DISINFECTION OF WASTEWATER EFFLUENTS: COMPARE ADVANCED OXIDATION PROCESS (AOP) WITH PERACETIC ACID AND ULTRAVIOLET RADIATION

BY

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Disinfection of Wastewater Effluents:

Compare Advanced Oxidation Process (AOP) with Peracetic Acid and Ultraviolet Radiation

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Esteban Rodrigo Madrid

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

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ABSTRACT

Wastewater treatment facilities are often faced with the need to find disinfection methods that meet increasingly stringent guidelines for wastewater effluent discharged to the environment. Currently, advanced oxidation processes (AOPs) are being researched as a technique to enhance the disinfection of pathogenic organisms in order to establish an effluent quality that is safe for public health. Wastewater effluent collected from the North End Water Pollution Control Centre (NEWPCC), Winnipeg, Canada, were disinfected with peracetic acid (PAA), ultraviolet (UV) light, and/or the combination of both (PAA/UV). The main parameter that influences UV light disinfection is UV transmissivity (UVT). The NEWPCC effluent UVT averages 47.3 ± 4.2% during normal treatment plant conditions. Thus, without altering the physical properties of wastewater effluent, this research assessed disinfection of the indicator organism – fecal coliform bacteria – by using the disinfectants individually or in combination (PAA/UV). Concentrations of PAA used were 0, 2, and 8 mg/L, whereas the fluences (dosages) of UV radiation used were 0, 5, 10, 20, and 40-mWs/cm^2. Wastewater effluent was taken from the final effluent conduit on two occasions; effluent collected during a dry weather condition was designated as Phase 1, whereas effluent collected during a wet weather condition was designated as Phase 2.

Results obtained from treatment of Phase 1 effluents indicate that UV and both AOP treatments tested sufficiently inactivated fecal coliform bacteria to the standard discharge limit (200-MPN/100ml). The UV alone treatment required a fluence of 28-mWs/cm^2 of radiation, whereas the 2 mg PAA/L AOP treatment required 22-mWs/cm^2. The latter treatment produced a 21% saving in UV energy costs when compared to UV alone. The UV treatment supplemented with 8 mg PAA/L out-performed all other treatment methods. It produced the standard effluent quality using 10-mWs/cm^2, which amounts to a 64% to energy savings compared to UV alone. The rapid inactivation of fecal coliform in treatments with both disinfectants could be accounted to synergism. Due to the positive response to indicator inactivation in Phase 1 tests, the feasibility of using AOP for treating Phase 2 effluents was evaluated. Phase 2 tests indicate that within the fluence limit provided disinfection of fecal coliform to the standard guideline was not achieved.

Separate disinfection tests were performed on wastewater effluent with somatic coliphage ϕX174 and reovirus seeded into it. These particles' susceptibility to the disinfection agents were assessed as well. Comparison of UV disinfection to 2 mg/L AOP and 8 mg/L AOP were evaluated. It was determined that low UV fluence (<10 mWs/cm^2) was sufficient to provide total reduction of somatic coliphage, whereas reovirus particles showed strong resistance to UV disinfection. An 8 mg PAA/L combined with 10 mWs/cm^2 treatment could reduce reovirus particles by 5 logs, which is virtually a pathogen free effluent.

Comparison of somatic coliphage and reovirus inactivation to poliovirus by UV disinfection was compared. A five log reduction of poliovirus is postulated by the National Water Research Institute (NWRI). This value is considered adequate disinfection for treated wastewater effluent designated for reuse. Results indicate that coliphages showed greater susceptibly to UV disinfection, more so than poliovirus particles, whereas reovirus showed high resistance to UV radiation. Specifically, a 15 mWs/cm^2 of UV radiation inactivated somatic coliphage by five log reduction, whereas, 35 mWs/cm^2 UV radiation was required to produce a five log reduction of reovirus.

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1. INTRODUCTION

1.1 Disinfection

The purpose of disinfection is to eliminate all the disease-causing organisms from the desired medium (WERF, 1995). In this study, the medium was wastewater effluent. For most of the twentieth century, disinfection of wastewater effluents was provided by chlorine, due to its low cost, ease of handling, and its ability to provide a disinfecting residual. During the mid-1970s, however, it was discovered that chlorine disinfection produced unwanted disinfection by-products (EPA, 2004). These compounds are organo-chlorinated compounds that include trihalomethanes (THMs). These compounds have been found to be acutely toxic to various species of fishes and aquatic organisms and possibly carcinogenic to humans (Liberti et al., 2002; Jolly et al., 1990; Whitby et al., 1984; Ward and DeGraeve, 1978; Oliver and Carey, 1976). These compounds are hazardous to natural ecosystems and public health wherever discharged. It is therefore important to search for innovative, alternative treatment methods that do not pose such risks.

Recently, UV radiation has received attention as an alternative to chlorine for disinfecting wastewater effluents. UV has several advantages with the added benefit of being cost-comparable and environmentally friendly compared to other disinfection methods (Savoye et al., 2001; Savolainen, 1991; Whitby et al., 1984).

Depending on the initial bacterial concentration in wastewater, a 4-5 $\log_{10}$ reduction is recommended for inactivating fecal indicator bacteria to comply to standard regulation (typically a measure of 200 Most Probable Number (MPN) per 100ml fecal coliforms) (WERF, 1995). For virus inactivation, the National Water Research Institute

(NWRI) has established five log reduction is required to achieve elimination of poliovirus from treated wastewater reuse (NWRI, 2003).

With these standards in mind, this bench-scale study will examine some of the factors that the North End Water Pollution Control Centre (NEWPCC), a wastewater treatment facility in Winnipeg, would have to assess if a decision to introduce a new disinfection process to treat their effluent is made.

1.2 Winnipeg's NEWPCC Secondary Treatment Facility: Identifying the Problem

The NEWPCC is one of three treatment facilities managed by the City of Winnipeg, Canada. The NEWPCC treats the city's northern residential wastewater, most of the industrial wastewater and during the summer, leachate from the local landfill. Currently, this treatment facility provides secondary treatment with a pure oxygen activated sludge plant with no disinfection to the final effluent. Currently, however, the NEWPCC is in the process of implementing an ultraviolet (UV) disinfection system.

Wastewater effluent has a number of characteristics that define its quality. These include total suspended solids (TSS), total organic carbon (TOC), soluble organic carbon (SOC), and turbidity. These factors influence the UV transmissivity (UVT) through the wastewater. This parameter (i.e. UVT) is one of the most important parameters for establishing a well designed disinfection system. AWWARF and AWWA (1992) showed that 85-95% transmissivity are good to excellent indicator for an efficient UV disinfection system. A historical mean of NEWPCC effluent parameters described above

is shown in Table 1-1. It can be determined that effluent discharge from the NEWPCC has a relatively low average UVT value (47.3 ± 4.2%).

Manitoba Conservation requires that the standard guideline for maximum allowable fecal coliform (FC) concentration discharged into watersheds be no greater than 200-MPN/100ml (Ralley, 2004). A study conducted during the summer of 2003 determined that 35mJ/cm^2 of UV dose (UV fluence) was adequate to achieve provincial guidelines for dry-weather periods (Earth Tech, 2004). However, this energy level is insufficient to disinfect higher FC concentrations which occur during higher plant flows, such as during wet weather periods. A wet weather event constitutes to plant flows that exceed 380 Million litres per day (MLD). The plant receives combined sewage during rainfall events. Combined sewage inflows to the plant can result in increased concentration of TSS, TOC, turbidity and decreased UVT to the treated effluent during wet weather periods.

Table 1-1. NEWPCC historical data on water quality parameters of Wastewater samples collected between 1996 to 2002, during dry weather periods.

Water Quality Parameters	1996 - 2002
	Mean
Raw Sewage Flow Rate	231 MLD
TSS	11 mg/L
UVT	46 %
Filtered UVT	55 %
TOC	24 mg/L
SOC	21 mg/L

1.3 Advanced Oxidation Processes (AOPs)

Methods that combine multiple disinfection procedures are collectively known as advanced oxidation processes (AOP) (IWA, 2004). These methods have been developed to enhance the treatment capability to eliminate toxic pollutants or biological organisms (IWA, 2004). Some important AOP that use UV as the catalyst are listed in Table 1-2.

Peracetic acid (PAA) combined with UV is an important AOP that is gaining recognition for its synergistic effects on biological inactivation (Chen et al., 2005; Caretti and Lubello, 2003). Several researchers have determined that peracetic acid alone is an effective disinfectant for treating combined sewer overflow (CSO) and biologically treated effluents (Gehr et al., 2002; EPA, 1999). The NEWPCC currently receives combined sewage and produces biologically treated effluent. Therefore this plant might benefit from using PAA. These are attractive technologies because they do not produce harmful disinfection by-products into the receiving system (Liberti and Notarnicola, 1999; WERF, 1995).

Table 1-2. Advanced oxidation processes (AOP) combining UV exposure, currently researched or used in treatment of wastewater effluents

AOP	Abbreviation	Reference
Peracetic Acid and Ultraviolet radiation	PAA / UV	Chen et al., 2005; Caretti and Lubello, 2003
Ozone and Hydrogen Peroxide	O_3 / H_2O_2 / UV	IWA, 2004
Ozone and Ultraviolet radiation	O_3 / UV	Venosa et al., 1984
Hydrogen Peroxide and Ultraviolet radiation	H_2O_2 / UV	Lubello et al., 2002
Titanium dioxide and Ultraviolet radiation	TiO_2 / UV	IWA, 2004
Ultrasound and Ultraviolet radiation	US/ UV	IWA, 2004

1.4 Objectives

The research described in this thesis will assess the disinfection of indicator bacteria and viral/coliphage particles in wastewater effluents. The primary portion of the thesis, identified as Part 1 will focus on fecal coliform (FC) bacteria inactivation from wastewater effluents. The secondary part of this research, Part 2, tested virus/coliphage particle inactivation by exposure to the two disinfecting agents seeded into wastewater effluents. Objectives one, two and three are associated to part 1 and objective four and five are related to part 2:

1) To evaluate the disinfection performance of UV, PAA or PAA/UV combination on fecal coliform inactivation from wastewater effluents collected during dry weather conditions.
2) To evaluate the disinfection performance of UV, PAA or PAA/UV combination on fecal coliform inactivation from wastewater effluents collected during wet weather conditions.
3) To determine and compare the cost-effectiveness between the various treatment methods.
4) To evaluate the effects of UV and PAA/UV combination on inactivation of somatic coliphage ϕX174 and reovirus.
5) Compare the inactivation of the above to particles with inactivation of poliovirus by UV found in literature.

2. LITERATURE REVIEW

2.1 Ultraviolet (UV) Radiation

2.1.1 UV Disinfection Theory

Low-pressure mercury lamps produce a nearly monochromatic emission spectrum, with 80 to 90% of the energy emitted at 253.7nm (Figure 2-1) (Kuo et al., 2003). DNA base pair molecules coincidently produce a peak absorbance for UV radiation within the low pressure peak emission spectrum. The DNA absorbencies occur between 250nm to 260nm wavelength (Figure 2-1) (Kuo et al., 2003). It is this factor that makes UV radiation an effective tool for disinfection of microorganisms. UV does not directly destroy microorganisms, but rather it prevents them from replicating. The mechanism works by UV catalysis of two adjacent thymine molecules producing a dimer (Friedberg, et al., 1995). The dimer(s) prevents the proper attachment of replication proteins to the DNA molecule. An analogous reaction occurs in RNA molecules. Instead of thymine dimers, uracil dimers frequently are produced (Friedberg, et al., 1995).

Typically, vegetative bacteria are the most vulnerable to UV radiation, followed by viruses and bacterial spores (Masschelein, 2002). Encysted protozoa show the greatest resistance to UV light. Some microorganisms are capable of repairing the irradiated DNA, a process known as reactivation (Mechsner et al., 1991; Harris et al., 1987).

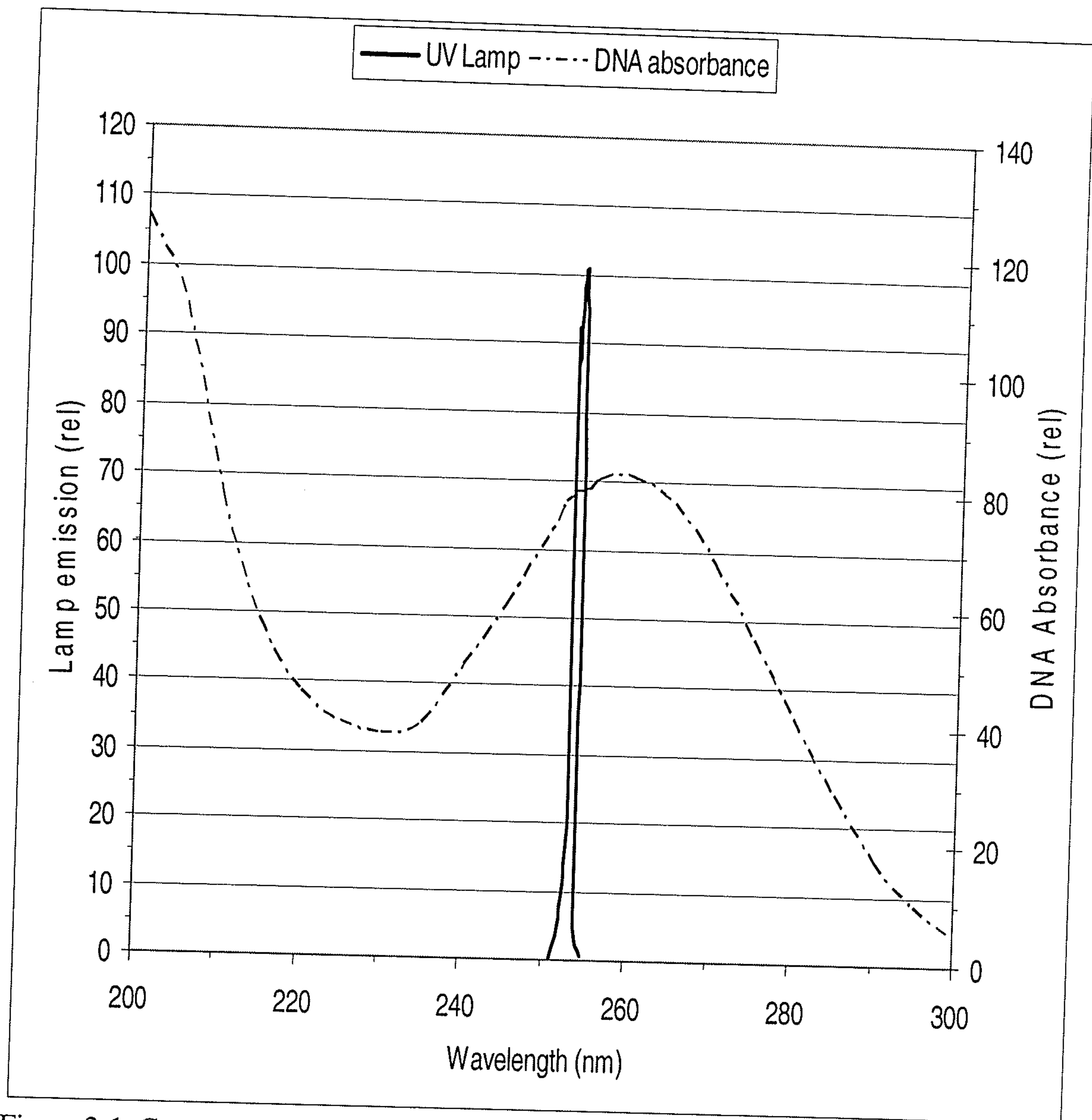


Figure 2-1. Comparison of UV lamp emission spectra emitted from low-pressure mercury lamp to the UV absorption spectra of DNA.

2.1.2 Effects of Effluent Quality on UV Disinfection

Wastewater quality has a considerable impact on the efficiency of UV disinfection. Wastewater characteristics that are of particular importance are UV transmissivity (UVT) and suspended solids (WERF, 1995), which co-dependently influence the degree of applied dose (fluence) required to inactivate microorganisms. UVT is defined as the percentage of UV light, at 253.7nm wavelength, transmitted through a 1-cm path-length of medium (WERF, 1995). Particulate matter influences microbial disinfection by scattering, and/or absorbing UV light, reducing its irradiance directed toward them, or by directly shielding them from the germicidal effects of the light (WERF, 1995). As transmission of UV light through wastewater decreases, the average UV irradiance (intensity) also decreases (WERF, 1995). Thus a fluence level required to maintain sufficient disinfection of pathogens is affected a decrease in irradiance transmitted. If the irradiance decreases a longer contact time is required to maintain the same fluence level (see equation 2.2, section 2.1.3).

Mounting evidence indicates that microorganisms embedded in particulate matter are protected from the germicidal effects of UV radiation, resulting in a reduced disinfection rate (Örmeci and Linden, 2002; Loge et al., 1999; Liberti and Notarnicola, 1999; Lazarova et al., 1998; Loge et al, 1999; WERF, 1995; Savolainen, 1991; Qualls et al., 1983). To the same effect, bacterial clumps have also been shown to harbour viable organisms, shielding them from UV light (Blatchley et al., 2001). The resulting effect is that microorganisms partially exposed to UV light are not completely inactivated and can repair their DNA (Lindenauer and Darby, 1994).

Table 2-1. Components found in wastewaters that absorb UV light.

Organic Compounds	Inorganic Compounds
Colouring agents*	Bromine
Organic dyes*	Chromium
Humic acids*	Cobalt*
Tea/Coffee	Iodide
Benzene	Iron*
Anisol	Manganese
Phenyl propane	Nickel
Phenolic compounds	Sulfates
Toluene	

* Strong UV absorbers (WERF, 1995)

2.1.3 UV Disinfection Model and Fluence (Dose) Response Curves

For simplicity sake, inactivation rate of microorganisms using ultraviolet light is approximated by first order kinetics with a log base 10 (eq. 2.1) (Scheible, 1987). This model indicates that microbial inactivation is an exponential function with respect to the applied UV fluence.

$$N = N_0^{(-k \cdot Igt)} \qquad (2.1)$$

Where

N = Surviving culture density after UV exposure (MPN/100ml)
N_0 = Culture density of microorganisms prior to UV exposure (MPN/100ml)
k = Inactivation rate (decay) constant ($cm^2/\mu Watt \cdot sec$)
I_g = Germicidal irradiance emitted by lamp (i.e. Radiant power energy) ($\mu Watt/cm^2$)
t = Exposure Time (sec)

The above model, presented as a fluence-response curve, shows a rapid reduction in microbial density as the applied fluence increases (Figure 2-2, Part A). The fluence is determined as the product of applied irradiance and contact time (eq.2.2).

$$\text{Fluence } (\mu W \cdot s/cm^2) = \text{Irradiance } (\mu W/cm^2) \times \text{Time (sec.)} \qquad (2.2)$$

A study conducted by Oliver and Cosgrove (1975) determined that the inactivation of microorganisms was dependent on the applied fluence and not on the irradiance. The authors observed that the reciprocal of the products of irradiance and contact time produced "virtually the same" microbial inactivation.

The inactivation rate constant (k) describes that the survival ratio changes with increasing UV-fluence. The specific k value varies from location to location, for a particular species of microorganism, depending on the wastewater characteristics particular to that site.

Deviation from the first order kinetics model is attributed to UV shielding by particles present in wastewaters (Gehr et al., 2003; Qualls et al., 1983). An active residual concentration of bacteria persists, regardless of the increase in UV fluence. This phenomenon is known as tailing (Figure 2-2, Part B). Tailing is also produced by naturally occurring more resistant bacteria. Virus particles have shown similar effects when exposed to increasing levels of UV radiation (Coombs, 2005). Emerick et al. (2000) determined that particles with an average diameter greater than 10-μm could protect bacteria from the germicidal effects of UV light.

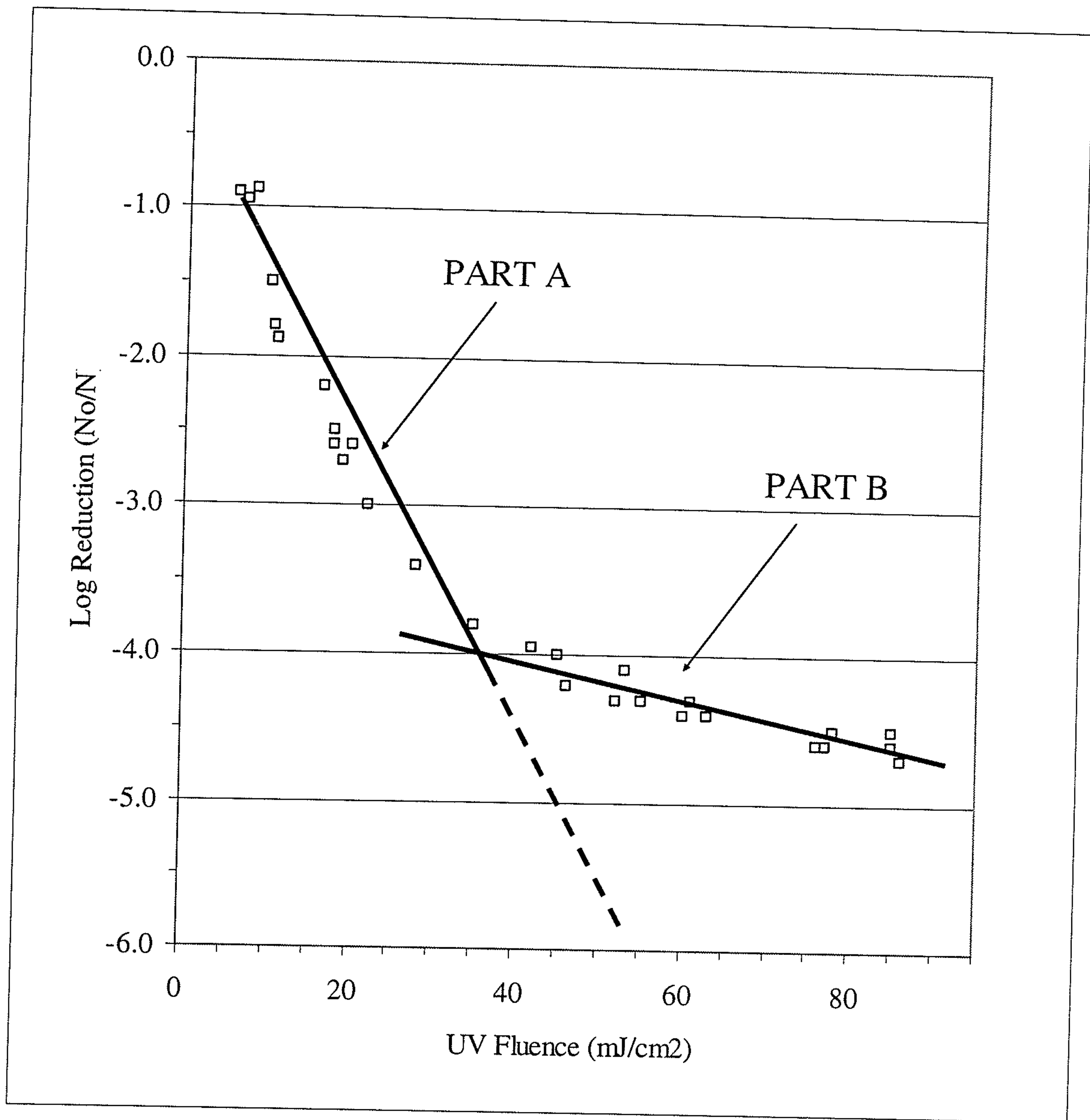


Figure 2-2. Fluence-response curve describing inactivation of microorganisms from wastewater effluent using UV light. Part A and Part B refer to first order inactivation and residual tailing, respectively.

2.2 Peracetic Acid (PAA)

2.2.1 Review of Peracetic Acid Disinfection

Traditionally, PAA is a chemical oxidant used in a variety of food and agricultural industries to disinfect their products (Rudd and Hopkinson, 1989; Fraser et al., 1984). This chemical was, however, introduced as an alternative to chlorine disinfection of wastewater effluents (Lefevre, et al., 1992; Baldry et al., 1991; Baldry and French, 1989). It has recently been recommended as an adequate disinfectant for combined sewer overflows (EPA, 1999) and biologically treated wastewaters (Gehr et al., 2002).

The range of PAA treatment studies presented by a number of researchers is extensive. Studies show a range in results, extending from a high log reduction (5.5) in FC concentration using very high PAA concentrations (500mg/L) with low contact times (CT) (Lazarova et al., 1997) to low log reduction (1.8) when 2 mg PAA/L for 10minutes (Caretti and Lubello, 2003). To facilitate analysis, the research is summarized in Table 2-2.

Other wastewater indicators important to the disinfection are poliovirus. The National Water Research Institute (NWRI) requires that 4 log reduction of poliovirus be removed from treated wastewater effluents for reuse (NWRI and AWWARF, 2003). Somatic coliphages are included in studies of different disinfection methods (Table 2-2). Lazarova et al. (1997) studied both MS2 and somatic coliphages testing phages inactivation up to 120min CT. They found that 10mg/L PAA reduced somatic coliphages by 5logs using 60min CT. It was further reduced by 2.5 logs with an additional 60min CT. MS2 bacteriophage were found to display extremely high resistance to treatment showing a 3.5 log reduction after treatment with 500 mg/L for a duration of 120 min.

These authors determined that poliovirus was more resistant than somatic coliphages, but less than MS2. Rajala-Mustonen et al. (1997) focused on somatic coliphage resistance to PAA treatments. These authors found that 25mg/L PAA-5minutes contact period produced a small 2 log removal of somatic coliphages.

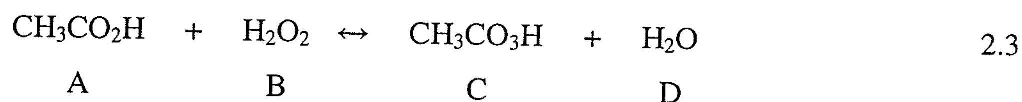
Table 2-2. Summary of PAA inactivation of indicator organisms (particles) disinfected from wastewater effluents.

Indicator Tested	PAA Dosage (mg/L)	Contact Time (min.)	Log Red.	Reference
[*]FC	2	-	2.8	Chen et al., 2005
FC	4	-	4.2	
FC	2	10	1.8	Caretti and Lubello, 2003
FC	2	30	2.8	
FC	8	10	3.2	
FC	8	30	4.2	
[**]TC	2	10	1.5	
TC	8	10	2.7	
FC	4.5	60	4	Gehr et al., 2003
FC	2	10	2.7	Wagner et al., 2002
FC	2	60	4.5	
FC	5	10	5	
TC	10	30	3.4	Liberti and Notarnicola, 1999
TC	400	20	5.2	
TC	10	10	2.6	Liberti et al., 1999
TC	10	60	3	
TC	100	5	4	
TC	500	5	>5	
FC	500	<5	[$]TR (~5.5)	Lazarova et al., 1997
FC	10	<10	3	
FC	5	10	3	
FC	5	60	4	
Phage ϕX174	10	120	7.5	
MS2	500	120	3.5	
Phage ϕX174	25	5	2	Rajala-Mustonen et al., 1997
Phage ϕX174	50	10	>3	
TC	2	30	1.4	Lefevre et al., 1992
TC	2	60	1.8	
TC	8	30	2.8	
TC	8	60	3.6	

[*]FC – Fecal coliforms [**]TC – Total coliforms [$]TR – Total Reduction

2.2.2 Peracetic Acid: Chemistry and Theoretical Basis of Disinfection

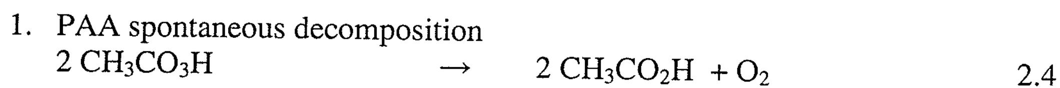
Peracetic acid (PAA) is a quaternary equilibrium mixture containing peracetic acid, acetic acid, hydrogen peroxide, and water. Stabilizers are added to the solution to prevent degradation of the chemical mixture. The balanced equation is shown below:



$$\underset{A}{CH_3CO_2H} + \underset{B}{H_2O_2} \leftrightarrow \underset{C}{CH_3CO_3H} + \underset{D}{H_2O} \qquad 2.3$$

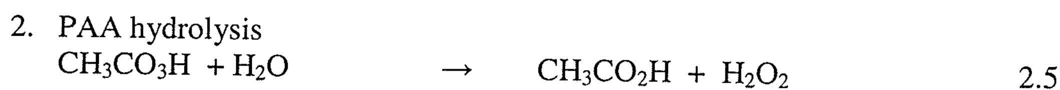
Wagner et al. (2002) suggests that PAA, represented by part C in the latter portion of equation 2.3, is the dominant biocidal agent in the solution. Hydrogen peroxide (H_2O_2) may also be involved to a lesser extent. Liberti and Notarnicola (1999) and Lefevre et al. (1992) state that PAA disinfects by oxidation with the release of "active" oxygen. They suggest that oxygen molecules oxidize sulphydryl and/or sulfur bonds in outer cellular proteins, thereby destroying the ability of the membrane to transfer solutes in and out of the cell. In wastewater effluents, oxygen may be produced by the following mechanisms of decay (Lafevre et al., 1992; Yuan et al., 1997):

1. PAA spontaneous decomposition



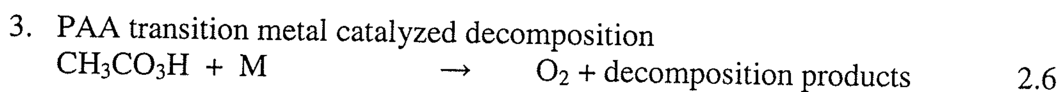
$$2\ CH_3CO_3H \rightarrow 2\ CH_3CO_2H + O_2 \qquad 2.4$$

2. PAA hydrolysis



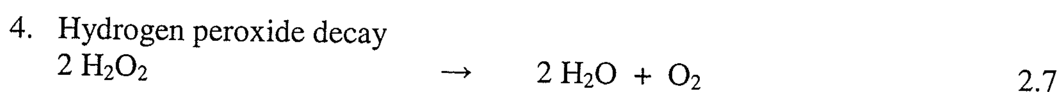
$$CH_3CO_3H + H_2O \rightarrow CH_3CO_2H + H_2O_2 \qquad 2.5$$

3. PAA transition metal catalyzed decomposition



$$CH_3CO_3H + M \rightarrow O_2 + \text{decomposition products} \qquad 2.6$$

4. Hydrogen peroxide decay



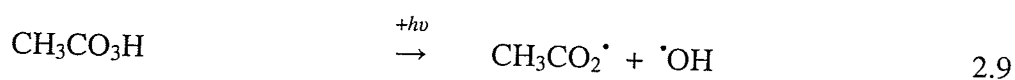
$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2 \qquad 2.7$$

Yuan et al. (1997) found that at a pH range between 5.5 and 8.2 units PAA was mostly consumed by the spontaneous decomposition mechanism of decay (eq. 2.4) and the hydrolysis decomposition mechanism was negligible (eq. 2.5). If the hydrolysis reaction takes place, the formation of H_2O_2 must be at a very high concentration to provide a

significant effect on disinfection (Wagner et al., 2002), especially since it is not reactive under neutral pH (Yuan et al., 1997).

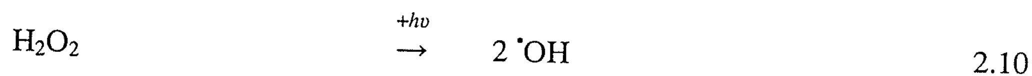
When PAA is combined with UV radiation as in an advanced oxidation process (AOP), disinfection is enhanced by the production of hydroxyl radicals (Lubello et al., 2002). The radiation serves as a photo-catalyst to activate this reaction. The formation of these compounds is predicted as follows:

Hydroxyl radical formation from PAA



$$CH_3CO_3H \xrightarrow{+h\upsilon} CH_3CO_2^{\bullet} + {}^{\bullet}OH \qquad 2.9$$

Hydroxyl radical formation from H_2O_2



$$H_2O_2 \xrightarrow{+h\upsilon} 2\ {}^{\bullet}OH \qquad 2.10$$

Where, $h\upsilon$ indicates activation energy

Caretti and Lubello (2003) indicate that $CH_3CO_2^{\bullet}$ rapidly decomposes into $CH_3^{\bullet}$ and CO_2. The hydroxyl radical is known to affect all biomolecules, at the primary, secondary, or tertiary structural level.

The synergy involved in the disinfection process can be estimated with equation 2.11 (Chen et al., 2005), shown below:

$$\text{Synergy} = I_r - (I_{r1} + I_{r2}) \qquad 2.11$$

Where, I_r is the inactivation of indicator caused by exposure to the combined disinfection treatment

I_{r1} is the inactivation of indicator cause by exposure to <u>first</u> disinfecting agent alone

I_{r2} is the inactivation of indicator cause by exposure to <u>second</u> disinfecting agent alone

2.2.3 Effects of Effluent Quality on PAA Disinfection

Lefevre et al. (1992) and Liberti et al. (1999) compared the effect of TSS concentrations on PAA dose delivery with differing results. Lefevre et al. (1992) found that disinfection of total coliforms using 5-mg PAA/L had a same influence on disinfection despite a large range in TSS concentration between treatments. A 1-log reduction difference was shown between the 10 mg TSS/L and 100 mg TSS/L treatments. Liberti et al. (1999), on the other hand, indicate that the wastewater samples had to be filtered to ≤10 mg TSS/L for 10 mg PAA/L to have a significant effect on disinfection rate. Other components in wastewater, such as organic matter have shown to be responsible for poor inactivation by PAA (Gehr et al., 2003; Leberti and Notarnicola, 1999), although the specific concentrations of organics were not discussed.

3.0 Methods and Materials

3.1 Collecting and Processing Wastewater Samples

Investigation of wastewater effluent disinfection was carried out in two parts. The main focus of this thesis (part I) involved assessing the inactivation of fecal coliform bacteria, indigenous to the North End Water Pollution Control Centre (NEWPCC) wastewater effluents, Winnipeg, Canada; whereas Part II involved the assessing the inactivation of reovirus and somatic coliphage seeded into the wastewater effluents after disinfection with the various agents.

Part I was further separated into two sections, Phase 1 and Phase 2. Phase 1 involved the disinfection of wastewater effluents collected during dry weather conditions, whereas Phase 2 involved the disinfection of wastewater effluents collected during wet weather conditions. Wastewater effluent collected during a storm event represented a worst-case scenario in the facility's ability to provide a level of disinfection that adequately complies with the regulation standard. Under these conditions, the facility's secondary by-pass system commences operation.

For each of three collection periods, a six liter effluent sample was taken from the final effluent channel using a Bristol automatic sampler. The treatment facility's laboratory technician analyzed turbidity, total organic carbon (TOC), soluble organic carbon (SOC), and total suspended solids (TSS) in accordance with standard methods (APHA et al., 1995). The remainder of the stock effluent was transported in a cooler, containing frozen ice packs, to the University of Manitoba for disinfection tests. All treatment tests are listed in Table 3-1. The UV transmissivity (UVT) was measured at the University using a portable spectrophotometer (P254C, Trojan Technologies), as well.

All tests conducted at the University were performed in triplicate. Treatments followed Caretti and Lubello (2003) and Liberti et al. (1999) recommendations that PAA precede UV disinfection PAA was added as pure concentrated solution to all samples, rather than in diluted form. The indicator fecal coliform (FC) bacteria concentration from each treatment was analyzed by the multiple tube fermentation (MTF) technique (APHA et al., 1995). Testing was completed within 24 hrs of collection and samples were stored in a cool (4 °C) and dark place.

Table 3-1. Summary of treatments carried out on wastewater effluent samples collected from the NEWPCC facility.

Weather Condition	PAA Concentration (mg/L)	PAA Contact Time (min.)	UV-Fluence (mWs/cm^2)	Treatment Abbreviation
	0	-		UV-D
Dry Flow (Phase 1)	2	-	[0, 5, 10, 20, 40]	2 AOP-D
	8	-		8 AOP-D
	2	[0, <1, 2, 4, 8]	-	2 PAA-D
	8		-	8 PAA-D
	0	-		UV-W
Wet Flow (Phase 2)	2	-	[0, 5, 10, 20, 40]	2 AOP-W
	8	-		8 AOP-W
	2	[0, 1, 3, 6, 12]	-	2 PAA-W
	8		-	8 PAA-W

3.2 Fecal Coliforms Bioassay Treatments and Testing

Using the stock wastewater effluent retrieved from the treatment plant, 100ml aliquots were added into amber-coloured jars. The amber colour jars were used to prevent any stray light from reaching the sample, potentially confounding the results. The test order was determined randomly using a random-number generator. The PAA concentrations tested were 0, 2 and 8-mg/L and UV fluences tested were 0, 5, 10, 20, and 40-mWs/cm^2. The exposure time for the PAA treated samples were equivalent to the exposure time given to UV irradiated samples. The PAA/UV AOP treatment samples were immediately irradiated with UV light after PAA addition. Wastewater samples receiving no PAA and no UV irradiation, the control treatment, were used to determine the initial fecal coliforms concentration. On completion of each test, the PAA residuals were quenched (or neutralized) with final concentrations of both, 100 mg/L of sodium thiosulfate followed by 50 mg/L of catalase (Wagner et al, 2002). The results were plotted on a $\log_{10}$ fluence (dose) response curve.

Two-paired t-tests were used to determine the significance of each disinfection test results as compared to the initial FC concentration. Separate two-paired t-tests were used for comparing AOP treatment disinfection improvement over the reference treatment (UV alone). Both tests used a 5% significance level. UV radiation has been well established as an appropriate method for fecal bacteria disinfection, thus in this study, the UV alone treatment was referred to as the *reference treatment* when compared to AOP performance tests.

3.3 UV Disinfection Procedure

A bench scale collimated beam apparatus, containing a single advantage-5 low-pressure mercury lamp (internal P/N 605055) was used to generate the UV radiation (Figure 3-1). The UV lamp, which mainly emits radiation at 253.7nm, was located above a collimating tube. The tube collimates the radiation ensuring only radiation that is perpendicular to the wastewater surface impinges it. The wastewater effluent sample is contained within a UV absorbing crystallization dish, so radiation striking the sample remains within the sample. A 50 ml aliquot sample in the dish was placed on a magnetic stirrer and continually mixed, without forming a vortex, for the duration of radiation exposure. The fluence was determined as the product of the irradiance (intensity) and exposure time. Using a pre-determined fluence value and obtaining the average irradiance emitted by the lamp, the contact time required to make the desired fluence (dose) was determined as follows:

$$\text{Exposure Time (sec.)} = \text{Fluence } (\mu W{\cdot}s/cm^2) \,/\, \text{Average Irradiance } (\mu W/cm^2) \qquad 3.1$$

International Light radiometer (Model No.-IL1400A) measured the irradiance emitted by the lamp at the level equal to the surface to water interface at the centre (E_0) and at 0.5cm increments, up to 3cm away from the centre, along the abscissa and ordinate (Bolton and Linden, 2003). Correction factors were used to adjust the measured irradiance to better reflect an average (germicidal) irradiance ($I_{avg\text{-}g}$) transmitted through the entire sample (Bolton and Linden, 2003). The correction factors included the petri factor (Pf), water quality factor (Wf), reflection factor (Rf), and the divergence factor (Df).

$$I_{avg\text{-}g} = E_0 \times Pf \times Wf \times Rf \times Df \qquad 3.2$$

A detailed description of each correction factor is presented in *Appendix A*.

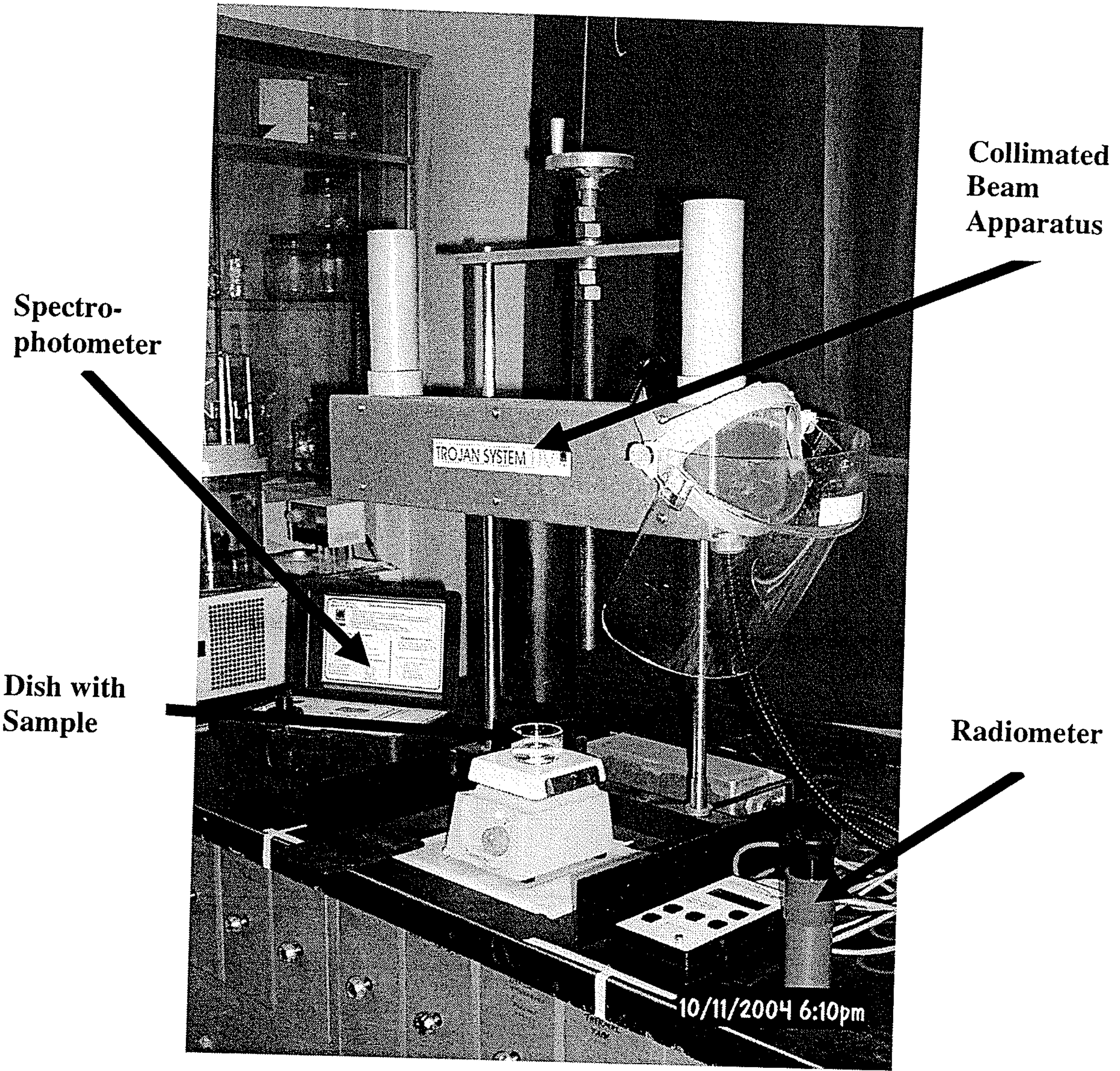


Figure 3-1. Collimated beam apparatus encasing a low-pressure mercury lamp used in biological assays.

3.4 Peracetic Acid Residual Assays

A colorimetric assay developed by Pütter and Becker (1983), modified by Wagner et al. (2002), was used to measure PAA concentration confirming the initial quantity added was the correct concentration. This assay cannot distinguish between the two peracids used. Thus, peroxycompound concentration represents both peracetic acid and hydrogen peroxide compounds. A small quantity of effluent sample containing PAA was combined with reacting agents. The mixture contains sodium phosphate buffer, horse-radish peroxidase (HRP), and 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfuric acid) di-ammonium salt (ABTS). The peroxidase enzymes, in the presence of both peracids, oxidize ABTS compounds to $ABTS^{+}$, its ionized form (Wagner et al., 2002). Absorbance of $ABTS^{+}$ is directly proportional to the peracid concentration present in the solution. A Biochrom spectrophotometer (Ultrospec 4300 pro), set to 405 nm wavelength, measured the absorbencies. A detailed preparation of the reagents for this assay is outlined in *Appendix A*. A negative control analyzed for the presence of naturally occurring peracids in wastewater effluent.

3.5 Somatic Coliphage and Reovirus Bioassay Treatments and Testing

Somatic coliphage ϕX174 (ATCC - 13706-B1) and reovirus were seeded into wastewater effluents collected from the NEWPCC. Effluent samples were placed into amber-coloured jars. A random-number generator selected the sequence order of experimentation.

The UV fluences tested were 0, 10, 20, and 40mWs/cm2 and the PAA concentrations for the AOP treatments tested were 0, 2, and 8mg/L. PAA concentrations were added prior to UV irradiation. For AOP treatments, post UV disinfection (i.e. 40mWs/cm^2) PAA residuals were quenched with 100mg/L of sodium thiosulfate and 50mg/l of catalase. The results were plotted on a $\log_{10}$ fluence (dose) response curve. Two paired t-tests were used to determine the effects of each disinfection procedure on phage/virus particle inactivation.

4. Results

4.1 Part I: Wastewater Effluent Samples Collected During Dry Weather (Phase 1) and Wet Weather (Phase 2) Conditions

Water quality parameters obtained from wastewater analyzed from phase 1 and phase 2 effluents are shown in Table 4-1. There is strong indication that phase 2 effluent was of poorer effluent quality than phase 1 effluent. It can be determined that TSS, turbidity, TOC, SOC, and FC concentrations from phase 2 effluents were 7.5x, 3x, 5x, 2x, 1.5x and 30x greater than phase 1 effluents, respectively. The UVT, on the other hand, obtained in phase 2 effluents were reduced by 1.5x compared to phase 1 effluents.

A result of poorer effluent quality requires that phase 2 effluents receive a greater UV exposure time to obtain the same fluence, as compared with phase 1 effluents. For instance, the maximum irradiation time, to obtain 40 mWs/cm^2, for phase 1 and phase 2 samples were 7.3 and 10.5 minutes, respectively. This discrepancy in time does not affect the inactivation of microorganisms irradiated because disinfection is a function of fluence and not contact time.

Table 4-1. Quantification of wastewater quality from samples collected on dry and wet weather conditions.

Parameter	Phase 1 Dry Weather Flow	Phase 2 Wet Weather flow
Raw inflow	251 MLD	714 MLD
TSS	10 mg/L	76 mg/L
Turbidity	4.1 NTU	14 NTU
TOC	20 mg/L C	41 mg/L C
SOC	19 mg/L C	27 mg/L C
UV Transmissivity	55%	33%
Initial FC Geometric Mean Concentration	2.86×10^5 MPN/100ml	8.85×10^6 MPN/100ml

4.1.1 Disinfection of Fecal Coliform Bacteria using UV, PAA, and/or PAA/UV AOP

4.1.1.1 Phase 1: UV treatment

UV-disinfected effluents from samples collected during dry weather conditions produced an inactivation quality that complied with the discharge limit. The 200-MPN/100ml standard guideline limit for FC discharge into the river is 2.3 represented in log units. This level of disinfection was achieved using 28-mWs/cm^2 of UV radiation (Figure 4-2). The UVT for this wastewater effluent was 55%, a value greater than the average. The 40 mWs/cm^2 UV radiation resulted in a FC geometric concentration of 33-MPN/100ml. This value was significant within 5% ($P<0.05$).

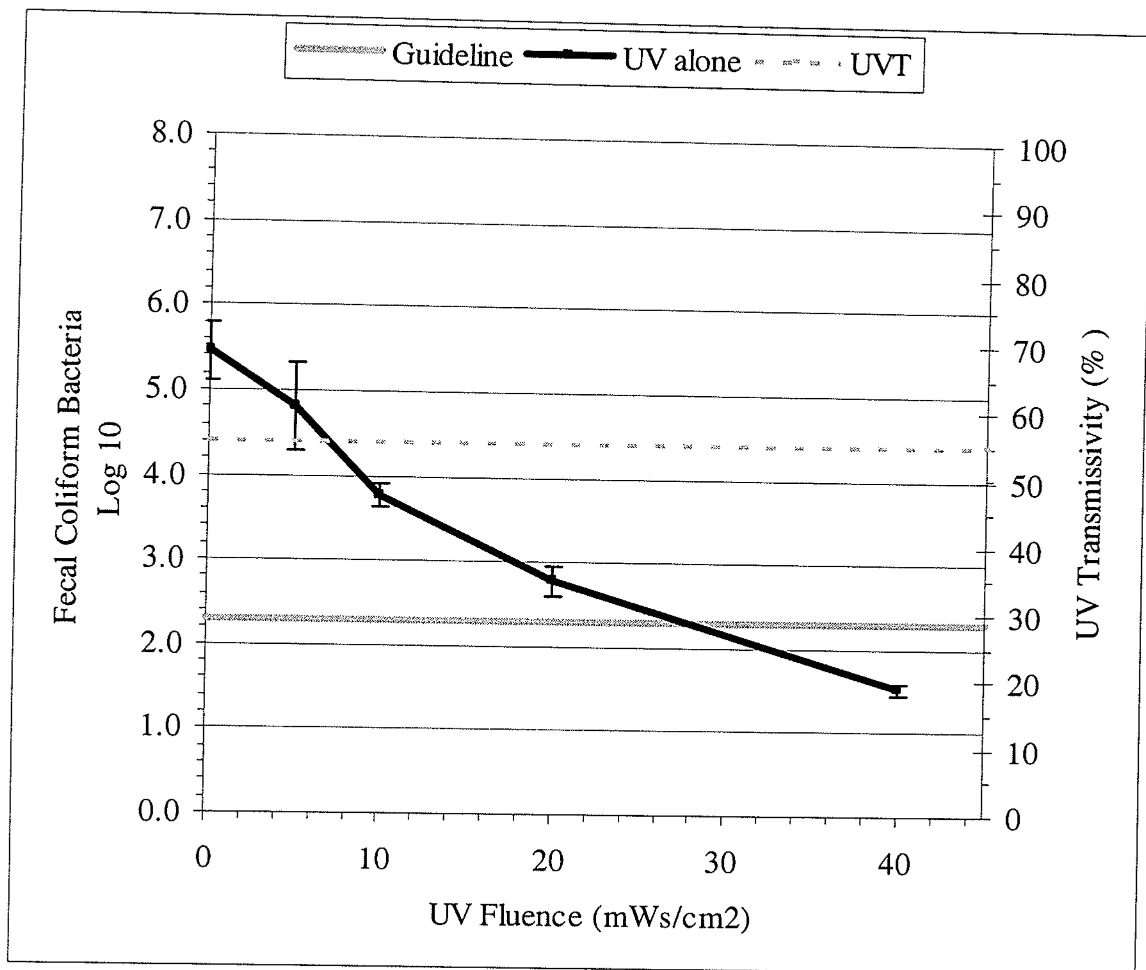


Figure 4-1. Survival of fecal coliforms bacteria after treatment with UV on effluent samples collected during a dry weather condition.

4.1.1.2 Phase 1: PAA treatment

The PAA contact times were specifically selected to accurately compare inactivation of fecal coliform concentration with inactivation results after exposure to the other two disinfection methods (i.e. UV alone and PAA/UV process), based on time.

Treatments with PAA alone were found to increase FC disinfection as the contact time (CT) increased (Figure 4-2). The lowest contact time (1min) for 2 mg/L and 8 mg/L treatments produced 0.1 and 0.2 log reductions, respectively. Within the maximum contact time (<8min.) the 2 mg/L treatment produced 0.7 log reduction. From an initial geometric mean concentration of 2.86×10^5 MPN/100ml, it was reduced to 54,000 MPN/100ml. The 8 mg PAA/L alone treatment, on the other hand, produced a 2.7 log reduction after the same amount of exposure time, leaving a 500 MPN/100ml FC concentration. Thus, using low concentrations and low contact times, PAA solution is not effective for inactivating FC to the standard guidelines.

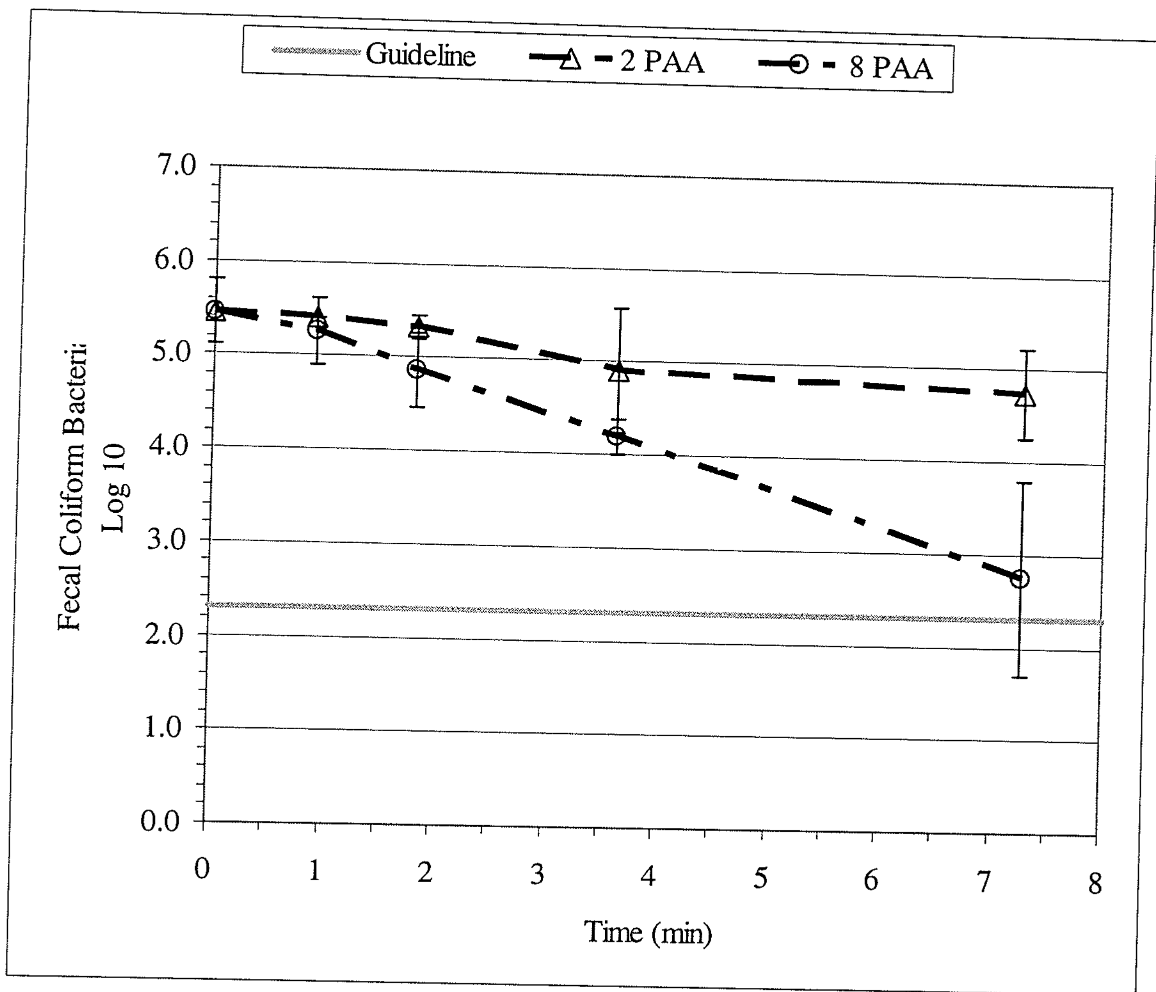


Figure 4-2. Survival of FC bacteria after treatment with two dosages of PAA. (0.9 min = $5 mWs/cm^2$; 1.8 min = $10 mWs/cm^2$; 3.6 min = $20 mWs/cm^2$; 7.3 min = $40 mWs/cm^2$)

4.1.1.3 *Phase 1: PAA/UV (AOP) treatment*

The combined treatment methods out-performed the two other single forms of treatment (Figure 4-3). The 2 mg PAA/L AOP treatment produced a rapid reduction in FC density, achieving the target level when combined with 22-mWs/cm^2 of UV radiation. This is equivalent to 21 % less energy requirement compared with the reference treatment (UV alone). This advanced oxidation process for disinfecting was between 10 times to 190 times better compared to UV alone treatment (Table B-1). The improvement occurred as the fluence increased. When this combined operation was compared to PAA alone treatment, the combined operation was 33 to 600 times more effective (Table B-1). Comparative analyses of the 8 mg PAA/L AOP supplement treatment to either UV alone or PAA alone treatments showed similar results to the above AOP treatment. Specifically, the 8 mg/L AOP treatment was in the range of 20 times to several hundred times more effective at disinfection than UV alone; when compared to PAA alone, this treatment was up to 480 times more effective at inactivating fecal coliform. This treatment provides significantly improved energy conservation compared to the other AOP treatment. It required 64% less energy when combined with 10-mWs/cm^2 of radiation ($P<0.05$) (Figure 4-3).

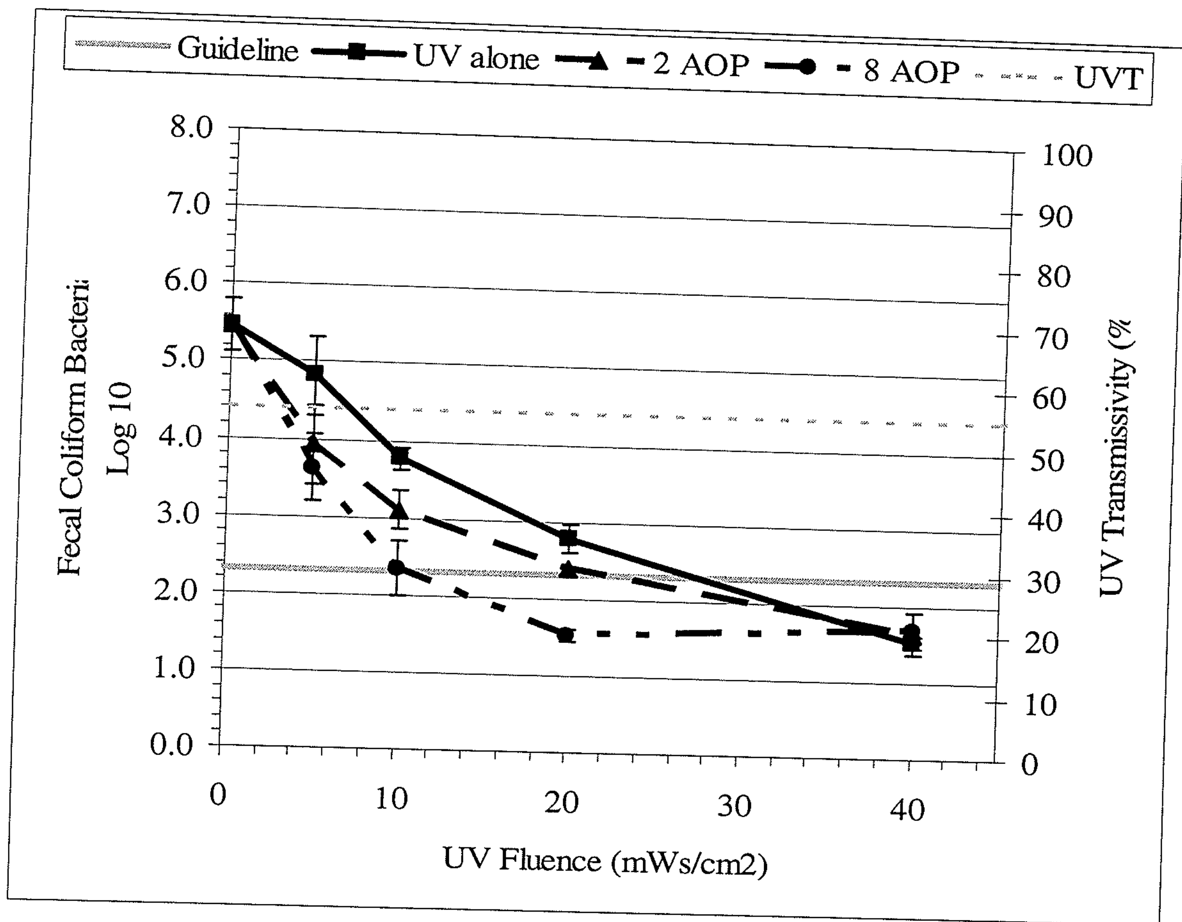


Figure 4-3. Survival of FC bacterial concentrations after treatment with UV, 2mg/L AOP and 8mg/L AOP.

The combined effects of PAA/UV treatment showed synergism effect for disinfection. The mechanism of FC inactivation between the two AOP treatments and the summation of the individual disinfectants were compared. The separate, yet, summed inactivation (as log reduction) of FC showed to be less than the combined effects in the AOP. For instance, 2 mg PAA/L treatment summed with 5, 10, or 20 mWs/cm^2 UV fluence resulted in 0.7, 1.8 and 3.2 log reduction, respectively. The combined operation at these fluences, on the other hand, showed 1.5, 2.4 and 3.2 log reductions for 5, 10, or 20-mWs/cm^2 UV fluence, respectively. These were 0.8 and 0.6 logs greater than the independent sum for 5 and 10mWs/cm^2 fluence, respectively (Figure 4-4). At 20-mWs/cm^2 the actual inactivation value for disinfection was equivalent to the added results. A similar trend was observed with 8 mg PAA/L combined treatment process. However, the 8 mg PAA/L AOP treatment was more efficient than the other AOP treatment (Figure 4-5). At the 20 mWs/cm2 fluence, both inactivation processes compared were equivalent.

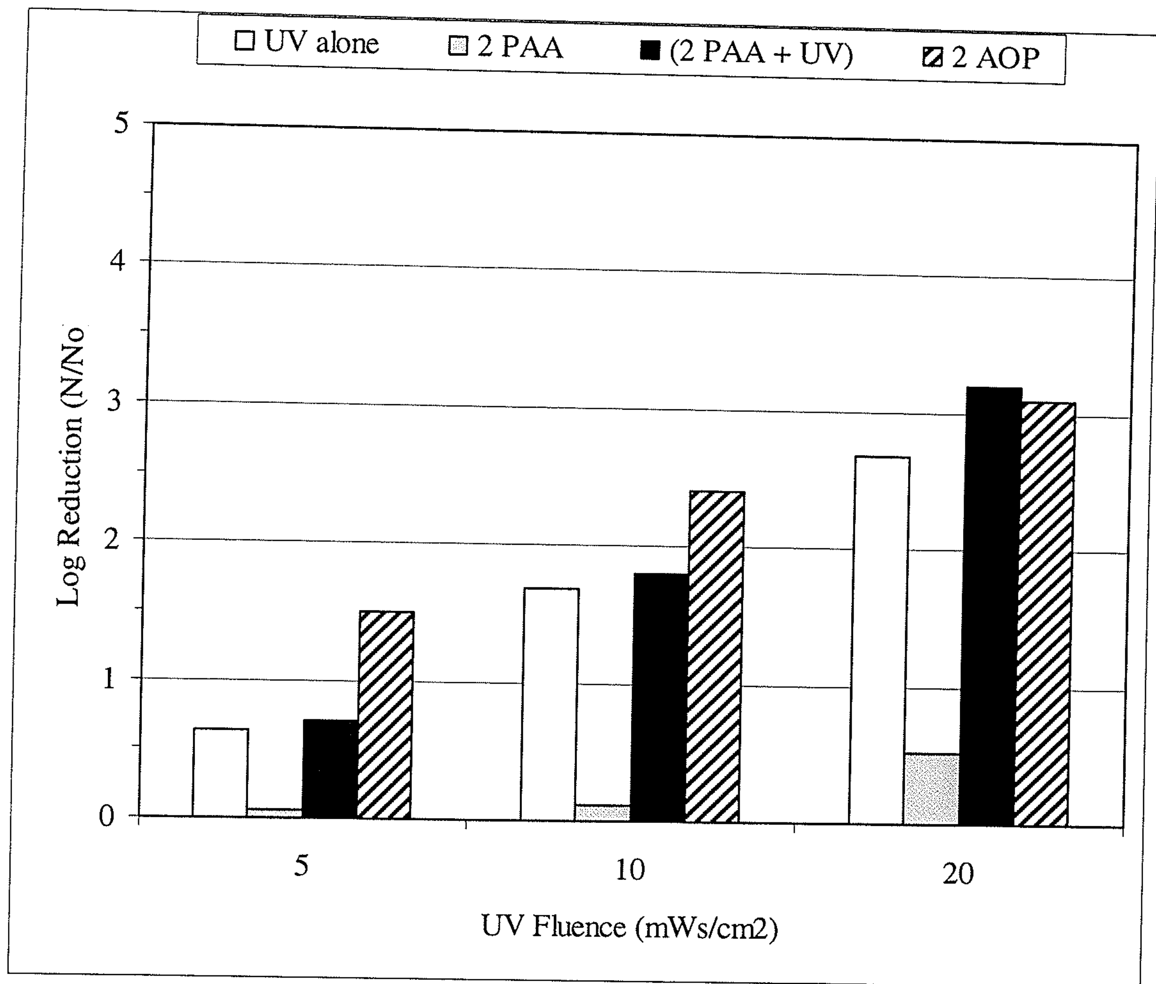


Figure 4-4. Comparing inactivation effect to determine synergy using 2 mg/L PAA supplement.

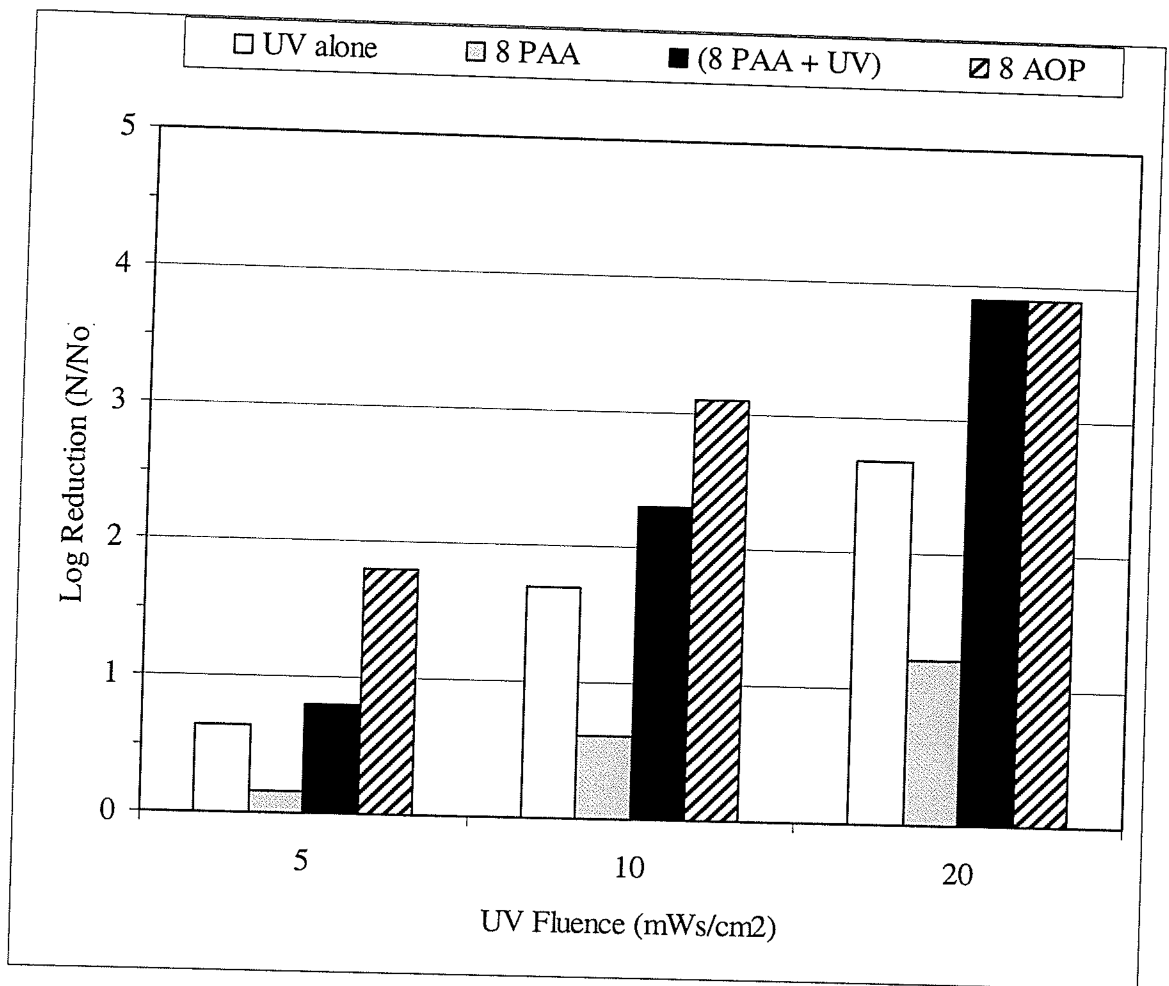


Figure 4-5. Comparing inactivation effect to determine synergy using 8 mg/L PAA supplement.

4.1.1.4 Phase 2: UV treatment

Effluents collected during wet weather conditions (phase 2) and treated with UV alone did not adhere to the discharge limit (Figure 4-6). The initial FC concentration was $8.85x10^6$-MPN/100ml. The maximum UV disinfection treatment provided (40-mWs/cm^2) produced a 4.0 log reduction ($P<0.05$), which was equivalent to 800-MPN/100ml - geometric mean. A 4.6 log reduction would be required to comply with the standard. The UVT value obtained for this treatment was 33%.

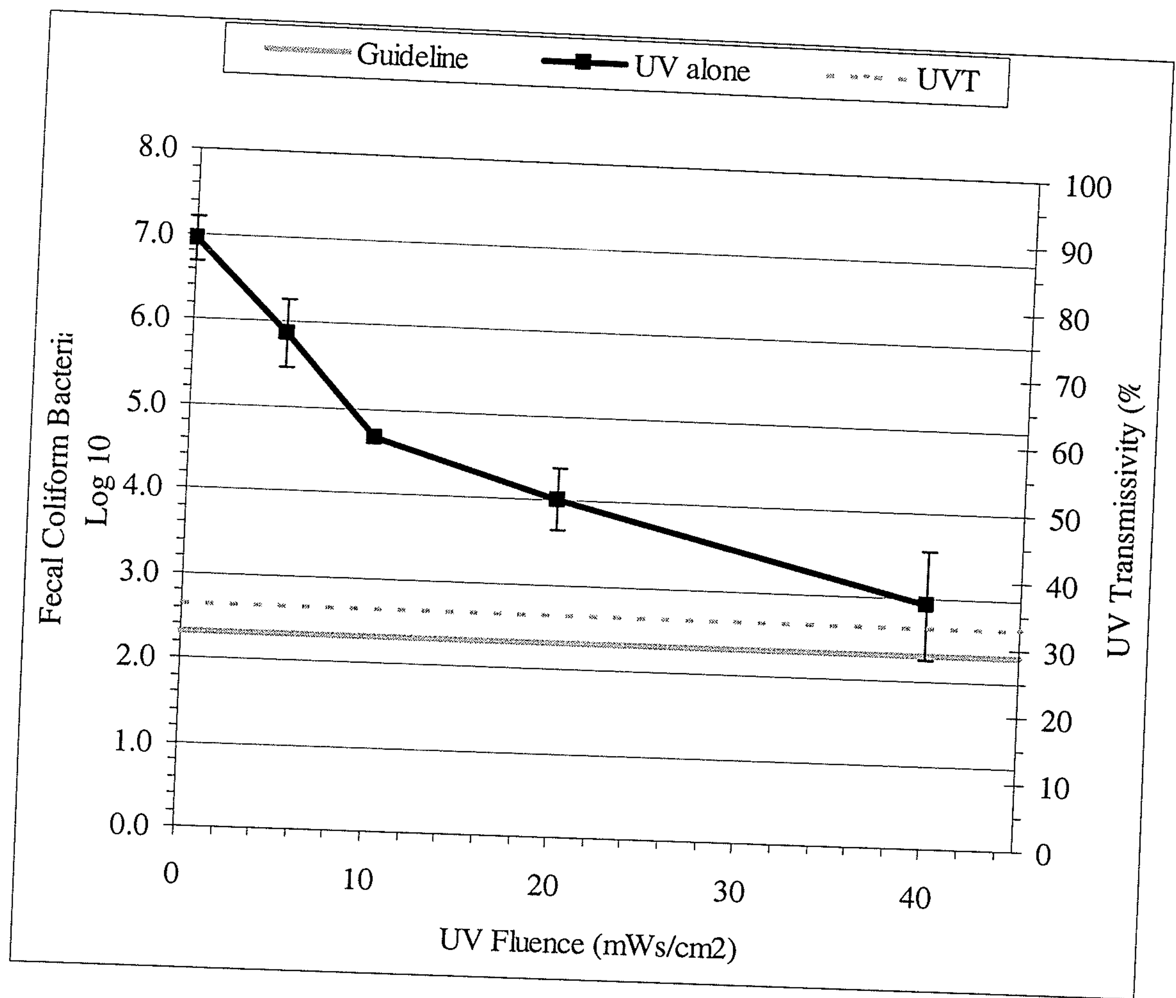


Figure 4-6. Survival of fecal coliforms bacteria after treatment with UV on effluent samples collected during WET weather conditions.

4.1.1.5 *Phase 2: PAA treatment*

The 2 mg PAA/L treatment was ineffective at inactivating FC bacteria. It did not meet the criteria for discharge limit when using the highest contact time (<12 minutes). For the 8 mg PAA/L treatment, a trend could not accurately be determined due to an error at the 5.2-minute interval. As a result, this treatment was excluded from analysis (Figure 4-7).

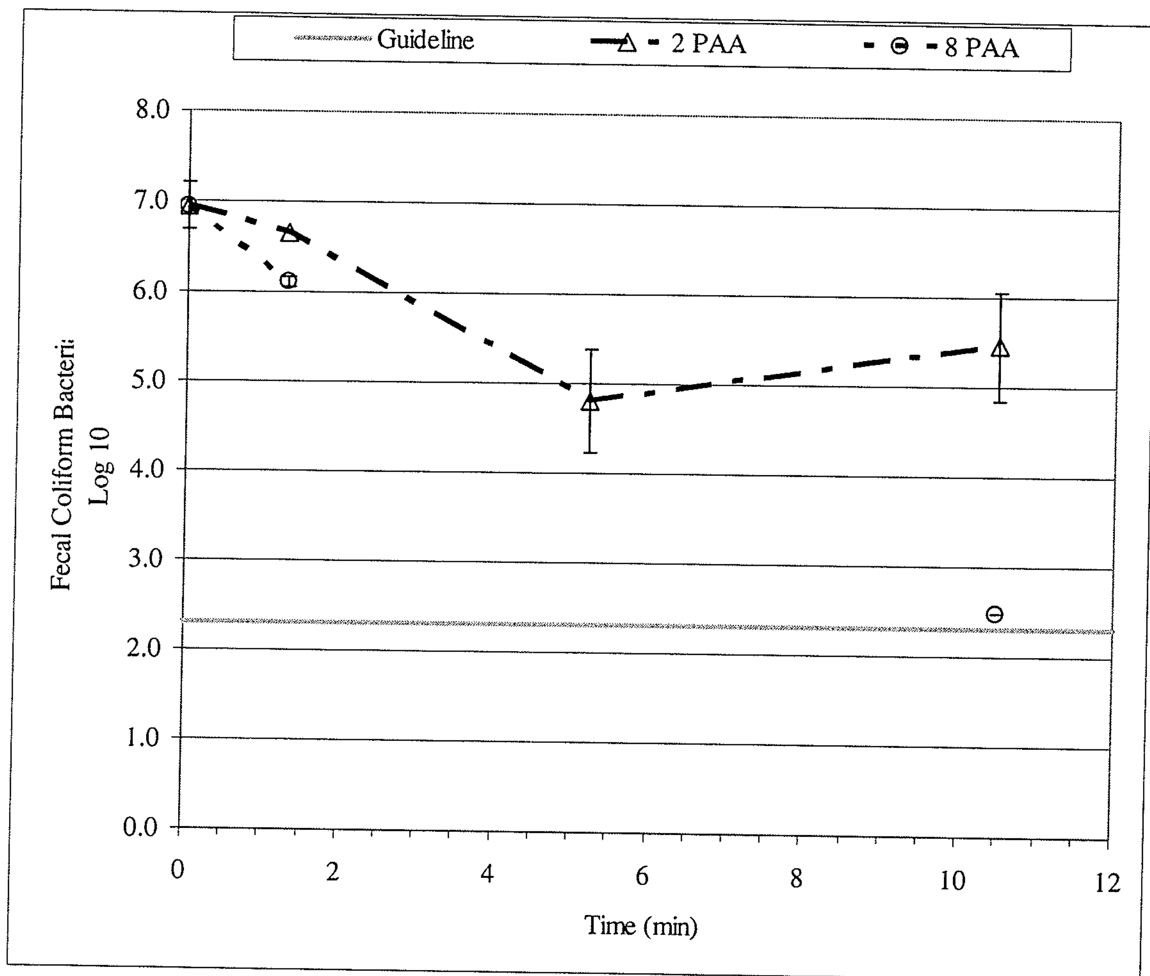


Figure 4-7. Survival of fecal coliforms bacteria after treatment with PAA. (1.3 min = 5 mWs/cm^2; 2.6 min = 10 mWs/cm^2; 5.2 min = 20 mWs/cm^2; 10.5 min = 40 mWs/cm^2)

4.1.1.6 Phase 2: AOP treatment

The 2 mg PAA/L and 8 mg PAA/L AOP treatments indicate as being effective in reducing the initial concentration of indicator bacteria, from 8.8x106-MPN/100ml, to 500-MPN/100ml and <300-MPN/100ml, respectively (2mg/L, P=0.0230; 8mg/L, P=0.0230) (Figure 4-8). However, both AOP treatments did not produce an effluent quality that met provincial standards, even after exposure to the highest UV fluence, 40mWs/cm^2. At this fluence the 2 mg PAA/L and 8 mg PAA/L AOP treatments produced 3.8 and 3.8 log reduction, respectively. These treatments, when compared with UV alone reference treatment, were both not significant in enhancing disinfection potential (2-mg/L, P>0.05; 8mg/L, P>0.05).

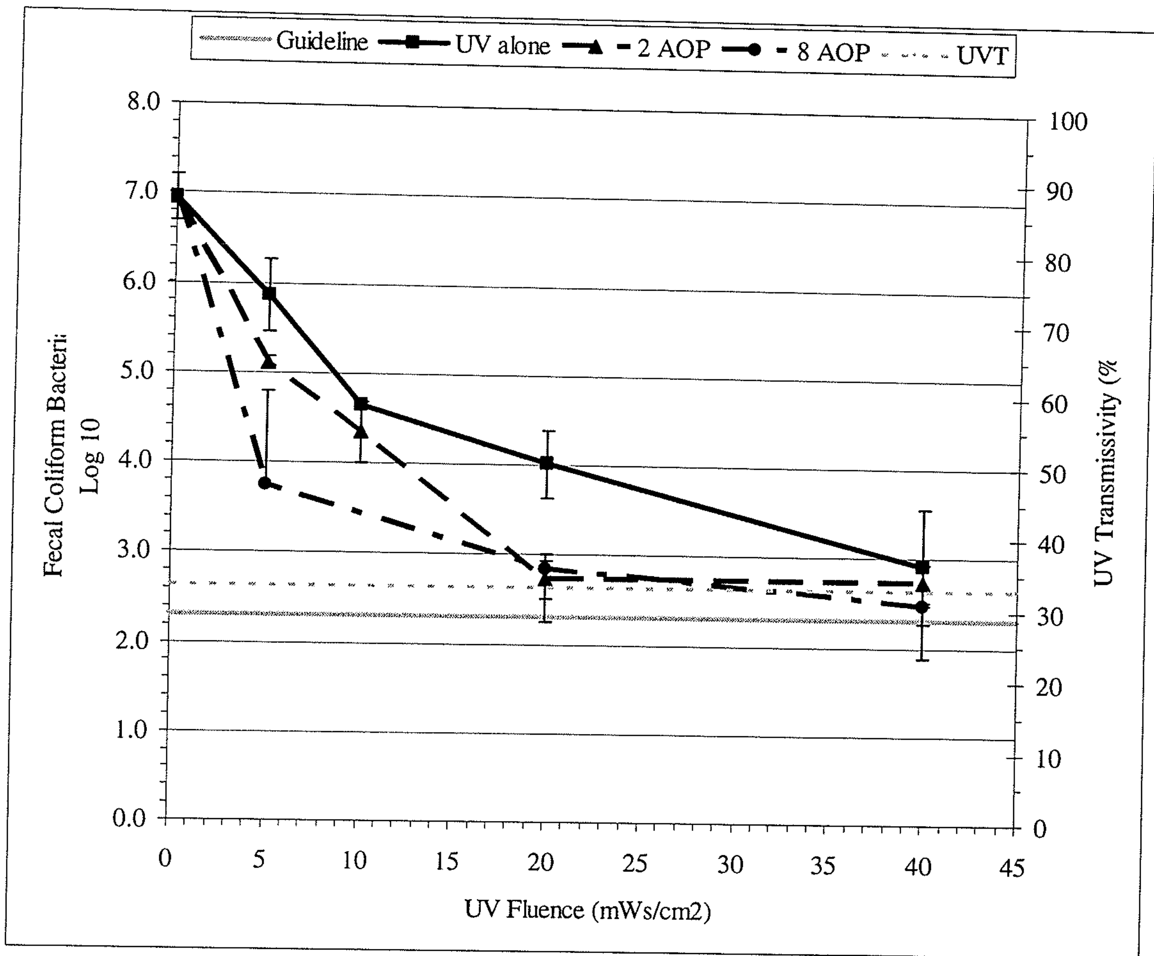


Figure 4-8. Survival of FC bacteria after treatment with UV, 2 mg PAA/L AOP and 8 mg PAA/L AOP.

4.1.2 PAA Residual Assay Results

The following values confirm that PAA concentrations mixed into the samples were accurate. These concentrations were analyzed by colourimetric assay discussed in *Appendix A*.

Phase 1 - 2 mg PAA/l target concentration
Actual concentration = 2.01 mg/L

Phase 1 - 8 mg PAA/L target concentration
Actual concentration = 7.96 mg/L

Phase 2 - 2 mg PAA/l target concentration
Actual concentration = 2.09 mg/L

Phase 2 - 8 mg PAA/L target concentration
Actual concentration = 7.57 mg/L

4.1.3 Cost Comparison Between Treatment Methods

Cost effective analysis for UV alone, 2 mg/L and 8 mg/L PAA/UV advanced oxidation process (AOP) treatments for conditions that reduce FC density to standard regulation were compared. The calculations were based in Canadian funds and on yearly average flow rate of 220 MLD. PAA costs were estimated at 2.7 CAN$/kg (Solvay Interox, Houston, USA). Transportation of the chemical would be provided by a 34,000 L truck several times a year. And an 8% interest rate over a 20 year period is included for capital recovery calculations.

Capital cost for installation of a full scale UV system at the North End Water Pollution Control Centre (NEWPCC) is estimated at 32.9 Million dollars (Earth Tech, 2004). The capital recovery for this system, thus, is 3.35 Million dollars per annum. The operation and maintenance (O&M) costs for this UV system, which includes electrical power usage, lamp replacement and cleaning, are estimated as 1.05 Million dollars per year (Earth Tech, 2004).

Investment in PAA supplement to UV procedure, on the other hand, requires 26.48 Million dollars and 12.18 Million dollars capital for 2 mg PAA/L and 8 mg PAA/L treatments, respectively. The capital costs cover installation of the UV disinfection system, although at a reduced size due to reduced energy expenditure required to disinfect, and the PAA contact basin. The O&M costs for both AOP treatments are calculated as 4.51 Million dollars for 2 mg PAA/L and 5.63 Million dollars for 8 mg PAA/L treatments.

Cost comparison of the three treatment methods is shown in Table 4-2 below. The total estimated yearly cost – including capital recovery and O&M costs – for UV alone

and 2 mg/L PAA/UV, and 8 mg/L PAA/UV were 4.41 Million dollars, 7.20 Million dollars and 6.87 Million dollars, respectively.

Table 4-2. Cost effective analysis comparing UV alone and PAA/UV AOP treatments at dose necessary to disinfect FC bacteria to standard regulation. An 8 % interest rate for a 20 year period is included in capital recovery costs.

	Canadian Dollars
UV TREATMENTS	35 mWs/cm^2 of Fluence
Capital Cost – UV facility	$ 32,900,000
Capital Recovery	$ 3,350,000
Operation & Maintenance	$ 1,057,000
Total Investment	**$ 4,407,000**
PAA/UV COMBINED PROCESS	
	2 mg PAA/L
Capital Cost	
UV facility	$ 25,991,000
PAA Contact basin	$ 494,000
Sub Total	$ 26,485,000
Capital Recovery	$ 2,697,000
Operation & Maintenance	
UV energy (includes 21 % savings)	$ 835,000
PAA Chemical	$ 3,521,000
PAA Transportation	$ 155,000
Sub total	$ 4,511,000
Total Investment	**$ 7,208,000**
	8 mg PAA/L
Capital Cost	
UV facility	$ 11,844,000
PAA Contact basin	$ 342,000
Sub Total	$ 12,186,000
Capital Recovery	$ 1,241,000
Operation & Maintenance	
UV energy (includes 64 % savings)	$ 380,000
PAA Chemical	$ 4,630,000
PAA Transportation	$ 622,000
Sub Total	$ 5,632,000
Total Investment	**$ 6,873,000**

4.2 Part II: Somatic Coliphage ϕX174 and Reovirus Seeded into Wastewater Effluents

4.2.1 Somatic Coliphage Disinfection using UV and AOP Treatments

The initial coliphage concentration seeded into the wastewater effluent samples was $5x10^6$ PFU/ml. Ten mWs/cm^2 of UV energy significantly reduced the initial coliphage concentration by 4.3 logs leaving 240 PFU/ml (Figure 4-9) ($P<0.05$). Using the same fluence but supplemented with either 2 mg/L or 8 mg/L of PAA, the value was reduced by 4.7 or 5.2 logs, respectively (2, $P<0.05$; 8, $P<0.05$). The results indicate that as the PAA concentration increases the effectiveness to disinfect increases as well. The remainder of the treatments produced total reduction, except values treated with 2 mg/L PAA combined with 20-mWs/cm^2, which produced a 5.4 log reduction.

A Comparison between both AOP treatments to UV alone were found to be statistically insignificant for somatic coliphage disinfection ($P>0.05$). This indicates that there was no difference between the AOP treatments with the UV reference treatment. Thus, the additional disinfection provided by supplementing PAA with UV does not enhance the disinfection of UV alone. Disinfection with UV is sufficient to provide adequate inactivation of somatic coliphage with 10 mWs/cm^2 of UV energy.

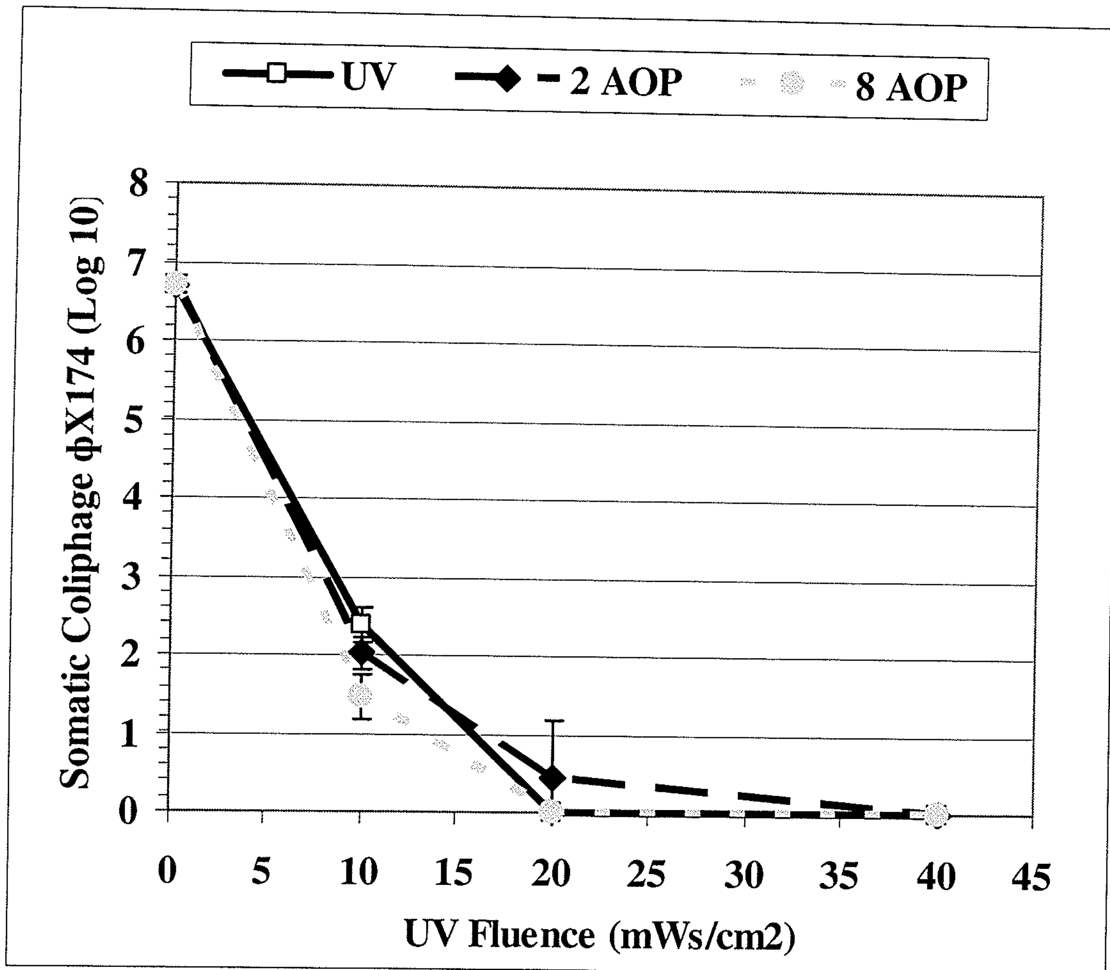


Figure 4-9. Survival of somatic coliphage using UV, 2mg/L AOP and 8mg/L AOP.

4.2.2 Comparing Reovirus Disinfection using UV and AOP Treatments

The initial reovirus concentration seeded into samples of wastewater was $8.6x10^6$ PFU/ml. The UV treatment increased the log removal of reovirus as fluence increased. At 20 mWs/cm^2 UV energy, 0.9 logs were reduced (Figure 4-10) ($P<0.05$). This value increased to a maximum of 3.0 log units after 40 mWs/cm^2 ($P<0.05$). Thus, a five log removal was not achieved with UV alone within the limit provided.

When the same effluent sample was exposed to 2 mg PAA/L supplemented with UV, disinfection was found to be similar to UV alone disinfection (i.e. first order). Using 10 mWs/cm^2 of UV radiation, reovirus concentration was reduced by less than 0.1 log ($P<0.05$). An increase in fluence to 40 mWs/cm^2 produced a 3.7 log reduction ($P<0.05$). Again five log removal could not be achieved within the limit provided. However, UV disinfection could be enhanced by supplementing the process with 8 mg PAA/L. This treatment complementary with approximately 35-mWs/cm^2 of UV radiation provided a five log reduction in reovirus concentration (Figure 4-10).

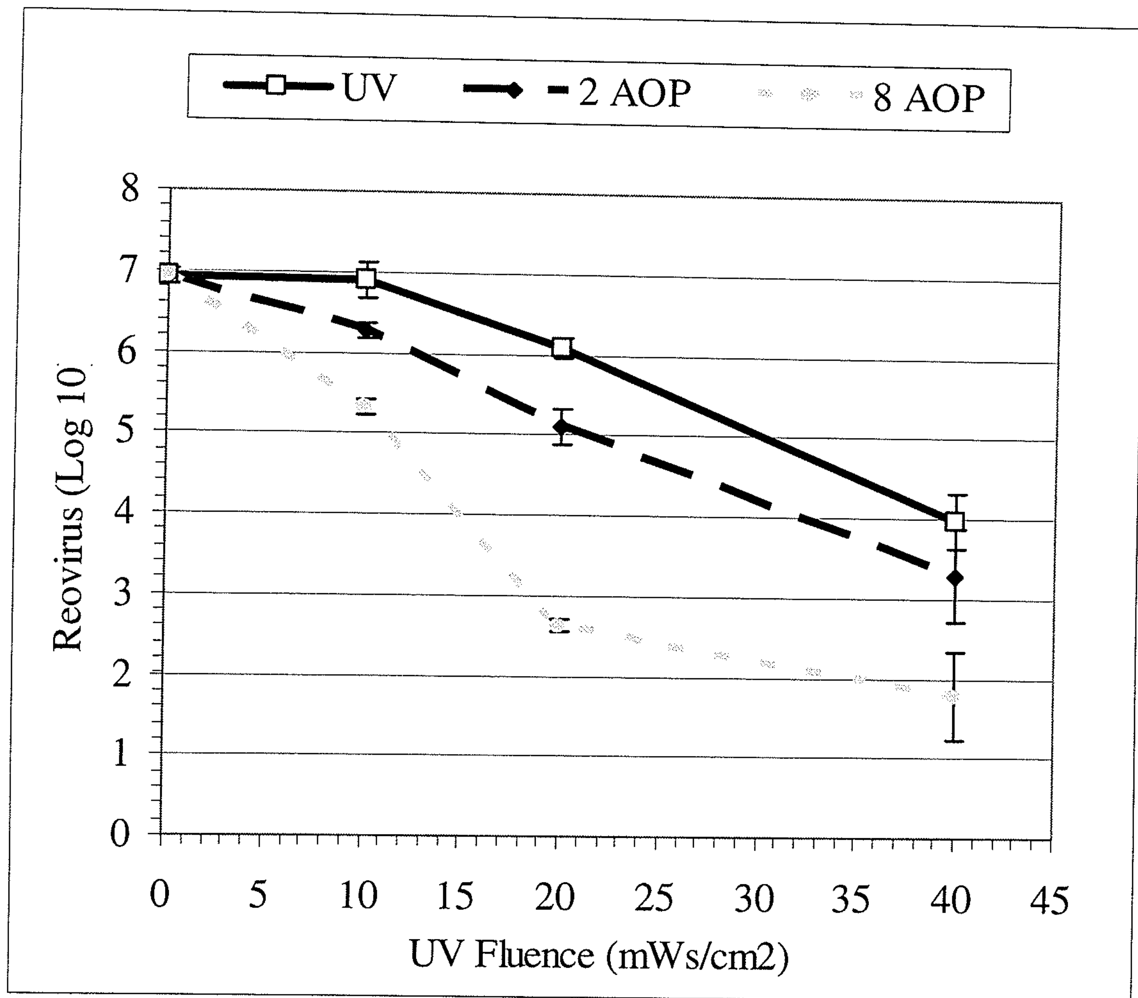


Figure 4-10. Survival of reovirus using UV, 2mg/L AOP and 8mg/L AOP

5. DISCUSSION

5.1 Part I: Wastewater Effluents Collected during Dry Weather (Phase 1) and Wet Weather (Phase 2) Conditions

5.1.1 Objective 1: Disinfection Performance of Various Treatments on Effluents Collected During Dry Weather Conditions

5.1.1.1 Phase 1: UV treatment

Disinfection of wastewater effluents with UV radiation inactivated FC more effectively when PAA was used, but disinfection with UV alone was less efficient than when the combined operation was used. The fluence-response curve indicates that the FC concentration could be reduced to a level that complies with the regulation standard when 28-mWs/cm^2 of UV energy was introduced (Figure 4-1). This fluence value falls within the standard error of the result indicated in the Earth Tech (2004) report. As a comparison, the effluent collected for the Earth Tech (2004) report was taken from the same wastewater treatment facility and the bench scaled tests were followed in the same format as the current study.

Figure 4-1 indicates that there was little tailing involved. This is an indication that the particle-associated bacteria were mostly affected due to low TSS concentration. Gehr et al. (2003) obtained similar results when comparing 10, 20, and 40 mWs/cm^2 fluences for disinfecting FC in effluents collected from the City of Montreal Wastewater Treatment Plant. The water quality parameters obtained from their effluent tests contained substantially lower concentrations for TSS and UVT than values obtained from NEWPCC effluents. The data obtained from this study more closely relate to disinfection

data presented in a study by Gehr et al. (2002) when comparing the biologically disinfected effluents. Specifically, the Gehr et al. (2002) study compared inactivation of FC bacteria from various treatment plants with two types of treatment processes – biological treatment vs. physicochemical treatment. For instance La Prairie treatment facility uses biological treatment processes, whereas the Montreal Urban Community (MUC) facility utilizes physicochemical treatment methods. The effluent quality released by the MUC treatment plant indicates as being of higher TSS and turbidity concentration as well as being relatively lower UVT values than the biologically treated facilities. Fecal coliform inactivation from the MUC treated effluents proved to be easier to disinfection, despite a poorer effluent quality produced by this treatment facility. It is thus, suspected that bacteria exposed to biological activated sludge treatment processes affects the bacteria's ability to resist UV disinfection. This suggests that method of wastewater treatment used by the facility will have an influence on bacteria resistance to UV disinfection.

5.1.1.2 Phase 1: PAA treatment

Treating wastewater effluents with PAA alone proved inadequate for disinfecting FC to a concentration that complies with the discharge limit (Figure 4-2). Low testing concentrations, 2 mg/L or 8 mg/L, combined with low contact times (<10minutes) were not sufficient to produce the desired outcome. These results corroborate other research where one of factors, either PAA dose or contact time, must be alternatively high to produce the desired results (Caretti and Lubello, 2003; Gehr et al., 2003; Wagner et al., 2002; Liberti et al., 1999; Lazarova et al., 1997; Lefevre et al., 1993). Increasing the PAA

concentration has a large drawback in terms of high operation and maintenance costs. Extending the contact time for disinfection is more favourable, especially if the treatment facility provides adequate spacing. An example of this is a long retention time in the outfall conduit. With respect to the NEWPCC treatment facility, the maximum retention time allowable in the outfall conduit is 16 minutes for average flow conditions (200 MLD). Alternatively, the West End Water Pollution Control Centre (WEWPCC) provides for greater than 30 minutes retention time, which allows for longer PAA contact time for disinfection. The PAA treatment method could be improved by providing rigorous mixing during and extending contact time (Liberti et al., 1999). Additionally, disinfection could be improved by adding PAA solution prior to irradiating sample with UV. This would enhance disinfection complementing both disinfection processes into an advanced oxidation process (AOP). This is the focus of the next section.

5.1.1.3 Phase 1: AOP treatment

Both AOP treatments were very efficient in producing FC concentrations that comply with standard effluent discharge (Figure 4-3). Combining both disinfectants enhanced the disinfection efficacy over using either of the two disinfectants alone. The 2 mg/L PAA treatment combined with 22-mWs/cm^2 of UV radiation produced 3.2 log reduction required to meet the standard. This combination effectively reduced the energy requirement for UV disinfection by 21%. By comparison, Caretti and Lubello (2003) obtained total inactivation of FC bacteria (approximately 4 log reduction) using a 2 mg /L AOP, however the UV fluences required to produce the 4 log reduction was far greater (165 and 192-mWs/cm^2) than required in current research. The rational for higher

fluences used in the Caretti and Lubello (2003) study could not be determined. A separate study, Chen et al. (2005) showed even better disinfection results than the current study in that a 4.0 log reduction of FC occurred when 2 mg PAA/L was supplemented with 10-mWs/cm^2 UV fluence. The discrepancy in these results could perhaps be explained by the 30 minutes delay to UV exposure, after PAA addition, given by the Chen et al. (2005) method.

Combining 8 mg/L PAA with UV energy enhanced the disinfection of FC over all other treatment methods tested. Specifically, this PAA concentration combined with 10 mWs/cm^2 of UV radiation was needed to meet the standard FC discharge guideline. This resulted in 64% less energy use to meet the guideline. This combined treatment produced results comparable to other forms of pre-treatment used to inactivate FC, such as sand filtration or micro-filtration (Qualls et al., 1985; Qualls et al., 1983; and Severn, 1980).

The AOP treatment appears to be more effective in inactivating FC than either of the individual disinfectants (Table B-1, B-2, *Appendix B*). It is suspected that PAA weakens bacteria with an initial shock, making it more susceptible to UV irradiation damage. The enhancement in disinfection efficiency, however, is suggested to result from the UV induced photo catalyzing reaction of PAA, and to a lesser extent to the action of H_2O_2, conducing to hydroxyl radical formation (Chen et al., 2005; Caretti and Lubello, 2003). Results show that irrespective to the PAA concentration used, combination of agents caused rapid inactivation of FC bacteria though synergistic effect (Figures 4-4, 4-5), although the disinfection reaction was at a faster rate when the higher PAA concentrations was used. This synergistic process peaks at the 20-mWs/cm^2 UV fluence level, after which the disinfection response turns additive. This result can be attributed to

the direct availability of viable FC bacteria remaining in the irradiated sample. With fewer bacteria present, the inactivation rates between the AOP treatments and the reference treatment ultimately overlap. Furthermore, under irradiation with higher fluences, >20 mWs/cm^2, the effect of inactivation in the AOP treatments was primarily caused by UV light rather than the hydroxyl radical. This could be observed by comparing the summed individual effects to the combined effects as depicted in Figure 4-4, 4-5.

5.1.2 Objective 2: Disinfection Performance of using AOP Compared with UV on Effluents Collected During Wet Weather Conditions

During wet weather periods, the effluent quality is typically worsened by a higher loading rate of untreated fecal contaminants and greater concentration of TSS flowing through the system (Table 4-1). A poorer effluent quality makes disinfection with UV more ineffective. Figure 4-7 shows that within the fluence limits provided, UV did not provide sufficient energy to meet the standard.

In section 5.1.1, it was determined that AOP treatments enhance UV disinfection of FC from effluents collected from a dry weather period. Thus, it is hypothesized that AOP could enhance UV disinfection of FC from effluents collected during wet weather periods. It was determined that due to the very poor effluent quality, with 76 mg/L TSS and 33 % UVT, resulted in AOP treatments being ineffective for FC disinfection. The concentration of TSS obtained from this sample was atypical. The average TSS is during a wet weather period is 24 mg/L and UVT is 35 %. The data indicate that an enhancement were statistically insignificant ($P>0.05$; Figure 4-7). It is hypothesized, from these results, that vigorous mixing of PAA prior to UV irradiation might improve the disinfection process.

5.1.3 PAA residual assay

Residual assay tests confirm that final PAA concentrations (2 mg/L; 8 mg/L) mixed into wastewater samples were accurate.

5.1.4 Objective 3: Cost Effectiveness Analysis: Comparing AOP to either Individual Disinfectant

A decision of cost-effectiveness between UV alone and PAA/UV advanced oxidation process treatments is shown in Table 4-2. An 8 % interest rate for a period of 20 years is used in the capital recovery calculation.

Previous section (5.1.1.3) shows that the PAA supplement to UV disinfection produces a faster inactivation of fecal coliforms to the standard regulation (200 MPN fecal coliform/100 ml) than using UV alone. The little benefit of reducing UV energy costs, provided by PAA supplemental to UV, is grossly outweighed-by the very high purchase and transportation costs of PAA solution. This is especially the case for the 8 mg/L AOP treatment because this treatment could save more than half of the UV energy cost, but overall it still is 1.5 times more costly. Until the PAA O&M costs are reduced it is more economical to use UV alone for disinfection, especially since electricity costs in Manitoba are low. It would be more efficient to increase the energy input to improve FC inactivation than to supplement the effluent with PAA prior to UV disinfection to meet the standard.

Less than 2 mg PAA/L concentration is required for the combined process to be an economically viable option. This treatment system, however, would require a larger contact basin. The NEWPCC does not have the space available for accommodating this system. The South End Water Pollution Control Centre, on the other hand, has the space available to install a larger contact basin. At the West End Water Pollution Control Centre, instead of placing a contact basin, the PAA contact period could be placed along the outflow conduit prior discharge to Assiniboine River.

5.2 Part II: Objective 4: Disinfection of Somatic Coliphage ϕX174 Compared to Reovirus Seeded in Wastewater Effluent

5.2.1 Inactivation of Somatic Coliphage using UV and PAA/UV Treatment

In accordance to National Water Research Institute (NWRI) for standard guideline for disinfection indicates a 5 log reduction of poliovirus is required for elimination of pathogenic organisms from wastewater effluents for purposes of reuse (NWRI, 2003).

UV disinfection was very effective at inactivating somatic coliphage ϕX174 (Figure 4-9). A low fluence, <10 mWs/cm^2, removes 4.3 log of the initial coliphage concentration (6.7-log value). Comparable to other UV disinfection studies, somatic coliphage, however, are less resistant to the radiation than is poliovirus (AWWARF and AWWA, 2000). Somatic coliphage, thus, would not be a good indicator of fecal contamination (i.e. pathogenic presence) especially since pathogenic organism concentration discharged can be highly variable from day to day.

Comparing the inactivation effects between the PAA/UV combined effects to UV alone found that treatment with UV alone was sufficient to inactivate coliphage particles. The additional PAA provides no enhancement to the inactivation of microorganisms. Rajala-Mustonen et al. (1997), in contrast, reports a reduced effect by combining PAA with UV for disinfection of somatic coliphage (RNA strain). Specifically, 20-mWs/cm^2 UV produced a 3.5 log reduction of somatic coliphage, whereas when it is supplemented with 20 mg PAA/L there is no log reduction after 20-mWs/cm^2. Their study indicates, however, a spike in a 4 log reduction after treatment with 60-mWs/cm^2 when combined

with a 20 mg/L PAA. It is hypothesized that the difference in results might be based on higher values of turbidity, causing an increase in UV absorbance by the particles in the effluent.

5.2.2 Inactivation of Reovirus using UV and PAA/UV Treatment

Disinfection of reovirus, on the other hand, showed strong resistance both to UV disinfection, as well as, to the PAA supplemented treatments (Figure 4-10). The inactivation of the reovirus with UV alone followed first order kinetics. This indicates that TSS concentration did not influence the inactivation rate and that the particles were not incorporated into the flocs. Natural resistance of reovirus to disinfection occurs as well (Figure 4-10). The UV treatment supplemented with 8 mg PAA/L indicates this effect. The inactivation rate of this treatment is reduced showing the tailing effect and the five log reduction value could be obtained by supplementing 35 mWs/cm^2 of UV fluence with 8 mg PAA/L.

Comparison of reovirus inactivation using with that poliovirus UV disinfection alone indicates those reoviruses are more resistant (AWWARF and AWWA, 2000). Figure 4-10 indicates that to obtain the five log reduction of virus greater than 40-mWs/cm^2 of UV radiation is required. Since, reoviruses are ubiquitous in nature (Coombs, 2002; Milde et al., 1995) they might be useful as an indicator in disinfection of fecal contaminated waters.

6. CONCLUSIONS

6.1 Part I

6.1.1 Phase 1 Results

- PAA at concentrations ≤8 mg/L is inadequate for disinfection of FC in effluents discharged by NEWPCC during normal operations.
- 2 mg PAA/L pre-treatment to UV reduces energy requirements by 21% compared to UV alone. 8 mg PAA/L pre-treatment to UV reduces energy requirements by 64% compared to UV alone.
- The faster inactivation of FC to the standard guideline compared to UV alone was produced by synergistic effect conduced by hydroxyl radical formation.
- By disinfection efficiency standards, inactivation of FC to standard regulation was as follows form most efficient to least efficient:

  8 mg PAA/L + 10 mWs/cm^2 > 2 mg PAA/L + 22 mWs/cm^2 > UV alone (28 mWs/cm^2)
- Activated sludge treated wastewater produced better effluent quality than the physicochemically treated wastewater; UV disinfection of FC from activated sludge wastewater effluents, however, were more difficult to inactivate.
- Evaluation of disinfection based on cost effectiveness, it was determined that treatment with the following disinfectants are set up from most efficient to least efficient:

  UV alone (28 mWs/cm^2) > 2 mg PAA/L + 22 mWs/cm^2 > 8 mg PAA/L + 10 mWs/cm^2

6.1.2 Phase 2 Results

- UV treatment did not meet the effluent standard for FC, possibly caused by poor effluent quality which interfered with the UV disinfection process.
- AOP treatments were not statistically different from the UV reference treatment. In this work, it could not accurately be determined whether the AOP treatments were more effective than the UV alone in improving the efficacy for UV disinfection.

6.2 Part II

- UV inactivates Somatic Coliphage ϕX174 very quickly; < 10 mWs/cm^2 provides total reduction (approximately 6 log reduction). PAA disinfection supplement does not improve inactivation of these particles.
- Reovirus is very resistant to UV disinfection; > 40 mWs/cm^2 would be required to obtain a five log reduction. When supplemented with 8 mg/L PAA disinfection occurs at 35-mWs/cm^2.
- Compared to the literature, reovirus is more resistant and Somatic Coliphage ϕX174 are less resistant to UV disinfection when compared with poliovirus disinfection.

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NOMENCLATURE

Irradiance and fluence rate are terms commonly used to describe the intensity of radiation, with subtle differences. *Irradiance* describes the amount of direct radiant energy incident, from a single direction, onto a surface area (e.g. collimated beam set-up) (Bolton and Linden, 2003). *Fluence rate,* the second concept, refers to the radiant energy directed over a surface area, incident from multiple directions (e.g. flow-through reactor). In a well-designed collimated beam set up, both the fluence rate and irradiance are synonymous. The units are expressed as W/m^2, but also widely accepted is mW/cm^2. Secondly, the terms *radiant exposure* and *fluence* describe the "dose" in UV systems depending on whether a single or multiple directed light source is used. The meaning for the term "dose" is typically used for describing the total energy absorbed by a given chemical (Bolton and Linden, 2003). In the UV disinfection process, an infinitesimal portion of the UV radiation is actually absorbed; the remainder virtually passes right through the microorganism (Bolton and Linden, 2003). Thus, the term fluence is more appropriate to describe the incident UV energy as opposed to the absorbed UV energy (Bolton and Linden, 2003).

LIST OF ABBREVIATIONS

AOP	Advanced oxidation process
FC	Fecal coliforms
CT	Contact time
PAA	Peracetic acid
SOC	Soluble organic carbon
TC	Total coliforms
TOC	Total organic carbon
TSS	Total suspended solids

Appendix A

A-1.0 Water Quality Characteristics

A-1.1 Total Suspended Solids (TSS)

Analysis following Standard Methods (APHA, AWWA, WEF. 1995).

Apparatus

- 100 ml graduated cylinder
- Porous crucible
- 934AH Whatman Glass microfibre filters Cat No 1827 032
- Vacuum pump
- Desiccator and desiccator stones
- 103 °C oven

A-1.2 Total Organic Carbon (TOC) and Soluble Organic Carbon (SOC)

Analysis following Standard Methods (APHA et al., 1995).

Apparatus

- 85% phosphoric acid - preservative
- Tekmar Dohrmann Apollo 9000 analyzer
- Vacuum pump
- GF/B Whatman Glass microfibre filters Cat No 1821 047

A-1.3 Turbidity

Analysis following Standard Methods (APHA et al., 1995).

Apparatus

TD-40 Turner Nephlometer

A-1.4 UV Transmissivity

Apparatus

- Trojan P254C Spectrophotometer
- Quartz Cuvette
- Deionized Water

A-2.0 UV Collimated Beam Testing

Reagents

- 1-N Sulphuric acid

Apparatus

- Collimated Beam Apparatus (Trojan Technologies)
- 1mm increment graph paper
- International Light Radiometer (Model No. IL1400A)
- 50x Crystallization Dishes (Fisher Scientific)
- 4x Graduated Cylinders
- 24x 1cm length Stir bars
- P254 Trojan Spectrophotometer
- Quartz cuvette
- Aluminum foil
- Autoclave and indicator tape
- Magnetic Stirrer
- UV cover slip
- Fisher Scientific Stop Watch

A-2.1 Procedure for Collimated Beam Testing

Set up Apparatus

- Place collimated beam apparatus on a horizontal surface.
- Adjust lamp distance to the desired height
- Using radiometer, locate the center of the UV intensity.
- Mark the center intensity point on graph paper and secure paper to table surface.
- Place magnetic stirrer on to irradiating surface.
- Turn on lamp 15-minutes prior to use.
- Important: Before testing, calibrate UV lamp to germicidal average UV irradiance with correcting factors, which is used for determining fluence dose.

A-2.2.0 Correcting Factors for Germicidal Average Irradiance (G_{avg})

A-2.2.1 Petri Factor (P*f*)

- Turn on radiometer; maintain protective cap and "zero" the radiometer.
- Place radiometer over the central position and record the value.
- Important: Record the intensity of the lamp in a spiral rotation away from the centroid position at 5-mm increments (up to 3cm away) along the abscissa and ordinate.
- Record the values onto the Excel spreadsheet.

The Petri Factor (P*f*) is defined as a ratio of the average incident irradiance, projected over the irradiating surface, to the incident irradiance at the centre of the

sample dish (Bolton and Linden, 2003). The purpose of this factor is to provide an average irradiance over the entire irradiating surface that best reflects the irradiance projected at the centre of the dish. Radiometer readings were recorded along the irradiating surface, up to 3-cm from the central axis, in 0.5 cm increments along the abscissa and ordinate equivalent to an area that covers the crystallization dish surface. A well-designed collimated beam set-up should have a P*f* greater than 0.90 (Bolton and Linden, 2003).

A-2.2.2 Divergence Factor (D*f*)

- The distance of the lamp to the water surface is calculated.

This factor adjusts for deviations in incident UV irradiance, created by non-parallel collimated rays that diverge from the normal; this divergence becomes greater as the distance from the lamp increases. The D*f* is calculated as the ratio between the distance (L) from the lamp to the water to air interface and the sum distance of "L" and the wastewater sample path-length. (L + *d*).

A-2.2.3 Reflectance Factor (R*f*)

- Record a 2.5 % reflectance.

The reflectance factor (R*f*) incorporates only the proportion or radiation remaining after the incident light is reflected from the wastewater surface. Electromagnetic radiation parallel to the normal, reflects 2.5% of the incident energy (Bolton and Linden, 2003)

A-2.2.4 Water Quality Factor (W*f*)

- Add a sample of wastewater into a 1-cm path length cuvette vial.
- Measure and record the UV light transmitted through the cuvette vial.

Collimated UV light that enters the wastewater sample attenuates in accordance to the Beer-Lambert Law. In a completely mixed sample, the Water Quality Factor (W*f*) was derived by integrating the Beer-Lambert Law through the whole sample depth (Bolton and Linden, 2003; Morowitz, 1950). The W*f* was calculated as:

$$Wf = \frac{1 - 10^{-\alpha d}}{\alpha\, d \ln(10)} \qquad \text{M-1.2}$$

Where

W*f* = the water quality factor

α = the absorption coefficient for a 1-cm path-length = (log(100/%T))

d = depth of irradiated wastewater sample

A-2.3 The germicidal average irradiance (G_{avg}) is calculated as

$$(G_{avg}) = (I_{centre} \times Pf) \times (Df) \times (Rf) \times (Wf) \qquad \text{M-1.3}$$

Where,

I_{centre} = Irradiance measured at the centre of the irradiating dish (mWs/cm^2)

Other parameters described above

A-2.4 Collimated Experimental Procedure

- Turn on UV lamp 15-minutes prior to use.
- Place magnetic stirrer under UV lamp rays.
- Measure 50-ml of treated sample, using graduated cylinder, and dispensed into crystallization dish.
- Use UV cover slip to block UV light.
- Place 1 stir bar into dish; Place dish on magnetic stirrer.
- As the cover slip is removed immediately initiate the stopwatch.
- At the end of the allotted time cover the UV rays with cover slip

A-3.0 Peracetic Acid (PAA) Assay

- Ultrospec 4300 pro Spectrophotometer
- 50 cuvette permit 405 nm transmittance
- Nichpet 1000 – 5000-μl pipette and pipette tips
- Nichpet 100 – 1000-μl pipette and pipette tips
- Nichpet 20 – 200-μl pipette and pipette tips
- Nichpet 0.5 – 10-μl pipette and pipette tips
- 12x 250-ml capacity flask
- Autoclave and indicator tape
- Aluminum foil
- 120x 7-ml capacity amber coloured vials and vial rack

Reagents

Reagent	Use
-Sodium Phosphate Buffer (SPB)	- for standard calibration and experimental runs
-Horse Radish Peroxidase (HRP)	- for standard calibration and experimental runs
-2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfuric acid) di-ammonium salt (ABTS)	- for standard calibration and experimental runs
-Sodium Thiosulfate	- for experimental runs only
-Catalase	- for experimental runs only
-PAA sample	- for standard calibration and experimental runs

A-3.1.0 Preparation of Reagents

A-3.1.1 Sodium Phosphate Buffer (SPB) Preparation

- STORED UP TO A WEEK – Store in 4 °C
- Prepare a 0.067 M solution from monobasic and dibasic sodium phosphate
- Dissolve 13.8g (monobasic sodium phosphate) into 500ml volumetric flask
- Dissolve 2.68g (dibasic sodium phosphate) into 50ml volumetric flask
  Combine 294-ml of monobasic with 41-ml of dibasic; top off to 1-L to make the 0.067-M SPB solution

A-3.1.2 HRP Preparation

- PREPARE DAILY – Store in 4 °C
- HRP is an enzyme extracted from bovine liver.
- Dissolve 5mg of HRP power into 1ml of deionized water (Use 20ml container); this makes a 5mg/ml solution. Mix thoroughly with autoclaved inert apparatus
- Dilute 0.1 ml of the above solution into 10ml of deionized water (use 20ml container); this makes a 0.05mg/ml solution

A-3.1.3 ABTS Preparation

- CAN BE STORED UPTO 3 DAYS – Store in 4 °C
- Dissolve 0.11g of ABTS into 10ml of 0.067M sodium phosphate buffer (SPB)

A-3.1.4 Sodium Thiosulfate Preparation

- Functions to cease PAA oxidation; a concentration of 100mg/L is adequate.
- CAN BE STORED IN 4 °C FOR 3 TO 6 MONTHS
- Make a 2.5 in 100 dilution of 2N (i.e. 158,110-mg/L) stock solution in deionized water; this makes a 4000-mg/L of a working solution.
- Add 25-µl of this solution into sample vials to quench 1-ml wastewater samples contained within.

$$(158{,}110\text{mg/L})\ (2.5\text{ml}) = (x)\ (100\text{ml})$$
$$x = 4000\text{-mg/L}$$

$$(4000\text{mg/L})\ (0.025\text{mL}) = (x)\ (1\text{ml})$$
$$x = 100\text{mg/L}$$

A-3.1.5 Catalase Preparation

- functions to cease H_2O_2 oxidation; require 50mg/L
- CAN BE STORED IN 4 °C FOR 3 TO 6 MONTHS
- Dissolve 200-mg into 100mL of deionized water; this makes a 2,000-mg/L working solution
- Add 25µl of this solution into sample vials to quench 1-ml wastewater samples contained within.
-

$$(2{,}000\text{-mg/L})\ (0.025\text{mL}) = (x)\ (1\text{ml})$$
$$x = 50\text{mg/L}$$

A-3.1.6 PAA sample

- Because this assay cannot distinguish between peracetic acid and hydrogen peroxide peracids, assay is performed in mM units instead of mg/L.
- Virgin concentration of PAA solution, 8,300mM (or 133,200mg/L), is diluted to produce 0.97mM (or 15mg/L) of PAA working solution.
- Working solution is used immediately after prepared.
- Using working solution, a standard curve is produced
-

$$(8{,}300\text{mM})\ (0.0117\text{-ml}) = (x)\ (100\text{ml})$$
$$x = 0.97\text{mMmg/L}$$

A-3.2.0 PAA residual assay Procedure

A-3.2.1 Standard Curve

- Add the specific volume of SPB, HRP, ABTS, and PAA together, as indicated in Table A-1.
- IMPORTANT: The PAA volume is added last and the solution is allowed to react for a duration of 6-minutes.
- After six minutes the solution's absorbance is measured at a 405-nm wavelength.
- A Ultrospec 4300 pro Spectrophotometer

Table A-1. Volumes necessary to produce the PAA residual assay standard curve.

	Buffer Solution volume (µl)	HRP volume (µl)	ABTS volume (µl)	PAA volume (µl)	Standard Conc. (mM)
1	4,000	400	400	0	0
2	3,960	400	400	40	0.0081
3	3,850	400	400	150	0.0303
4	3,740	400	400	260	0.0526
5	3,630	400	400	370	0.0748
6	3,520	400	400	480	0.0967

A-3.2.2 Experimental Runs – Confirm PAA Concentration

- 2 and 8mg/L of PAA solution, which convert to 0.124mM and 0.496mM, respectively are used
- Negative controls are included in the tests.
- Each vial contains 3,520 SPB, 400 HRP, and 400 ABTS.
- Add 480-µl of wastewater effluent containing the PAA compounds (except for negative control) to each reaction vial.
- Negative control receives no PAA to the wastewater sample.
- Measure absorbencies using a Ultrospec 4300 pro Spectrophotometer

A-4.0 Biological Assay –Enumeration of Fecal Coliform Bacteria

Analysis followed in accordance with Standard Methods (APHA, AWWA, WEF. 1995).

Apparatus

- Nichpet 1000 – 5000-µl pipette and pipette tips
- Nichpet 100 – 1000-µl pipette and pipette tips
- Peristaltic pump
- Culture tubes and autoclave resistant caps
- Durham vials
- Autoclave and indicator tape
- Aluminum foil
- Applicator transfer sticks

Reagents

- Bromcresol purple
- Magnesium Chloride
- Phosphate buffer solution
- Buffer Dilution Water

Culture Media

- Lauryl Sulfate Broth (LSB)
- EC medium

A-4.1.0 Preparation of Reagents and Culture Media

Reagents

A-4.1.1 Bromcresol Purple

- Dissolve 1g of Bromcresol purple powder into 100ml of deionized water.
- This makes a 10mg/L working stock concentration.

A-4.1.2 Buffer Dilution Water (BDW)

- Dilute 5-ml of Magnesium Chloride and 1.25-ml of phosphate buffer solution into 1-L deionized water.
- Dispense 10-ml of BDW into tubes and; loosely cover with heat resistant caps and sterilize for 15 minutes at 121°C.

Culture Media

A-4.1.3 Lauryl Sulfate Broth (LSB)

- Hydrate LSB medium into deionized water and mix thoroughly. pH should be 6.8±0.2 C after sterilization.
- add 0.01g/L bromcresol purple, to determine acid production, to LSB media
- Dispense 10-ml of broth medium into culture tubes; loosely cover with caps and then autoclave for 15 minutes at 121 °C.
- Arrange culture tubes in rows of 3 tubes.
- Mix test portions using vortex mixer.

- Sterilize in autoclave.
- When remove immediately press down on caps.
- Allow tubes to cool to room temperature prior to use. Discard unused media after 7 days.

A-4.1.4 EC Medium

- Dissolve EC medium into deionized water
- pH should be 6.9 ± 0.2 C
- Place one inverted Durham vial into each culture tube.
- Dispense 10-ml of hydrated EC medium into culture tube; loosely cover with heat resistant caps and then sterilize for 15 minutes at 121°C.
- Remove tubes from autoclave and immediately press down on caps.
- Allow tubes to cool to room temperature prior to use. Discard unused media after 7 days.

A-4.2 9221 B Standard Total Coliform Fermentation Technique

- Make a serial dilution, from $x10^1$ to $x10^6$ factors, into BDW using a 1-ml aliquot of the original treated sample, for each treatment.
- Set up LSB tubes in rows of three for the designated number of dilutions; This is done for each treatment.
- From each dilution tube add a 1-ml aliquot, three times into culture tubes containing LSB.
- Incubate inoculated tubes at 35 ± 0.5 °C
- after 24 ± 2hrs swirl each tube and examine for growth, or acidic reaction (shades of yellow).
- If no growth or reaction is evident, re-incubate and reexamine at end of 48 ± 3hrs.
- Record findings as positive or negative, based on growth and acid production

A-4.3 9221 E Fecal Coliform Presence

- All presumptive fermented tubes (+) (i.e. tubes showing any amount of gas, growth, or acidity within 48hrs of incubation are tested for FC.
- Gently rotate fermented tubes, with a sterile 3mm wooden applicator stick.
- Transfer growth from each presumptive tube to EC broth tubes.
- Place all EC tubes in incubator within 30min after inoculation
- Incubate tubes at 44 ± 0.5 °C for 24 ± 2 hrs
- Remove tubes from incubator and determine the number of positive tubes by observing change in colour and/or presence of gas collected in Durham vial.
- Document the number of positive tubes in accordance to the dilution factor for the tube.

A-4.4 Interpretation

- Gas production with growth in EC broth culture within 24±2hrs or less is a positive FC reaction.
- Failure to produce gas (with little or no growth constitutes a negative reaction).
- MPN is estimated from list of tables provided by Finstein (1972).

A-5.0 Reovirus Plaque Assay

PART A – Setting up indicator cells (12 well plate) DO DAY BEFORE ASSAY
- Each plate contains 12 wells, 2 wells are designated for each dilution
- Pipette mouse fibroblast cells into each well (1.25ml per well of 4 x 10^5 cells/ml)
- Incubate plates at 37°C for 1 day
- Remove plates from incubator and ensure cells are 90-95% confluent.

PART B - Serial Dilutions (96 well plate)
- Dispense 270ul of PBS (phosphate buffer solution) into wells
- Dispense 30ul of 10^0 "treatment sample" into well filled with 270ul PBS
- Make serial dilutions of 10^{-1} to 10^{-6} dilution factors from 10^0 by transferring 30ul into subsequent 270ul wells

- Discard overlay media from subconfluent 12-well plates, exposing cells
- Add 75ul aliquots of serial dilutant into plate well
- Leave plates for 45min to 1 hr and apply periodic rocking motion every 15minutes
- While waiting prepare Overlay media (see PART C)

- discard excess dilution from wells
- add 1.25 ml of overlay media into each well (total of 15ml required per plate)
- allow 10 minutes to solidify
- Incubate plates at 37 °C for 3 days
- On 3rd day of incubation, feed cells with 1ml fresh Overlay Media
- On 6th day of incubation stain cells with 0.04% neutral red (made by mixing equal volumes of 2% agar and 2x PBS, then add 2ml of 2% neutral red per 100ml); add 0.8ml stain per well
- Count plaques next day (day 7)

PART C - Overlay Media
- Calculate how much needed and liquefy 2% agar in microwave for 1 – 5 minutes depending on volume (USE ½ POWER SETTING)
- Place molten agar into 62°C water bath and allow to cool
- Once agar cooled to 62°C and plates (above) have incubated 1hr, pour equal volume completed Medium 199 (2x Medium 199 + 2.5% FCS + 2.5% VSP serum + 2x l-glutamine + 2x Penicillin/Streptomicin) into molten agar
- Add 1x Amphotericin-B
- Add 0.4ml of 7.5% Sodium bicarbonate per 100ml mixed Overlay
- Immediately (but slowly) pipette Overlay Media onto infected cells, let cool, incubate

APPENDIX B

Table B-1. Inactivation performance of 2 mg PAA/L AOP treatment compared with either of two disinfectants.

PAA Conc. 2mg/L	DRY	
	2AOP-UV	2AOP-2PAA
UV Fluence	Ratio	
5	10	33
10	29	199
20	102	440
40	191	640

Table B-2. Inactivation performance of 8 mg PAA/L AOP treatment compared with either of two disinfectants.

PAA Conc. 8mg/L	DRY	
	8AOP-UV	8AOP-8PAA
UV Fluence	Ratio	
5	21	45
10	97	166
20	806	484
40	187	13

Table B-3. P-values comparing various test treatments with control treatment from phase 1 effluents.

Treatments	UV fluence (or time equivalence)			
	5	10	20	40
UV-D	0.0841	0.0484	0.0471	0.0469
2 AOP-D	0.0498	0.0473	0.0470	0.0469
2 PAA-D	0.2559	0.1958	0.2041	0.0666
8 AOP-D	0.0483	0.0470	0.0469	0.0469
8 PAA-D	0.2622	0.0743	0.0511	0.0474

Table B-4. P-values comparing test treatments with UV reference treatment from phase 1 effluents.

Treatments	UV fluence (or time equivalence)			
	5	10	20	40
2 AOP-D	0.1598	0.0133	0.0593	0.2468
8 AOP-D	0.1476	0.0140	0.0269	0.1971

Table B-5. P-values comparing various test treatments with control treatment from phase 2 effluents.

Treatments	UV fluence (or time equivalence)			
	5	10	20	40
UV-W	0.0421	0.0366	0.0364	0.0363
2 AOP-W	0.0369	0.0365	0.0363	0.0363
2 PAA-W	0.0987	-	0.0369	0.0386
8 AOP-W	0.0364	-	0.0363	0.0363
8 PAA-W	0.0465	-	-	0.0363

Table B-6. P-values comparing test treatments with UV reference treatment from phase 2 effluents.

Treatments	UV fluence (or time equivalence)			
	5	10	20	40
2 AOP-W	0.0685	0.1068	0.1198	0.2442
8 AOP-W	0.0603	-	0.0924	0.2045

Table B-7. P-values comparing AOP treatments to inactivate somatic coliphage ϕX174 with control treatment.

Treatments	UV fluence (or time equivalence)		
	10	20	40
UV Reference	0.0089	0.0089	0.0089
2 AOP	0.0089	0.0089	0.0089
8 AOP	0.0089	0.0089	0.0089

Table B-8. P-values comparing AOP treatments to inactivate somatic coliphage ϕX174 with UV reference treatment.

Treatments	UV fluence (or time equivalence)		
	10	20	40
2 AOP	0.0679	0.2113	-
8 AOP	0.0332	-	-

Table B-9. P-values comparing AOP treatments to inactivate reovirus with control treatment.

Treatments	UV fluence (or time equivalence)		
	10	20	40
UV Reference	0.4998	0.0115	0.0094
2 AOP	0.0133	0.0096	0.0094
8 AOP	0.0098	0.0094	0.0094

Table B-10. P-values comparing AOP treatments to inactivate reovirus with UV reference treatment.

Treatments	UV fluence (or time equivalence)		
	10	20	40
2 AOP	0.0718	0.0131	0.1403
8 AOP	0.0502	0.0117	0.0937

Table B-11. Concentration of Fecal coliform bacteria after treatment with UV, PAA and AOP on phase 1 effluents.

Treatment		T1 MPN/100ml	T2 MPN/100ml	T3 MPN/100ml	Geometric Mean	Standard Deviation
UV-D						
Fluence (mWs/cm^2)	0	450,000	115,000	450,000	2.9.E+05	1.9.E+05
	5	25,000	45,000	250,000	6.6.E+04	1.2.E+05
	10	7,000	7,000	4,000	5.8.E+03	1.7.E+03
	20	400	900	600	6.0.E+02	2.5.E+02
	40	30	30	40	3.3.E+01	5.8.E+00
2 AOP-D						
Fluence (mWs/cm^2)	0	450,000	115,000	450,000	2.9.E+05	1.9.E+05
	5	9,500	2,500	25,000	8.4.E+03	1.2.E+04
	10	900	900	2,500	1.3.E+03	9.2.E+02
	20	250	250	250	2.5.E+02	0.0.E+00
	40	30	90	30	4.3.E+01	3.5.E+01
2 PAA-D						
Time (minutes)	0.0	450,000	115,000	450,000	2.9.E+05	1.9.E+05
	0.9	250,000	250,000	250,000	2.5.E+05	0.0.E+00
	1.8	250,000	250,000	150,000	2.1.E+05	5.8.E+04
	3.6	25,000	45,000	450,000	8.0.E+04	2.4.E+05
	7.3	95,000	15,000	110,000	5.4.E+04	5.1.E+04
8 AOP-D						
Fluence (mWs/cm^2)	0	450,000	115,000	450,000	2.9.E+05	1.9.E+05
	5	11,000	1,500	4,500	4.2.E+03	4.9.E+03
	10	90	450	250	2.2.E+02	1.8.E+02
	20	30	30	40	3.3.E+01	5.8.E+00
	40	30	90	40	4.8.E+01	3.2.E+01
8 PAA-D						
Time (minutes)	0.0	450,000	115,000	450,000	2.9.E+05	1.9.E+05
	0.9	140,000	95,000	450,000	1.8.E+05	1.9.E+05
	1.8	140,000	25,000	110,000	7.3.E+04	6.0.E+04
	3.6	25,000	14,000	11,000	1.6.E+04	7.4.E+03
	7.3	950	40	4,500	5.6.E+02	2.4.E+03

Table B-12. Concentration of Fecal coliform bacteria after treatment with UV, PAA and AOP on phase-2 effluents.

Treatment		T1 MPN/100ml	T2 MPN/100ml	T3 MPN/100ml	Geometric Mean	Standard Deviation
UV-W						
Fluence (mWs/cm^2)	0	4,500,000	11,000,000	14,000,000	8.85.E+06	4.9E+06
	5	1,400,000	1,100,000	250,000	7.3.E+05	6.0E+05
	10	45,000	45,000	45,000	4.5.E+04	0.0E+00
	20	9,500	4,500	25,000	1.0.E+04	1.1E+04
	40	300	400	4,500	8.1.E+02	2.4E+03
2 AOP-W						
Fluence (mWs/cm^2)	0	4,500,000	11,000,000	14,000,000	8.85.E+06	4.9E+06
	5	110,000	140,000	140,000	1.3.E+05	1.7E+04
	10	25,000	9,500	45,000	2.2.E+04	1.8E+04
	20	1,500	2,500	4,500	2.6.E+03	1.5E+03
	40	900	400	400	5.2.E+02	2.9E+02
2 PAA-W						
Time (minutes)	0.0	4,500,000	11,000,000	14,000,000	8.85.E+06	4.9E+06
	1.4	4,500,000	4,500,000	4,500,000	4.5.E+06	0.0E+00
	2.9	No Value	No Value	No Value	No Value	No Value
	5.8	140,000	14,000	140,000	6.5.E+04	7.3E+04
	11.6	110,000	150,000	1,400,000	2.8.E+05	7.3E+05
8 AOP-W						
Fluence (mWs/cm^2)	0	4,500,000	11,000,000	14,000,000	8.85.E+06	4.9E+06
	5	400	45,000	9,500	5.6.E+03	2.4E+04
	10	No Value	No Value	No Value	No Value	No Value
	20	450	950	750	6.8.E+02	2.5E+02
	40	300	300	300	3.0.E+02	0.0E+00
8 PAA-W						
Time (minutes)	0.0	4,500,000	11,000,000	14,000,000	8.85.E+06	4.9E+06
	1.4	1,400,000	1,400,000	1,100,000	1.3.E+06	1.7E+05
	2.9	No Value	No Value	No Value	No Value	No Value
	5.8	No Value	No Value	No Value	No Value	No Value
	11.6	300	300	300	3.0.E+02	0.0E+00

Table B-13. Comparisons of Fecal coliform bacteria log values, and log reduction using UV, PAA and AOP using 2 mg PAA/L and 8 mg PAA/L dosages.

Treatment		DRY		WET	
		Log values	Log reduction	Log values	Log reduction
UV alone					
Fluence (mWs/cm^2)	0	5.5	-	6.9	-
	5	4.8	0.6	5.9	1.1
	10	3.8	1.7	4.7	2.2
	20	2.8	2.9	4.0	2.9
	40	1.5	3.9	2.9	4.0
2 AOP					
Fluence (mWs/cm^2)	0	5.5	-	6.9	-
	5	3.9	1.5	4.4	2.5
	10	3.1	2.4	4.3	2.6
	20	2.4	3.1	3.4	3.5
	40	1.6	3.8	2.7	4.2
2 PAA					
Time (minutes)	0/0	5.5	-	6.9	-
	1/1	5.4	0.1	6.7	0.3
	2/3	5.3	0.1	No Value	No Value
	4/6	4.9	0.6	4.8	2.1
	7/12	4.7	0.7	5.5	1.5
8 AOP					
Fluence (mWs/cm^2)	0	5.5	-	6.9	-
	5	3.6	1.8	3.7	3.2
	10	2.3	3.1	No Value	No Value
	20	1.5	3.9	2.8	4.1
	40	1.7	3.8	2.5	4.8
8 PAA					
Time (minutes)	0/0	5.5	-	6.9	-
	1/1	5.3	0.2	6.1	0.8
	2/3	4.9	0.6	No Value	No Value
	4/6	4.2	1.3	No Value	No Value
	7/12	2.7	2.7	2.5	4.5

Table B-14. Concentration of somatic coliphage after treatment with UV and AOP.

Treatment	Time (min.)	T1 (PFU/ml)	T2 (PFU/ml)	T3 (PFU/ml)	Geometric Mean (PFU/ml)	Standard Deviation
UV						
0	0.0	6,000,000	5,800,000	3,800,000	5.1E+06	1.2E+06
10	1.9	280	360	140	2.4E+02	1.1E+02
20	3.9	TR	TR	TR	TR	-
40	7.8	TR	TR	TR	TR	-
2mg/L AOP						
10	1.9	80	180	80	1.0E+02	5.8E+01
20	3.9	20	TR	TR	2.7E+00	-
40	7.8	TR	TR	TR	TR	-
8mg/L AOP						
10	1.9	20	60	20	2.9E+01	2.3E+01
20	3.9	TR	TR	TR	TR	-
40	7.8	TR	TR	TR	TR	-

T = Trial

Table B-15. Concentration of reovirus after treatment with UV and AOP.

Treatment	Time (min.)	T1 (PFU/ml)	T2 (PFU/ml)	T3 (PFU/ml)	Geometric Mean (PFU/ml)	Standard Deviation
UV						
0	0.0	11,865,000 10,605,000	7,413,000 7,276,500	7,833,000 7,833,000	8.6E+06	1.9E+06
10	1.9	14,700,000 14,700,000	5,670,000 6,573,000	5,600,000 5,600,000	7.9E+06	6.6E+06
20	3.9	1,701,000 1,480,500	1,302,000 1,071,000	979,650 882,000	1.2E+06	3.2E+05
40	7.8	24,360 23,520	6,017 5,177	5,597 6,993	9.4E+03	9.3E+03
2mg/L AOP						
10	1.9	2,100,000 2,383,500	2,100,000 1,953,000	1,533,000 1,396,500	1.9E+06	3.8E+05
20	3.9	206,850 176,400	170,100 136,500	63,000 84,000	1.3E+04	3.4E+06
40	7.8	6,857 7,413	1,953 2,100	420 560	1.9E+03	3.1E+03
8mg/L AOP						
10	1.9	170,100 201,600	237,300 294,000	170,100 201,600	2.1E+05	4.7E+04
20	3.9	513	365	393	4.2E+02	7.9E+01
40	7.8	264	30	27	6.0E+01	1.4E+02

T = Trial

Table B-16. Comparisons of Reovirus and Somatic Coliphage ϕX174 log values, and log reduction using UV, and AOP using 2 mg PAA/L and 8 mg PAA/L dosages.

Treatment	Coliphage		Reovirus	
	Log values	Log reduction	Log values	Log reduction
UV alone				
0	6.71	-	6.94	-
10	2.38	4.32	6.90	0.04
20	0.00	Total Reduction	6.08	0.86
40	0.00	Total Reduction	3.98	2.96
2mg/L AOP				
0	6.71	-	6.94	-
10	2.02	4.69	6.27	0.66
20	0.43	5.48	5.11	1.83
40	0.00	TR	3.28	3.65
8mg/L AOP				
0	6.71	-	6.94	-
10	1.46	5.25	5.32	1.62
20	0.00	TR	2.62	4.31
40	0.00	TR	1.78	5.16