A in Figure 4). This phenomenon, known as tailing, has been observed in several collimated beam tests using drinking water (*Polioviruses, Rotaviruses, E. coli, C. parvum* and *Giardia muris*) and also for naturally occurring bacteriophages, and bacteria in continuous flow systems of wastewater treatment (Hijnen et al. 2006). In most cases tailing is observed only after at least 99% of the microorganisms are inactivated, and is more common in the more UV sensitive microorganisms. For the most resistant organisms (*Adenoviruses, MS2 phages*, bacterial spores and *Acanthamoeba spp.*), tailing is not observed (Hijnen et al. 2006). There is no consensus on the cause of tailing (Cerf 1977) or how it should be taken into account in predicted UV reactor performance (Hijnen et al. 2006). Several causes have been hypothesised, such as experimental bias, hydraulics, aggregation of microorganisms, or presence of a resistant subpopulation. The theory of variable permanent resistance

suggests that the degree of resistance to disinfection varies between individuals present in a suspension of apparently the same microorganisms and this difference in the degree of resistance is permanent (Cerf 1977). In the case of an inactivation curve with serious tailing, extrapolation of low dose inactivation to high dose inactivation may result in serious over-prediction of inactivation in a full-scale UV reactor. The first-order model can be used only for the dose range that yields a linear relationship with the inactivation in the experiment. A conservative approach for reactor design purposes, is to assume that the maximum inactivation is the inactivation observed at the highest dose in the linear region of the experimental inactivation curve (Hijnen et al. 2006). Inactivation curves may also be sigmoidal, characterized by both shoulder and tailing phenomena (Curve D in Figure 4) (Cerf 1977).



Figure 4: Sketch of typical inactivation curves, (A) tailing, (B) shoulder, (C) linear, and (D) tailing and shoulder

2.5 INDICATOR ORGANISMS

2.5.1 Surrogate Microorganisms

An evaluation of the effectiveness of disinfection processes and the concern for public health requires developed and standardized methods for microbial indicators to provide a reliable measurement of the concentration of pathogenic microorgainsms present (Grabow 2001). A microbial indicator has to meet the following basic requirements to be used as models/surrogates in wastewater disinfection processes.

- They should be present in water environments whenever target microorganisms are present
- They should be present in the same or higher numbers than target microorganisms
- They should be at least as resistant as target microorganisms to water disinfection processes
- They should be particular for faecal or sewage pollution
- They should preferably not be able to replicate in water environments
- They should preferably be non-pathogenic and detectable by simple, rapid and inexpensive methods (Grabow 2001).

The above criteria are for the use of indicator microorganisms for determining the safety of water and treated wastewater. The criteria for the use of indicator microorganisms for validating the performance of UV reactors are not the same. The water and wastewater industry in North America (the US in particular) has adopted MS2 coliphage as the surrogate microorganism of choice for biodosimetry studies of UV reactors (USEPA 2003b). But MS2 is not present in water environments and has to be cultured in the laboratory and be seeded to the water sample or water flow. In Europe, *Bacillus subtilis* is preferred for biodosimetry studies.

The factors supporting the choice of these surrogates include the near-linear response curve to increasing UV doses (at least for MS2 coliphage), highly reproducible inactivation data, ease of propagation of large numbers of organisms, and simple procedures for organism enumeration (Bukhari et al. 2004). In addition, the fact that the
value of the inactivation rate constant (k) of MS2 coliphate is on the same order of magnitude as the rate constant observed for most pathogenic viruses, strongly supports the use of this microorganism as surrogate for virus inactivation by UV irradiation. Only adenoviruses are more resistant to UV. Vegetative bacteria, such as *E. coli*, and encysted protozoa, such as *Cryptosporidium parvum* cysts and *Giardia lamblia* cysts, are less resistant to UV than are viruses with dose requirement of less than 20 mJ/cm² for 3 log reduction (Hijnen et al. 2006). In comparison, a dose of 60 mJ/cm² is required for 3 log inactivation of MS2 coliphage.

Several studies have reported that the UV resistance of naturally-occurring, or indigenous strains of bacteria is greater than the UV resistance of laboratory cultured strains. For example, the results of collimated beam tests show that bacteria (faecal coliforms, *salmonella typhi* and *enterococci*) naturally present in wastewater were more resistant to UV, than the same species of bacteria that were cultured in the laboratory, and seeded artificially into the wastewater (Hijnen et al. 2006).

2.5.2 Fecal Coliforms

Total coliforms, fecal coliforms, *Escherichia coli*, *Fecal streptococci*, and *Enterococci spp.*, are the most commonly tested fecal bacteria indicators of wastewater contamination because they are present in human and animal feces. All, except for *E. coli*, are composed of a number of species of bacteria that share common characteristics such as shape and habitat. Most are not pathogenic, but they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems (USEPA, 1997). Coliform bacteria are members of the Enterobacteriacae family. They are facultative anaerobic, gram-negative non-sporeforming rods that can ferment lactose and produce gas within 48 hours at 35°C (Standard Methods 1998).

Fecal coliform consists of *E. coli*, *Citrobacter*, *Enterobacter* and *Klebsiella* species. These gram negative bacilli (rod shaped bacteria) are found in the digestive tracts of all warm-blooded animals. Since they are discharged with feces, they are associated with pathogens that infect the intestinal tract of warm-blooded animals, such as *Vibrio cholera* bacteria or hepatitis A virus. Total coliform bacteria counts are also used to test for fecal water contamination. These organisms are less precise as fecal contamination indicators because many can live and reproduce in soil and water, without having a human or warm-blooded host (Markowitz 2005).

E. coli is a single species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. USEPA recommends *E. coli* as the best indicator of the health risk due to contact with recreational water, or from ingestion of drinking water. Some states have changed their water quality standards from fecal coliform to *E. coli* and are monitoring accordingly. Fecal coliforms are still being used in many states as the indicator bacteria (USEPA 1997).

2.5.3 Coliphages

Due to particular difficulties related to detection of viruses of public health interest in the aquatic environment, including the present limitations of virus estimation and identification methods, there is the need for model organisms to test the effectiveness of virus removal from water. In this respect, coliphages share several morphological and biochemical properties with enteroviruses, a group of viruses that cause enteric diseases in humans. New and well-developed methods for coliphage detection and enumeration are available, offering the possibility for their use as indicators for viral pathogens (Grabow 2001; Bourrouet et al. 2001; Mooijiman et al. 2001).

Bacteriophages (phages) are viruses that infect bacteria. Coliphages are bacteriophages that grow and multiply using many subspecies of E. *coli* as host cells. Virus reproduction was first indicated by work with phages. Coliphages are valuable models or surrogates

for enteric viruses in basic genetic research, as well as in water quality assessment because they share many fundamental properties and features with enteric viruses. In addition, the coliphages and enteric viruses in the environment, originate from the same source, the feces of humans and animals (Grabow 2001). A major advantage of phages is compared to viruses; they are detectable by simple, inexpensive and rapid techniques. Therefore, phages are particularly useful models to assess the behaviour, and survival of enteric viruses in the environment, and as surrogates to assess the resistance of human viruses to water treatment and the disinfection process.

Somatic coliphages and F-specific RNA coliphages are the most commonly used viral indicators in water quality assessment. Somatic coliphages infect *E. coli* and other related bacteria through the cell wall, and they have DNA. Found in human and animal feces, they are numerous in wastewaters and are easy to detect. F-specific RNA (also called F+) coliphages also infect *E. coli* and other related bacteria, but via sex pili. An example of the effecting organism, often used for testing of UV disinfection reactor performance is the F-specific RNA virus MS2. It is also found in human and animal faeces. Laboratory experiments with individual coliphages confirmed that many of them survive longer in natural water environments than enteric viruses, and are at the very least, as resistant as enteric viruses to commonly used disinfectants such as chlorine (Metcalf & Eddy 2002; Standard Methods 1998; Farahbakhsh et al. 2004, Bourrouet et al. 2001; Grabow 2001; Lazarova and Savoye 2004).

2.5.4 UV inactivation of Fecal Coliforms and Coliphages

Bourrouet et al. (2001) compared the inactivation efficiency of indigenous fecal bacteria and a single type of bacteriophage (somatic coliphage) present in a full-scale UV disinfection process, under exposure to different UV doses. They suggested that bacterial indicators may be suitable for virus inactivation control when the UV doses delivered by the reactor was less than equal to approximately 40 mJ/cm². Inactivation of both fecal coliform (FC) and somatic coliphage (SC) was very similar (1.15 to 1.25 log-units) when the UV system was operating at a UV dose of 40 mJ/cm². Inactivation of FC and SC, however, tended to diverge as the UV dose delivery reached 80 mJ/cm². FC, with a 2.8 log inactivation was more sensitive to UV than SC, with a 1.6 log inactivation. The main conclusion was that the inactivation of SC and FC had similar values at a low UV dose (40 mJ/cm²), but was considerably different with increase of UV doses over 80 mJ/cm² where bacterial indicators were more sensitive than bacteriophages. Bacterial indicators alone may not be suitable for validating virus inactivation of wastewater. This can be explained by the intrinsic differences in their resistance to disinfectants (Tree et al. 1997). The production of high quality virus-free effluents, is required for irrigation purposes to minimize risk for human health (Lazarova and Savoye 2004).

Thompson et al. (2003) reported that a LPHO UV disinfection pilot unit treating wastewater achieved effective reduction of the indigenous total and fecal coliform bacteria (to 2 MPN/100 mL) at a dose of 60 mJ/cm². The UV pilot unit also achieved a 4 log inactivation of seeded MS2 coliphage, at a dose of approximately 69 mJ/cm².

Numerous laboratory experiments have recently reported laboratory strains of coliphage are less resistant to UV than naturally occurring coliphages (Dizer et al. 1992; Tree et al. 1997). The reduction of the naturally present bacteria and viruses should therefore be used to evaluate the efficiency of UV reactors. Future work is required to establish whether laboratory cultured organisms are ideal representatives of wild type populations in wastewater effluent. Oppenheimer et al. (1997) used indigenous fecal coliform and seeded MS2 coliphage as indicators to evaluate a full-scale UV system. A UV dose of 75 mJ/cm² reduced the concentration of fecal coliform and MS2 coliphage by 4 log. Dizer et al. (1993) used naturally occurring coliphages to evaluate a pilot scale unit, and the concentration of indigenous coliphages in the influent decreased by 1 to 2 log at a UV dose of 47 mJ/cm².

2.6 UV REACTORS

2.6.1 UV Reactor Components and Configurations

UV lamps, quartz sleeves, supporting structure for the UV lamps and the quartz sleeves, ballasts for supplying regulated power to the UV lamps and power supply are the principal components of a UV disinfection system (Metcalf & Eddy 2002). Ultraviolet disinfection reactors can be classified into open channel, and closed channel configurations. Closed channel reactors are usually used in drinking water while open channel systems are typically used in municipal wastewater treatment plants. There are two basic designs for the position of the lamps in closed reactor systems used in drinking water treatment; horizontal and parallel to the water flow, or horizontal and perpendicular to water flow systems. The lamps are enclosed in a reaction chamber that is completely enclosed, except for the inlet and outlet (Metcalf & Eddy 2002; Hydromantis & Stantec 2003).

In open channel reactors used in wastewater applications, the lamps can be arranged either horizontally and parallel to the flow, or vertically and perpendicular to the flow. Open channel UV systems with horizontal lamps are the most common UV disinfection configurations in the municipal wastewater treatment plants. The lamps used are low pressure and can be either low or high intensity. Medium pressure systems are also used, for example the TROJAN 4000 system at Gold Bar WWTP uses MP lamps mounted vertically and perpendicular to the flow.

Horizontal systems are comprised of modules of lamps (or racks of UV lamps) that are mounted side by side in banks in an open channel. The modules span the width of the channel to form a bank. Each module consists of a metal support frame that holds a specified number of evenly spaced lamps encased in quartz sleeves. A module usually contains 8 or 16 lamps. The optimum lamp spacing is a project-specific decision depending on minimum UV transmittance of the wastewater, UV output of lamps at the end of their lifetime, quartz sleeve diameter and UV dose requirement. The standard spacing of 75 mm (3 in) between the centres of UV lamps is the most frequently used lamp configuration employing low pressure lamps (Metcalf & Eddy 2002; Hydromantis & Stantec 2003).

UV lamps are enclosed within cylindrical quartz sleeves in order to maintain optimal operating temperature at the lamp wall and to protect the lamp from breaking. Lamp sleeves may fracture, foul and their transmittance decreases as they age. Lamp fouling occurs due to the deposit of inorganic, organic and biological solids on the quartz sleeves surrounding the lamps. Both off-line chemical cleaning, and on-line mechanical cleaning are available for cleaning lamp sleeves. Mechanical wipers (Figure 5) and physical-chemical wipes are two types of wipers used in on-line mechanical cleaning systems. The proper function of the wiping systems is very critical because the performance of the UV disinfection system relies on the wiping tools (Cabaj et al. 2005).

UV sensors (Figure 6) are photosensitive detectors used to measure UV fluence rate at a point within the UV reactor (USEPA 2003; Hydromantis & Stantec 2003) and to ensure that lamps are in operation. UV sensors are used to verify the performance of the UV system and also, in some systems, to control UV dose by control systems.

UV disinfection systems normally have multiple channels and multiple banks per channel, which provide redundancy. Thus, cleaning and maintenance tasks can be conducted without interrupting the operation of the UV system. This also allows controlling UV dose delivery by shutting lamps or entire banks on or off (Hydromantis & Stantec 2003). Sizing of UV systems to meet the required microbial reduction targets depends on three parameters: wastewater flow rate, UV transmittance of the water at a wavelength of 254 nm, and disinfection requirements (Leuker 1999; Hydromantis & Stantec 2003). In practice, fluctuations in wastewater transmittance are the most common reason for the unsatisfactory performance of UV systems (Leuker 1999).



Figure 5: UV Lamp with Wiper from Alberta Capital Region WWTP UV System



Figure 6: UV Sensor placed in Alberta Capital Region WWTP UV Reactor

2.6.2 UV Dose Distributions

The fluence rate varies specially within a UV reactor because of absorbance of UV within the wastewater. The fluence rate is highest at points close to the lamp, and decreases with distance from the lamps. The lower the UV transmittance of the water being disinfected, the higher is the expected inhomogeneity of the fluence rate field. An ideal UV reactor has plug flow with perfect radial mixing where very little mixing occurs in the direction of flow, but complete mixing occurs perpendicular to the flow, and across the fluence rate gradient. In this way, fluence rate gradient has no effect on inactivation because all microorganisms receive the same average UV fluence rate and for identical exposure times. In a real UV reactor, the water is not perfectly mixed across fluence rate gradients (i.e. across the depth and width of the reactor) and flow dispersion occurs lengthwise. As each microorganism enters the UV reactor, it takes a unique path through the reactor and the inhomogeneous fluence rate field. Microorganisms traveling along pathways far from the lamps will receive a lower UV dose than microorganisms travelling along pathways closer to the lamps. As a result, a distribution of dose values will exist among the microorganisms leaving the reactor (Cabaj et al. 1996).

Fluence rate gradient combined with imperfect radial mixing induces a dose distribution, as not all microorganisms traveling through the reactor are exposed to the same fluence rate. Also the flow pattern inside the reactor highly affects the residence time of the microorganisms in certain regions of the irradiation field. Axial dispersion within a reactor also contributes to a dose distribution, as not all microorganisms have the same retention time within the fluence rate field. Some microorganisms may spend a longer time in reactor, with a longer UV exposure, than others. As a result, each of the individual microorganisms receives a different UV dose. This makes the efficiency of microbial inactivation occurring in the reactor susceptible to the affects of shortcircuiting, axial dispersion, and imperfect radial mixing. Reactor geometry, hydraulics, water quality, lamp age and orientation all can influence the UV dose distribution in a reactor. It is necessary to take into account the distribution of dose values among the microorganisms passing through the reactor for the accurate measurement of UV dose for the disinfection of water. Cabaj et al. (1996) hypothesized a normal distribution of dose among the microorganisms to perform UV dose calculations. The better performance is achieved when the dose distribution is narrower, rather than wider. The theoretical limit of performance results in an ideal plug flow that has no distribution. Because the target levels of inactivation in disinfection are usually fairly high (i.e. > 99 or 99.9%), small deviations from ideal flow (especially short-circuiting) can seriously limit performance.

2.6.3 Measurement of UV Dose Delivered

One of the remaining technical challenges with UV technology is to accurately determine the delivered UV dose. Determining the UV dose with accuracy is important for two reasons. First, UV reactors must not be under-designed, causing inadequate inactivation of the target organism. Secondly, reactors must not be over-designed leading to unnecessarily high operation costs, due to excess energy supply (Templeton et al. 2006). Current techniques of UV reactor validation provide only a measure of the average UV dose-not the entire dose distribution (Bohrerova et al. 2005). The average UV dose must be inferred, as it cannot be measured directly because UV disinfection leaves no measurable disinfectant residual in the water (Bohrerova et al. 2005; Hijnen et al. 2006).

There are two basic approaches developed by researchers in order to determine dose delivery in a UV reactor: numerical and experimental (Qualls and Johnson 1983; Ducoste et al. 2005). The first uses computational models and the second uses biodosimetry with seeded (or naturally occurring) microorganisms. The first method (computational) can be sub-divided into CFD-type (Computational Fluid Dynamics) models that attempt to describe the detailed fluid flow and fluence rate fields, and the empirical or semi-empirical models based on simplifying assumptions of plug flow and an average fluence rate.

2.6.3.1 Empirical Calculation Methods for Determining Dose in UV Reactors

UV reactors used in wastewater disinfection are typically open channel with relatively high length-to-width ratios, compared to the closed channel reactors common for disinfection of drinking water. In addition, if the lamps are positioned parallel to the flow in open channel reactors, plug flow is approached. This simplifies UV dose modeling. The plug flow assumption indicates no dispersion along the main axis of the reactor from inlet to outlet, except complete mixing across fluence rate gradients perpendicular to the main axis of the reactor. The other critical component of the UV dose calculation, is the UV fluence rate modeling. Commercially available UV fluence rate models use the lamp power, the UV transmittance (UVT) of the water, the lamp sleeve radius, the lamp arc length, lamp spacing and channel dimensions as inputs for calculating the UV fluence rate distributions. These models calculate the reduction in UV fluence rate with distance away from the lamp and the net fluence rates resulting from multiple lamps (Templeton et al. 2006). Effects such as water transmittance, and light divergence will result in reduction in UV fluence rate as with distance from the lamp.

Traditionally, the point source summation (PSS) model described by Qualls and Johnson (1983) was the mathematical model used to describe the UV fluence rate. This model assumes that a UV lamp is comprised of a series of small point or volume sources which independently emit light in all directions. The receptor is considered to be a point or small sphere within the reactor. This model uses the Beer-Lambert absorption law to account for the attenuation of light as it is transmitted through a medium. The fluence rate determined at any point in the irradiated volume is calculated as the sum of the fluence rates to each of the point sources along the lamp. The lamp should be divided into 1001-point sources in order to approximate the emission of a linear lamp reasonably (Bolton 2000).

Simple PSS-based fluence rate models use the average cross-sectional UV fluence rate in combination with the assumption of ideal plug flow, to calculate an average UV dose received by all microorganisms travelling through the reactor. The average UV exposure time of a microorganism in the reactor is calculated as the average residence time, τ , in the irradiation zone ($\tau = V/Q$, where V = volume of the irradiation zone and Q = volumetric flow rate). However, this approach has a fundamental error. It assumes that the high UV fluence rate very near the lamps is distributed evenly throughout the irradiation volume within the reactor, including regions far from the lamps, i.e. near the reactor walls. Though it is reasonable to assume that microrganisms are evenly distributed through the cross-section of flow at the inlet of the reactor, the assumption that they will mix completely across fluence rate gradients is not necessarily accurate. Microorganisms that receive a greater UV dose in areas of high UV fluence rate are not inactivated more than once. Also, due to reactor geometry, the majority of the surviving

microorganisms are the ones that travel through the low fluence rate areas. Therefore using the average UV fluence rate to calculate UV dose, would over-estimate the actual dose by neglecting the survival of microorganisms that flow through the low fluence areas.

Templeton et al. (2006) developed a simplified computational model to estimate the UV dose delivered to wastewater, and compared the model outputs, with pilot-scale biodosimetry data. The model assumed plug flow and used the output from a twodimensional, point source summation UV fluence rate model to account for the crosssectional distribution of UV fluence rate within the reactor. These researchers used MS2 coliphage as a biodosimeter which was added to an inlet tank, and mixed by recirculating the water through the tank. UV dose was measured under different simulated operating conditions using MS2 coliphage dose response curve. UVT values of 55% and 65%, were created to represent typical wastewater UVT values by iteratively adding coffee powder and mixing it into the water. Flow through the UV reactor was varied between 760 and 2840 L/min by gradually opening a valve upstream of the UV reactor. Results indicated that the simplified model over-estimated the UV dose delivery within the reactor at the highest tested flow rates (greater than 1500 L/min). Since the UV fluence rate distribution in the UV reactor is independent of flow rate, deviation from plug flow assumption was the most likely reason for this outcome. Further support for this flow deviation hypothesis, was provided as a model that better predicted the UV dose delivery at the 65% UVT than at 55% UVT consistently. A lower UVT contributed to even less UV fluence rate reaching the reactor walls, resulting in greater MS2 survival in dose-limiting areas. Consequently, wider deviation between the model and the biodosimetry-derived UV dose values were observed. Templeton et al. (2006) concluded that more sophisticated models of the flow distribution must be considered for precise quantitative estimation of UV doses, even in cases where open channel UV reactors are expected to exhibit plug flow.

The experimental method validating UV reactors using a bioassay with challenge microorgansims, is known as biodosimetry (Qualls and Johnson 1983; USEPA 2003). Biodosimetry is used to test the performance or to validate dose delivery of large UV reactors, resulting in the determination of a single reduction equivalent dose (RED) for a particular reactor under defined conditions (Bohrerova et al. 2005). The measuring procedure starts with the bench-scale development of the inactivation curve (calibration curve) for the challenge microorganisms which are to be used in the biossay. This is done by exposing the microorganisms to a series of well-defined, and accurately measured UV doses using a collimated beam apparatus. A solution of challenge microorganisms is mixed into the water in a tank, or is seeded continuously into the water flowing into the operating UV reactor to be tested. In the latter case, complete mixing of the microorganism with the feed stream is usually achieved by means of a static mixer or similar mixing device. The concentration of microorganisms in the flowing water up stream, N_0 and down stream, N, of UV reactor is then measured (Figure 7) and the inactivation, $-\log N/N_0$, is determined. The resulting inactivation is then compared to the calibration curve to determine the reduction equivalent dose (RED) for the UV reactor running under that specific set of operating conditions (Cabaj et al. 1996). The biossay is typically repeated for different sets of operating conditions achieved by varying water flow rate and transmittance, and UV lamp power (Leuker 1999).



Figure 7: Typical Biodosimetry Set up

Biodosimetry depends on the use of a UV dose-inactivation calibration curve of the challenge organism against which inactivation rate from full-scale testing of UV system

can be compared. An equation describing the dose-inactivation relationship is usually determined by regression analysis of the calibration curve, and this is used to calculate the RED for the UV reactor bioassay (Sommer et al. 1997). It is important to note that for real UV reactors, the value of the RED determined experimentally depends on the challenge microorganism. Two microorganisms with different resistance to UV will yield different values of RED for the same reactor-operating test conducted under identical conditions. This difference increases, as does the broadness or spread of the dose distribution produced by the reactor (Cabaj et al. 1996; USEPA 2003; Ducoste et al. 2005). In the case of ideal plug flow UV reactor, there is no dose distribution and the REDs of all microorganisms will be the same regardless of their UV resistance. The resistance of the challenge microorganisms (i.e. MS2 coliphage or B. subtilis spores) will typically be different than the resistance of the target pathogen (i.e. C. parvum). For a real reactor that has a dose distribution, the RED of the challenge microorganism and the target pathogen will most likely be different. Therefore, using the RED of the challenge microorganisms will result in overestimating, or underestimating the inactivation of the target pathogen. This over or underestimation is called the RED bias. The RED of the challenge microorganism will only be equal to the RED delivered to the target microorganism, if both have the same microbial dose response curve, or the flow regime in the reactor is close to perfect plug flow with complete transverse mixing across fluence rate gradients. But real UV reactors do not exhibit ideal plug flow characteristics. As a result, the USEPA (2003) suggests computing a RED bias factor to account for the difference between the expected dose delivered to the target pathogen, and the actual dose measured using a challenge microorganism during biodosimetry. The RED bias is designed to convert the RED of the challenge microorganism, to the RED of the target microorganism (Ducoste et al. 2005). The bias can be computed by comparing the first order rate constants (k) of the two microorgansims from collimated beam experiments.

To assess reactor performance in drinking water treatment, it is generally necessary to seed challenge microorganisms into the inlet of the reactor during a biodosimetry test, because the concentration of naturally occurring microorganisms are usually far too low. In wastewater treatment, the situation is different. This means cultivating large quantities

of the microorganisms, and postulating a method of how to add them to the water to become well-mixed upstream of the UV reactor. This is one of the limitations of biodosimetry on full-scale UV reactors. For drinking water, there are two or three facilities in North America that have been established to do this kind of testing but it is expensive. In the current study, naturally occurring indicators (fecal coliform and coliphage) in wastewater were used as biodosimetry microorganisms for a full-scale wastewater UV reactor. Using naturally occurring (or indigenous) microorganisms (fecal coliform or coliphage) is a big advantage, because it is not necessary to culture and add microorganisms to the reactor. The objective of the current study was to test the potential for using indigenous microorganisms for doing biodosimetry on a full-scale, wastewater UV reactor.

Biodosimetry is a relatively expensive and time consuming process and the range of operating conditions that may be examined on-site is often limited. In addition, many microorganisms exhibit a non-linear response to the level of UV dose due to inactivation saturation and minimal sensitivity thresholds (Figure 4). These non-linear effects can make the mean UV dose a poor estimate for the actual efficiency of the reactor (Bohrerova et al. 2005). When inactivation in a pilot-scale UV unit determined using biodisometery was compared with inactivation in a controlled bench-scale reactor, differences were observed. Inactivation in the full-scale reactor was always higher than that predicted based on the bench-scale data (Bukhari et al. 2004). Also calculated doses based on information from the supplier of the UV equipment for continuous-flow systems frequently do not match those obtained by biodosimetry (Hijnen et al. 2006). The various biodosimetery studies performed to date have pointed out a discrepancy between UV doses predicted using simple models, and the actual disinfection performance of UV systems in practical applications. For this reason, there has been recent interest in more complex mathematical models that incorporate more realistic descriptions of the flow dynamics into the UV dose calculations (Leuker 1999).

While biodosimetry is still essential to determine the effective dose in continuous-flow UV systems in water treatment practice, the use of computational fluid dynamics (CFD) improves the description of reactor hydraulics and dose distribution. CFD as a numerical modeling technique, is an efficient way to calculate dose accurately for commercially available reactors. Several studies have been performed to assess the performance of CFD UV models (Ducoste et al. 2005). CFD is based on solving the governing equations of fluid flow. With CFD, a numerical description of the process flow geometry is defined by representing each location in space with a set of grid points. Fluid characteristics such as velocities and turbulent quantities are then determined at each grid point by numerical solution of the fundamental differential equations of fluid flow (Bohrerova et al. 2005). In principle, sophisticated CFD modeling can be used to describe the often complex flow patterns that exist in continuous-flow UV reactors. However, the complexity and cost of CFD modeling still limits its wide application in the water and wastewater industries (Templeton et al. 2006).

2.7 WATER QUALITY CONSIDERATIONS

2.7.1 Wastewater Quality Affecting UV Performance

Constituents in the wastewater subjected to treatment have an impact on the effectiveness of a UV disinfection system. UV transmittance (UVT), suspended solids concentration, and constituents that can foul the UV lamp sleeves are the important water quality factors to be considered in UV treatment performance. UVT is the most important water quality parameter impacting UV reactor performance. As UVT decreases, the fluence rate decreases throughout the reactor for constant lamp radiant output. This results in a reduction in the average UV dose reaching the microorganisms. Transmittance is reduced by the presence of materials that can either absorb or scatter UV light. In wastewater this includes dissolved and colloidal organic and inorganic compounds, as well as suspended solids. Iron is considered to be the most important inorganic compound due to its high absorbence of UV in the germicidal range (USEPA 2003; Hydromantis & Stantec 2003).

Suspended solids present in wastewater can reduce the effectiveness of a UV system by shading microorganisms from UV light, scattering and absorbing UV light, and embedding bacteria, which may shield them from UV exposure (USEPA 2003; Hydromantis & Stantec 2003). Turbidity is an optical property that is related to particulate material present in water or wastewater (i.e. clay, slit, finely divided organic and inorganic matter, microorganisms). Turbidity is used as an indicator of water quality, and is measured in Nephelometric Turbidity Units (NTU) by detecting the amount of visible light scattered at 90° by particles present in a sample compared to the amount scattered by a reference suspension (AWWA 1999).

Microorgansim inactivation by UV has been found to decrease significantly as the concentration of particles increases (Loge et al. 1999; Shaban et al. 1997; Dizer et al. 1992). Inactivation of microorganisms by UV was found to decrease when the turbidity of a tap water was > 5 NTU even when the effect of absorbance was accounted for in determining UV dose (Shaban et al. 1997). This suggests that particles in the water that resulted in turbidity were harbouring some of the microorganisms within their structure and sheltering them from UV exposure. Interestingly turbidity protected *E. coli* but not coliphage at test contact times.

In another study, the reduction of indigenous bacteria and coliphages decreased by more than 50% due to the presence of suspended solid. Filtration of the wastewater was found to improve the performance of UV disinfection significantly (Lazarova and Savoye 2004; Dizer et al. 1992). Microorganisms excreted in feces are often aggregated, attached to solids or encapsulated in cell debris. Approximately 25% of coliforms discharged with treated water are associated with particles > $3-5 \mu m$ (George et al. 2002). This may result in protection of microorganisms embedded in fecal material from UV exposure (Tree et al. 1997).

2.7.2 Impact of Particles on UV Inactivation

Wastewater effluents contain particles that are able to scatter, as well as absorb the UV light. Particle absorbance in wastewater can significantly decrease the overall available UV radiation for disinfection (Linden and Darby 1998). Increased particle content reduces UV transmittance, thus requiring a longer period of exposure to deliver the same target UV dose. The UV dose calculations based on conventional absorbance measurements of unfiltered samples on a UV spectrophotometer, will result in underestimation of UV dose because the effect of UV light scattering is not accounted for. UV absorbance of turbid wastewater samples is affected by three components: absorbance due to soluble substances, absorbance due to particles and scattering due to particles. Suspended particles can absorb and scatter the UV light. They can also harbour bacteria, thus protecting them from UV light (Figure 8).



Figure 8: Particle Shading and Incomplete Penetration (Adapted from Loge et al. 1996)

A coliform bacterium is approximately 1 to 2 μ m in size. Filtration of wastewater samples through 8 μ m pore size filters was found to result in the removal of particles large enough to harbour coliforms and shelter them from UV exposure (Qualls et al. 1983). The inactivation curve of filtered effluent showed improved disinfection by 3 or 4 log units. In addition, filtration of samples indicated that most of the UV absorbance was due to dissolved components rather than suspended particles (Qualls et al. 1983). The effect of lower filter pore sizes (lower than 8 μ m) was not examined by Qualls et al. (1983). A minimum particle size of 10 μ m was found to be the critical size for particle shielding of coliform in wastewater (Emerick et al. 2000, Metcalf & Eddy 2002). In another study particle- and non-particle associated environmental coliforms were separated and exposed to the same dose of UV. The coliforms associated with particles were found to be more resistant to UV (Örmeci and Linden 2002).

Suspended particles can scatter light away from light path and the detector during UV absorbance measurement by conventional spectroscopy. Measurement of total radiant energy, scattered or unscattered, is possible through use of an integrating sphere attachment to a standard spectrophotometer. Spectrophotometric methods indicated that integrating sphere spectroscopy is the most appropriate mean for measuring UV absorbance of wastewater for the purposes of UV irradiance calculations in collimated beam UV exposure experiments. The UV absorbance measurements using the conventional method are higher than those using the integrating sphere method. Conventional absorbance measurements on filtered secondary effluent wastewater samples with average suspended solids concentration of 14.3 mg/L resulted in overestimation of the UV absorbance by 25%. Use of conventional absorbance measurements on unfiltered samples resulted in underestimation of the UV dose by up to 50% of the actual UV dose in a reactor (Linden and Darby 1998). When the samples were filtered before the conventional spectrophotometric absorbance measurements and filtered absorbance was used to determine the UV fluence rate within a UV reactor, the PSS model provided an overestimated value of up to 190% depending on the concentration of suspended solids.

Jolis et al. (2001) concluded that particles greater than 8 to 10 μ m are more critical to UV inactivation than smaller particles. Suspended particles smaller than 7 μ m do not shield coliform bacteria effectively from UV radiation, and removal of particles larger than 7 μ m substantially increased the coliforms UV inactivation rate. Jolis et al. (2001) also

indicated that researchers should not compare inactivation data obtained from largely dissimilar suspended particle distributions present in treated wastewater samples, as different studies estimated different first order inactivation rates from collimated beam dose-response curves.

The simple first-order kinetic relationship of Equation (8) has been used by many researchers to describe the log-linear inactivation of dispersed coliform bacteria in batch systems. This general form of the equation is adequate and applicable for disperse microorganisms only, as it assumes that all members of a mixed population of bacteria are exposed to the same UV fluence rate. Not only will a microorganism embedded in a particle receive a reduced UV fluence rate, but also its fluence rate may be different from the fluence rate received by a microorganism embedded in other particles. However, if the total number of particle-embedded coliform bacteria exposed to one specific fluence rate were known, Equation (8) could be used to describe the UV dose-response of that sub-population. By making two assumptions, Emerick et al. (2000) developed a model to predict the number of particle-associated coliform bacteria after UV dose delivery. First, one particle with at least one live embedded coliform bacterium is being counted as one coliform bacterium, regardless of the actual number of coliform bacteria embedded within the particle. Second, the probability of inactivating the shielded coliform bacterium is independent of the size of the particle containing the organism. The model proposed by Emerick et al. (2000) is described by Equation (9):

$$N_{P} = \frac{N_{P,0}}{kH} (1 - e^{-kH})$$
 Equation (9)

where:

- N_P = total number of particles containing at least one surviving fecal coliform bacterium after delivery of UV dose of H
- $N_{P,0}$ = total number of particles containing at least one viable fecal coliform bacterium before UV dose delivery of *H*
- k = first-order UV inactivation rate constant (cm² mJ⁻¹), and
- $H = \text{average UV dose, mJ/cm}^2$

The cumulative dose-response curve of dispersed and particle-associated coliform inactivation can be modeled by the summation of equations (8) and (9) which yields:

$$N = N_{D,0}e^{-kH} + \frac{N_{P,0}}{kH}(1 - e^{-kH})$$
 Equation (10)

where:

 $N_{D,0}$ = total number of dispersed viable fecal coliform bacterium before UV dose delivery of *H*

Figure 9 depicts the UV dose-inactivation response curve described by Equation (10). The first-order rate constant, k, can be evaluated by determining the slope of the curve at low UV doses (<10 mJ/cm²) in which the inactivation curve is dominated by dispersed microorganisms. $N_p(0)$ can then be estimated by fitting equation (10) to the tailing region of the curve which is dominated by the particle-associated coliform bacteria. Emerick et al. (2000) also investigated the second assumption of this model; bacteria did not have a greater survival rate in particles greater than 40 µm, than in particles smaller than 20 µm due to the uniformity of coliform bacteria distribution throughout all particles containing coliform bacteria. They concluded that size is not significant in determining shielding of coliform bacteria at sizes greater than the critical particle size (Emerick et al. 2000).



Figure 9: Inactivation of coliform bacteria as a function of average UV dose, (Adapted from Emerick et al. 2000)

2.8 SUMMARY

Biodosimetry is a powerful tool to determine germicidal dose values in continuous-flow UV reactors used for disinfection. It is possible to measure the disinfection performance of large flow-through UV reactors using non-pathogenic indicator microorganisms as biodosimeters (Cabaj et al 2002). The level of inactivation of the indicator in the UV reactor at a specified set of operating conditions results in the reduction equivalent dose (RED). The wider the dose distribution and the lower the UV resistance of the microorganisms, the lower is the measured RED (Cabaj et al. 1996). Calculation of dose in UV reactors, requires accurate descriptions of both the flow and UV fluence rate field. Because of the complexity and uncertainty in describing flow and fluence rate field, independent verification of dose-delivery with biodosimetry is still essential for large, multi-lamp UV reactors. The theoretically calculated UV dose and the experimentally determined UV dose are often different in UV reactors (Leuker 1999).

When considering a biodosimetry approach of a wastewater UV reactor, the high concentration of naturally present microorganisms, such as coliform bacteria and coliphages, would allow on-site verification of the efficacy of UV systems in practice without the need to add indicator microorgansims. In this thesis, naturally occurring indicators (fecal coliform and coliphage) were used as biodosimetry microorganisms. The objective of the current study was to test the feasibility of using these microorganisms for doing biodosimetry on a full-scale wastewater UV reactor. The current study attempted to use a biodosimetry approach based on indigenous fecal coliform to provide an independent evaluation of the dose delivery in the UV reactor system at the municipal wastewater treatment plant operated by Alberta Capital Region Wastewater Commission. The objective was to compare the RED of the reactor measured using biodosimetry, to the dose computed and reported by the UV reactor monitoring system under variety of operating conditions and delivered doses. No previous studies were found in the literature in which indigenous microorganisms were used to determine dose delivery in a large-scale wastewater UV reactor using a biodosimetry approach.

This, therefore, is a unique approach in UV reactor validation studies for wastewater treatment.

The concentration of particulate matter is typically much greater in secondary effluents than in filtered drinking water and has two major effects on UV disinfection. The tailing that is characteristic of coliform UV inactivation-dose response curves in secondary wastewater, is believed to be due to the embedding of the bacteria within particulate matter, and shielding from UV exposure (Qualls et al. 1983; Loge et al. 1999; Emerick et al. 2000; Emerick et al. 1999; Madge and Jensen 2006). Many studies have shown that the UV inactivation increased when the wastewater was filtered prior to UV exposure (Jolis et al. 2001; Madge and Jensen 2006). In some cases, filtration also eliminated tailing from the dose-response curve (Qualls et al. 1983; Madge and Jensen 2006). The presence of microorganisms embedded in particles and the resulting tailing could potentially confound determination of dose using biodosimetry. When tailing is present at high UV doses, a large increase in dose results in only a small increase in microorgansims inactivation. A secondary objective of current study, therefore, was to explore the use of sample membrane filtration to remove particle-associated coliform bacteria and to reduce the effect of tailing on the UV dose determination.

3 EXPERIMENTAL MATERIALS AND METHODS

This chapter explains the experimental materials and methods that were used in biodosimetric analysis of the Alberta Capital Region UV reactor performance using indigenous coliphage and fecal coliform. Coliphage experiments were conducted during the summer of 2004. Reactor performance analysis based on biodosimetry technique using indigenous fecal coliform as an indicator, was completed in April 2006, while the test period was from summer 2005 till spring 2006. This chapter contains 5 main sections that describe:

- 1. Microbiology Procedures
- 2. Collimated Beam Experiment Procedures
- 3. Coliphage Experiments
- 4. Performance Testing of the Alberta Capital Region UV Reactor
- 5. Statistical Analysis

3.1 Microbiology Procedures

From this point forth, sterile means that broth, media or bottles were sterilized by autoclaving at 121°C for at least 15 minutes on a steam cycle unless otherwise stated. The autoclave was routinely tested using *Bacillus thermophilus* test strips.

3.1.1 Coliphage Analysis

3.1.1.1 E. coli Host Production

Escherichia coli strain 13706 was purchased from the American Type Culture Collection (ATCC). This bacteria as a host is essential for detection and enumeration of coliphage. *E.coli* was revived, cultured and then preserved in slants for the next use. At first time, the thawed content of the purchased vial was transferred to a test tube of sterile 6 mL nutrient broth, was mixed well and was incubated at 37° C on an incubator shaker (Innova 4080, New Brunswick Instruments Co. Inc., Edison, NJ) at 180 rpm for 12 to 18 hours. About 0.1 mL of *E. coli* broth was transferred aseptically to each of several fresh tube of

sterile 6 mL nutrient broth. The inoculated test tubes were incubated at 37° C on an incubator shaker at 180 rpm for 6 hours. The *E. coli* ATCC 13076 that revived and cultured in this way was used immediately for coliphage analysis.

E. coli cultures were also grown and preserved on agar slants as *E. coli* broth has to be used immediately and cannot be preserved for the later use. *E. coli* colonies cultured on agar slants can be prepared easily over and over to provide *E. coli* colonies for the period of the experiment. Nutrient broth, 0.5% NaCl and 1.5% solidifying agar were added to purified water, and the mixture was boiled while stirring. Then, about 6 ml of the solution was transferred into each of several test tubes. After closing test tube caps properly (not too loose and not too tight), agar test tubes were autoclaved at 121°C for 15 min. The agar test tubes then were placed at a 5° angle was left to solidify. The agar slants were preserved in a fridge at 4°C after complete media solidification. Agar slants were inoculated from *E. coli* broth (prepared as above) and were incubated at 37 °C for 48 hours. The cultured slants then were preserved in a fridge at 4°C. For each coliphage assay, test tubes of 9 mL nutrient broth were inoculated from *E. coli* colonies grown on the agar slants and were incubated at 37°C on an incubator shaker at 180 rpm for only 12 hours.

3.1.1.2 Coliphage Detection and Enumeration

For the purpose of enumeration of naturally occurring coliphage in wastewater, a modified Double Agar Layer (DAL) procedure was used as described by Grabow (2001). *E. coli* ATCC 13706 was used as a host culture as described above. Normal Petri dishes of 90 mm were used due to the relatively large number of indigenous coliphage present in tested wastewaters. The DAL method is described briefly. Bottom agar was prepared by adding 30 g of trypticase soy broth (TSB) and 15 g of solidifying agar to 1000 mL of de-ionized water. The solution was brought to a boil with constant stirring and was then autoclaved at 121°C for 30 minutes. The bottom agar was cooled in water bath at 50°C and about 20 ml was poured aseptically into each of several 90 mm diameter presterilized plastic Petri dishes. After solidifying, the plates were inverted and stored in a

refrigerator at 4°C. The top agar layer (soft agar) was prepared by adding 30 g of TSB and 7.5 g of solidifying agar to 1000 mL de-ionized water. The mixture was brought to a boil while stirring continuously. One hundred mL of the soft agar solution was poured into each of several 200 to 250 mL flasks. The soft agar flasks were autoclaved at 121°C for 15 min and then kept in water bath at 50°C. The soft agar was prepared freshly each day and used within 24 hours. Fresh 12 hours old host bacteria culture was prepared following the procedure explained in section 3.1.1.1. Three mL of the host culture was added aseptically to 100 mL of soft agar, mixed completely and let to stand for 3 minutes in the water bath at 50 °C. One hundred mL of the room temperature wastewater sample was added to the mixture of soft agar and host culture. The flask content was mixed very gently and kept in the water bath for 3 minutes. Bottom agar plates were removed from the refrigerator and allowed to warm to room temperature. Approximately 10 mL of the soft agar mixture was added to each bottom agar plate. After the soft agar was solidified, the plates were inverted and incubated at 37 °C for between 8 to 12 hours. The plates were removed from the incubator and the number of plaques, areas of zero E. coli host growth, was enumerated. Plate counts between 30 to 300 plaques per plate were reported except for samples after UV exposure where very few plaques were detected per 100 mL of sample. The concentration of plaque forming units (PFU/100 mL) was then determined. Figure 10 shows somatic coliphage plaques obtained for Gold Bar WWTP upstream UV sample using the DAL technique. Coliphage assays were performed on the both upstream and downstream of UV system samples.



Figure 10: Somatic coliphage plaques obtained from the Gold Bar WWTP sample tested using the double agar-layer technique

3.1.2 Fecal Coliform Analysis

Fecal coliform bacteria concentration of samples was determined using the membrane filtration procedure and MF-C medium according to 9222 D of Standard Methods (Clesceri et al. 1998). The most important part was to select the sample size and proper dilution of secondary effluent in order to give counts between 20 and 60 fecal coliform colonies per membrane. Therefore, serial dilutions of secondary effluent wastewater were prepared using milk bottles containing 90 mL of sterile peptone dilution water (Fisher Scientific, Part no. 290061057). A serial dilution began by first shaking the 500 mL wastewater sample. A 10 mL aliquot of the well-mixed wastewater was transferred quantitatively, and aseptically into 90 mL of sterile peptone to yield 100 mL of 10⁻¹ diluted sample. The 10⁻¹ dilution bottle was shaken and 10 ml was transferred into second dilution milk bottle containing 90 ml of sterile peptone to yield 100 ml of 10⁻² diluted sample, and so on through the dilution series. Then, the whole content of each milk bottle was filtered through a membrane filter (Fisher Scientific, Part No. E04WG047S1, Gamma radiation pre-sterilized, Diameter: 47mm, 0.45µm pore size, gridded surface, White) starting with the lowest concentration. Vacuum filtration apparatus was used and

the filter holders were rinsed with peptone water after each filtration. Each membrane was aseptically placed on MF-C agar in a 47 mm plastic sterile Petri dish. The dish was covered, inverted and incubated at 44.5 °C for 24 hours. Coliform contamination was checked before testing each sample by filtering 20 to 30 mL dilution peptone water to make sure that fecal coliform was not introduced through dilution solution, growth media and that the aseptic methods were satisfactory. The number of metallic blue colonies on each plate was counted and the number of colony forming units (CFU) per 100 mL was calculated based on the dilution. Figure 11 shows fecal coliform colonies obtained for Alberta Capital Region WWTP secondary effluent using the membrane filtration technique. Plate counts greater than 60 colonies per plate were reported TMTC (too many to count) and plate counts fewer than 20 colonies per plate were reported TFTC (too few to count) except for samples after UV exposure where very few colonies were detected per 100 mL of sample. Wherever the counts were valid (between 20 and 60) for two serial dilutions, an average was reported.



Figure 11: Fecal coliform colonies obtained from the Alberta Capital Region WWTP sample tested using the membrane filter technique

3.2 Collimated Beam Experiment Procedures

3.2.1 Collimated Beam Apparatus

A collimated beam apparatus manufactured by Calgon Carbon Corporation, USA was used to apply the UV dose to the samples. A collimated beam apparatus allows the delivery of a constant and known irradiance (fluence rate) to the surface of the liquid sample and precise control of exposure time. This collimated beam apparatus consists of a 30 W low- pressure (LP) mercury arc lamp (Ster-L-Ray Germicidal Lamp, model G12T6L 15114, Atlantic Ultraviolet Corporation, Hawpange, NY) housed in a metal box. The irradiation of samples was controlled using a gas-operated pneumatic shutter located beneath the lamp with a quartz window located beneath the shutter and at the top of the collimating tube. UV exposure duration was measured with a stopwatch. A Petri dish containing the water sample was placed below the shutter where the distance between the lamp and the surface of the liquid sample was 460 mm. A PVC tube 400 mm long and 60 mm diameter was located beneath the shutter opening, and was used to collimate the UV light. The distance between the end of the collimation tube and the sample surface was 30 mm. See Figure 3 in section 2.3.1 for a simple sketch of the collimated beam apparatus.

3.2.2 Exposure Procedures

For a typical UV reactor performance test, five samples of wastewater were collected from upstream of the reactor. Also, at each set of operating condition five samples from downstream of the reactor were collected within an approximate 2 to 3 minutes time interval. Downstream samples were collected during the same time period as upstream samples were collected. Upstream and downstream samples were collected in randomized order, over a period of approximately 40 to 45 minutes from points near the middle of the channel at mid-depth. Two sets of operating conditions were tried in one day of experimentation, with about a 30 minute allowance for stabilization of each operating condition. Exact sampling locations, sampling spots and sampling procedure are described in section 3.4.4.

Secondary effluents contain particles that may scatter, as well as absorb the UV light. The absorbance at 254 nm of each wastewater suspension was measured using a UV-Vis spectrophotometer (UV-2401PC, Shimadzu Corp., Columbia, Maryland) equipped with an integrating sphere attachment (ISR 2200) and using a 10 mm quartz cell. The integrating sphere measures forward- and side-scattered light, and provides a more accurate analysis of true solution absorbance when particles are present (Linden and Darby 1998). On average, absorbance determined using conventional spectroscopy measurements of the unfiltered wastewater was approximately 50% greater than absorbance determined using the integrating sphere.

On each experiment day, sub-samples of undiluted secondary wastewater collected from upstream of the UV reactor were exposed to three controlled UV doses of 5, 10, and 20 mJ/cm² from a low-pressure mercury arc lamp using a collimated beam apparatus (RayoxTM by Calgon Carbon Inc., Pittsburgh, PA) using the standard procedure of Bolton and Linden (2003). Twenty-mL aliquots of wastewater samples were placed in 50 mm ID glass Petri dishes, resulting in a liquid depth of approximately 8 mm. The Petri dishes were placed at the centre of the beam where UV irradiance is greatest. The samples were stirred continuously but gently at the same degree during the exposure periods by means of small magnetic bar (Teflon coated, 10×3 mm) and magnetic stir plate. The irradiance at the liquid surface and at the centre of the UV beam was measured before, and after each exposure using a calibrated radiometer and detector (P-9710 Optometer and UV-3710 Irradiance Detector, Gigahertz Optic, Newburyport, MA). The exposures were conducted in random order with respect to dose and each exposure was carried out in duplicate. The UV exposures were carried out in random order to avoid the effect of systematic error on the results. Following the UV exposure, 10 mL of exposed sample was transferred from the Petri dish to the first dilution bottle. The dilution bottles containing exposed samples were covered with aluminium foil to prevent photoreactivation of inactivated coliforms. A dilution series was prepared and FC bacteria were enumerated as described earlier. For each collimated beam experimental sample, a minimum of two culture plates were conducted at a time. Multiple exposures were

required to obtain 100 mL of UV irradiated samples at doses of 10 and 20 mJ/cm² for microbiology analysis giving valid plate counts.

To determine a depth-average irradiance (fluence rate), the centre-surface irradiance measurement was corrected for radial variation, surface reflection and depth attenuation, using standard procedures described in section 2.3.2. The depth-averaged irradiance was obtained by applying a Reflection factor, Petri factor, Water factor and Divergence factor. Corrections for reflection at the air-liquid interface, the radial variation over the surface area, water absorbance and beam divergence provided an estimate of the depth averaged fluence rate to which each microorganism was exposed.

3.2.3 WASTEWATER SAMPLE PRE-TREATMENT BY MEMBRANE FILTRATION

Association between microorganisms and particles present often results in tailing of UVdose inactivation curves of secondary wastewater (Qualls et al. 1983; Emerick et al. 2000). If the delivered UV reactor dose was within the tailing region of the fecal coliform UV dose-inactivation curve, a large change in delivered dose would result in little change in microorganism inactivation. This would tend to reduce the accuracy of the biodosimetry analysis. To address this issue, a series of preliminary experiments were conducted in which three samples of the secondary effluent were collected from upstream of the reactor within an approximately 10 minute time interval. The samples were combined to produce a composite sample. Sub-samples of the composite sample were exposed to pre-calculated doses of UV in the range of 0 to 30 mJ/cm² in the laboratory using the collimated beam apparatus (RayoxTM by Calgon Carbon Inc., Pittsburgh, PA) (described in section 3.2.2). Samples from the collimated beam experiments were filtered through either 5 or 10 µm pore size polycarbonate track-etched (PCTE) membrane filters prior to microbial analysis (but after UV exposure). Millipore IsoporeTM polycarbonate membrane filters (Fisher Scientific, Part no. TMTP 047 00, TCTP 047 00, Diameter: 47mm) were chosen for this operation because they are hydrophilic and non-hygroscopic.

Figure 12 is a photomicrograph of the surface of the Millipore Isopore polycarbonate membrane filters used for these experiments. The photograph of the clean filter surface taken by a standard light microscope shows the uniformity of the filter pores.



Figure 12: Photomicrograph of the surface Millipore IsoporeTM Polycarbonate Membrane Filter (10 µm), magnification: 400 X

Other researchers have found that a minimum particle size of about 10 µm is required to harbour coliform bacteria in wastewater from exposure to UV light (Emerick et al. 2000). The objective of the filtration step was to reduce the concentration of particle-associated bacteria and, thus, mitigate the effect of tailing on the UV inactivation curve. Vigorous agitation steps, such as vortex mixing were avoided to keep particles from breaking. The filtration was carried out using a vacuum filtration apparatus and Gelman filter holders, at consistent vacuum pressure of 14 kPa. The filtered water was collected in Erlenmeyer vacuum filtration flasks that were wrapped in tinfoil to minimize the potential for photoreactivation. The fecal coliform analysis was performed as described in section 3.1.2. Fecal coliform concentration of secondary effluent samples was determined while each serial dilution was repeated twice more in order to have 3 replicates of each dilution as shown in Figure 13.



Figure 13: Sketch of a serial dilution of a sample for membrane filtration

On the same day of the experiment, secondary effluent wastewater samples that were filtered through 5 and 10 μ m PCTE membrane filters were prepared as described above. A 150 mL aliquot of filtered sample was placed in a clean 200 mL beaker that was washed with particle-free water. The number of particles passing through the PCTE membranes was measured using a Hiac-Royco Particle Counting System (HRLD-150 Sensor, Model 8000A Particle Counter). The cumulative and differential particles for (2 to 5, 5 to 8, 8 to 10, 10 to 14, 14 to 20, and > 80 μ m) were reported by the particle counter. The smallest countable particle on this instrument was 2 μ m. Particle concentration and size distribution of the unfiltered secondary effluent wastewater sample was also measured.

3.3 Coliphage Experiments

To perform coliphage analyses, samples of secondary effluent were collected at locations upstream and downstream of the UV reactors at variety of wastewater treatment plants: Edmonton Gold Bar, Kelowna Wastewater Treatment Facility and South End Water Pollution Control Centre operated by the City of Winnipeg. Samples from Gold Bar Wastewater Treatment Plant located at south side of the North Saskatchewan River in the City of Edmonton, were placed on ice and shipped to the laboratory within an hour and analyzed on the same day as collection (described in section 3.1.1). Edmonton's Gold Bar Wastewater Treatment Plant provides primary treatment, secondary treatment upgraded to biological nutrient removal process in 1996, and ultraviolet disinfection opened in 1998. The City of Kelowna Wastewater Treatment facility also incorporated biological nutrient removal step and the effluent from the secondary clarifiers is pumped to the filters before UV disinfection. The South End Water Pollution Control Centre, located at 100 Ed Spencer Drive in the City of Winnipeg, provides primary treatment and activated sludge treatment and uses Trojan's UV system for tertiary treatment since 1999.

Samples from the City of Kelowna and Winnipeg Wastewater Treatment Plants, were collected by plant employees and shipped to the University of Alberta Environmental Engineering laboratory within 24 hours. Coliphage analyses were carried out on the wastewater samples as soon as their arrival (described in section 3.1.1). In summer 2004, when preliminary study of UV reactor performance using indigenous coliphage was conducted, Alberta Capital Region Wastewater Treatment Plant did not have the UV reactor installed. ACRWWC planned to convert its secondary clarifiers to nutrient removing bioreactors within a year, therefore above WWTPs which offer variety of treatment processes, were selected for preliminary coliphage study. Sufficient concentration of coliphage in the secondary effluent is required for accurate UV reactor performance testing using biodosimetry. In addition, to determine if there was a measurable change in the naturally occurring coliphage concentration in wastewater samples as with sample storage time, a coliphage assay was conducted every 24 hours for 72 hours on sub-samples of the same original wastewater sample (described in section 3.1.1).

Edmonton's Gold Bar secondary effluent samples were used for collimated beam experiment in the laboratory. Undiluted secondary effluent samples were exposed to the UV doses of 15, 30, and 45 mJ/cm² as described in section 3.2.2.

3.4 Performance Testing of the Alberta Capital Region UV Reactor

3.4.1 Alberta Capital Region Wastewater Treatment Plant

The Alberta Capital Region Wastewater Commission (ACRWC) is an example of local cooperation, which provides wastewater treatment services to 13 municipalities in the Alberta Capital Region. The annual average wastewater flow to the plant in 2005 was 72,831 m³/d and the plant secondary treatment has run at the capacity of 87,500 m³/d since December 2005. The estimated service population was about 193,000 in 2005. Recent plant operation data is summarized in Table 1. The Commission was formed in 1985 and is governed by the Municipal Government Act. The plant treatment processes were upgraded on June 1, 2005 in order to comply with Approval No. 488-01-100 set by Alberta Environment, which regulates all Commission facilities and operations. The upgrade included conversion of the original conventional activated sludge process to biological nutrient removal (BNR) process to meet nutrient removal regulations. Also, a UV disinfection system was installed to reduce effluent fecal coliform to less than 200 counts/100 mL, making it safe for contact recreation before discharging the treated wastewater to the North Saskatchewan River. A process diagram of the facility is depicted in Figure 14.

 Table 1 : Alberta Capital Region Wastewater Treatment Plant Monthly Operation Data, monthly average values (2006)

| Month | Flow (m ³ /d) | Effluent CBOD (mg/L) | Influent TSS (mg/L) | Effluent TSS (mg/L) | Effluent Fecal Coliform (CFU/100 ml) |
|-------|-----------------------------|----------------------------|---------------------------|---------------------------|--|
| | | | | | |
| March | 69,280 | 4 | 421 | 4 | 11 |

Reference: http://www.acrwc.ab.ca/Data.html, 2006-11-23



Figure 14: Flow Sheet of the Plant showing the location of the UV unit and the sampling points, (Reference: <u>http://www.acrwc.ab.ca/Plantmain.html</u>, 2006-11-23)

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3.4.2 WEDECO UV Reactor System

Alberta Capital Region WWTP UV reactor designed by WEDECO Inc., has been sized for a peak flow of 140 MLD, an average flow of 70 MLD and a wastewater transmittance at 254 nm of 55% (10 mm path length). The Alberta Environment disinfection performance requirement is less than 200 FC/ 100mL (based on a 30 day geometric mean of daily samples). All lamps within the UV system are of identical design (type, length, diameter, power, output etc.). They are low-pressure mercury amalgam, high intensity variable output (LPHO) type with an arc length of 143 cm. The UV disinfection system is composed of 2 UV reactor channels operated in parallel, each containing 2 banks of UV lamps (Figure 15). Therefore, there are 4 banks of UV lamps in total. Each bank contains 5 modules of UV lamps and each module is composed of 18 lamps. Thus, the total number of lamps is 360. Figure 15 shows a simple sketch of the Alberta Capital Region UV reactor system that depicts the layout of the channels, banks, modules, and lamps. Each UV module consists of a dual (side-by-side) row configuration of UV lamp assemblies (Figure 16), 9 pairs of lamp assemblies in the vertical direction, giving a total of 18 lamps per module (2 assemblies \times 9 lamps per assemblies = 18 lamps). The UV modules are installed horizontally and parallel to the water flow in stainless steel fixed frames. The electrical and electronic components of the UV system are located in the UV control panel, which includes the control and electronic ballast. The UV fluence rate is a measure of the UV output of the lamps and is measured using a UV fluence rate sensor (Figure 16) mounted in the central module in the bank. It is used for online monitoring of the lamp output for the UV dose calculation. Additionally, since the UV fluence rate sensor directly measures the UV output of the lamps it can be used to monitor the operational status of the lamps (full power, half power, off). For instance with the lamps operating at full power any decrease in the measured UV fluence rate could be attributed to lamp aging, lamp scaling or a reduction in UV transmittance. In addition to indicating lamp status, the sensor can also monitor reduction in lamp output. This may be an effective optimization and / or maintenance tool, indicating the effectiveness of the automatic wiping system and when a scheduled lamp change is due. Each UV module is
equipped with an automatic wiping system with selectable wiping frequency and number of strokes. Reactor design specifications are summarized in Table 2.

| DESCRIPTION | UNITS | DATA |
|-----------------------------------|----------------------|--|
| DESIGN FLOW: | | |
| Peak Flow | 10 ⁶ L/d | 140 |
| Average Flow | | 70 |
| UV DOSE: | µW-s/cm ² | >30,000 [†] |
| TOTAL SUSPENDED SOLIDS: | mg/l | 20.0 |
| BOD | mg/l | 20.0 |
| UV TRANSMITTANCE RANGE (253.7nm): | % | 55 (minimum) |
| EFFLUENT FECAL COLIFORM | CFU/100 mL | |
| STANDARD: | | |
| 30 day geometric mean | | < 200.0 |
| EFFLUENT TEMPERATURE: | min/Max °C | 10/18 |
| CONFIGURATION: | <u>.</u> | |
| Number of lamps | | 360 |
| Number of channels | | 2 |
| Number of banks per channel | | 2 |
| Number of modules per bank | | 5 |
| Number of lamps per module | | 18 |
| CHANNEL DIMENSIONS: | mm | ······································ |
| Width along the UV banks | | 1190 |
| Width along the weir | | 1600 |
| Water depth | | 1190 |
| Total depth | | 1465 |
| Approx. length | | 11500 |
| ELECTRICAL LOAD: Maximum | KW | 108/113.7 |

Table 2 : Design Specifications of the Capital Region UV Reactor

[†]Note: The stated dose of >30,000 μ W-s/cm² was calculated at peak flow with the following factors: 0.70 Lamp aging, 0.92 Quartz transparency, 0.9 Overall safety factor. Dose calculation is based on lamp power output at the end of the guaranteed lamp life.



Figure 15: Alberta Capital Region UV Disinfection System General Arrangement



Figure 16: UV Module consisting of a dual (side-by-side) row configuration of UV lamp assemblies

3.4.3 Dose Calculation Method

A complete ultraviolet disinfection system, WEDECO Inc. model TAK55M 9-5(6)/143 \times 2i2W in two banks per two channels, was the subject of the current study. UV dose calculations are carried out by software in the WEDECO control system and are reported on the control panel and to the Alberta Capital Region Plant SCADA system (Supervisory Control and Data Acquisition system). A Point Source Summation Method is used to calculate the fluence rate field in the UV reactor. The fluence rate at any x, y, z point in the reactor is calculated based on the UV-C output of the lamp and the x, y, z coordinate. Plug flow with complete lateral mixing is assumed, which means that the exposure time, t_d is equal to V/Q, where V = the irradiation volume and Q = volumetric flow rate. The average fluence rate is then calculated and multiplied by the exposure time (V/Q) to obtain the delivered UV dose. The preliminary testing of the UV reactor performance and on-site monitoring of the control panel for hours at the Alberta Capital Region Plant, provided an opportunity to determine the possible operating range of the UV reactor system. For example, the lowest calculated UV dose that can be studied is 15 mJ/cm^2 . This dose is achieved when the entire plant effluent is diverted through a single UV reactor channel with one bank in operation, and with the lamp power at 50%. Table 3 outlines the operating ranges (i.e. lamp power 50 to 100%, one or two lamp banks on, 100 % or 50% plant flow through one channel) and the approximate dose range that resulted. High flow means that 100% of the plant effluent was diverted through UV reactor channel one. Low flow means that the plant effluent was split into two channels and only channel one was examined.

| Conditions | Flow rate | UV lamp power | Number of banks in operation | UV Dose (mJ/cm ²) [†] |
|------------|-----------|------------------|------------------------------------|---|
| 1 | Low | 50% | 1 | 20 |
| 2 | High | 50% | 1 | 15 |
| 3 | Low | 100% | 1 | 30 |
| 4 | High | 100% | 1 | 25 |

Table 3 : Examined Operating Conditions for UV Reactor Performance Validation

[†]Based on UV reactor control system

3.4.4 Sample Collection and Preparation

Samples of secondary effluent were collected at locations upstream and downstream of the UV reactor at the Capital Region Wastewater Treatment Plant as shown in Figure 14. The upstream wastewater samples were collected at a point in the UV reactor channel immediately upstream of the first bank of UV reactor lamps, through a cover in the floor grating. The downstream wastewater samples were collected at a point in the UV reactor channel downstream of the second UV reactor bank. Both samples were collected from points at approximately mid-depth and mid-width, in the channel using a 1.8 m aluminium subsurface grab sampler rod with a bottle attached to the end with a VelcroTM strap. The subsurface grab sampler was lowered into the channel with a sterile empty sample bottle attached, and was removed when the sample bottle was almost full. A depth mark on the rod made it possible to sample from the same depth reproducibly. Sample bottles were 500 mL wide-mouth polypropylene bottles with polypropylene linerless screw closures (Fisher Scientific, Part no. 02-896E), were opaque (to avoid photoreactivation) and were autoclavable. Sample bottles were chosen and prepared according to Standard Methods (9030 B -18). Samples were collected and transported following sampling procedure described in Standard Methods (9060 A- 3). Wastewater samples were placed on ice during transportation to the laboratory and were analyzed on the same day as collection to avoid changes in the microbial population. Wastewater samples were analyzed within 6 hours of collection according to Standard Methods (9060 B- 1.a) and were stored at 4 °C until use in experiments. In a typical UV reactor test, five samples of wastewater from upstream of the reactor and five from downstream of the reactor (consistently from channel one) were collected in randomized order.

3.4.5 Calculation of Inactivation

Microorganism inactivation in both collimated beam experiments and in the flowing water through full-scale UV reactors, was measured as the log inactivation ratio, described as:

Log Inactivation = - log
$$(\frac{N}{N_{o}})$$
 Equation (11)

where N_0 was the average of viable microorganism concentration up stream of UV reactor (before UV exposure) and N was the average of viable microorganism concentration down stream of UV reactor (after UV exposure).

3.4.6 Calculation of the RED

Reduction Equivalent Dose (RED) is determined by comparing the inactivation measured as microorganisms passed through the full scale UV reactor, to the UV dose inactivation relationship developed at the same day in the collimated beam experiments in the laboratory. In this study, the UV dose–inactivation response model was developed to compute the reduction equivalent dose (RED). Non-linear least-squares regression was used to predict the parameter values of the UV dose–inactivation response model. Solver tool in a Microsoft ExcelTM spreadsheet (2002) was used to determine values of model parameters such that sum of square errors was minimized.

3.4.7 Organization of the Full-scale UV Reactor Performance Tests

The objective was to measure the dose delivery of the UV reactor under a variety of operating conditions using a biodosimetry approach based on inactivation of indigenous fecal coliform bacteria. The reduction equivalent dose (RED) measured using biodosimetry was to be compared to the dose computed, and reported by the UV reactor monitoring system (provided by the UV reactor vendor) under a variety of operating conditions and delivered doses. Reactor channel one, which is identical to channel two, was selected for performance testing and sampling for this study. The upstream wastewater samples were always collected at a point upstream of the first bank (bank A) of UV reactor lamps, and the downstream wastewater samples were always collected at a point upstream of the second UV reactor bank (bank B) in channel one. The control program enabled the facility operator to control and set the UV dose at the desired testing levels

outlined in Table 3. The operator adjusted the calculated dose to the set point required for the tests by varying the power level and the flow rate and then allowing 30 minutes for the reactor to adjust to the new conditions. At each operating condition, the following information was recorded: UV fluence rate, calculated UV dose, number of lamp banks in operation, lamp power level, and water flow rate. Also TSS data were obtained from the Alberta Capital Region Wastewater Treatment Plant laboratory. All recorded on-site information can be found in Appendix A.

Based on the constraints outlined in Table 3, four operating conditions were examined to provide a range of expected UV doses. These four test conditions were carried out in duplicate on different experimental days. Two sets of conditions were completed on each experimental day. In order to streamline the analysis, five samples of wastewater were collected from upstream, and five samples from downstream of the UV reactor at each operating condition (See section 3.4.4 for exact sampling procedure). Each sample was analyzed for FC once (i.e. total of 5 measurements from each sample location). This sampling plan captured the total process/analytical variability. Parallel to the full-scale experiments, samples of wastewater effluent collected from upstream of the UV reactor were exposed to doses of 5, 10 and 20 mJ/cm² in the collimated apparatus in duplicate in order to verify the dose response (See section 3.2.2 for details).

3.5 Statistical Analysis

A Microsoft ExcelTM spreadsheet (2002) was used to analyze data and experimental results. Microsoft Excel provides a set of data analysis tools such as ANOVA, regression, solver, etc. By providing the data and parameters for each analysis, the tool uses the appropriate statistical functions and then displays the results in an output table.

4 ANALYSIS OF RESULTS AND DISCUSSION

The experimental results obtained in this study and discussions are presented in this chapter. This chapter is grouped into 5 sections:

- 1. Coliphage as an indicator of disinfection performance of wastewater UV reactors;
- 2. Effect of wastewater sample pre-treatment by membrane filtration on fecal coliform dose-response;
- 3. Preliminary UV reactor performance testing using indigenous fecal coliform bacteria at the Alberta Capital Region Wastewater Treatment Plant;
- 4. Performance testing and determination of the reduction equivalent dose (RED) in the Capital Region Wastewater Treatment plant UV reactor; and
- 5. Interpretation of the measured RED

4.1 COLIPHAGE AS AN INDICATOR OF DISINFECTION PERFORMANCE OF WASTEWATER UV REACTORS

The primary objective of the coliphage study was to assess the usefulness of naturally occurring coliphage as an indicator for biodosimetry testing of the large-scale UV reactors used in municipal wastewater treatment. This testing was done during the summer of 2004, in anticipation of performance testing of the Alberta Capital Region Wastewater Treatment Plant (ACRWWTP) UV reactor system that was installed in May of 2005. This section is organized into 4 sub-sections: assessment of coliphage concentration in various wastewater treatment plants effluents, UV inactivation of coliphage in wastewater effluent in collimated beam experiments, impact of storage time on coliphage viability, and conclusions of the coliphage experiments.

4.1.1 Presence of Coliphage in Various Wastewater Treatment Plants

The coliphage measurement results are summarized in Table 4. The Edmonton Gold Bar Watewater Treatment Plant was sampled four times, while the other three wastewater treatment plants were sampled only once. These coliphage measurements indicated that coliphage were consistently present in all of the secondary effluent samples examined. However, there were very few coliphage remaining in the UV treated secondary effluents from Edmonton Gold Bar, Kelowna or Winnipeg. For the Edmonton and Kelowna facilities, the measurable log inactivation was limited to about 2.5 to 3.0 log at normal UV reactor operating conditions due to the limited coliphage concentration in the secondary effluent. In fact, in four sampling events, no coliphage were detected in the UV treated effluent, and only a lower limit on log inactivation could be calculated. In these two plants, therefore, the coliphage are not likely to be present in sufficient concentration in the secondary effluent to allow accurate quantitation of true log inactivation. This is a requirement for UV reactor performance testing using biodosimetry.

In the Winnipeg and Alberta Capital Region facilities, the coliphage concentration was an order of magnitude greater than in the Edmonton and City of Kelowna facilities. At the Winnipeg facility, a log inactivation of > 3.60 was calculated. It is important to note that the two facilities with the lowest coliphage concentration (Edmonton and Kelowna) employed biological nutrient removal (BNR) activated sludge systems while Winnipeg and Alberta Capital Region (in 2004) employed conventional activated sludge. It is possible that the extended aeration times of BNR facilities might have resulted in better removal of coliphage from the wastewater. This was an important consideration for UV reactor performance testing at the Alberta Capital Region facility, because they were planning to upgrade to a BNR process prior to the performance testing. It was likely, therefore, that the coliphage concentrations at Alberta Capital Region would decrease after the upgrade.

| WWTP | Test Date | Upstream of the UV Reactor | Down Stream of the UV Reactor | Log Inactivation | Calculated UV Dose in Reactor [†] (mJ/cm ²) |
|---------------------------|-----------|----------------------------------|--|---------------------|--|
| Edmonton Gold Bar | 7-Jul-04 | 1250 | 0 | > 3.10 | 27 |
| Edmonton Gold Bar | 14-Jul-04 | 1879 | 0 | > 3.27 | 27 |
| Edmonton Gold Bar | 5-Aug-04 | 1798 | 1 | 3.25 | 28 |
| Edmonton Gold Bar | 12-Aug-04 | 530 | 0 | > 2.72 | 27 |
| City of Kelowna | 20-Jul-04 | 529 | 0 | > 2.72 | unknown |
| Winnipeg | 17-Aug-04 | >20,000 | 5 | > 3.60 | unknown |
| Alberta Capital Region | 28-Aug-04 | >20,000 | N/A [‡] | N/A | N/A |

Table 4: Coliphage concentration (PFU /100 ml) in secondary effluent samples from three wastewater treatment plants in Western Canada

Reported by the UV reactor control system

[‡] Data not available - UV reactor system was not installed at time of measurement

4.1.2 UV Inactivation of Coliphage in Collimated Beam Experiments

Measurement of dose delivery in the UV reactor using biodosimetry based on inactivation of indigenous coliphage depends on the UV dose-inactivation curve developed in laboratory UV exposure experiments. The reduction equivalent dose (RED) of the UV system is determined by comparing the measured coliphage inactivation to the UV dose-inactivation curve developed in the collimated beam experiments. The UV-dose inactivation curve of coliphage, is characterized by the concentration of coliphage in the secondary effluent and the UV dose-inactivation characteristics of the coliphage.

A coliphage UV inactivation plot was developed by exposing samples of wastewater effluent collected from the Edmonton Gold Bar Wastewater Treatment plant on Aug 12, 2004, to controlled doses of UV radiation in duplicate using a collimated beam apparatus. The collimated beam apparatus was used to deliver UV doses of 15, 30, and 45 mJ/cm^2 to the secondary effluent sub-samples. Figure 17 shows the log inactivation plotted against the germicidal UV dose for coliphage. Because there was only data point at 45 mJ/cm² and the inactivation result was beyond detection, it was hard to prove the existence of tailing from coliphage inactivation plot (Figure 17). The initial concentration of coliphage in the wastewater sample sample (N_0) was 530 PFU/100 mL. At the higher two UV doses, very few coliphage were detected in the irradiated samples, and no coliphage plaque were detected on the plates from the 45 mJ/cm² UV experiment, making quantitative enumeration difficult. Corresponding measurements at the full-scale UV reactor at the Edmonton Gold Bar Wastewater Treatment Plant indicated complete coliphage inactivation at calculated UV doses of less than 30 mJ/cm² (Table 4). Farahbakhsh and Smith (2003) reported 2.3 to 3.4 coliphage log inactivation by the Gold Bar UV system, but they did not mention the secondary effluent coliphage concentration (N_0) and the applied UV dose. Another pilot plant study reported coliphage removal of 1 to 2 logs at UV dose of 47 mJ/cm² and indicated that concentrations of coliphage naturally present in secondary effluent were relatively low. A suspension of laboratory culture coliphage, therefore, was introduced to the UV reactor for performance testing purposes (Dizer et al. 1993).



Figure 17: Indigenous coliphage UV dose-inactivation response determined in collimated beam experiments plot (Each point on the graphs represents the average of two collimated beam UV exposures)

4.1.3 Impact of Sample Storage Time on the Coliphage Viability

The objective of this controlled experiment was mainly to study whether the storage time between sample collection and coliphage analyses has significant impacts on the viability of coliphage in the wastewater. Jolis (2002) reported an increase in the resistance of MS2 coliphage to UV radiation after 18 days of sample storage. A decrease in viability of the coliphage over time could contribute to this observation, giving lower coliphage log inactivation at the same applied UV dose.

A sample of Alberta Capital Region wastewater effluent was analyzed for naturally occurring coliphage concentration 24, 48 and 72 hours after sample collection, to determine if the coliphage concentration decreased with sample storage. The original sample was stored in a refrigerator at 4°C. This test was important because it may be necessary to ship or store samples when conducting UV reactor performance testing, particularly when the facility and the laboratory are in different cities. Samples are

typically shipped in coolers containing ice packs, therefore, the 4°C test temperature was appropriate. As described earlier, wastewater samples of City of Kelowna and Winnipeg were shipped to the University of Alberta Environmental Engineering laboratories in the current study.

The results of this experiment are shown in Table 5. An ANOVA analyses indicated that there was no change in the viable coliphage concentration over time. (p- value = 0.78, n = 9). Coliphage counts with time separation of 24 hours, and ANOVA test result can be found in Appendix B. The outcome of this controlled experiment indicated that there was no decrease in coliphage viability after 72 hrs. of storage.

 Table 5 : Coliphage concentration (PFU/100mL) in secondary effluent sample as a function of sample storage time

| Sample storage time | Replicate 1 | Replicate 2 | Replicate 3 | |
|---------------------|-------------|-------------|-------------|--|
| 24 hrs | 57,000 | 81,000 | 65,000 | |
| 48 hrs | 68,000 | 57,000 | 66,000 | |
| 72 hrs | 65,000 | 73,000 | 63,000 | |

4.1.4 Conclusions of Coliphage Measurements

The presence of coliphage in fecal matter means that they can serve as indicators of fecal contamination and may indicate the concurrent presence of human enteric viruses (Sundram et al. 2002). In the current study, coliphage enumeration in secondary effluent samples from four different wastewater treatment plants confirmed the presence of naturally occurring coliphage at all four plant locations. However, low coliphage concentrations in the secondary effluents of the plants using biological nutrient removal (BNR) activated sludge systems, led to the use of naturally occurring fecal coliform bacteria as an indicator instead of coliphage for biodosimetry testing of the Alberta Capital Region Wastewater Treatment Plant (ACRWWTP) UV reactor. UV inactivation of coliphage in the collimated beam experiments, showed that naturally occurring coliphage may serve as useful indicators of UV reactor performance at relatively low UV

doses (i.e. less 30 mJ/cm²). At higher doses (greater than 30 mJ/cm²) no surviving coliphage were detected in the wastewaters of the Kelowna and Edmonton treatment plants, making quantitation of inactivation difficult. This occurred mainly because the concentration of coliphage in the secondary effluent upstream of UV treatment at these plants was low. The concentration of coliphage in the secondary effluent upstream of UV treatment at ACRWWTP was considerably higher than that at Kelowna and Edmonton. The concentration of fecal coliform in ACRWWTP secondary effluent, however, decreased by approximately 10 times greater after the facility was upgraded in the spring of 2005 (personal communication with ACRWWTP laboratory staff). The same ratio was expected for reduction of indigenous coliphage after incorporating biological treatment since the fecal coliform are the natural host of coliphage. Thus, the use of naturally occurring coliphage as an indicator was not pursued further and fecal coliform bacteria were used for performance testing of the ACRWWTP UV reactor system as will be described in the following sections.

4.2 WASTEWATER SAMPLE PRE-TREATMENT BY MEMBRANE FILTRATION

Laboratory fecal coliform UV inactivation tests were conducted on the wastewater effluent collected from Alberta Capital Region WWTP (prior to UV treatment) using the collimated beam apparatus and the procedure described in section 3.2.2. To examine the effects of sample pre-filtration on the UV dose-inactivation curve, samples of the secondary effluent exposed to UV in collimated beam experiments were filtered through 10 μ m pore sized membranes prior to microbial analysis but after UV exposure. The objective of the filtration step was to remove coliform bacteria that were associated with particulate matter from the analysis, and to thus reduce the tailing in the inactivation curve. Two sets of inactivation curves were generated using samples of secondary effluent collected from upstream of the UV reactor on two different days (3 wastewater samples collected and combined on each day). In each set, inactivation was measured at controlled UV doses of 5, 10, 15, 20 and 30 mJ/cm² for both pre-filtered and unfiltered samples. At UV doses greater than 30 mJ/cm² very few coliform were detected in the

irradiated sample fecal coliform culture plates, which made quantitative enumeration difficult.

The results of the two sets of collimated beam experiments are shown in Figure 18. Details of the UV exposures and fecal coliform analysis with and without sample prefiltration are present in Appendix C. Each data point is based on the results of duplicate UV exposure experiments and determinations of inactivation. There was little difference in the fecal coliform UV inactivation curves determined for the pre-filtered samples when compared to the unfiltered samples. A statistical comparison using ANOVA confirmed that there was no difference at the 95% confidence level (p-value = 0.59, n = 40). Details of the ANOVA can also be found in Appendix C. A normal probability plot of the ANOVA residuals and residual plots versus estimates and factor levels (provided in Appendix C), demonstrated that the residuals were independent and normally distributed and the ANOVA assumptions were satisfied. It was concluded that pre-filtration through 10 μ m filters effect did not mitigate the tailing region of the fecal coliform inactivation curve.



Figure 18: UV dose-inactivation curves of fecal coliform with and without sample prefiltration through 10 µm PCTE membrane filters for two secondary effluent (pre-UV) wastewater samples collected from the ACRWWTP. (Each point on the graphs represents the average of two collimated beam UV exposures)

The effect of sample pre-filtration through 5 μ m membrane filters on UV inactivation was also examined and compared to the results of sample pre-filtration through 10 μ m membrane filters. For this experiment, UV exposures were conducted on the same wastewater sample collected on the same day. For the purpose of this examination, a UV dose of 10 mJ/cm² was chosen because the expected level of inactivation at this dose lies within the linear portion of the UV dose response curve (Figure 18). A dose of 20 mJ/cm² was also chosen because at this dose the expected inactivation lies in the tailing region of the inactivation curve, yet the level of inactivation could still be quantified accurately. In this experiment, samples of wastewater were exposed to UV light at the two doses (10 and 20 mJ/cm²) and were then divided into the three sub-samples. The first sub-sample was filtered through 5 μ m PCTE membranes prior to fecal coliform analysis. The second sub-sample was not filtered prior to analysis. This experimental design is summarized in Table 6.

| UV Dose | membrane filter | membrane filter | No Filtration | |
|-------------|-----------------|-----------------|---------------|--|
| (mJ/cm^2) | (5 µm) | (10 μm) | | |
| 10 | 2 | 2 | 2 | |
| 20 | 2 | 2 | 2 | |

Table 6 : The experimental design to examine the effects of wastewater sample prefiltration through 5 μm and 10 μm PCTE and membrane filters on UV inactivation, The numbers in the table represent the number of replicate UV exposures.

The results of the inactivation experiments are summarized in Figure 19. Detailed results can be found in Appendix C. Fecal coliform UV inactivation was almost identical for all samples. An ANOVA indicated that there was no statistical difference between the three groups at the 95% confidence level (p-value = 0.69). The ANOVA can also be found in Appendix C.



Figure 19: UV inactivation of fecal coliform with sample pre-filtration through 5 and 10 µm PCTE membrane filters and without sample pre-filtration

The results of the pre-filtration experiments suggest that the tailing in the UV inactivation curve that was evident in Figure 18, was not due to association of the fecal coliform

bacteria with particles greater than 5 μ m. These results were unexpected because Qualls et al. (1983) reported that the inactivation curve of the secondary effluent samples passing through 8 μ m showed evidence of tailing only beyond 4.5 log inactivation units. Unlike the current study, they filtered the secondary effluent samples prior to UV exposure. In current study, secondary effluent samples were filtered after UV exposure and prior to FC analysis to simulate the actual ACRWWTP treatment process.

It is possible that tailing in the UV inactivation curve of the fecal coliform bacteria present in the Capital Region WWTP effluent, was due to association with particulate matter of less than 5 μ m in size and that removal of the tailing effect by sample pre-filtration would, therefore, be difficult. It is also possible that the tailing was due to other causes such as aggregation of microorganisms, presence of a resistant subpopulation or both (Cerf 1977).

At the same time, the results of these experiments showed that the UV dose-inactivation relationship for the fecal coliform bacteria present in the ACRWWTP was approximately linear up to doses of 15 to 20 mJ/cm² and also that inactivation as measured in the collimated beam experiments was reproducible. Sample pre-filtration, therefore, was determined to be ineffective and unnecessary, at least for UV doses up to 20 mJ/cm², and was not used in the subsequent UV reactor performance study.

Particle concentration and size distribution measurements were made on the unfiltered wastewater samples, and wastewater samples filtered through 5 and 10 μ m membranes to determine if there was measurable change in particle size (described in section 3.2.3). Particle size distribution curves for samples of unfiltered wastewater, filtered wastewater through 10 μ m membrane filters and filtered wastewater through 5 μ m membrane filter are shown in Figure 20.



Figure 20: Particle size distribution curves of secondary effluents (without filtration, after filtration through 10 μ m PCTE membrane filters, after filtration through 5 μ m PCTE membrane filters)

The purpose of particle size distribution (PSD) measurements was to check if particles and aggregated fecal coliform bacteria larger than the filter pore size were caught on filters effectively. Figure 20 shows that filtration resulted in a significant change in the PSD and there were far fewer particles in the size range of 10 μ m or greater. Also, filtration through 5 μ m PCTE membrane filter resulted in considerably fewer particles in the size range of 5 μ m or greater remained in the filtered wastewater sample. This finding suggests relatively effective cut-off size of the filtration process, which of course was expected. However, it is not consistent with the UV inactivation findings where tailing was apparent even after filtration prior to FC analysis. The association of fecal coliform bacteria with particulate matter of less than 5 μ m in size, and the presence of a resistant subpopulation possibly explains the difficulty of removing the tailing effect by sample pre-filtration.

4.3 PRELIMINARY UV REACTOR PERFORMANCE TESTING

Preliminary performance testing of the UV reactor was conducted as soon as an upgraded control program that provided the control of the lamp power input (i.e. no UV dose set point) was installed on the ACRWWTP UV reactor system. The control program was provided by the UV reactor system manufacturer. Three different combinations of wastewater flow rate, and UV lamp power input were examined initially to establish the approximate levels of coliform inactivation that might be expected at different reactor UV doses. The flow rate, UV lamp power conditions, and the resulting calculated fluence rate and dose are provided in Table 7. The results of three sets of UV reactor experiments carried out on the same day are provided in Figure 21. Inactivation measured across the UV reactor is plotted as a function of the UV dose calculated by the reactor control system. Each data point is based on analysis of three wastewater samples collected from upstream, and three samples collected from downstream of the UV reactor. Three coliform analyses were conducted on each sample (i.e. total of 9 coliform measurements per sample location). Detailed fecal coliform enumeration information counts for this experiment can be found in Appendix D.

| Test Date | Wastewater Flow Rate Through the UV Reactor Channel 10 ⁶ L/d | Number of UV Lamp Banks in Operation | UV Lamp Power (% of Full Power) | Calculated UV Fluence Rate (mW/cm ²) | Calculated UV Dose (mJ/cm ²) |
|------------|--|--|---------------------------------------|---|--|
| | 15 | 1 | 500/ | 7 | 1.5 |
| 17-Jan-06 | 65 | I | 50% | 7 | 15 |
| 17-Jan- 06 | 35 | 1 | 50% | 9 | 32 |
| 17-Jan-06 | 32 | 2 | 50%, 50% [†] | 10,8 [†] | 71 [‡] |

Table 7 : On-site operating conditions for initial UV reactor performance testing

[†]Two banks were in operation and each number belongs to one bank

[‡] The summation of UV doses resulted from each bank in operation



Figure 21: Inactivation of fecal coliform in the UV reactor versus UV dose computed by the control system

The general shape of the inactivation curve in Figure 21, linear inactivation at low UV dose with tailing at higher doses, is similar to what was measured in the collimated beam experiments (Figure 18). However, the doses required for specific levels of inactivation are different. For example, 1 log inactivation was measured at a calculated UV dose of

approximately 15 mJ/cm² in the UV reactor (Figure 21). In the collimated beam apparatus, the same level of inactivation was achieved at 5 to 6 mJ/cm² (Figure 18). For 3 log inactivation, a dose of approximately 36 to 37 mJ/cm² was required (Figure 21) in the UV reactor while a dose of 10 to 15 mJ/cm² was required in the collimated beam apparatus (Figure 18). These preliminary results suggested that the RED determined in performance testing with fecal coliform, will be considerably lower than the UV dose computed and reported by the UV reactor control system. Also, at the highest UV dose of 71 mJ/cm², the fecal coliform concentration in the UV treated effluent ranged from 3 to 11 CFU/100 mL. At this coliform concentration level in the UV treated effluent, quantification of inactivation is difficult and the results should be considered semiquantitative. At a UV dose of 32 mJ/cm² the coliform concentration was 15 to 29 CFU/100 mL which is near the bottom of the quantifiable range. It should be noted that the dose-inactivation curve of Figure 18 was determined using samples of wastewater that were collected during a time period that did not correspond to the time period of the UV reactor tests of Figure 21. It is possible, therefore, that differences in the level of inactivation were due to differences in the wastewater characteristics. Although, given the relatively good reproducibility of the fecal coliform inactivation curves generated using different wastewater samples (Figure 18), this does not seem to be a likely explanation for the differences that were observed between the collimated beam and UV reactor experiments. Nevertheless, a rigorous comparison between the collimated beam test results and the UV reactor performance, therefore, could not be made.

4.4 RED DETERMINATION IN THE UV REACTOR

The preliminary testing period provided an opportunity to determine the possible operating range of the UV reactor system with the upgraded control system installed. For example, the lowest calculated UV reactor dose that could be studied was 15 mJ/cm². This dose was achieved when the entire plant effluent flow was diverted through a single UV reactor channel (a high flow rate condition) with one bank in operation, and with the lamp power at the lowest setting of 50%. When the plant effluent flow rate was split equally between the two channels (a low flow rate condition), with one bank in operation

and the lamp power at 100%, the calculated UV dose was approximately 30 mJ/cm^2 . The exact UV dose varied from day-to-day with the actual total plant effluent flow rate and the UV transmittance of the water.

An experiment was designed in which four combinations of flow rate (low and high) and lamp power (50% and 100%) were tested as described in Table 3. The low flow rate conditions corresponded to a nominal flow rate of 33 to 37 MLD through the UV reactor channel and the high flow rate corresponded to a nominal flow rate of 54 to 67 MLD. At these conditions, the calculated UV dose varied between 15 and 33 mJ/cm². Each of the four operating conditions was tested independently twice, on separate days. For each test, 5 samples of wastewater were collected from upstream of the UV reactor and 5 samples were collected from downstream of the UV reactor after the reactor had stabilized at the selected operating condition. The fecal coliform concentration was determined in each sample once (no replicate measurements). Detailed fecal coliform enumeration information counts for the real UV reactor performance testing can be found in Appendix D. The single analysis of five samples captures the entire variation in the measurement and is more efficient than repeat analysis of fewer samples. The drawback is that sampling variation cannot be separated from analytical variation. The 95% confidence interval on a mean for fecal coliform concentration in the wastewater collected from upstream of the UV reactor was [6,390 CFU/100 mL - 10,440 CFU/ 100 mL]. This indicates that the true value of the mean lies between the upper and lower confidence interval. The normal probability plot of the fecal coliform concentration in the secondary effluent wastewater upstream of the UV reactor (provided in Appendix D), showed that the concentration was an approximately normally distributed random variable. Table 8 shows the results of the UV reactor testing.

In addition, on each experiment day sub-samples of the wastewater collected from upstream of the UV reactor were exposed to three controlled UV doses (5, 10 and 20 mJ/cm^2) in the laboratory using the collimated beam apparatus. The results of these collimated beam experiments, provided in Figure 22, indicate that the UV dose-inactivation response was relatively consistent during the time period of the experiment.

In Figure 22, each data point is an average of two independent UV exposures done on the experimental day. An analysis of variance (ANOVA) indicated there were no statistical differences between the UV dose-inactivation response on the different days at the 95% confidence level (*p*-value = 0.20). Therefore, the results were pooled to generate an average UV dose-inactivation response function for the testing period. Table 9 shows the results of the UV dose-inactivation relationship that was determined using the collimated beam apparatus.

| Test Date | Flow Rate | Lamp Power | Calc. UV Dose | FC ((CFU/1 | Conc. 100 mL) | FC Conc. Std. Mea Deviation | | Measured Log | Measured RED [§] | |
|-----------|---------------------|---------------|------------------------------------|--------------------------------|---------------------|-----------------------------------|-----|---------------------------|------------------------------|--|
| | 10 ⁶ L/d | % | (mJ/cm ²) [†] | UP Stream N ₀ | Down Stream N | N_0 | N | Inactivation [‡] | (mJ/cm ²) | |
| | | | | | | | | | | |
| 3-Feb-06 | 66 | 50% | 15 | 4,590 | 130 | 814 | 9.9 | 1.55 | 6.1 | |
| 3-Feb-06 | 59 | 100% | 25 | 4,590 | 17 | 814 | 5.1 | 2.44 | 9.8 | |
| | | | | | | | | | | |
| 17-Feb-06 | 54 | 50% | 21 | 9,060 | 183 | 421 | 8.7 | 2.12 | 8.4 | |
| 17-Feb-06 | 67 | 100% | 21 | 9,060 | 69 | 421 | 12 | 1.69 | 6.7 | |
| | | | | | | | | | | |
| 13-Mar-06 | 37 | 50% | 21 | 7,230 | 22.5 | 577 | 5.4 | 2.51 | 10.2 | |
| 13-Mar-06 | 33 | 100% | 33 | 7,230 | 11 | 577 | 3.8 | 2.82 | 11.7 | |
| | | | | | | | | | | |
| 23-Mar-06 | 36 | 50% | 21 | 12,800 | 95 | 480 | 5.4 | 2.13 | 8.5 | |
| 23-Mar-06 | 34 | 100% | 29 | 12,800 | 40 | 480 | 6.3 | 2.50 | 10.1 | |
| | | | | | | | | | | |

Table 8 : Results of UV reactor testing

[†]UV dose reported by UV reactor control system on field control panel

[‡]fecal coliform inactivation based on upstream and downstream coliform concentrations $(-\log N/N_0)$

[§]reduction equivalent dose calculated using measured log inactivation and UV-dose inactivation model (Figure 22, Equations (10) and (12))

| UV Dose | Lower 95% | Upper 95% | Mean |
|-----------------------|---------------------|---------------------|--------------|
| (mJ/cm ²) | confidence interval | confidence interval | inactivation |
| 5 | 1.09 | 1.43 | 1.26 |
| 10 | 2.37 | 2.63 | 2.49 |
| 20 | 3.24 | 3.59 | 3.41 |

Table 9 : Results of fecal coliform UV inactivation determined using the collimated beam apparatus



Figure 22: Fecal coliform UV dose-inactivation curve determined in collimated beam experiments for UV reactor performance testing

The UV-dose inactivation response was modeled using the kinetic model proposed by Emerick et al. (2000) for inactivation of coliform bacteria in wastewater (described previously in section 2.7.2). The model assumes that the coliform bacteria population is composed of a sub-population of freely-dispersed microorganisms that are fully-exposed to the UV fluence rate field in the liquid, and another sub-population of microorganisms

that are attached to particles and receive varying degrees of UV exposure. The mathematical form of the Emerick et al. model, repeated here for convenience, is:

$$N = N_{D,0}e^{-kH} + \frac{N_{P,0}}{kH}(1 - e^{-kH})$$
 Equation (10)

where:

N = total number of viable coliform after exposure to UV dose H

 $N_{D,0}$ = total number of dispersed viable fecal coliform bacterium before UV dose delivery of *H*

 $N_{P,0}$ = total number of particles containing at least one viable fecal coliform bacterium before UV dose delivery of *H*

k = first-order UV inactivation rate constant (cm² mJ⁻¹), and

 $H = UV \text{ dose } (mJ/cm^2)$

The first term on the right hand side of Equation (10) represents inactivation of freelydispersed microorganisms while the second term represents inactivation of particleassociated microorganisms. The model can be completed by defining the fraction of particle-associated microorganisms that form conlonies (CFU) in the wastewater before UV exposure as:

$$\alpha = \frac{N_{P,0}}{N_{D,0} + N_{P,0}}$$
 Equation (12)

Equations (10) and (12) were fit to the UV dose-inactivation data of Figure 22 using nonlinear least-squares regression. The best-fit model is represented by the solid line in Figure 22. The values of the best-fit model coefficients were k = 0.592 cm² mJ⁻¹ and $\alpha = 0.0041$. Particle associated CFU, therefore, represented about 4% of the total population.

The UV dose-inactivation response model of Figure 22 was used to compute the reduction equivalent dose (RED) for the eight UV reactor trials described in Table 8. For each test, the value of dose, H, was calculated using the measured inactivation based on

upstream and downstream fecal coliform concentrations (column 9 in Table 8), and Equation (10). The computed value of H, referred to as the measured RED, ranged from 6.1 mJ/cm² to 11.7 mJ/cm² (column 10 in Table 8).

In Figure 23, the measured RED is compared to the UV dose calculated by the reactor control system and reported on the field control panel. A few anomalies in the computed reactor dose were observed. For example, in the February 17 trials, the computed dose was 21 mJ/cm² at both 100% and 50% lamp power settings. The reason for this anomaly was not clear and could not be investigated since the functioning of the reactor control system and the computational logic was not available. It may possibly be explained by a sudden increase of wastewater flow through the plant. Nevertheless, it was clear that the measured RED was consistently lower than the computed reactor UV dose. On average, the ratio of the measured RED to the computed UV dose was 0.30. This implies that the RED of the UV reactor measured using indigenous fecal coliform bacteria was approximately 70% lower than the dose computed and reported by the UV reactor control system. Further, this ratio was independent of the volumetric flow rate and lamp power level.



Figure 23: Comparison of measured reduction equivalent dose (RED) and the dose calculated by the UV reactor control system. (The dark solid line is a least-squares regression fit of the data. The dotted line represents a perfect match between measured RED and calculated dose.)

4.5 INTERPRETATION OF THE MEASURED RED

The results of the UV reactor testing suggest that biodosimetry testing of the UV reactor using indigenous fecal coliform microorganism will result in measured doses that are considerably lower than the computed UV dose. This does not necessarily suggest an error in computation of the UV reactor dose by the control system. The offset between the biodosimetry measured dose and the calculated dose, may be explained in terms of the UV dose distribution and the bias involved in determining the reduction equivalent dose (RED bias). In an ideal UV reactor, there is no mixing in the direction of water flow (i.e. plug flow) but there is complete mixing across UV fluence rate gradients (i.e. perpendicular to the direction of flow). This type of idealized flow pattern guarantees that each microorganism that enters the reactor has the same residence time, and is exposed to the same average UV fluence rate field. As a result, each microorganism receives an identical UV dose. Many UV reactor dose calculation programs make this idealized flow assumption. A UV fluence rate field is calculated based on the output of the lamps, the UV transmittance of the water and a multiple-point source summation approximation of the UV lamps. The average fluence rate is then computed, and this is multiplied by the hydraulic detection time (V/Q) of the reactor to yield the dose. The ACRWWTP UV reactor uses a similar approach for dose calculation.

For an ideal reactor, the calculated UV dose and RED determined in a biodosimetry test are identical. The ideal reactor also results in the best possible disinfection performance for a given average UV dose. In a real reactor, mixing across fluence rate gradients is less than complete, and the microorganisms receive a distribution of doses around an average. The disinfection performance reflects the shape of dose distribution. A reactor that delivers a broader dose distribution will result in lower inactivation of a particular microorganism than one that delivers a narrower dose distribution, even though the average doses are the same in both reactors.

A well-designed reactor will have a narrow dose distribution and efficient dose delivery. In a real UV reactor with relatively wide dose distribution, the RED determined using biodosimetry will always be different from the computed dose. This difference is referred to as the RED bias. The degree of deviation will depend on the intrinsic resistance of the microorganism used in the biodosimetry test as well as on the shape of the dose distribution. For a given reactor operating condition, the RED determined using a more UV resistant microorganism will be greater than the RED determined using a more UV sensitive microorganism (Wright et al. 2006). If the reactor UV dose distribution and the UV dose-inactivation characteristics of a test microorganism are known, the microorganism survival ratio (N/N_0) in the reactor can be computed by integrating the dose distribution probability density function, P(H), and the UV survival-ratio function, G(H), as follows (Cabaj et al. 1996; Wright 2001)

$$\left[\frac{N}{N_0}\right]_{reactor} = \int_0^\infty P(H)G(H)dH$$
 Equation (13)

As an illustration, consider a case where the dose delivered by a hypothetical real UV reactor is normally distributed. The fecal coliform inactivation in this reactor can be calculated by solving Equation 13 numerically where G(H) is given by the fecal coliform UV dose inactivation kinetic model (Figure 22, Equations 10 and 13). In order to solve Equation (13) numerically, P(H) of each dose was calculated using normal distribution function, as follows:

$$P(H) = \frac{1}{\sigma\sqrt{2\pi}} e^{-0.5(\frac{H-\mu}{STD})^2}$$
 Equation (14)

where:

 σ = standard deviation of UV dose distribution μ = average UV dose H = UV dose (mJ/cm²)

Fecal coliform inactivation at each UV dose, $-\log (N/N_0)$ was calculated using the Emerick (2000) model (Equations (10) and (12)) where k = 0.59 and $\alpha = 0.004$. Then, the survival ration, G(H), at each dose was determined as N/N_0 . This calculation was performed from a dose of H = 1 to H = 50 mJ/cm² using a step size of $\Delta H = 0.5$ mJ/cm². Finally, inactivation of fecal coliform by the UV reactor was integrating Equation (13) numerically as follows:

$$\left[\frac{N}{N_0}\right]_{reactor} = \sum_{1}^{50} P(H)G(H)\Delta H$$
 Equation (15)

The RED was then determined by referring $-\log N/N_0$ calculated for the reactor to the UV dose-response curve of Figure 22. This was substituting the value of N/N_0 calculated from Equation (15) into the Emerick (2000) model and solving for *H* by trial and error. Calculations were performed using solver tool in Microsoft Excel 2003 TM spreadsheet.

Figure 24 shows the hypothetical reactor UV dose distribution function, P(H), with an average UV dose of 20 mJ/cm² and a standard deviation of 6 mJ/cm². The calculation spreadsheet for this case is provided in Appendix E. The resulting fecal coliform inactivation in the reactor was 3.05 which yielded an RED of 13.31 mJ/cm² (67% of the average dose of 20 mJ/cm²). Table 10 provides the results of additional calculations that illustrate how the deviation between the RED and the computed average dose increases with the standard deviation, σ , of the dose distribution.



Figure 24: A hypothetical normal UV dose distribution with an average dose of 20 mJ/cm² and a standard deviation of 6 mJ/cm²

| Average Dose (mJ/cm ²) | Standard Deviation (mJ/cm ²) | Inactivation | RED (mJ/cm ²) | Ratio RED/Average Dose |
|---------------------------------------|--|--------------|---------------------------|------------------------------|
| 20 | 15 | 2.18 | 8.7 | 0.44 |
| 20 | 10 | 2.35 | 9.45 | 0.47 |
| 20 | 6 | 3.05 | 13.31 | 0.67 |
| 20 | 5 | 3.38 | 17.71 | 0.88 |
| 20 | 4.75 | 3.45 | 20 | 1.00 |

Table 10 :Illustration of the effect of dose distribution on fecal coliform RED values

The deviation between the RED and the computed dose observed in the Capital Region UV reactor trials (Table 8, Figure 23), is consistent with a non-ideal dose distribution. Moreover, the computations in Table 10 suggest that the dose distribution in the reactor would need to be relatively broad to account for the observed ratio of the RED to average computed dose of 0.30.

This simplified analysis illustrates the concept of RED bias and how the difference between the observed RED and computed dose can be explained in terms of dose distribution. It also illustrates how the RED bias complicates the definition of the performance of a real reactor system. It is unrealistic to expect perfect mixing and, therefore, an ideal dose distribution in a real UV reactor. As a result, the RED will always be different from the average dose computed based on the assumption of perfect mixing. However, the degree of deviation will be a function of the dose distribution and reflects the overall performance of the reactor.

Ideally, performance of a UV reactor should be described in terms of a dose distribution (i.e. a mean dose and a standard deviation or a dose distribution function.). The dose distribution in a full scale UV reactor is determined by a complex interaction of the fluence rate field and the hydrodynamics and is difficult to predict without the use of computational fluid dynamic (CFD) models. Alternatively, the UV dose of a reactor can be defined in terms of the RED for a particular test microorganism. Most importantly, the concept of RED bias should be understood in regards to the expected RED performance.

5 CONCLUSIONS AND RECOMMENDATIONS

- 1. Indigenous fecal coliform analysis was used to determine the dose delivery in the Alberta Capital Region Wastewater Treatment Plant UV reactor, however, given the concentration of fecal coliform in the secondary effluent the method was only useful up to reactor UV doses of approximately 35 mJ/cm². At higher doses, the number of surviving fecal coliform was small and difficult to quantify. Doses of less than 15 mJ/cm² were difficult to test due to limitations on the lamp power levels and maximum wastewater flow rates. The practical range of doses that could be evaluated at the ACRWWTP, therefore, was 15 to 35 mJ/cm² (calculated dose). Based on this study, the use of indigenous FC bacteria for performance testing and dose determination in the wastewater UV reactors is recommended because RED measured using a UV-sensitive challenge microorganism better reflected the lower end of the reactor's dose distribution (Wright et al. 2006) and there was sufficient concentration of FC bacteria present in the secondary effluent (before UV treatment).
- 2. Sample pre-treatment by membrane filtration had little effect on the tailing of the fecal coliform UV dose-inactivation curve. However, in the range of the measured reduction equivalent doses (RED) 6 to 12 mJ/cm² (collimated beam dose) the dose-inactivation curve was relatively linear and was reproducible. Tailing in the dose-inactivation curve should not be an important issue as long as the measured reduction equivalent doses are less than 15 to 20 mJ/cm². Sample pre-filtration is not recommended for future performance testing of the UV reactor at Alberta Capital Region Wastewater Treatment Plant. This conclusions may not necessarily hold for all wastewater treatment plants.
- 3. The reduction equivalent dose (RED) of the UV reactor measured using indigenous fecal coliform bacteria was approximately 70% lower than the dose computed and reported by the UV reactor control system. The ratio of RED to computed dose was independent of reactor operating conditions. The deviation

between computed RED and calculated dose is consistent with the concept of RED bias and influence of the dose distribution on RED. The degree of deviation between RED and calculated dose that was observed in the UV reactor systems suggests that the standard deviation of the dose distribution was large. The effect of dose distribution on the measured RED must be considered in validation of the UV reactor performance. Ideally, the expected performance of the reactor should be expressed not in terms of an averaged calculated dose, but in terms of the RED of the chosen challenge microorganism. The concept of RED bias should be understood in regards to the expected RED performance. This RED bias, therefore, must be considered carefully when evaluating the performance of wastewater UV reactors using biodisimetry with indigenous fecal coliform bacteria.

- 4. Indigenous somatic coliphage can be used to determine the dose delivery in full-scale wastewater UV reactors if the concentration in the secondary effluent is high enough (greater than 20,000). Coliphage are considered to be more resistant to UV radiation than bacteria (Dizer 1993; Shaban 1997; Tree et al. 1997; Bosch st al. 1989) and, should, therefore, allow for testing of a greater range of UV doses. However, since the coliphage concentrations in the tested BNR secondary effluents (Goldbar WWTP and City of Kelowna WWTP) were not high enough, using indigenous coliphage as an indicator in the biodosimetry testing of the Alberta Capital Region UV reactor was not pursued. Sufficient concentration of coliphage in secondary effluent is a requirement for UV reactor performance testing using biodosimetry. Though, there was coliphage present in all tested secondary effluents the concentration was not sufficient to allow accurate quantitation of log inactivation.
- 5. Use of a more UV-resistant test microorganism should be considered in future reactor testing as this will allow a broader range of operating conditions to be evaluated. A possible candidate is indigenous aerobic spore formers (Wight 2001; Leinan et al. 2002). The concentration of this group of microorganisms in

secondary effluent may be sufficient to permit meaningful biodosimetry but this can only be verified by testing.

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Appendix A: Alberta Capital Region Wastewater Treatment Plant UV Reactor Information (Reported on Control Panel)

| T4 D-4- | Flow Rate | Lamp | Calculated UV | UV Fluence | Number | TSS |
|------------|---------------------|-------|----------------------------|----------------------------|----------|--------|
| I est Date | 10 ⁶ L/d | Power | Dose (mJ/cm ²) | Rate (mW/cm ²) | of Banks | (mg/L) |
| | | | | | | |
| 3-Feb-06 | 66 | 50% | 15 | 6 | 1 | 4 |
| 3-Feb-06 | 59 | 100% | 25 | 9 | 1 | 4 |
| | | | | | | |
| 17-Feb-06 | 54 | 50% | 21 | 7 | 1 | 4 |
| 17-Feb-06 | 67 | 100% | 21 | 9 | 1 | 4 |
| | | | | | | |
| 13-Mar-06 | 37 | 50% | 21 | 6 | 1 | 4 |
| 13-Mar-06 | 33 | 100% | 33 | 9 | 1 | 4 |
| | | | | | | |
| 23-Mar-06 | 36 | 50% | 21 | 5 | 1 | 4 |
| 23-Mar-06 | 34 | 100% | 29 | 9 | 1 | 4 |

Table A- Recorded on-site UV reactor information on test days for full-scale UV reactor performance testing

Appendix B: Raw Data for Determination of the Effect of Sample Storage Time on Coliphage Viability

| Alberta Capital Region effluent sample after 24 hrs. | | | | | | | |
|--|-------------|-------------|-------------|------|--|--|--|
| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. | | | |
| 0.1 | 69 | 67 | 36 | 57 | | | |
| 0.01 | 5 | 2 | 10 | - | | | |
| 0.1 | 70 | 97 | 76 | 81 | | | |
| 0.01 | 0 | 0 | 0 | - | | | |
| 0.1 | 70 | 67 | 58 | 65 | | | |
| 0.01 | 7 | 4 | 2 | - | | | |
| 100 | 67,700 | | | | | | |

Table B1- Coliphage counts with time separation of 24 hours

Alberta Capital Region effluent sample after 48 hrs.

| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. |
|------|-------------|-------------|-------------|------|
| 0.1 | 56 | 76 | 72 | 68 |
| 0.01 | 6 | 7 | 5 | - |
| 0.1 | 51 | 72 | 49 | 57 |
| 0.01 | 7 | 7 | 5 | - |
| 0.1 | 54 | 58 | 87 | 66 |
| 0.01 | 9 | 4 | 8 | - |
| 100 | 63,800 | | | |

Alberta Capital Region effluent sample after 72 hrs.

| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. |
|------|-------------|-------------|-------------|------|
| 0.1 | 53 | 73 | 69 | 65 |
| 0.01 | 10 | 5 | 7 | - |
| 0.1 | 74 | 70 | 75 | 73 |
| 0.01 | 0 | 0 | 0 | - |
| 0.1 | 67 | 62 | 61 | 63 |
| 0.01 | 7 | 4 | 2 | - |
| 100 | 67,100 | | | |

Table B2- ANOVA for the coliphage detection data as with storage time Anova: Single Factor

SUMMARY

| Groups | Count | Sum | Average | Variance |
|----------|-------|--------|----------|----------|
| Column 1 | 3 | 203000 | 67666.67 | 1.49E+08 |
| Column 2 | 3 | 192500 | 64166.67 | 15361111 |
| Column 3 | 3 | 205500 | 68500 | 23694444 |

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---------------------|-----------|----|----------|---------|----------|----------|
| Between Groups | 31722222 | 2 | 15861111 | 0.25258 | 0.784657 | 5.143249 |
| Within Groups | 376777778 | 6 | 62796296 | | | |
| | | | | | | |
| Total | 408500000 | 8 | | | | |

Appendix C: Experimental Data and Information for Wastewater Sample Pre-

treatment by Membrane Filtration

| Table C1- Log mactivation Results | | | | | | | | |
|-----------------------------------|--------|---------|---------|---------|---------|--|--|--|
| Sample | Dose 5 | Dose 10 | Dose 15 | Dose 20 | Dose 30 | | | |
| Unfiltered | 0.76 | 1.59 | 2.86 | 3.68 | 4.28 | | | |
| Unfiltered | 0.86 | 2.39 | 3.50 | 3.88 | 4.58 | | | |
| Unfiltered | 1.31 | 2.54 | 3.43 | 3.51 | 3.91 | | | |
| Unfiltered | 1.13 | 2.69 | 3.61 | 3.91 | 3.91 | | | |
| Pre-Filtered | 0.74 | 2.89 | 4.11 | 4.11 | 4.11 | | | |
| Pre-Filtered | 0.93 | 3.11 | 3.21 | 4.11 | 4.41 | | | |
| Pre-Filtered | 1.01 | 2.90 | 3.12 | 3.43 | 3.43 | | | |
| Pre-Filtered | 0.90 | 2.60 | 3.43 | 3.25 | 3.73 | | | |

Table C1- Log Inactivation Results

Table C2- ANOVA for UV inactivation of pre-filtered samples and unfiltered samples Anova: Two-Factor With Replication

| SUMMARY Unfiltered | Dose 5 | Dose 10 | Dose 15 | Dose 20 | Dose 30 | Total |
|-----------------------|--------|---------|---------|---------|---------|-------|
| Count | 4 | 4 | 4 | 4 | 4 | 20 |
| Sum | 4.07 | 9.21 | 13.40 | 14.98 | 16.68 | 58.33 |
| Average | 1.02 | 2.30 | 3.35 | 3.74 | 4.17 | 2.92 |
| Variance | 0.06 | 0.24 | 0.11 | 0.03 | 0.11 | 1.44 |

| Pre-Filtered | | | | | | |
|--------------|------|-------|-------|-------|-------|-------|
| Count | 4 | 4 | 4 | 4 | 4 | 20 |
| Sum | 3.57 | 11.50 | 13.87 | 14.90 | 15.67 | 59.52 |
| Average | 0.89 | 2.88 | 3.47 | 3.72 | 3.92 | 2.98 |
| Variance | 0.01 | 0.04 | 0.20 | 0.20 | 0.19 | 1.37 |

| Total | | | | | |
|----------|------|-------|-------|-------|-------|
| Count | 8 | 8 | 8 | 8 | 8 |
| Sum | 7.64 | 20.72 | 27.27 | 29.87 | 32.35 |
| Average | 0.95 | 2.59 | 3.41 | 3.73 | 4.04 |
| Variance | 0.04 | 0.22 | 0.14 | 0.10 | 0.14 |

| ANOVA | | | | | | |
|---------------------|-------|----|-------|--------|---------|--------|
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Sample | 0.03 | 1 | 0.03 | 0.29 | 0.59 | 4.17 |
| Columns | 49.05 | 4 | 12.26 | 102.04 | 0.00 | 2.69 |
| Interaction | 0.80 | 4 | 0.20 | 1.67 | 0.18 | 2.69 |
| Within | 3.61 | 30 | 0.12 | | | |
| | | | | | | |
| Total | 53.50 | 39 | | | | |



Figure C1- Normal probability plot for ANOVA residuals of UV inactivation of fecal coliform with and without sample pre-treatment



Figure C2- Residual plot versus ANOVA estimates



Figure C3- Residual plot versus factor levels (UV Dose)



Figure C4- Residual plot versus factor level (Pre-Filtered- Unfiltered)

Table C3- UV inactivation results for wastewater pre-filtered through 5 and 10 μm membrane filters. A wastewater sample was collected and the sub-samples were filtered

| Sample | Dose 10 | Dose 20 |
|-----------------|---------|---------|
| Filtered (5µm) | 3.01 | 3.86 |
| Filtered (5µm) | 2.17 | 3.86 |
| Filtered (10µm) | 2.73 | 4.13 |
| Filtered (10µm) | 2.39 | 4.13 |
| No Filtration | 2.67 | 3.75 |
| No Filtration | 2.58 | 3.75 |

| and exposed | to | UV. |
|-------------|----|-----|
|-------------|----|-----|

Table C4- ANOVA for UV inactivation of pre-filtered samples through 5 and 10 μm

membrane filters and unfiltered samples

Anova: Two-Factor With Replication

| SUMMARY <i>Filter 5µm</i> | Dose 10 | Dose 20 | Total |
|------------------------------|---------|---------|-------|
| Count | 2 | 2 | 4 |
| Sum | 5.18 | 7.72 | 12.90 |
| Average | 2.59 | 3.86 | 3.23 |
| Variance | 0.35 | 0.00 | 0.66 |

| Filter 10µm | | | |
|-------------|------|------|-------|
| Count | 2 | 2 | 4 |
| Sum | 5.12 | 8.26 | 13.38 |
| Average | 2.56 | 4.13 | 3.35 |
| Variance | 0.06 | 0.00 | 0.84 |

| No Filtration | | | _ |
|---------------|------|------|-------|
| Count | 2 | 2 | 4 |
| Sum | 5.25 | 7.50 | 12.75 |
| Average | 2.63 | 3.75 | 3.19 |
| Variance | 0.00 | 0.00 | 0.42 |

| Total | | _ |
|----------|-------|-------|
| Count | 6 | 6 |
| Sum | 15.55 | 23.48 |
| Average | 2.59 | 3.91 |
| Variance | 0.08 | 0.03 |

| A | N | 0 | ٧ | 'A |
|---|---|---|---|----|
|---|---|---|---|----|

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---------------------|------|----|------|-------|---------|--------|
| Sample | 0.05 | 2 | 0.03 | 0.39 | 0.69 | 5.14 |
| Columns | 5.24 | 1 | 5.24 | 75.83 | 0.00 | 5.99 |
| Interaction | 0.10 | 2 | 0.05 | 0.75 | 0.51 | 5.14 |
| Within | 0.41 | 6 | 0.07 | | | |
| Total | 5.81 | 11 | | | | - |

Appendix D: Experimental Data and Information for the UV Reactor Performance

Testing

Table D1- Fecal coliform counts for the preliminary UV reactor performance testing

| | UP Stream UV | | | | | | |
|-----|--------------|-------------|-------------|------|--|--|--|
| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. | | | |
| 1 | 102 | 127 | 116 | - | | | |
| 0.1 | 20 | 18 | 19 | 19 | | | |
| 100 | 19,000 | | | | | | |

UP Stream UV Replicate 2 Replicate 3 ml **Replicate 1** Ave. 103 129 137 1 -0.1 23 16 21 23 100 21,000

UP Stream UV Replicate 1 Replicate 2 Replicate 3 ml Ave. 1 98 125 128 0.1 16 15 13 8 13,000 100

Dose 15 mJ/cm² **Replicate 1 Replicate 2 Replicate 3** Ave. ml TMTC TMTC TMTC 100 TMTC 132 150 10 141 14 21 19 1 22 0.1 1 2 2 -1,900 100

| Dose 15 mJ/cm ² | | | | | | |
|----------------------------|-------------|-------------|-------------|------|--|--|
| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. | | |
| 100 | TMTC | TMTC | TMTC | TMTC | | |
| 10 | 160 | 138 | 149 | - | | |
| 1 | 25 | 14 | 23 | 21 | | |
| 0.1 | 1 | 5 | 8 | - | | |
| 100 | 2,100 | | | | | |

Dose 15 mJ/cm² **Replicate 2 Replicate 3** ml **Replicate 1** Ave. TMTC 100 TMTC TMTC TMTC 137 10 125 112 14 17 1 14 23 0.1 1 3 0 -100 1,700

| Dose 32 mJ/cm ² | | | | | | |
|----------------------------|-------------|-------------|-------------|------|--|--|
| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. | | |
| 100 | 17 | 16 | 35 | 23 | | |
| 10 | 0 | 0 | 4 | - | | |

Dose 32 mJ/cm²

| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. |
|-----|-------------|-------------|-------------|------|
| 100 | 10 | 40 | 37 | 29 |
| 10 | 1 | 0 | 0 | - |

Dose 32 mJ/cm²

| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. |
|-----|-------------|-------------|-------------|------|
| 100 | 23 | 7 | 15 | 15 |
| 10 | 1 | 0 | 0 | - |

Dose 71 mJ/cm²

| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. |
|-----|-------------|-------------|-------------|------|
| 100 | 4 | 2 | 2 | 3 |

Dose 71 mJ/cm²

| mi | Replicate 1 | Replicate 2 | Replicate 3 | Ave. |
|-----|-------------|-------------|-------------|------|
| 100 | 4 | 8 | 1 | 4 |

Dose 71 mJ/cm²

| ml | Replicate 1 | Replicate 2 | Replicate 3 | Ave. |
|-----|-------------|-------------|-------------|------|
| 100 | 6 | 16 | 10 | 11 |

| Lamp power 100%, High flow Dose 25 mJ/cm² | | | |
|--|------|---|--|
| mL 100 10 | | | |
| Sample 1 | 25 | 3 | |
| Sample 2 | 18 | 2 | |
| Sample 3 | 15 | 0 | |
| Sample 4 | 12 | 0 | |
| Sample 5 | 14 | 0 | |
| Ave. | 16.8 | - | |

Table D2- Fecal coliform counts for the real UV reactor performance testing

Lamp power 50%, High flow Dose 15 mJ/cm²

| mL | 100 | 10 | |
|----------|-----|----|--|
| Sample 1 | 145 | 15 | |
| Sample 2 | 135 | 13 | |
| Sample 3 | 121 | 12 | |
| Sample 4 | 124 | 9 | |
| Sample 5 | 125 | 16 | |
| Ave. | 130 | 13 | |

Lamp power 100%, Low flow Dose 33 mJ/cm²

| mL | 100 | 10 | |
|----------|-----|----|--|
| Sample 1 | 15 | 1 | |
| Sample 2 | 13 | 0 | |
| Sample 3 | 8 | 1 | |
| Sample 4 | 18 | 1 | |
| Sample 5 | 16 | 1 | |
| Ave. | 14 | - | |

Lamp power 50%, Low flow Dose 21 mJ/cm²

| mL | 100 | 10 | |
|----------|-----|----|--|
| Sample 1 | 25 | 2 | |
| Sample 2 | 35 | 3 | |
| Sample 3 | 27 | 2 | |
| Sample 4 | 20 | 0 | |
| Sample 5 | 28 | 2 | |
| Ave. | 27 | - | |

Lamp power 100%, High flow

| Dose_21 | mJ/cm | |
|----------|-------|------|
| mL | 100 | 10 |
| Sample 1 | 144 | 26 |
| Sample 2 | 162 | 20 |
| Sample 3 | 154 | 19 |
| Sample 4 | 167 | 17 |
| Sample 5 | 158 | 24 |
| Ave. | - | 21.2 |

Lamp power 50%, High flow Dose 21 mJ/cm²

| mL | 100 | 10 |
|----------|-----|-----|
| Sample 1 | 75 | 5 |
| Sample 2 | 53 | 6 |
| Sample 3 | 76 | 9 |
| Sample 4 | 62 | 6 |
| Sample 5 | 84 | 8 |
| Ave. | 70 | 6.8 |

Lamp power 100%, Low flow Dose 29 mJ/cm²

| mL | 100 | 10 | |
|----------|------|----|--|
| Sample 1 | 32 | 5 | |
| Sample 2 | 39 | 7 | |
| Sample 3 | 30 | 6 | |
| Sample 4 | 43 | 4 | |
| Sample 5 | 28 | 1 | |
| Ave. | 34.4 | - | |

Lamp power 50%, Low flow

| Dose 21 mJ/cm ⁻ | | |
|----------------------------|------|-----|
| mL | 100 | 10 |
| Sample 1 | 95 | 11 |
| Sample 2 | 99 | 10 |
| Sample 3 | 89 | 7 |
| Sample 4 | 98 | 11 |
| Sample 5 | 87 | 9 |
| Ave. | 93.6 | 9.6 |



Figure D- Normal probability plot for fecal coliform concentration in wastewater samples collected from upstream of the UV reactor

Appendix E: A Sample Calculation for Inactivation of Fecal Coliform by UV Reactor

| μ | 20 | α |
|----|-----|---|
| σ | 6 | k |
| | | |
| ΔH | 0.5 | |

| α | 0.004 |
|---|-------|
| k | 0.592 |

| | | | Calculated FC | | | |
|----------|----------|--|------------------------------------|-----------|-------------|---|
| | | | Inactivation | G(H) | | Integral |
| <u>D</u> | P(D) | | = -log (<i>N/N</i> ₀) | $= N/N_0$ | P(D)G(H) | <i>P</i> (<i>D</i>) <i>G</i> (<i>H</i>)Δ <i>H</i> |
| 1 | 0.000221 | | 0.256414848 | 0.554096 | 0.000122408 | |
| 1.5 | 0.000287 | | 0.384509211 | 0.412563 | 0.000118253 | 6.01652E-05 |
| 2 | 0.000369 | | 0.512502091 | 0.307254 | 0.000113475 | 5.79321E-05 |
| 2.5 | 0.000473 | | 0.640367222 | 0.228893 | 0.000108169 | 5.5411E-05 |
| 3 | 0.000601 | | 0.768071204 | 0.17058 | 0.000102435 | 5.26509E-05 |
| 3.5 | 0.000758 | | 0.895571547 | 0.127183 | 9.63786E-05 | 4.97033E-05 |
| 4 | 0.00095 | | 1.022814182 | 0.094882 | 9.01065E-05 | 4.66213E-05 |
| 4.5 | 0.001182 | | 1.149730345 | 0.070839 | 8.37224E-05 | 4.34572E-05 |
| 5 | 0.001461 | | 1.276232666 | 0.052938 | 7.7326E-05 | 4.02621E-05 |
| 5.5 | 0.001793 | | 1.402210346 | 0.039609 | 7.10097E-05 | 3.70839E-05 |
| 6 | 0.002185 | | 1.52752328 | 0.029681 | 6.48573E-05 | 3.39668E-05 |
| 6.5 | 0.002645 | | 1.651995026 | 0.022285 | 5.89421E-05 | 3.09498E-05 |
| 7 | 0.003179 | | 1.775404656 | 0.016772 | 5.33259E-05 | 2.8067E-05 |
| 7.5 | 0.003795 | | 1.897477648 | 0.012663 | 4.80586E-05 | 2.53461E-05 |
| 88 | 0.004499 | | 2.017876352 | 0.009597 | 4.3178E-05 | 2.28092E-05 |
| 8.5 | 0.005297 | | 2.136190988 | 0.007308 | 3.871E-05 | 2.0472E-05 |
| 9 | 0.006193 | | 2.251932791 | 0.005598 | 3.46689E-05 | 1.83447E-05 |
| 9.5 | 0.00719 | | 2.364531704 | 0.00432 | 3.10587E-05 | 1.64319E-05 |
| 10 | 0.00829 | | 2.473341743 | 0.003362 | 2.7874E-05 | 1.47332E-05 |
| 10.5 | 0.009492 | | 2.577657516 | 0.002644 | 2.51012E-05 | 1.32438E-05 |
| 11 | 0.010793 | | 2.67674483 | 0.002105 | 2.27197E-05 | 1.19552E-05 |
| 11.5 | 0.012188 | | 2.769886372 | 0.001699 | 2.07033E-05 | 1.08557E-05 |
| 12 | 0.013667 | | 2.856439763 | 0.001392 | 1.90217E-05 | 9.93125E-06 |
| 12.5 | 0.015221 | | 2.93590062 | 0.001159 | 1.76415E-05 | 9.16579E-06 |
| 13 | 0.016833 | | 3.007959202 | 0.000982 | 1.65275E-05 | 8.54225E-06 |
| 13.5 | 0.018488 | | 3.072537976 | 0.000846 | 1.56439E-05 | 8.04284E-06 |
| 14 | 0.020164 | | 3.129800728 | 0.000742 | 1.49548E-05 | 7.64966E-06 |
| 14.5 | 0.021841 | | 3.180130797 | 0.00066 | 1.44256E-05 | 7.34509E-06 |
| 15 | 0.023493 | | 3.224084161 | 0.000597 | 1.40232E-05 | 7.1122E-06 |

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| 15.5 | 0.025095 | 3.262328667 | 0.000547 | 1.37168E-05 | 6.93501E-06 |
|------|----------|-------------|----------|-------------|-------------|
| 16 | 0.026621 | 3.295582043 | 0.000506 | 1.34783E-05 | 6.7988E-06 |
| 16.5 | 0.028044 | 3.324558609 | 0.000474 | 1.32825E-05 | 6.69021E-06 |
| 17 | 0.029339 | 3.349930015 | 0.000447 | 1.31072E-05 | 6.59743E-06 |
| 17.5 | 0.030481 | 3.372300962 | 0.000424 | 1.29339E-05 | 6.51028E-06 |
| 18 | 0.031449 | 3.392197887 | 0.000405 | 1.27469E-05 | 6.42018E-06 |
| 18.5 | 0.032222 | 3.410067306 | 0.000389 | 1.2534E-05 | 6.32021E-06 |
| 19 | 0.032787 | 3.426280392 | 0.000375 | 1.22862E-05 | 6.20504E-06 |
| 19.5 | 0.03313 | 3.441140976 | 0.000362 | 1.19972E-05 | 6.07084E-06 |
| 20 | 0.033245 | 3.454894892 | 0.000351 | 1.16636E-05 | 5.9152E-06 |
| 20.5 | 0.03313 | 3.467739371 | 0.000341 | 1.12845E-05 | 5.73702E-06 |
| 21 | 0.032787 | 3.479831713 | 0.000331 | 1.08609E-05 | 5.53633E-06 |
| 21.5 | 0.032222 | 3.491296905 | 0.000323 | 1.03958E-05 | 5.31418E-06 |
| 22 | 0.031449 | 3.502234077 | 0.000315 | 9.89388E-06 | 5.07243E-06 |
| 22.5 | 0.030481 | 3.512721836 | 0.000307 | 9.36068E-06 | 4.81364E-06 |
| 23 | 0.029339 | 3.5228226 | 0.0003 | 8.80276E-06 | 4.54086E-06 |
| 23.5 | 0.028044 | 3.532586076 | 0.000293 | 8.2272E-06 | 4.25749E-06 |
| 24 | 0.026621 | 3.542052016 | 0.000287 | 7.64129E-06 | 3.96712E-06 |
| 24.5 | 0.025095 | 3.551252391 | 0.000281 | 7.0523E-06 | 3.6734E-06 |
| 25 | 0.023493 | 3.560213092 | 0.000275 | 6.46723E-06 | 3.37988E-06 |
| 25.5 | 0.021841 | 3.568955262 | 0.00027 | 5.89263E-06 | 3.08997E-06 |
| 26 | 0.020164 | 3.577496326 | 0.000265 | 5.33439E-06 | 2.80675E-06 |
| 26.5 | 0.018488 | 3.585850795 | 0.00026 | 4.79768E-06 | 2.53302E-06 |
| 27 | 0.016833 | 3.594030889 | 0.000255 | 4.28683E-06 | 2.27113E-06 |
| 27.5 | 0.015221 | 3.602047017 | 0.00025 | 3.8053E-06 | 2.02303E-06 |
| 28 | 0.013667 | 3.609908152 | 0.000246 | 3.35568E-06 | 1.79024E-06 |
| 28.5 | 0.012188 | 3.617622121 | 0.000241 | 2.9397E-06 | 1.57385E-06 |
| 29 | 0.010793 | 3.625195829 | 0.000237 | 2.5583E-06 | 1.3745E-06 |
| 29.5 | 0.009492 | 3.63263543 | 0.000233 | 2.21165E-06 | 1.19249E-06 |
| 30 | 0.00829 | 3.639946471 | 0.000229 | 1.89931E-06 | 1.02774E-06 |
| 30.5 | 0.00719 | 3.647133992 | 0.000225 | 1.62025E-06 | 8.79888E-07 |
| 31 | 0.006193 | 3.654202609 | 0.000222 | 1.373E-06 | 7.48311E-07 |
| 31.5 | 0.005297 | 3.661156586 | 0.000218 | 1.15573E-06 | 6.32182E-07 |
| 32 | 0.004499 | 3.667999882 | 0.000215 | 9.66361E-07 | 5.30523E-07 |
| 32.5 | 0.003795 | 3.674736192 | 0.000211 | 8.02625E-07 | 4.42247E-07 |
| 33 | 0.003179 | 3.681368983 | 0.000208 | 6.62176E-07 | 3.662E-07 |
| 33.5 | 0.002645 | 3.687901523 | 0.000205 | 5.42649E-07 | 3.01206E-07 |
| 34 | 0.002185 | 3.694336897 | 0.000202 | 4.41718E-07 | 2.46092E-07 |
| 34.5 | 0.001793 | 3.70067803 | 0.000199 | 3.5715E-07 | 1.99717E-07 |
| 35 | 0.001461 | 3.7069277 | 0.000196 | 2.86834E-07 | 1.60996E-07 |

| | 1 | | | • | |
|------|----------|-------------|----------|-------------------|-------------|
| 35.5 | 0.001182 | 3.713088553 | 0.000194 | 2.28814E-07 | 1.28912E-07 |
| 36 | 0.00095 | 3.719163112 | 0.000191 | 1.81304E-07 | 1.0253E-07 |
| 36.5 | 0.000758 | 3.725153786 | 0.000188 | 1.42692E-07 | 8.09989E-08 |
| 37 | 0.000601 | 3.73106288 | 0.000186 | 1.11546E-07 | 6.35595E-08 |
| 37.5 | 0.000473 | 3.7368926 | 0.000183 | 8.66116E-08 | 4.95395E-08 |
| 38 | 0.000369 | 3.742645062 | 0.000181 | 6.67972E-08 | 3.83522E-08 |
| 38.5 | 0.000287 | 3.748322295 | 0.000179 | 5.11681E-08 | 2.94913E-08 |
| 39 | 0.000221 | 3.753926249 | 0.000176 | 3.89312E-08 | 2.25248E-08 |
| 39.5 | 0.000169 | 3.759458794 | 0.000174 | 2.94206E-08 | 1.7088E-08 |
| 40 | 0.000129 | 3.764921733 | 0.000172 | 2.20831E-08 | 1.28759E-08 |
| 40.5 | 9.7E-05 | 3.770316797 | 0.00017 | 1.64634E-08 | 9.63661E-09 |
| 41 | 7.27E-05 | 3.775645655 | 0.000168 | 1.21907E-08 | 7.16352E-09 |
| 41.5 | 5.41E-05 | 3.780909914 | 0.000166 | 8.96577E-09 | 5.28912E-09 |
| 42 | 4E-05 | 3.786111121 | 0.000164 | 6.54927E-09 | 3.87876E-09 |
| 42.5 | 2.94E-05 | 3.791250771 | 0.000162 | 4.75165E-09 | 2.82523E-09 |
| 43 | 2.14E-05 | 3.796330305 | 0.00016 | 3.42405E-09 | 2.04392E-09 |
| 43.5 | 1.55E-05 | 3.801351112 | 0.000158 | 2.45063E-09 | 1.46867E-09 |
| 44 | 1.12E-05 | 3.806314536 | 0.000156 | 1.74204E-09 | 1.04817E-09 |
| 44.5 | 7.96E-06 | 3.811221874 | 0.000154 | 1.22992E-09 | 7.4299E-10 |
| 45 | 5.65E-06 | 3.816074379 | 0.000153 | 8.62455E-10 | 5.23094E-10 |
| 45.5 | 3.98E-06 | 3.820873264 | 0.000151 | 6.00666E-10 | 3.6578E-10 |
| 46 | 2.78E-06 | 3.8256197 | 0.000149 | 4.15495E-10 | 2.5404E-10 |
| 46.5 | 1.93E-06 | 3.830314823 | 0.000148 | 2.85453E-10 | 1.75237E-10 |
| 47 | 1.33E-06 | 3.834959729 | 0.000146 | 1.94777E-10 | 1.20057E-10 |
| 47.5 | 9.12E-07 | 3.839555481 | 0.000145 | 1 <u>.</u> 32E-10 | 8.16941E-11 |
| 48 | 6.21E-07 | 3.844103109 | 0.000143 | 8.88466E-11 | 5.52116E-11 |
| 48.5 | 4.19E-07 | 3.848603611 | 0.000142 | 5.93937E-11 | 3.70601E-11 |
| 49 | 2.81E-07 | 3.853057952 | 0.00014 | 3.94339E-11 | 2.47069E-11 |
| 49.5 | 1.87E-07 | 3.857467071 | 0.000139 | 2.60033E-11 | 1.63593E-11 |
| 50 | 1.24E-07 | 3.861831877 | 0.000137 | 1.703E-11 | 1.07583E-11 |
| 50.5 | 8.14E-08 | 3.866153251 | 0.000136 | 1.10772E-11 | 7.02681E-12 |

| SUM | 0.000883779 |
|---------------------|-------------|
| Log Inactivation | 3.053656435 |

RED 13.31885192

0.999333