An investigation of UV disinfection performance under the influence of turbidity & particulates for drinking water applications

by

Guo Liu

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Applied Science
in
Civil Engineering

Waterloo, Ontario, Canada, 2005

AUTHOR'S DECLARATION FOR ELECTRONIC SUBMISSION OF A THESIS

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

UV disinfection performance was investigated under the influence of representative particle sources, including wastewater particles from secondary effluent in a wastewater treatment plant, river particles from surface water, floc particles from coagulated surface water, floc particles from coagulated process water in a drinking water treatment plant, and soil particles from runoff water (planned). Low-pressure (LP) and medium-pressure (MP) UV dose-response of spiked indicator bacteria *E. coli* was determined using a standard collimated beam apparatus with respect to different particle sources.

Significant impacts of wastewater suspended solids (3.13~4.8 NTU) agree with the past studies on UV inactivation in secondary effluents. An average difference (statistical significance level of 5% or α=5%) of the log inactivation was 1.21 for LP dose and 1.18 for MP dose. In river water, the presence of surface water particles (12.0~32.4 NTU) had no influence on UV inactivation at all LP doses. However, when the floc particles were introduced through coagulation and flocculation, an average difference (α=5%) of the log inactivation was 1.25 for LP doses and 1.12 for MP doses in coagulated river water; an average difference (α=5%) of the log inactivation was 1.10 for LP doses in coagulated process water.

Chlorination was compared in parallel with UV inactivation in terms of particulate impacts. However, even floc-associated *E. coli* were too sensitive to carry out the chlorination

experiment in the laboratory, indicating that chlorine seems more effective than UV irradiation on inactivation of particle-associated microorganisms. In addition, a comprehensive particle analysis supported the experimental results relevant to this study.

Acknowledgements

My sincere appreciation goes to my co-supervisors, Dr. Peter M. Huck and Dr. Robin M. Slawson, for their excellent guidance and invaluable advice throughout this study and the completion of the thesis.

I wish to express my gratefulness to Dr. Michele Dan Vyke and Dr. William B. Anderson, for their encouragement and support during the research and the preparation of this thesis.

I would like to thank all the NSERC Chair staff and students, as well as the fellows in the Water Resources Group, for their support throughout the program; special thanks go to Bruce Stickney and Mark Sobon, for their technical support and effort in the laboratory.

Finally, my deep appreciation goes to my beloved wife, Fengli Ju and our parents, for their endless devotion, patience, and love. To them I dedicate this thesis.

Table of Contents

List of Figures

List of Tables

List of Acronyms and Abbreviations

ANONA---------- Analysis of variance

BOM---------- biodegradable organic matter

CFU---------- colony forming unit

CM---------- complete mixing

CPS ---------- critical particle size

CT or C×T---------- residual chlorine concentration (mg/L) × contact time (minute)

DBPs---------- disinfection byproducts

DOC ---------- dissolved organic carbon

DPA---------- dynamic particle analyzer

DWE building -------- Douglas Wright Engineering building at the University of Waterloo

E. coli---------- *Escherichia coli*

E. cloacae---------- *Enterobacter cloacae*

GAC ---------- granular activated carbon

HAV ---------- Hepatitis A virus

HPC---------- heterotrophic plate count

LP UV ---------- low-pressure UV

LSD ---------- least significant difference

MF ---------- membrane filter

MLD ---------- million liters per day

MP UV ---------- medium-pressure UV

MQ water ---------- ultra-pure water by Milli-Q UV plus (MilliPore Corp.)

MTF ---------- multiple tube fermentation

NOM---------- natural organic matter

NTU ---------- nephelometric turbidity units

PBS ---------- phosphate buffered saline

PF ---------- plug flow

PSD ---------- particle size distribution

QA/QC ---------- quality assurance/quality control

RED---------- reduction equivalent dose

RSD---------- relative standard deviation

SEM ---------- scanning electron microscopy

SS ---------- suspended solids

SD---------- standard deviation

Total coliforms ------- total coliforms

TSS ---------- total suspended solids

USEPA ---------- US Environmental Protection Agency

UV ---------- ultraviolet

UVA ---------- UV absorbance at specific wavelengths

UVDGM ---------- Ultraviolet Disinfection Guidance Manual

UVT ---------- UV transmittance

WWTP ---------- wastewater treatment plant

WTP ---------- water treatment plant

Chapter 1

Introduction

1.1 Background

UV light has been widely used to disinfect effluent from wastewater treatment facilities in meeting the discharge regulations. As opposed to chlorine disinfection, UV inactivation was considered to be cost-effective (Scheible and Bassel, 1981), and formed little toxic residuals or disinfection byproducts (DBPs) that would be discharged to the receiving water body (Ward and DeGrave, 1978; Whitby and Scheible, 2004). Because of the considerable level of suspended solids present in the effluent from wastewater treatment plants, a wide range of research has been triggered to investigate whether UV light can inactivate microorganisms (e.g. total coliforms as indicator bacteria) effectively under the influence of suspended solids (Qualls *et al.*, 1985; Loge *et al.*, 1996, 1999; Parker and Darby, 1995; Emerick *et al.*, 1999, 2000). Results of the research in wastewater have indicated that clumping or particle association shielded microorganisms from UV irradiation. Consequently, some of the affected microorganisms escaped UV inactivation and survived successfully.

The application of UV disinfection in drinking water has been boosted in North America recently, since it was shown that UV light can inactivate *Cryptosporidium* oocysts effectively based on infectivity, even at very low doses (Clancy *et al.*, 1998; Bukhari *et al.*, 1999; Clancy *et al.*, 2000; Shin *et al.*, 2001). The second advantage of UV disinfection is the

minimal DBPs formation. Liu *et al.* (2002) reported that low pressure and medium pressure UV lamps did not have a significant impact on the formation of DBPs at doses less than 500 mJ/cm^2. The recommended UV dose for the purpose of disinfection in drinking water treatment plants in North America is 40 mJ/cm^2 (NWRI/AWWARF, 2000), which is well below 500 mJ/cm^2.

In drinking water treatment systems, there are many sizes of facilities in North America that supply potable water by providing extensive watershed protection, water quality monitoring, and disinfection. These "unfiltered" systems meet the filtration avoidance criteria of the Surface Water Treatment Rule (SWTR, 40 CFR 141.71, USEPA, 1979), which allow unfiltered turbidity of up to 5 NTU. The proposed Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) assigned the same UV dose requirement for both unfiltered and post-filtration systems. Additionally, the US Environment Protection Agency (USEPA) only included inactivation data from studies where turbidity was less than 1 NTU (USEPA, 2003).

A better understanding of source water turbidity and particulates on UV disinfection robustness is therefore critical for the unfiltered systems. Many research projects (Womba *et al.*, 2002; Craik, 2002; Oppenheimer *et al.*, 2002; Templeton *et al.*, 2003; Passantino *et al.*, 2004; Batch *et al.*, 2004) have been undertaken to investigate the impact of turbidity and particulates on UV performance in drinking water systems. Results of this research, as

opposed to wastewater research, have shown that the influence due to natural turbidity and particulates was insignificant on the pattern of UV inactivation. Nevertheless, significant shielding effects were found after the coagulation process in which it was hypothesized that some microorganisms would be partly or completely embedded in these coagulated particles (formed floc). For this new and practical topic with respect to UV inactivation, further research and development are necessary to provide in-depth knowledge and understanding.

1.2 Objectives

The principal objectives of this thesis were as follows:

1. Determine the dose-response of indicator bacteria by low-pressure UV irradiation:

- under the influence of wastewater particles
- under the influence of surface water particles
- under the influence of coagulated surface water particles
- under the influence of coagulated process water particles (from a full-scale drinking water treatment plant)
- under the influence of runoff water samples (planned)

2. Determine the dose-response of indicator bacteria by medium-pressure UV irradiation:

- under the influence of wastewater particles
- under the influence of coagulated surface water particles

3. Compare chlorination versus UV inactivation in terms of the impact of particles

4. Integrate and depict the significant impacts of particulates on UV dose-response through comprehensive particle analysis

1.3 Research Approach

Indicator Bacteria — a natural source of coliform bacteria and a laboratory grown *E. coli* were chosen as the candidates of indicator bacteria. A preliminary experiment was conducted to determine which was more suitable.

Particulate Sources — particles representative of wastewater were from the secondary effluent of the Waterloo Wastewater Treatment Plant in the Regional Municipality of Waterloo; particles relevant for drinking water were obtained from the municipal intake location and the flocculation tank of the Mannheim Water Treatment Plant in the Regional Municipality of Waterloo.

Water Quality Parameters — water quality parameters of interest relevant to the experiments included dissolved organic carbon (DOC), turbidity, total suspended solids (TSS), and UV absorbance (UVA)/UV transmittance (UVT).

UV Sources — a bench-scale collimated beam apparatus equipped with either a low-pressure (LP) mercury vapour lamp or a medium-pressure (MP) mercury vapour lamp was used to deliver the designated UV dose.

Particle Analysis — a dynamic particle analyzer (DPA 4100, Brightwell Technologies Inc.) with Micro-Flow Imaging technology was used for sizing and imaging the particles of interest.

1.4 Organization of Thesis

Chapter 1 — background information is provided to show the causes that motivate and drive the present research. The principal objectives of this thesis and approach are listed.

Chapter 2 — a broad and comprehensive literature review provided the fundamental knowledge relevant to this thesis topic. A thorough understanding of UV inactivation and its affecting factors are introduced. Particulate impact, one of the key factors, is then highlighted and expanded in terms of the association between particles and microorganisms. The consequences of particulate impact are illuminated for both chlorination and UV inactivation. Future research needs are also addressed.

Chapter 3 — experimental design and setup are emphasized with respect to the target microorganisms, various particulate sources, LP/MP UV irradiation, chlorination, and particle analysis. The corresponding materials and experimental methods are detailed.

Chapter 4 — objectives and results of critical preliminary experiments are presented, including the selection of indicator bacteria, settling of bacteria with particles, and attachment of bacteria with particles.

Chapter 5 — there are three parts in the final results in terms of the applied disinfectant. For each particulate source, the dose-response of indicator bacteria is explicitly demonstrated for LP UV irradiation, MP UV irradiation, and chlorination. Particle analysis is incorporated with the interpretation of the results.

Chapter 6 — overall conclusions are summarized based on the experimental results. The potential applications of this study and future research are recommended at the end.

Chapter 2

Literature Review

2.1 Organization

The literature review consists of the following three sections:

UV inactivation

The fundamental aspects of UV light are described and discussed. How to define and calculate UV dose with respect to LP and MP are presented. The mechanism of UV inactivation of microorganisms is introduced briefly, as well as the photo and dark repair after exposure. Finally, the UV dose-response of microorganisms and related dynamic models are depicted.

Factors affecting UV inactivation

A general list of factors affecting UV inactivation is discussed in terms of UV dose-response of microorganisms. The impact of UV light absorbance and scattering is highlighted in the present research. The wavelength of UV light is briefly described. The state of microorganisms affecting UV inactivation is emphasized as the key point.

Particles and microorganisms

The interactions between particles and microorganisms of interest are reviewed. The consequences of association on the disinfection processes (chlorination, UV inactivation) are then discussed. The role of particle size and its distribution function are interpreted.

2.2 UV Inactivation

2.2.1 UV Light

According to photochemistry, UV light is the region of the electromagnetic spectrum that lies between x-rays and visible light (Figure 2.1). The spectrum can be divided into four ranges: vacuum UV (100 to 200 nm), UV-C (200 to 280 nm), UV-B (280 to 315 nm), and UV-A (315 to 400 nm) (Meulemans 1986).

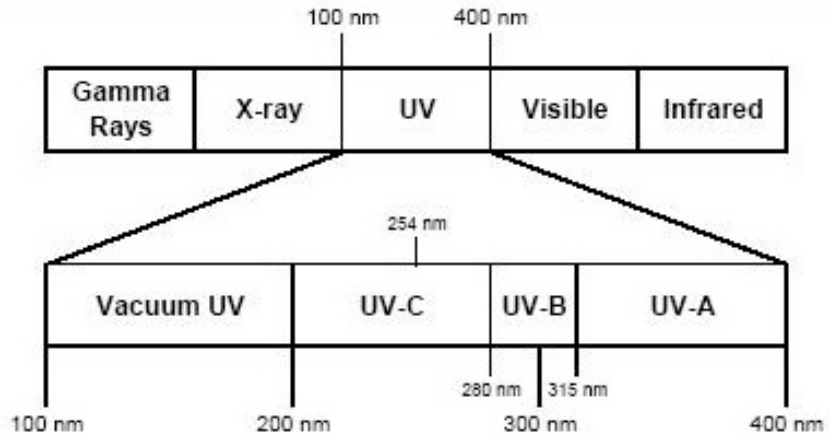


Figure 2.1 UV light in electromagnetic spectrum (courtesy of UVDGM, 2003)

The UV-A range causes tanning of the skin while the UV-B range causes the skin to burn and is known to eventually induce skin cancer. The UV-C range is so-called "germicidal range" since it is absorbed by proteins, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), and can lead to cell mutations and/or cell death, therefore it is effective in inactivating pathogens. The vacuum UV range is so powerful that it is absorbed by almost all substances including water and air (Bolton, 1999). Typically, the practical germicidal wavelength for UV light ranges between 200 and 300 nm (Bolton, 1999), i.e. essentially UV-C and UV-B.

Applying a voltage across mercury vapour can generate UV light, resulting in a discharge of photons. The specific wavelengths of light emitted from the photon discharge and the light output depend on the concentration of mercury atoms associated with the mercury vapour pressure. USEPA (2003) concluded that mercury at low vapour pressure (near vacuum; 0.01 to 0.001 torr, 2×10^{-4} to 2×10^{-5} psi) and moderate temperature (40 ºC) produces essentially monochromatic UV light at 253.7 nm (LP UV light). Mercury at higher vapour pressures (100 to 10,000 torr, 2 to 200 psi) and higher operating temperatures (600 to 900 ºC), produces UV light over a broad spectrum (polychromatic) with an overall higher intensity (MP UV light). The relative spectral emittance from LP and MP UV lamps is shown in Figure 2.2.

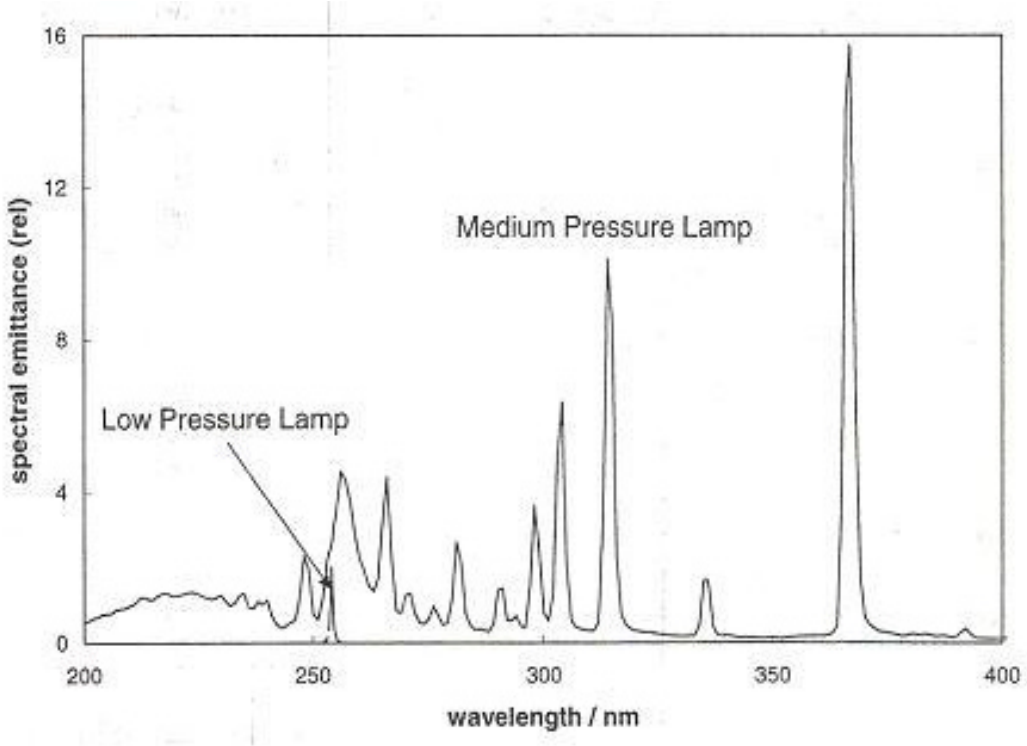


Figure 2.2 Relative spectral emittance from LP and MP UV lamps (Bolton, 1999)

Absorption, reflection, refraction, and scattering all interfere with the travel of UV light. The reflection, refraction, and scattering only change the direction of UV light which is still capable of inactivating microorganisms, whereas the absorbed UV light is no longer available. In commonly used bench-scale equipment, referred to as collimated beam apparatus, these interactions are between emitted UV light and beam components, petri dishes, and samples being irradiated. UV absorbance (UVA) or UV transmittance (UVT) is the parameter accounting for the impact of absorption and scattering. All these factors should

be appropriately accounted for in UV dose determination. More details are discussed in Sections 2.2.2 and 3.2.8.

2.2.2 Definition and Determination of UV Dose

The UV dose is defined as the product of UV intensity expressed in milliWatts per square centimeter (mW/cm^2) and the exposure time of the fluid or particle to be irradiated expressed in seconds (s) (NWRI/AWWARF, 2000). Units commonly used for UV dose are mJ/cm^2 (equivalent to $mW \times s/cm^2$) in North America and J/m^2 in Europe.

So far it is only possible to determine the UV dose when using a collimated beam apparatus because both the average intensity delivered to target microorganisms and the exposure time can be accurately measured and calculated. Conversely, UV dose determination is far more complicated in a continuous flow UV reactor. A detailed description is beyond the scope of this thesis, however, the procedure is summarized in the next paragraph.

Briefly speaking, UV dose distribution in a continuous flow UV reactor is subject to non-ideal hydraulic characteristics and non-uniform intensity profiles within the reactor. Ideally, all target microorganisms passing the reactor will receive the identical dose only if the reactor is plug flow (PF) with complete mixing (CM) perpendicular to that PF, which does not generally exist in a real UV reactor. There are two methods to estimate the delivered UV dose in a reactor. One is the so-called reduction equivalent dose (RED) based on

biodosimetry (Qualls and Johnson, 1983), which is defined by measuring the inactivation level of a challenge microorganism with a known UV dose-response. Hence, the RED for a UV reactor is equal to the UV dose that achieves the same inactivation level of the challenge microorganism in a collimated beam apparatus during the biodosimetry testing. Another approach is to employ Computational Fluid Dynamics (CFD) modeling in determining the hydraulic characteristics of a UV reactor, and then integrate this information with UV dose determination.

Since the bench-scale collimated beam apparatus was used in this study, the fundamental principles, equations, and calculation spreadsheets of UV dose determination are listed and detailed in Section 3.4.3 in terms of the specific configuration in this study.

2.2.3 UV Inactivation of Microorganisms

UV light inactivates microorganisms by damaging their DNA or RNA, thereby preventing reproduction, which differs distinctly from chemical disinfectants such as chlorine and ozone. Chemical disinfectants inactivate microorganisms by destroying or damaging cellular structures, interfering with metabolism, and hindering biosynthesis and growth (Snowball and Hornsey, 1988).

Only the absorbed UV light can induce a photochemical reaction. Nucleotides absorb UV light from 200 to 300 nm, which enables the photochemical reaction that leads to the damage

of nucleic acids. The UV absorption by nucleic acids has a peak near 260 nm (see Section 2.3.2).

Wright and Sakamoto (2001) broadly reviewed the experimental data for UV inactivation of microorganisms and tabled the UV dose required to achieve the inactivation of bacteria, viruses, and protozoa (Table 2.1).

All data in the tables are for microorganisms suspended in water and irradiated using a collimated beam apparatus with LP UV light at 254 nm. The UV sensitivity of microorganisms varies from species to species. Of the pathogens of interest in water, viruses are most resistant to UV inactivation followed by bacteria, *Cryptosporidium* oocysts and *Giardia* cysts. The most UV resistant viruses of concern are adenovirus Type 40 and 41.

Table 2.1 UV dose required to achieve incremental log inactivation (Wright and Sakamoto, 2001)

Reference	Microorganism	Type	UV Dose (mJ/cm^2) per Log Reduction of						
			1	2	3	4	5	6	7
Wilson et al, 1992	Aeromonas hydrophila ATCC7966	Bacteria	1.1	2.6	3.9	5	6.7	8.6	
Wilson et al, 1992	Campylobacter jejuni ATCC 43429	Bacteria	1.6	3.4	4	4.6	5.9		
Harris et al, 1987	*Escherichia coli* ATCC 11229	Bacteria	2.5	3	3.5	5	10	15	
Chang et al, 1985	*Escherichia coli* ATCC 11229	Bacteria	3	4.8	6.7	8.4	10.5		
Sommer et al, 1998	*Escherichia coli* ATCC 11229	Bacteria	3.95	5.3	6.4	7.3	8.4		
Sommer et al, 1998	*Escherichia coli* ATCC 29222	Bacteria	4.4	6.2	7.3	8.1	9.2		
Wilson et al, 1992	*Escherichia coli* O157:H7 ATCC 43894	Bacteria	1.5	2.8	4.1	5.6	6.8		
Sommer et al, 1998	*Escherichia coli* Wild Type	Bacteria	4.4	6.2	7.3	8.1	9.2		
Wilson et al, 1992	Klebsiella terrigena ATCC 33257	Bacteria	4.6	6.7	8.9	11			
Wilson et al, 1992	Legionella pneumophila ATCC 43660	Bacteria	3.1	5	6.9	9.4			
Tosa and Hirata, 1998	Salmonella anatum (from human feces)	Bacteria	7.5	12	15				
Tosa and Hirata, 1998	Salmonella derby (from human feces)	Bacteria	3.5	7.5					
Tosa and Hirata, 1998	Salmonella enteritidis (from human feces)	Bacteria	5	7	9	10			
Tosa and Hirata, 1998	Salmonella infantis (from human feces)	Bacteria	2	4	6				
Wilson et al, 1992	Salmonella typhi ATCC 19430	Bacteria	1.8	4.8	6.4	8.2			
Chang et al, 1985	Salmonella typhi ATCC 6539	Bacteria	2.7	4.1	5.5	7.1	8.5		
Tosa and Hirata, 1998	Salmonella typhimurium (from human feces)	Bacteria	2	3.5	5	9			
Wilson et al, 1992	Shigella dysenteriae ATCC29027	Bacteria	0.5	1.2	2	3	4	5.1	
Chang et al, 1985	Shigella sonnei ATCC9290	Bacteria	3.2	4.9	6.5	8.2			
Chang et al, 1985	Staphylococcus aureus ATCC25923	Bacteria	3.9	5.4	6.5	10.4			
Chang et al, 1985	Streptococcus faecalis ATCC29212	Bacteria	6.6	8.8	9.9	11.2			
Harris et al, 1987	Streptococcus faecalis (secondary effluent)	Bacteria	5.5	6.5	8	9	12		
Wilson et al, 1992	Vibrio cholerae ATCC 25872	Bacteria	0.8	1.4	2.2	2.9	3.6	4.3	
Wilson et al, 1992	Yersinia enterocolitica ATCC 27729	Bacteria	1.7	2.8	3.7	4.6			
Mofidi et al, 1999	Cryptosporidium parvum oocysts, mouse infectivity assay	Protozoa	3.1	4.7	6.2				
Shin et al, 2000	Cryptosporidium parvum oocysts, tissue culture assay	Protozoa	1.3	2.3	3.2				
Rice and Hoff, 1981	Giardia lamblia cysts, excystation assay	Protozoa	> 63						
Karanis et al, 1992	Giardia lamblia cysts, excystation assay	Protozoa	40	180					
Linden et al, 2001	*Giardia lamblia* cysts, gerbil infectivity assay	Protozoa	<1	<1	<2	<3			
Carlson et al, 1985	Giardia muris cysts, excystation assay	Protozoa	77	110					
Craik et al, 2000	*Giardia muris* cysts, mouse infectivity assay	Protozoa	<2	<6	Plateau at 2.5-3 log inactivation				
Chang et al, 1985	Bacillus subtilis spores ATCC 6633	Spores	36	48.6	61	78			
Sommer et al, 1998	Bacillus subtilis spores ATCC 6633	Spores	29	40	51				

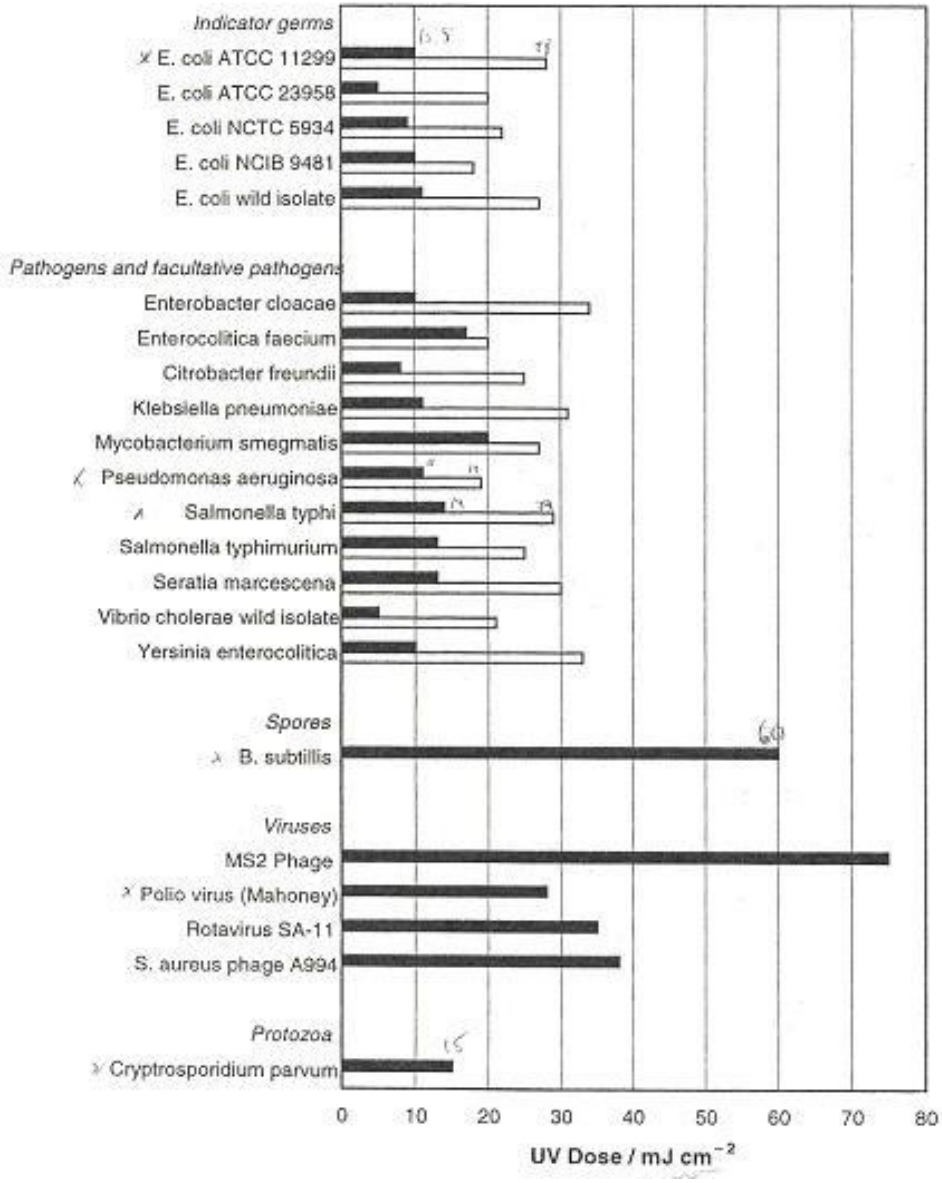
Table 2.1 UV dose required to achieve incremental log inactivation (cont'd)

Reference	Microorganism & Assay	Type	UV Dose (mJ/cm^2) per Log Reduction of						
			1	2	3	4	5	6	7
Sommer et al, 1998	B40-8 Phage (B.fragilis HSP-40 assay)	Phage	12	18	23	28			
Nieuwstad and Havelaar, 1994	MS2 (Salmonella typhimurium WG49 assay)	Phage	16.3	35	57	83	114	152	
Wiedenmann et al, 1993	MS2 DSM 5694 (*E. coli* NCIB 9481 assay)	Phage	4	16	38	68	110		
Wilson et al, 1992	MS2 ATCC 15977-B1 (*E. coli* ATCC 15977–B1 assay)	Phage	15.9	34	52	71	90	109	128
Tree et al, 1997	MS2 NCIMB 10108 (Salmonella typhimurium WG49 assay)	Phage	12.1	30.1					
Sommer et al, 1998	MS2 (*E. coli* K-12 Hfr assay)	Phage	21	36					
Rauth, 1965	MS2 (*E. coli* CR63 assay)	Phage	16.9	33.8					
Meng and Gerba, 1996	MS2 (*E. coli* 15977 assay)	Phage	13.4	28.6	44.8	61.9	80.1		
Snicer et al, 1998	MS2 (Standard Methods 9060)	Phage	5-32	24-50	44-72	64-93			
Battigelli et al, 1993	MS2 (*E. coli* C3000 assay)	Phage	35						
Oppenheimer et al, 1993	MS-2 (*E. coli* ATCC 15597)	Phage	19	40	61				
Sommer et al, 1998	ox174 Phage (*E. coli* WG5 assay)	Phage	2.2	5.3	7.3	10.5			
Battigelli et al, 1993	ox174 Phage (*E. coli* C3000 assay)	Phage	2.1	4.2	6.4	8.5	10.6	12.7	14.8
Oppenheimer et al, 1993	ox174 Phage (*E. coli* ATCC 15597)	Phage	4	8	12				
Meng and Gerba, 1996	PRD-1 (Salmonella typhimurium Lt2 assay)	Phage	9.9	17.2	23.5	30.1			
Meng and Gerba, 1996	Adenovirus 40 ATCC Dugan (primary liver carninoma cell line)	Virus	29.5	59.4	89.8	120.9			
Meng and Gerba, 1996	Adenovirus 41 ATCC TAK (primary liver carninoma cell line)	Virus	22.4	49.5	80.2				
Battigelli et al, 1993	Coxsackievirus B5 (Buffalo Green Monkey cell assay)	Virus	6.9	13.7	20.6				
Wilson et al, 1992	Hepatitis A HM175 (FRhK-4 cell assay)	Virus	5.1	13.7	22	29.6			
Wiedenmann et al, 1993	Hepatitis A virus (HAV/HFS/GBM assay)	Virus	5.5	9.8	15	21			
Battigelli et al, 1993	Hepatitis A virus HM-175 (FRhK-4 cell assay)	Virus	4.1	8.2	12.3	16.4			
Meng and Gerba, 1996	Poliovirus Type 1 LSc2ab (Buffalo Green Monkey cell assay)	Virus	4.0	8.7	14.2	20.6	28.1		
Harris et al, 1987	Poliovirus Type 1 ATCC Mahoney	Virus	6	14	23	30			
Chang et al, 1985	Poliovirus Type 1 LSc2ab (MA104 cell assay)	Virus	5.6	11	16.5	21.5			
Wilson et al, 1992	Poliovirus Type 1 LSc2ab (BGM cell assay)	Virus	5.7	11	17.6	23.3	32	41	50
Snicer et al, 1998	Poliovirus Type 1 LSc-1(BGMK host cell)	Virus				23-29			
Rauth, 1965	Reovirus-3 (Mouse L-60 assay)	Virus	11.2	22.4					
Harris et al, 1987	Reovirus Type 1 Lang strain	Virus	16	36					
Battigelli et al, 1993	Rotavirus SA-11 (MA-104 cell line assay)	Virus	7.6	15.3	23				
Chang et al, 1985	Rotavirus SA-11 (MA-104 cell line assay)	Virus	7.1	14.8	25				
Wilson et al, 1992	Rotavirus SA-11 (MA-104 cell line assay)	Virus	9.1	19	26	36	48		
Snicer et al, 1998	Rotavirus WA (MA-104 cell line assay)	Virus				50			

Some microorganisms are able to repair the damage done by UV light and regain infectivity. Repair of UV light-induced DNA damage can be accomplished by photoreactivation and/or dark repair (Knudson, 1985). Photoreactivation is dependent on many factors, including types, species, and strains of the target microorganism, inactivation levels following irradiation, the photoreactivating light, and nutrient state of the microorganism.

Knudson (1985) reported that photoreactivation increased the UV dose required to achieve 3 log inactivation of seven *Legionella* species by a factor of 1.1 to 6.3. Photoreactivation also increased the required dose by a factor of 1.2 to 3.5 for 4 log inactivation of twelve species of bacteria (Hoyer, 1998). The indicator bacteria *E. coli* (ATCC 11229) had been reported to be capable of photo-repair following LP UV irradiation by a number of studies (Harris *et al.*, 1987; Schoenen and Kolch, 1992; Hoyer, 1998; Sommer *et al.*, 2000; Zimmer and Slawson, 2002). Furthermore, Zimmer and Slawson (2002) reported that *E. coli* (ATCC 11229) and *E. coli* O157:H7 were able to effectively photo repair themselves up to 2.5 and 2.9 log respectively following a dose of 8 mJ/cm^2 LP UV irradiation, but limited or no photo repair was observed after MP UV exposure of 5 mJ/cm^2 (note that these doses are considerably below those commonly used in drinking water practice). The photoreactivation of *Cryptosporidium parvum* was not evident following either LP or MP irradiation (Shin *et al.*, 2001; Zimmer and Slawson, 2002). Apparently the repair mechanism can be inhibited by a higher UV dose. Bolton (1999) summarized the different doses for inactivating bacteria, spores, viruses and protozoa with or without photoreactivation in Table 2.2. The open and solid bars represent the inactivation with or without photoreactivation, respectively.

Table 2.2 UV dose required for 4 log inactivation of bacteria, spores, viruses and protozoa (Bolton, 1999)



Dark repair is the repair process that does not require reactivating light; this means that repair to damage caused by UV light can occur in the presence or absence of light. For example,

Knudsen (1985) showed that the required UV dose for 1-log inactivation of *E. coli* with dark repair was over two orders of magnitude larger than that of the strain without dark repair. Dark repair was minimal or not observed for *E. coli* (ATCC 11229) and *E. coli* O157:H7 following LP UV exposure of 5 mJ/cm^2 (Zimmer, 2002).

2.2.4 Indicator Bacteria in Past UV Studies

Pathogenic microorganisms can include bacteria, viruses and protozoa in a natural aqueous environment. Indicator organisms are commonly tested instead of pathogens as an indication of water contamination because it is impractical to analyze for pathogenic bacteria, viruses and protozoa in water on a routine basis due to time constraints and the expense associated with testing (APHA *et al.*, 1998). Prescott *et al.* (1993) stated that the characteristics of an ideal indicator organism include the following: they are present when a pathogen of concern is present, found in greater numbers, persist in the environment as long as the potential pathogens, do not reproduce in the environment, are harmless to humans, and the testing methods are easy to perform.

One group of indicator bacteria is the coliform bacteria, which are operationally defined as Gram-negative, non-spore-forming, aerobic and facultative anaerobic bacteria that ferment lactose and produce acid and gas (Black, 1999). The indicator coliform bacteria, including total coliforms, fecal coliforms, and *E. coli*, are commonly used in water and wastewater research. The presence of these bacteria indicates the related contamination with fecal material.

Given the objectives of this thesis, a reliable testing method is the greatest concern in the selection of the target microorganism. Testing methods for indicator bacteria are well established (APHA *et al.*, 1998).

In past studies regarding UV inactivation under the influence of suspended solids in wastewater, naturally present total coliforms and fecal coliforms have been broadly used as the target microorganisms (Qualls *et al.*, 1985; Harris *et al.*, 1987; Loge *et al.*, 1996, 1999; Jolis *et al.*, 2001; Ormeci and Linden, 2002; Parker and Darby, 1995; Emerick *et al.*, 1999, 2000). These past studies have served as a valuable resource for the present research.

Laboratory grown *E. coli* has also been widely used as indicator bacteria in studies of UV inactivation and their photoreactivation following irradiation (Zimmer and Slawson, 2002; Sommer *et al.*, 2000; Hoyer, 1998; Schoenen and Kolch, 1992; Harris *et al.*, 1987).

2.2.5 UV Dose-response and Related Models

UV dose-response of microorganisms is expressed as either log inactivation or log survival. Log inactivation is the proportion of microorganisms inactivated; it is the positive slope of a semi-log dose-response curve. Log survival is the proportion of microorganisms remaining; it is the negative slope of a semi-log dose-response curve. For convenience, all UV dose-response curves in this thesis are shown as log inactivation, i.e. the log inactivation of

microorganisms ($\log_{10}$) is shown as a function of UV dose (a linear scale). Based on the definition, the log inactivation can be calculated as:

$$Log\ Inactivation = \log_{10} \frac{N_0}{N} \qquad \text{Equation 2.1}$$

Where

N_0 = concentration of viable microorganisms before UV irradiation (cells/mL)

N = concentration of viable microorganisms after UV irradiation (cells/mL)

UV dose-response of many free-floating or dispersed microorganisms follows first order inactivation kinetics (Severin *et al.*, 1984) as Equation 2.2.

$$N = N_0 \exp(-kD) \qquad \text{Equation 2.2}$$

Where

k = first order inactivation coefficient (cm^2/mJ)

D = delivered UV dose (i.e. calculated average intensity × exposure time, in mJ/cm^2)

Combining Equation 2.1 and Equation 2.2, Equation 2.3 is obtained. The UV dose-response curve is linear with a positive slope for log inactivation with first order kinetics.

$$Log\ Inactivation = kD \times \frac{1}{\ln 10} \qquad \text{Equation 2.3}$$

The dose-response curves normally contain so-called “shoulder” or “tailing” regions. The term “shoulder” is defined by a period of lag time with little inactivation at lower doses before the linear region of the curve. The term “tailing” is defined by a period of lag time with little inactivation at higher doses after the linear region of the curve. Figure 2.3 shows various typical patterns of dose-response curves (Chang *et al.*, 1985).

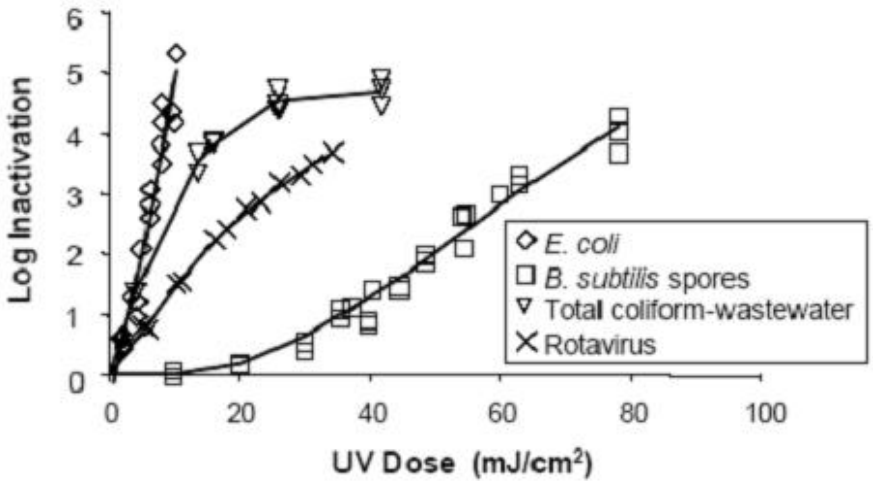


Figure 2.3 Typical patterns of UV dose-response curves (Chang *et al.*, 1985)

In Figure 2.3, *Bacillus subtilis* exhibited a “shoulder” followed by first order behavior. Generally, the “shoulder” is due to a delayed response of a microorganism when exposed to UV light. One of the models derived from the empirical curves was developed by Cabaj *et al.* (2001) to describe the UV dose-response curves with “shoulder” (Equation 2.4).

$$N/N_0 = 1 - [1 - \exp(-kD)]^d \qquad \text{Equation 2.4}$$

Where

d = intercept of the linear region of UV dose-response with the y-axis

In Figure 2.3, total coliforms in wastewater exhibited a “tailing” after first order behavior. Generally, the “tailing” is directly associated with the UV inactivation of a less resistant population following by a more resistant population of the target microorganisms. There are several possible explanations of this phenomenon.

First, the variation in resistance of individual microorganisms to UV irradiation may cause “tailing” because some microorganisms are more resistant than others. For instance, the target microorganisms are a group of microorganisms (e.g. total coliforms as indicator bacteria). Second, any shielding effect may render the microorganisms more resistant to UV irradiation. For the application in wastewater, Dietrich *et al.* (2003) concluded that UV inactivation of coliform bacteria in the “tailing” region was attributed to the inactivation of bacteria associated with particulate matter. For UV application in drinking water, Templeton *et al.* (2003) investigated the impact of particle-association and viral aggregation on UV inactivation of alum floc-associated MS2 coliphage. More extensive discussion of the interaction between particles and microorganisms is provided in Section 2.4.

For the "tailing" region of UV dose-response, Emerick *et al.* (2000) developed a model (Equation 2.5) to directly correlate the survival of shielded coliform bacteria with the total number of particles containing at least one coliform bacterium before the exposure.

$$N = N_{D0} \times \exp(-kD) + \frac{N_{P0}}{kD}\left[1 - \exp(-kD)\right] \qquad \text{Equation 2.5}$$

Where

N = concentration of viable coliform bacteria after UV exposure (cells/mL)

N_{D0} = concentration of viable dispersed coliform bacteria before UV exposure (cells/mL)

N_{P0} = total number of particles containing at least one bacterium before exposure (#/mL)

k = first order inactivation coefficient (cm^2/mJ)

D = delivered UV dose (i.e. calculated average intensity × exposure time, in mJ/cm^2)

2.3 Factors Affecting UV Inactivation

Generally speaking, UV intensity, temperature and pH are considered to have negligible impact on UV dose-response of microorganisms in water. Several other factors such as the wavelength of UV light, UV absorbance and scattering, and the state of the microorganisms affect UV dose-response of microorganisms.

2.3.1 UV Intensity, Temperature and pH

It has been reported that UV dose-response of microorganisms follows the Law of Reciprocity over an intensity range of 1 to 200 mW/cm^2 (Oliver and Cosgrove, 1975). For instance, the inactivation performance by a UV intensity of 2 mW/cm^2 and an exposure time of 50 seconds (i.e. UV dose of 100 mJ/cm^2) is equivalent to a UV intensity of 5 mW/cm^2 and an exposure time of 20 seconds.

It is well documented that UV inactivation is not directly affected by temperature and pH. Severin *et al.* (1983) reported that the UV dose needed for a given log reduction of *E. coli*, *Candida parapsilosis,* and *f2 bacteriophage* increased slightly as temperature decreased. Similarly, Malley (2000) found that the dose-response of MS2 was independent of temperature from 1 to 23°C. Although the pH of the water may vary, the pH within a cell is buffered to a relatively constant value. Therefore, UV dose-response is usually independent of the pH of the water. Malley (2000) also reported that the dose-response of MS2 was independent of the suspension pH from pH 6 to 9.

2.3.2 UV Wavelength

UV dose-response of microorganisms varies in the range of the so-called germicidal wavelength (200~300 nm) because microorganisms can absorb different amounts of UV light at different wavelengths in that range. At a given UV dose, a measured inactivation of a microorganism as a function of the wavelengths is similar to the DNA absorbance in the range of these wavelengths (Figure 2.4).

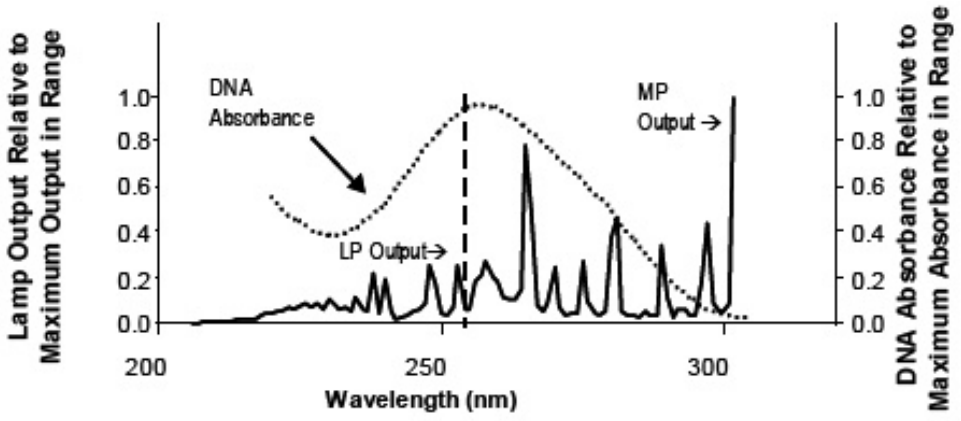


Figure 2.4 UV absorbance of DNA with LP and MP lamp output (Bolton, 1999)

The action spectrum has a peak near 260 nm and drops to zero near 300 nm. It is generally believed that microorganisms are most sensitive around 260 nm within the germicidal wavelengths. However, there are the exceptions, such as MS2 bacteriophage and Herpes simplex virus, whose UV susceptibilities are greater at the wavelengths below 230 nm (Linden *et al.*, 2001).

2.3.3 UV Absorbance and Scattering

UV attenuation in water is due to absorbance and scattering, which can be categorized as the soluble absorbance, the particulate absorbance, and the particulate scattering (Qualls *et al.*, 1985). The soluble absorbance and the particulate absorbance constitute the UV absorbance. Qualls *et al.* (1985) concluded that the soluble absorbance, the particulate absorbance and the particulate scattering were 47%, 41% and 21%, respectively for the secondary effluent (14 NTU) of a municipal wastewater treatment plant. Those authors developed a correlation

(R^2=0.81) between the UV absorbance and the COD and the turbidity in the wastewater samples.

The absorbed UV light is no longer available for inactivation and therefore the UV absorbance should be accounted for in the determination of delivered UV dose (this is discussed as the water factor, Section 3.2.8). UV absorbance at 254 nm (UVA) is a well-established water quality parameter that characterizes the reduction of incident UV light as it passes through water samples.

Since most particles in water may contribute to UV absorbance to some extent, Christensen and Linden (2001) recommended that UV absorbance measurement be made without filtering the sample, i.e. by using a modified version of Standard Method 5910B (APHA *et al.*, 1998). Therefore, the modified method accounts for the particulate scattering and the particulate absorbance that may interfere with UV irradiation. However, this direct absorbance measurement did not differentiate the scattering and the absorbance (Christensen and Linden, 2003). In terms of UV inactivation, the scattered UV light is still available for inactivating microorganisms, while the absorbed UV light is no longer available.

In wastewater, Loge *et al.* (1999) reported UV absorbance of the wastewater solids varied from 0.33 to 56.9 μm^{-1} (3,300 to 569,000 cm^{-1}) with the high absorbance associated with using iron to remove phosphorus in the activated sludge plant. Linden and Darby (1998) also

found that UV absorbance of the wastewater particles varied among the different treatment effluents.

The scattering of UV light is the change of light direction caused by interacting with a particle (Figure 2.5). Particulate scattering occurs in all directions including toward the source of incident light (back-scattering). Unlike the absorption of UV, the scattered UV light is believed still available to inactivate microorganisms. The problem of scattering is that it may interfere with the UV absorbance measurement when there is a high level of particulates present in the water.

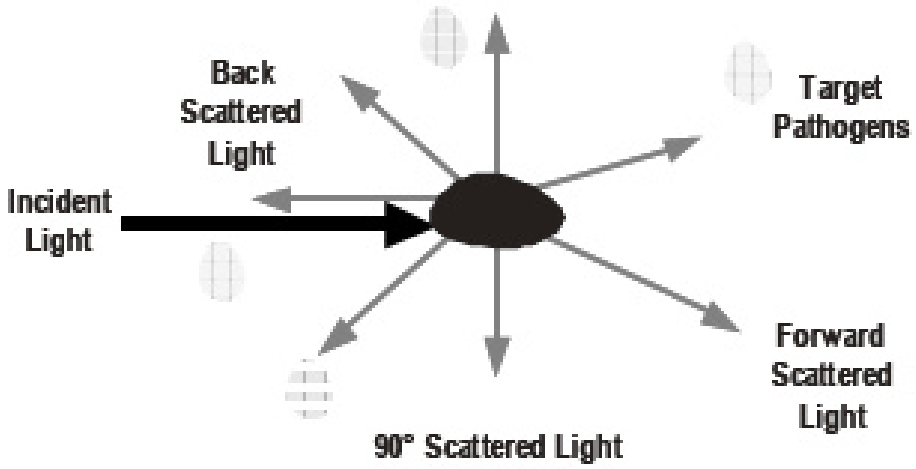


Figure 2.5 Scattering pattern of UV light on particles (UVDGM, 2003)

In drinking water, Christensen and Linden (2001) concluded that particulate scattering had a remarkable influence on the UVA measurement (modified Standard Method 5910B) when

the turbidity was up to 10 NTU in the collimated beam test. In later studies (Christensen and Linden, 2002, 2003), the authors reported that turbidity greater than 3 NTU resulted in an overly conservative UV dose determination by the direct absorbance measurement and an increase of turbidity from 1 to 10 NTU would decrease the average dose from 5 to 33% in the collimated beam test.

2.3.4 State of Microorganisms

Stagg *et al.* (1977) demonstrated that viruses present in sewage might exist in several physical states: suspended as individuals; aggregated in viral clumps; and associated with suspended solids. Because viruses display an affinity for silts, clay minerals, cell debris, and particulate organic matter, Bitton (1980) suggested that viruses be considered as biocolloids, which are subject to electrostatic attraction by a positively charged surface in a natural aquatic environment.

Analogous to the above statement, Emerick *et al.* (1999, 2000) concluded that many microorganisms of interest in wastewater were present in either a disperse state (i.e. not bound to others) or a clumped or particle-associated state (i.e. bound to other bacteria or particles). Two possible sources inducing bound microorganisms are biological floc (e.g. activated sludge) in wastewater treatment and chemical floc (e.g. coagulant) in drinking water treatment.

The state of microorganisms in water plays a critical role in two aspects: (1) accurate enumeration of these clumped and particle-associated microorganisms is difficult by commonly used methods such as the multiple tube fermentation and membrane filter test (APHA *et al.*, 1998), because clumps of bacteria are only counted as single colonies; (2) it may significantly change the disinfection kinetics of microorganisms, such as UV inactivation (Section 2.2.5) and chlorination.

Until recently, there has not been an effective method to quantify the aggregated or particle-associated microorganisms. Knowledge of the interaction between particles and microorganisms is still limited in terms of the fundamental mechanisms and their determining factors. More research is necessary to provide better insight into the phenomena.

Since this thesis is focusing on UV inactivation of indicator bacteria associated with particulate matter, the interaction between particles and microorganisms, and its consequence on chlorination and UV inactivation are reviewed in the following section.

2.4 Particles and Microorganisms

2.4.1 Interaction between Particles and Microorganisms

Colloidal particles are defined as finely divided solids larger than molecules but generally not distinguishable by the unaided eye. The principal natural sources of colloidal particles in water are soil-weathering processes and biological activity. Clays are the principal

component of colloidal particles produced by weathering, while algae, bacteria, and other higher microorganisms are the predominant types of colloidal particles produced biologically (Montgomery, 1985). Particles and microorganisms of interest are subject to electrostatic attraction by a positively charged surface, such as a chemical coagulant, when they are negatively charged in a natural aqueous environment (Montgomery, 1985). However, certain bacteria are capable of forming capsules, which is a layer of polysaccharide material that surrounds many bacteria cells and can act as a barrier to host defense mechanism; the chemical composition of each capsule is unique to the strain of bacteria that secreted it (Black, 1999).

Extensive research has been performed to investigate the association of microorganisms and particles, as well as how to extract microorganisms from particles. It is evident that a reliable method for extracting viable microorganisms from particles is essential for directly quantifying the particle-associated microorganisms and evaluating the impact of particle association on the disinfection processes.

It had been estimated that up to 90% of viruses found in wastewater are solid-associated (Bitton, 1980). Small viral aggregation or viruses attached to submicron particles (<0.22 μm) were the major portion of solid-associated virus in treated sewage (Hejkal *et al.*, 1981).

In municipal wastewater, substantial numbers of bacteria can be associated with fecal particles. Cairns (1993) found that particle-associated microorganisms accounted for over 1% of the total microbial count in raw wastewater. Berman *et al.* (1988) used a blending method to extract coliform bacteria from primary effluent. The coliform counts increased from 3 to 25 times after blending in four experiments, but decreased 5 times in one experiment. For secondary effluent, Parker *et al.* (1995) reported that homogenization for 1.5 minutes at 19,000 rpm at 4^{o}C with chemical extractant (final concentrations of 10^{-6} M Zwittergent 3-12, 10^{-3} M EGTA, 10^{-2} M Tris buffer, 0.1% peptone, and pH 7.0) resulted in the best recovery of particle-associated coliforms while sonication proved ineffective. Furthermore, Loge *et al.* (1999) developed an experimental technique for the in situ hybridization of a fluorescent 16S rRNA oligoncleotide probe to visualize and enumerate wastewater particle-associated coliform bacteria.

In untreated stormwater runoff, Schillinger and Gannon (1985) reported that about 10~20% of the fecal coliform cells present were adsorbed to the suspended particles (mostly greater than 30 μm) and that more than 50% of organisms remained in the dispersed state. Borst and Selvakumar (2003) also found that all microorganisms except *E. coli* showed a significant increase in measured concentration after blending runoff samples at 22,000 rpm.

In conventional drinking water treatment, GAC filters provide an effective physico-chemical process to remove contaminants. GAC particles, which are characterized as porous media,

can filter or absorb particles and microorganisms in water. Using scanning electron microscopy (SEM), Stewart *et al.* (1990) found that 85% of the GAC particles in the effluent of a water treatment pilot plant occluded less than 50 bacteria and 8% of these particles occluded numerous bacteria. Camper *et al.* (1985a) performed bacteria extraction from suspended GAC particles by blending the effluent of water treatment plants with the chemical extractants. These chemical extractants formed a solution (so-called Camper's Solution) with final concentrations of 10^{-6}M Zwittergent 3-12, 10^{-3}M EGTA, 10^{-2}M Tris buffer, and 0.01% peptone at pH 7.0 (Camper *et al.*, 1985a). The authors found that the coliform count increased nearly 1,200 times after 3 minutes blending at 16,000 rpm at 4^{o}C. Many researchers have employed the same or similar extraction techniques as Camper *et al.* (1985a) to extract bacteria from GAC particles (LeChevallier *et al.*, 1984; Pernitsky *et al.*, 1997; Camper *et al.*, 1985b, 1986; Stewart *et al.*, 1990).

In municipal drinking water distribution systems, the issue of bacteria associated with suspended particles is another research area of interest. Herson *et al.* (1987) showed the drinking water distribution system particles associated with E*nterobacter cloacae* by using SEM images. In examining two distribution systems, Ridgway and Olson (1981, 1982) found that a 1-mL sample could contain anywhere from 1,500 to 15,000 particle-associated bacteria, mostly in association with particles greater than 10 μm. LeChevallier *et al.* (1987) used a procedure similar to Camper *et al.* (1985a) to extract the bacteria attached to particles in drinking water distribution systems.

In surface water, Geesey and Costerson (1979) used SEM to find that typical surface water contained approximately 76% free-floating bacteria and 24% particle-associated bacteria attached to suspended sediments. LeChevallier *et al.* (1981) also used SEM to visualize some bacteria, which were either embedded in the particles or which appeared to be coated with amorphous material, or both, in surface water samples. Roper and Marshall (1979) reported that the number of particle-associated bacteria in estuarine sediments surviving environmental stresses, e.g. predation and solar radiation, was 2 to 3 orders of magnitude greater than the number of free-floating bacteria. Ramsay (1984) found that more than 70% of the bacteria attached to soil particles were released by blending the sample. That author also showed that the extraction recovery by sonication was greater than by either blending or shaking even though the prolonged exposure to ultrasound resulted in bacterial inactivation.

Camper *et al.* (1985a) proposed that a particular combination of chemical extractants might improve extraction efficiency by neutralizing surface charge, therefore enhancing the shearing mechanism, releasing the microorganisms with less impact on cell viability. Parker and Darby (1995) suggested that the fragmentation of large floc during homogenization, as evidenced by the change in particle size distribution (PSD), was most likely the responsible mechanism for the extraction of bacteria.

Selected relevant references are tabled in terms of different research objectives, particulate sources, target microorganisms, microbial methods and so on in chronological order in Table

2.3. The association of particles and microorganisms is common in drinking water and wastewater treatment. The most effective method for bacteria extraction seems quite site specific and depends on a variety of parameters. It was suggested that the appropriate extraction procedure be verified by preliminary experiments before practical application (Camper *et al.*, 1985a; McDaniel and Capone, 1985).

It is helpful to establish a potential correlation between the characteristics of the association and the general water quality parameters from an engineering point of view. Parker and Darby (1995), however, reported that all correlations between water quality parameters (e.g. SS, turbidity, and UVA) and the number of particle-associated coliform bacteria were statistically insignificant in secondary effluent of wastewater treatment plants.

Two possible sources of the particle-associated microorganisms are biologically induced floc (e.g. biosolids in activated sludge) in wastewater treatment and chemically induced floc (e.g. coagulant) in drinking water treatment. In municipal wastewater, biosolids are composed of various microbial groups associated with one another. In surface water of rivers and lakes, particulate floc may embed the microorganisms either naturally or as a result of point/non-point source pollution. In drinking water treatment, one of the key mechanisms is that particles and pathogens are enmeshed in the formed floc through coagulation and flocculation, and then removed by sedimentation and filtration.

Table 2.3 Reference List of Interaction between Particles and Microorganisms

References / Perspectives	LeChevallier *et al.* 1981	Ramsay 1984	Elley and Schleyer 1984
Objectives	Post-disinfection recovery (chlorine)	Enumeration of attached bacteria	Extract attached sedimentary bacteria
Particle Sources	Raw surface watershed	Sandy soil, silt loam soil	Sandy sediments from a lagoon
Target Organisms	Total coliforms	Bacteria	Bacteria
Microbio. Method	MF	Fluorescence microscopy and MPN	MF (AODC count)
Extraction method	Intermittent blending with chemical extractant	Shaking, blending and ultrasonication with chemical extractant	Homogenization and ultrasonication
Chem. extractant	10^{-6}M Zwittergent	Tris buffer	10^{-4}% Tween 80
Initial conc.	11~500 /100mL, then 1~3 log reduction	10^{8}~10^{10}/g	10^{7}~10^{8} /g
Remarks	1 minutes in 15-s interval, 20,000rpm	Ultrasonication better than either shaking or blending Ultrasonication may impair viability	Ultrasonication better than homogenization Ultrasonication destroy bacteria (proposal of factor 1.44)

Table 2.3 Reference List of Interaction between Particles and Microorganisms (cont'd)

References / Perspectives	LeChevallier *et al.* 1984	Camper *et al.* 1985a
Objectives	Post-disinfection recovery (chlorine)	Evaluation of desorption procedures
Particle Sources	GAC	GAC
Target Organisms	*E. coli* S. typhimurium Y. enterocolitica Shigella sonnei	HPC bacteria *E. coli*
Microbio. Method	Spread plate	Spread plate MF (Plate count)
Extraction method	Blending with chemical extractant	sonicator, blender, and homogenizer chemical and enzymatic comparison
Chem. extractant	10^{-6}M Zwittergent, 10^{-3}M EGTA, 10^{-2}M Tris buffer, 0.1% peptone	10^{-6}M Zwittergent, 10^{-3}M EGTA, 10^{-2}M Tris buffer, 0.01% peptone
Initial conc.	10^{8}~10^{9} /g	10^{6} E.coli/mL
Remarks	3min, 16,000rpm, pH7.0 and 4^{o}C	Homogenization better than blending and sonication; Chemicals greatly efficient, not enzymes 90% for *E. coli* to PAC, 3min, 16,000rpm, pH7.0 and 4^{o}C In 1985b and 1986, use 0.1% peptone

Table 2.3 Reference List of Interaction between Particles and Microorganisms (cont'd)

References / Perspectives	Bakken 1985	McDaniel and Capone 1985
Objectives	Separation and purification	Separation of aquatic bacteria
Particle Sources	Soil cultivated fields, spruce forest	Sediments from an intertidal mud flat
Target Organisms	Bacteria	Epibenthic bacteria
Microbio. Method	Fluorescence microscopy	MF (AODC count)
Extraction method	Repeat blending-centrifugation	Dilution & stir, homogenization, sonication, and two chemical treatment
Chem. extractant	N/A	Triton X-100 Sodium periodate
Initial conc.	10^8~10^9 /g	10^9~10^{10} /g
Remarks	N/A	Sonication better than homogenization, and both better than the others Recommendation of each procedure determined individually 1min, 20,000rpm

Table 2.3 Reference List of Interaction between Particles and Microorganisms (cont'd)

References / Perspectives	LeChevallier *et al.* 1987	Stewart *et al.* 1990	Parker and Darby 1995
Objectives	Enumeration of attached bacteria	Bacteriological analysis	Post-disinfection recovery (UV irradiation)
Particle Sources	DW effluents Distri. biofilm	GAC (DW effluents)	WW secondary effluents
Target Organisms	Total coliforms HPC bacteria	Total coliforms HPC bacteria	Total coliforms fecal coliforms
Microbio. Method	MF Spread plate	MPN MF	MTF (30 tubes)
Extraction method	Homogenizer with chemical extractant	Blending with chemical extractant	Blending and sonication with chemical extractant
Chem. extractant	10^{-6}M Zwittergent, 10^{-3}M EGTA, 10^{-2}M Tris buffer, 1.0% peptone	10^{-6}M Zwittergent, 10^{-3}M EGTA, 10^{-2}M Tris buffer, 0.01% Bacto-peptone	10^{-6}M Zwittergent, 10^{-3}M EGTA, 10^{-2}M Tris buffer, 0.1% peptone
Initial conc.	0.03~0.64 coli/100mL 10^{6} HPC/mL	1~5 coli/100mL 10^{4}~10^{5} HPC/mL	10^{4}~10^{6} /100mL 10^{3}~10^{5} /100mL
Remarks	2min, 22,000rpm, pH7.0 and 4^{o}C	1min, high level at pH7.0 and T<23^{o}C	Maximum at Blending at 1.5min, 19,000rpm, pH7.0 and 4^{o}C Sonication proved ineffective

Either naturally occurring or introduced during treatment, particle association has a potential impact on the resistance pattern of the associated microorganisms to disinfection.

2.4.2 Consequences for Chlorination and UV Inactivation

Particles are a great challenge for inactivating microorganisms using either chlorination or UV. Montgomery (1985) stated that one of the specific health-related characteristics of turbidity was the association of microorganisms with particulate material, with resulting interference with disinfection.

Particles affect the disinfection process in two distinct ways. One is that particles attenuate the applied disinfectant directly, e.g. react with chlorine as particulate chlorine demand or absorb UV light as particulate UV absorbance; the other is that particles associate with the target microorganisms, shielding them from disinfection.

The direct attenuation of chlorine or UV by particles is generally minor compared with the contribution to chlorine demand or UV attenuation by soluble substances (e.g. natural organic matter, NOM) in water or wastewater. In addition, there are well-established methods to quantify the influence in terms of chlorine demand or chlorine residual for chlorination, UVA/UVT for UV inactivation. The influence can be excluded or accounted for in the dose determination of chlorine and UV light.

Since there is a lack of effective methods for quantifying particle-associated microorganisms, there is much uncertainty in quantifying the impact of particle association on disinfection. The critical point related to the impact of particulate matter on disinfection should therefore be addressed in relation to the association between particles and target microorganisms.

Note there are two possible distinct mechanisms in the association between particles and microorganisms: adsorption or occlusion. The combined chlorine residual for inactivating occluded virus increased 4 times to achieve the same degree of inactivation for free virus or secondarily adsorbed virus (Hejkal *et al.*, 1979). The author also suggested a different mechanism of protection due to adsorption or occlusion. Thus, it is hypothesized that the microorganisms attached onto the surface of particles can still be fully exposed to the disinfectants, as opposed to those embedded in particles.

Chlorination

Hejkal *et al.* (1979) reported that a combined chlorine residual of 6.6 mg/L was necessary to achieve 50% inactivation in 15 minutes (pH 8.0, 22°C) in a particulate suspension containing occluded virus, compared to 1.4 mg/L for free-floating virus. Similar particulate shielding effects with respect to viruses were also evident in work by other contemporary researchers (Bitton *et al.*, 1972; Stagg *et al.*, 1977; Babich and Stotzky, 1980).

Sobsey *et al.* (1991) found that cell-associated HAV was always inactivated more slowly than dispersed HAV by free chlorine and monochloramine. The authors also recommended that values of CT or other disinfection criteria for inactivation of HAV and other enteric viruses be based on viruses associated with cells or other solids because they are better models for the likely state of viruses found in water.

Hoff (1978) showed that coliform bacteria associated with primary effluent particles were protected from chlorination. Coliform bacteria were recovered from the sewage effluent particles after disinfection by 0.5 mg/L chlorine (pH 5.0, 5 °C) for 60 minutes compared with more than 4 log inactivation of *E. coli* alone in 1 minute under the same conditions.

Stewart *et al.* (1990) found that bacterially colonized GAC particles released into the product drinking water were extremely resistant to either free chlorine or chloramines at 1.5 mg/L (pH 8.2, 23°C) even after 40 minutes contact time. LeChevallier *et al.* (1984) conducted a comprehensive study to compare the chlorine resistance pattern of GAC particles in three scenarios: (1) naturally occurring heterotrophic plate count (HPC) bacteria attached to GAC particles from an operating drinking water treatment plant; (2) cultured *E. coli* strain grown on GAC particles for 48-hour incubation; (3) cultured and washed *E. coli* strain attached to GAC particles for 20 minutes. Results showed that all of these attached bacteria were extremely resistant to chlorination. No significant inactivation was observed in every case at 1.4~1.6 mg/L free chorine (pH 7.0, 4°C) for 1 hour.

Herson *et al.* (1987) reported that the attachment of *E. cloacae* to drinking water distribution particles resulted in the protection of these microorganisms from chlorination and the effect was found to be dependent on both the level of chlorine in the system and the attachment time. Ridgway and Olson (1982) revealed the presence of attached HPC bacteria by SEM in drinking water distribution particles. The most resistant attached bacteria were able to survive even under 10 mg/L chlorine (pH 7.0, 23°C) for 2 minutes. LeChevallier *et al.* (1988a) concluded that the attachment of bacteria to biofilm, nutrient level, the age of biofilm, bacterial encapsulation, and growth conditions contributed to the increased resistance to chlorine from 2 to 10 times in chlorinated water supply systems. In a later study, LeChevallier *et al.* (1988b) found that monochloramine was better able to penetrate and inactivate biofilm bacteria than free chlorine relative to inactivation of the unattached bacteria.

LeChevallier *et al.* (1981) reported that coliform bacteria in water with turbidity of 1.5 NTU showed more than 4 log inactivation by 0.5 mg/L chlorine (pH 7.0, 10°C) for 1 hour, while a turbidity of 13 NTU resulted in only 1 log inactivation by 1.5 mg/L chorine for 1 hour. The authors also developed a statistical model to predict that an increase of turbidity from 1 to 10 NTU would result in an 8-fold decrease of chlorination efficiency based on experimental data in unfiltered surface water treatment facilities. Kaneko (1998) reported that a turbidity of 5 mg/L (spiked kaolin powder) significantly increased the CT values (pH 7.2, 30°C) for inactivating *E. coli* O157 and K12 from 0.032-0.035 to 0.04-0.05 mg·minutes/L for 2 log inactivation, and from 0.067-0.071 to 0.08-0.09 mg·minutes/L for 4 log inactivation.

UV Inactivation

It was well recognized that suspended solids in secondary effluent could interfere with UV inactivation of coliform bacteria in wastewater. Indicator coliform bacteria were of particular importance because they played a key role in meeting the discharge regulations for wastewater treatment facilities. Therefore, coliform bacteria associated with wastewater particles (fecal particles mostly) in secondary effluent have been widely investigated because the presence of suspended solids seems to be a significant obstacle to UV irradiation.

Qualls *et al.* (1983) reported that filtration of secondary effluents through an 8-μm filter removed the particulates responsible for the "tailing" in the UV dose-response relationship for total coliforms. The log inactivation increased from 3 log to over 4.5 log at 12 mJ/cm^2 after filtration. In a later study, Qualls *et al.* (1985) found that the UV dose-response curves of unfiltered and the corresponding filtrate (10-μm filter) of the wastewater samples significantly diverged at higher doses after 2 log inactivation in five secondary aerobic biological treatment effluents. The authors inferred that the non-particle-associated (i.e. dispersed) coliform bacteria with the capability of full exposure to UV irradiation were dominant in those samples. Consequently, it was concluded that the particle-associated coliform bacteria were only a small fraction, less than 1% (after 2 log inactivation), but they could be the critical factor limiting the further inactivation required for regulations (e.g. above 3 log inactivation).

Emerick *et al.* (1999) reported that roughly 0.7% of all associated particles with a diameter greater than 10 μm could completely shield coliform bacteria from UV irradiation in secondary wastewater effluents. The authors also inferred that chemically induced floc (e.g. by coagulant) created more UV resistant particle-associated coliform bacteria than those of biologically induced floc (e.g. by activated sludge).

Jolis *et al.* (2001) reported that a dose of approximately 80 mJ/cm^2 was necessary to consistently meet the wastewater reclamation coliform criteria if there were large particles present in the tertiary effluent due to the transient poor operation of the in-line filtration. Other researchers (Harris *et al.*, 1987; Parker and Darby, 1995; Loge *et al.*, 1996, 1999; Emerick *et al.*, 2000; Ormeci and Linden, 2002) also observed similar shielding effects on the wastewater particles in secondary effluents.

Although the concentration of the target microorganisms is limited in natural drinking water sources, significant results of wastewater research may also be applicable to the particle association occurring in drinking water disinfection. Therefore, further studies on particles, other than from sewage sources, are also crucial for drinking water applications.

In a wastewater matrix, it is relatively easy to acquire the "natural" particle-associated microorganisms due to the presence of high microbial concentrations and amorphous biological flocs. In drinking water sources, the concentrations of naturally occurring

microorganisms of interest, on the other hand, are typically around the detection limit. Hence, it is currently not practical to study the "natural" occurring particle-associated microorganisms representative of drinking water sources. To overcome this difficulty, many researchers have seeded or spiked the laboratory strain of target microorganisms in various drinking water sources under laboratory conditions. However, as opposed to the results of wastewater research, it has been shown that the presence of particles did not impact UV disinfection performance on the seeded microorganisms in drinking water applications.

Batch *et al.* (2004) investigated the effects of water quality on UV inactivation of spiked MS2 coliphage in 17 filtered water facilities (turbidity range from 0.01 NTU to 0.35 NTU) across the United States. Results showed that turbidity, particle count, and absorbance did not affect the process of inactivation, if those factors were properly taken into account in the LP or MP bench-scale dose determination. Linden *et al.* (2002) showed that the UV dose-response of seeded MS2 coliphage in filtered drinking waters is not altered by variations in turbidity of filtered water that already met regulatory requirements (40 CFR 141.73, USEPA, 1979). Passantino *et al.* (2004) reported that UV light was effective for inactivating the seeded MS2 bacteriophage in montmorillonite clay turbidity up to 12 NTU and algal content up to 42 μg/L as chlorophyll at the MP UV collimated beam test. A similar result was observed when the naturally occurring turbidity was applied to the reservoir waters. In another bench-scale and pilot-scale study, Oppenheimer *et al.* (2002) showed that turbidity ranging from 0.65 to 7 NTU did not affect the UV dose required for per log inactivation of seeded MS2, *Giardia muris*, or *Cryptosporidium parvum* in unfiltered waters. Craik *et al.*

(2002) observed very similar results of *Cryptosporidium parvum* and *Giardia* under UV inactivation. Womba *et al.* (2002) found that UV inactivation of seeded MS2 was not affected by turbidity if the impact of turbidity was accounted for in the UV dose determination at the bench- and pilot-scale test.

Another concern is the presence of algae may interfere with UV inactivation even the level of algae in water is currently not regulated. Womba *et al.* (2002) and Passantino *et al.* (2004) showed that algal counts up to 70,000 cells/mL and 42,000 cells/mL, respectively, did not affect UV inactivation of seeded MS2 bacteriophage at the bench-scale collimated beam test.

All the above seeded experiments, however, may not completely represent naturally occurring particle-microorganism associations because of two possible reasons: (1) seeded target microorganisms were more likely adsorbed or attached onto the particles instead of becoming absorbed or embedded into those particles during limited contact time; (2) those studies only investigated the impact of turbidity or particulate matter on UV absorbance and scattering rather than the particle-association or clump of microorganisms.

Another critical concern is amorphous chemical induced floc, i.e. aluminum or iron floc formed after typical process of coagulation and flocculation. It is necessary to investigate the impact of the induced coagulant-bound between particles and microorganisms on UV inactivation after coagulation and flocculation.

Petri *et al.* (2000) reported that coagulation of MS2 bateriophage by iron in ground water increased the UV dose by a factor of 2.5 to 3.5 to obtain same log inactivations of MS2 bacteriophage. Figure A.1 also shows that the settled alum floc and settled wastewater solids could effectively shield MS2 bacteriophage from UV inactivation (Malley, 2000). Templeton *et al.* (2003) observed similar results with respect to MS2 bacteriophage enmeshed in coagulated clay particles (kaolin) within a drinking water application. Because the essential mechanism of coagulation-flocculation is to destabilize and aggregate particles and microorganisms, formed floc most likely consists of amorphous particles and enmeshed microorganisms. More research is necessary to verify whether the formed floc occluded with target microorganisms can escape from downstream disinfection, such as chlorination and UV inactivation.

In addition, some researchers conducted parallel comparisons of chlorination and UV inactivation in wastewater. Ormeci and Linden (2002) concluded that chlorine appeared to be more effective for disinfecting the particle-associated coliform bacteria than UV inactivation under prolonged contact time in the secondary wastewater effluents. The author also inferred that contact time was the most important factor in determining the effectiveness of chlorination of particle-associated coliform bacteria, while the chlorine CT value alone was not a good indicator of chlorination effectiveness.

Dietrich *et al.* (2003) proposed that UV light penetrated into particles only through the pathways due to the macroporous structure of a particle, whereas chlorine was capable of penetrating into both the macroporous and microporous network of pathways of wastewater particles in the secondary effluent. For drinking water application, more research is necessary to compare the efficiency of chlorination and UV inactivation under the influence of particulate matter.

2.4.3 Role of Particle Size

Particles present in water are characterized by their size distribution, density, shape, and surface charge. A comprehensive particulate analysis is a direct approach to investigate the influence of particle-associated microorganisms as opposed to the surrogates of water quality parameters, such as turbidity and TSS. Currently, the particle counters are able to account for particulate number and size distribution.

The particle size has a profound effect on the reading of turbidity meters. Small particles (<0.1 μm) do not scatter visible light effectively, so water could contain large number of small particles or microorganisms but still give a low turbidity reading. The size of large particles (0.1~0.8 μm), such as clays or plankton, is near the wavelength of visible light (0.4~0.8 μm). These particles scatter light more effectively and yield higher turbidities (Berman *et al.*, 1988). Edzwald (1983) showed that 50 mg/L kaolinite gave a turbidity reading of about 80 NTU, while 50 mg/L humic acid gave a turbidity reading only slightly greater than 3 NTU.

Hoff (1978) proposed two factors regarding the significance of particles on disinfection processes: (1) the size of target microorganisms and particles, (2) relative innate disinfection resistance of the microorganisms themselves. The author assumed that the particles of interest present in water ranging upward from 0.03 μm (equal to the smallest size within virus, bacteria and pathogenic protozoa, as poliovirus) would possibly affect disinfection efficiency.

In wastewater, Berman *et al.* (1988) reported that the bacteria associated with particles (>7 μm) were 3 to 5 times more resistant than those associated with smaller particles in the primary effluent. Homogenization of the >7 μm fraction increased not only the number of <7 μm particles, but also the rate of inactivation to a level similar to that of the <7 μm fraction.

Many researchers (Qualls *et al.*, 1985; Emerick *et al.*, 1999; Dietrich *et al.*, 2003) tried to define the so-called critical particle size (CPS) for different target microorganisms (e.g. bacteria, virus) with respect to chlorination or UV inactivation. A general understanding is that any particle size below the CPS is not of concern while the particle size above the CPS is equally significant to determine the disinfection of the particle-associated microorganisms. All the documented experimental studies of CPS, however, were concluded or inferred from the divergence of dose-response curves before and after filtration by the known pore size membrane.

Comparing the UV dose-response of 10 μm filtered samples with the corresponding unfiltered ones in the secondary effluent of five aerobic biological treatment plants (Figure A.2), Qualls *et al.* (1985) suggested the CPS of particles shielding coliform bacteria from UV inactivation would be about 10 μm, i.e. all the particles greater than 10 μm diameter might equally contribute to that protection. Ridgway and Olson (1982) showed by scanning electron microscopy (SEM) that particles with attached bacteria retained by a 2-μm pore size filter were usually greater than 10 μm in two drinking water distribution systems. The number of bacteria attached to a single particle varied from 5 to 10 to as many as several hundred. Dietrich *et al.* (2003) concluded that the CPS of wastewater particles remained invariant with varying intensity of UV light (fixed dose with changed exposure time) applied in the bulk liquid medium, but varied with the bulk aqueous concentration of chlorine, based on model fit to the experimental data.

Particle size distributions (PSD) of several types of particles are reviewed as follows to provide some background information relevant to the present research.

Clay Particles

Commercial clay particles (kaolin, bentonite, montmorillonite, etc.) have been used in previous research, such as Stagg *et al.*, 1977; Hejkal *et al.*, 1979; Babich and Stotzky, 1980; Berman *et al.*, 1988; Templeton *et al.*, 2003; Passantino *et al.*, 2004. These studies focused on the association between virus (e.g. MS2 bacteriophage) and clay particles, and its affect on chlorination and UV inactivation. The selected literatures are summarized in Table 2.4.

Table 2.4 PSD of clay particles used in chlorination and UV inactivation of viruses

Perspectives / References	Associated viruses	Commercial species	Disinfectant
Bitton *et al.* 1972	Klebsiella aerogenes	Clay minerals, ~2 μm	UV
Stagg *et al.* 1977	Bacteriophage MS-2	Bentonite, 7~130 mg/L 95% count<2 μm	Chlorine
Babich and Stotzky, 1980	Bacteriophage	Kaolin, bentonite, 100 mg/L Count<2 μm	N/A
Templeton *et al.* 2003	Bacteriophage MS-2	Coagulated Kaolin, 12.5 mg/L 1~2 μm	UV

River Floc

McCoy and Olson (1987) reported that in New Zealand over 80% of the particles found in municipal drinking water systems were about 2 μm in size or smaller. However, Droppo and Ongley (1994) reported that the PSD of six rivers in southeastern Canada (Grand River included) had the mean particle size range from 8 μm to 10 μm. The predominant particle size ranged from 5 μm to 10 μm in the Grand River.

Sewage Particles

Several researchers (Parker and Darby, 1995; Emerick *et al.*, 1999, 2000) discussed the PSD of wastewater particles in the secondary effluent. The acquired PSD data varied from site to

site and it was difficult to compare them due to the different PSD interpretation and experimental method. Nevertheless, according to the Power-law function (Kavanaugh *et al.*, 1980), Neis and Tiehm (1997) reported that the β values for the secondary effluent in three German wastewater treatment plants ranged from 0.8~1.0, implying that the particles of 0.3~100 μm provided the predominant contribution to total particulate surface area and volume. Parker and Darby (1995), however, observed a trimodal volume distribution with the peak values at the size of 1 μm, 8 μm, and 35 μm in a wastewater treatment plant in Northern California.

2.5 Summary and Research Need

Summary

The practical germicidal wavelength for UV light ranges from 200 to 300 nm. UV lamps are commonly categorized as monochromatic LP UV light at 253.7 nm and polychromatic MP UV light with an overall higher output.

UV dose is defined as the product of UV intensity and the exposure time. So far it is only possible to determine the delivered UV dose using a collimated beam apparatus as both the average intensity delivered to target microorganisms and the exposure time can be accurately measured and calculated.

UV light inactivates microorganisms by damaging their DNA or RNA, thereby preventing reproduction of the microorganisms. UV dose-response of microorganisms is expressed as either log inactivation or log survival. The pattern of UV dose-response curves could be linear (first order kinetics) with “shoulder” or “tailing” in the application of water treatment.

UV intensity, temperature and pH have negligible impact on UV dose-response of microorganisms in water. Several other factors significantly affect the UV dose-response of microorganisms including the wavelength of UV light, UV absorbance and scattering, and the state of microorganisms.

Association of particles and microorganisms are common in the context of water and wastewater treatment. A reliable method for extracting viable microorganisms from particles seems quite site specific and depends on a number of parameters.

Association of particles and microorganisms can have a tremendous effect on disinfection processes, rendering the microorganisms more resistant to disinfectants such as chlorine and UV light. Chlorine seems more effective in disinfecting those particle-associated microorganisms than UV irradiation.

A general understanding of critical particle size (CPS) is that any particle size smaller than CPS is not of concern while particle size greater than CPS is equally significant to determine the disinfection of the particle-associated microorganisms.

Research Need

UV inactivation is an effective barrier to disinfect pathogens within both drinking water and wastewater treatment. Research has shown that suspended solids were able to influence the efficacy of UV inactivation in wastewater treatment facilities. One emerging question is whether particulate matter, other than from sewage sources, can shield pathogens, and allow them to escape UV inactivation.

The potential influence of particles has an impact on UV engineering practice from a regulation point of view. More research is necessary to explore the impact of particulate matter on UV inactivation for drinking water applications.

Chapter 3

Experimental Design and Methodology

3.1 Experimental Design and Approach

The overall objective of this research is to investigate the potential influence of different particles of interest on UV inactivation. Overall experimental design and approach includes three subsections: (1) three sets of preliminary experiments, (2) UV inactivation and chlorination, (3) particle analysis.

Since the concentration of naturally occurring microorganisms is limited in drinking water sources, the goal of the first preliminary experiment was to determine a suitable target microorganism for spiking into the samples and subsequent exposure to UV inactivation. Two candidates were natural total coliforms and laboratory grown *E. coli* (Section 2.2.4). Natural total coliforms were considered a better candidate for this study because they closely represent environmental strain coliform bacteria. The strain of *E. coli* ATCC 11229 was chosen because it was well documented in terms of UV dose-response, including potential photo and dark repair after irradiation (Zimmer and Slawson, 2002).

Either natural total coliforms or laboratory grown *E. coli* needs to be incubated to a minimum required concentrations (10^6 cells/mL, NWRI/AWWRF, 2000; Wright and Sakamoto, 2001) before spiking into the different particulate samples for UV inactivation. To achieve this

bacteria concentration, natural total coliforms, through an environmental surface water sample, were incubated in simulated natural nutrient environments. Laboratory grown *E. coli*, on the other hand, is well established to inoculate and maintain the required concentration in the laboratory.

The other two preliminary experiments aimed at separating or simulating the particle-associated bacteria under laboratory conditions by settling or attachment, respectively. This was necessary because there is no commonly accepted method to quantify the particle-associated microorganisms (Section 2.4.1).

Based on the principles of Stoke's Law, it was hypothesized that the settling velocity of particles would increase significantly if the particle size increased due to the association between bacteria and particles. *E. coli* and commercial kaolin particles were used in the experiments. Three types of samples were prepared for settling: *E. coli*, kaolin particles, and *E. coli* with kaolin particles. First, the settling pattern of *E. coli* or kaolin particles was determined separately after being suspended in water. Then the settling pattern of *E. coli* with kaolin particles was determined and compared to that of *E. coli* or kaolin particles only.

The colony forming units were hypothesized to decrease significantly following particle association because a group of clumped bacteria and particles only produces one colony. Petri *et al.* (2000) observed that the coagulation of the challenge microorganisms could result

in reduced colony counts in the UV reactor validation test. *E. coli*, kaolin particles, and surface water particles were used in the experiments. Two types of samples were prepared for attachment, including *E. coli* with kaolin particles and *E. coli* with surface water particles. The changing trend of *E. coli* concentration was determined in the samples during the contact time.

UV inactivation experiments were done by spiking *E. coli* into five representative particulate sources as follows. Chlorination experiments were done using identical samples as those that were used for the UV experiments, to compare the two disinfection methods in parallel.

- Fecal particles—secondary effluent from the wastewater treatment plant
- River sediments—surface water from the Grand River
- Floc particles—coagulated surface water from the Grand River
- Floc particles—coagulated process water from the water treatment plant
- Soil particles—runoff water from the Grand River (planned)

A standard bench-scale collimated beam apparatus (Bolton, 1999), equipped with either a low-pressure (LP) UV lamp or a medium-pressure (MP) UV lamp, was employed for the UV irradiation experiments. UV doses up to 40 mJ/cm^2 (LP or MP) were delivered to the prepared samples and UV dose-response of *E. coli* was determined in terms of the log inactivation (Section 2.2.5).

The UV doses were determined prior to each exposure. In a standard bench-scale collimated beam apparatus (LP or MP), the average intensity can be determined mathematically. Bolton and Linden (2003) concluded that several practical corrections are necessary to calculate the average intensity (E_{avg}) accurately and therefore determine the delivered UV dose in bench-scale UV experiments. Since there is no universally acceptable definition to differentiate between the terms "UV dose" and "delivered UV dose", both terms are used interchangeably to indicate the UV dose delivered to the target microorganisms in the bench-scale collimated beam apparatus in this study.

The chlorination experiments were performed using the free chlorine stock solution in a bench-scale batch reactor. Sodium thiosulfate was used to neutralize (dechlorinate) the chlorine residual at the reaction times. Chlorine dose-response of the *E. coli* was determined in terms of the log inactivation.

Unlike UV inactivation, chemical disinfectants, such as chlorine, have measurable residual concentration after a period of reaction time with the samples. Hence, the dose of chlorine is interpreted in terms of C×T values in mg·minute/L instead of UV doses in mJ/cm^2. The concept of C×T values is commonly used in chlorination applications. Briefly speaking, C×T is the product of the concentration of chlorine residual ('C') and the contact time ('T'). Similar to the definition of UV dose, a lower residual concentration with a longer contact time could generate the same C×T value.

Since *E. coli* used in this study was sensitive to chlorine (Section 5.3), the contact time was fixed at 1 minute, which was the minimum required operation time for chlorine demand measurement. Therefore, the C×T varied in response to the increments of stock solution that were added to the batch reactor (i.e. the concentration of chlorine within the reactor increased as more stock solution was added).

Comprehensive particle analysis is crucial for the present research. Evaluating the previous experimental results integrated with particle analysis is a helpful approach for a better understanding of the particle association. A dynamic particle analyzer (DPA) from Brightwell Technologies Inc. (2004) was used. The exceptional property of the DPA is so-called Micro-Flow Imaging technology, "Micro-Flow Imaging technology captures a digital image of every particle contained within a volume (typically one or more mL) as the sample flows through a micro-fluidic cell. The system software analyzes these images in real time to produce accurate particle size distributions and captures images meeting user-selected criteria." (Brightwell Technologies Inc., 2004). The model DPA 4100 that was used is a bench-scale laboratory analyzer for particle sizing and imaging.

Particles greater than 2 μm were counted and sized in terms of particle size distribution (PSD). The shape and visible porosity of particles were depicted by the imaging function.

3.2 Methodology (Materials and Methods)

3.2.1 Total Coliforms

Environmental total coliforms were obtained from raw surface water samples, collected in the Laurel Creek Conservation Area in the Grand River Watershed (GRCA, 2004). Water was collected in sterile 1000 mL polypropylene sample bottles (VWR) at the University of Waterloo and transported on ice to the laboratory. The sampling procedure followed Standard Method 9060A.3 and all the samples were stored in a cooler at 4^{o}C as described in Standard Method 9060B (APHA *et al.*, 1998).

The key point of using natural total coliforms was not to alter the characteristics of environmental strain coliform bacteria. Thus, total coliforms were incubated in the simulated environments with natural nutrients. The overall incubation process included two steps: water samples from the creek were directly incubated first; part of the incubated samples was inoculated to fresh assimilable natural nutrients to minimize the impact of environmental variables and then incubated again. The detail is described in sequence below.

First, the 100 mL creek water samples were incubated at 37^{o}C at 150 rpm for 24 to 48 hours (Incubator/Shaker, Innova^{TM} 4230, New Brunswick Scientific) in a 1000 mL capped shaking flask (VWR). Second, 10 mL aliquot suspension was inoculated to 90 mL of one of the four prepared nutrients, including 0.45-μm filtrate of the creek water, biodegradable organic

matter (BOM) cocktail, groundwater, and dechlorinated tap water. Third, the resulting 100 mL samples were incubated again at 37°C at 150 rpm at the Incubator/Shaker for another 24 to 48 hours in a different flask.

The four natural nutrients were prepared as follows:

Filtrate of the creek water—Creek water was filtered using a 0.45-μm membrane filter (GN-6, VWR). The filtrate was stored in the cooler at 4°C and used as a source of natural organic matter (NOM).

Cocktail of BOM—A synthetic BOM cocktail has been used in several other studies regarding the effect of BOM on drinking water biofilms (Urfer and Huck, 1999; Gagnon *et al.*, 2000; Gagnon and Huck, 2001). Briefly, the cocktail was made up of organic carbon, nitrogen (as $NaNO_3$) and phosphorus (as K_2HPO_4) in a molar ratio of C: N: P of 100:20:5 (Gagnon and Huck, 2001) in MQ water (Milli-Q UV Plus, 0.22 μm MilliPak-40 Ultra Pure Water system, MilliPore Corp.). The recipe for the organic carbon was 100 mg/L formaldehyde, 30 mg/L glyoxal, 400 mg/L formate, and 300 mg/L acetate (final concentrations). A 10× stock solution of the cocktail was prepared and stored in the cooler at 4°C.

Groundwater—Well water was acquired from the Biology 1 building on campus. The groundwater supply, which is used for fishery research in the Department of Biology, is independent of the municipal system without any chlorine residual.

Dechlorinated Tap Water—Tap water was dechlorinated by passing it through two parallel bench scale filters (5 cm inner diameter × 60 cm high glass columns containing Calgon Filtrasorb F-300 GAC). This was done to remove any free or combined chlorine that may have been present in the tap water (Anderson, 2004). The potential free chlorine residual in the filter effluent was detected using an amperometric titrator (Wallace & Tiernan Inc.) as described in Standard Method 4500-Cl.D (APHA *et al.*, 1998).

3.2.2 *Escherichia coli* (*E. coli*)

E. coli (ATCC 11229, Manassas, VA) was grown in the nutrient broth (VWR) at 37^{o}C at 150 rpm (Incubator/Shaker, Innova^{TM} 4230, New Brunswick Scientific). A 16 to 18 hours culture in stationary phase was selected for experimental purposes to closely represent cells in the environment. The typical growth curve can be found in Figure B.1 (Zimmer, 2002). Approximately 200 mL of nutrient suspensions with *E. coli* were stored in a 300 mL flask (VWR) and placed in the Incubator/Shaker at 37^{o}C at 150 rpm for inoculation every time before use.

One mL aliquots of the *E. coli* suspension were centrifuged (Sorvall FA-Micro, DuPont Canada, Mississauga, Canada) at 8000 ×g (10,000 rpm) for 2 minutes and the supernatant was aseptically drawn off. The pellet was resuspended in 1 mL 1× phosphate buffered saline (PBS, 0.01M, EMD^{TM}) and then centrifuged. As described above, the washing procedure was repeated twice to remove any nutrient medium and obtain an *E. coli* concentration of approximately 10^8 cells/mL. Thereafter 1 mL aliquots of the *E. coli* suspension in the PBS were ready for spiking in the samples.

3.2.3 Microbiological Methods

Total coliforms were enumerated by plate counts (MF technique) as described in Standard Method 9222B (APHA *et al.*, 1998), using mEndo LES agar (VWR) and GN-6 filters (0.45 μm, VWR). The plates were incubated at 37^{o}C for 24 hours and colonies characteristic of coliform bacteria were counted.

E. coli were enumerated by plate counts (MF technique) as described in Standard Method 9213D.3 (APHA *et al.*, 1998), using mTEC agar (VWR) and GN-6 filters (0.45 μm, VWR). The plates were first incubated at 37^{o}C for 2 hours and then incubated at 44.5^{o}C for 22 hours, and the colonies characteristic of *E.coli* were counted.

3.2.4 Settling of Bacteria with Particles

E. coli and kaolin particles (0.1~4 μm, Sigma-Aldrich, Canada) were used for the settling experiments at room temperature. The processes used to prepare three types of samples in

parallel, including *E. coli*, kaolin particles, and *E. coli* with kaolin particles, are described below.

A. 1 mL aliquots of the *E. coli* suspension (Section 3.2.2) were spiked into two 1000 mL flasks containing 500 mL MQ water each and mixed thoroughly. One flask was left stationary for static settling, while the other was continuously shaken at 100 rpm (Model M49235, Thermolyne, USA) as a control.

B. 0.035 to 0.05 g of sterilized kaolin particles were suspended in a 1000 mL flask containing 500 mL MQ water and mixed thoroughly. The flask was left stationary for static settling.

C. 1 mL aliquots of the *E. coli* suspension were spiked into four 1000 mL flasks each containing a 500 mL suspension of the kaolin particles (0.035 to 0.05 g) and were allowed to contact at 100 rpm for 0.5, 2.0, 4.0, and 8.0 hours. The flasks were left stationary for static settling.

For sample A, *E. coli* were enumerated in both the supernatant of the settled sample and the suspension of the control sample at intervals during the settling (0 to 24 hours). For sample B and C, TSS in the supernatants was measured (DR/2000 spectrophotometer, Hach) at intervals during the settling (0 to 24 hours).

3.2.5 Attachment of Bacteria with Particles

E. coli, kaolin particles, and river water particles were used for the attachment experiments at room temperature. River water samples were from the municipal intake location of the Mannheim Water Treatment Plant in the Regional Municipality of Waterloo (detail in Section 3.2.6). The potential attachment of *E. coli* with kaolin particles or river water particles was then evaluated.

E. coli concentration of the suspensions were determined at designated intervals throughout the contact time period. The growth of *E. coli* in the corresponding "particle-free" samples (MQ water without kaolin particles, filtrate of river water samples without the particles) was measured during the contact time as a control.

A. 1 mL aliquots of the *E. coli* suspension were spiked into two flasks containing either 500 mL MQ water or 500 mL kaolin suspension and both flasks were continuously shaken at 100 rpm (Model M49235, Thermolyne, USA).

B. 1 mL aliquots of the *E. coli* suspension were spiked into two flasks containing either 500 mL filtrate (0.45 μm, GN-6, VWR) of river water samples or 500 mL unfiltered river water samples and both flasks were continuously shaken at 100 rpm.

For samples A and B, *E. coli* was enumerated in the suspensions at intervals during the contact time (0 to 3.5 hours and 0 to 24 hours, respectively).

3.2.6 Sample Source and Preparation

Five categories of the sample sources (Section 3.1) were prepared for use in the UV inactivation and chlorination experiments. The sampling procedure followed Standard Method 9060A.3 and the collected samples were stored in the cooler at 4°C as described in Standard Method 9060B (APHA *et al.*, 1998). Thus, the temperature of the samples was 4°C at the time of analysis.

The corresponding "particle-free" samples were prepared by filtration. For each type of water sample, filtration was used to remove the particles, resulting in a set of filtered or unfiltered samples for each source. There was no broadly accepted pore size for filtration and many researchers used 7~10 μm for wastewater samples based on the concept of critical particle size (CPS, section 2.4.3). However, filtration using large pore sizes may not remove smaller particles that could have a potential impact on the experimental results. Therefore, experiments in this thesis used the common pore size in the microbiological assay, 0.45 μm, for filtration of all samples.

Apparently, the 0.45-μm filtration would also remove the background *E. coli* at the same time of removing the particles. In the unfiltered surface water, the background *E. coli* concentrations were low (22~89 cells/100 mL) and therefore not considered to impact analyses. In the unfiltered wastewater effluent, the background *E. coli* concentrations were

significant (about 10^4~10^5 cells/mL). Thus, the dose-response of these natural *E. coli* was determined separately before the spiking (Section 5.1.2) as a control.

The appropriate amounts of *E. coli* were spiked into both filtered and unfiltered samples, resulting in a concentration of approximately 10^6 cells/mL. An hour contact time between spiked *E. coli* and each sample was selected to minimize the effect of environmental variables. A longer contact time was not employed due to time constraints. The general sample preparation procedure is shown in Figure 3.1.

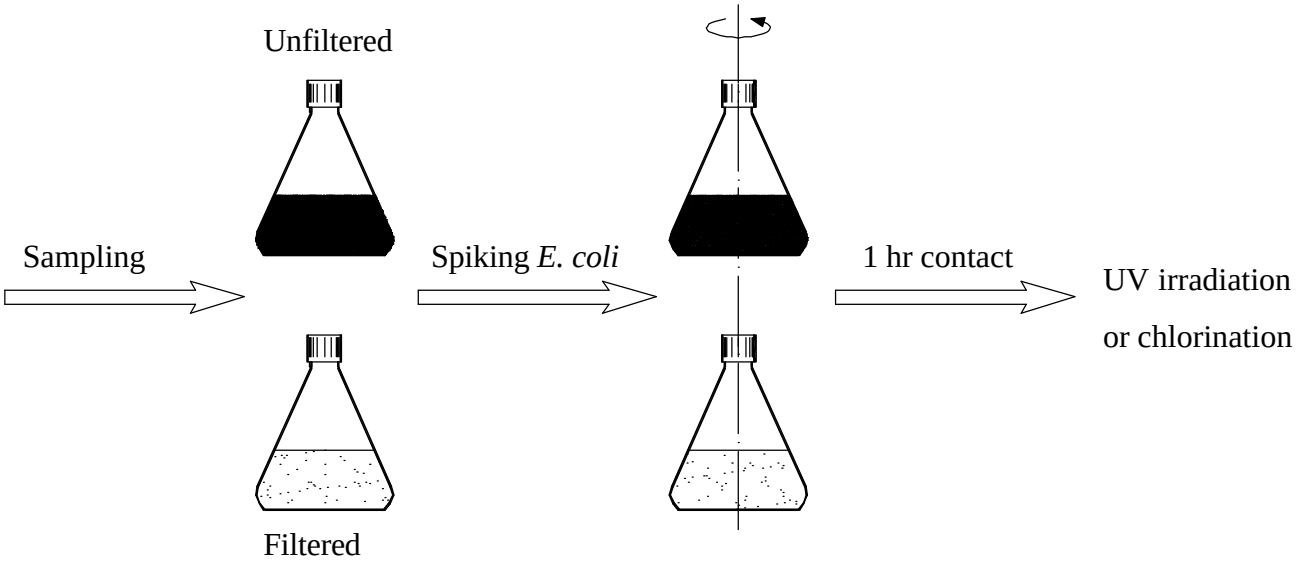


Figure 3.1 General preparation procedure

Besides MQ water samples, pairs of the prepared samples could be categorized as: filtered secondary effluent and unfiltered secondary effluent; filtered river water, unfiltered river water, and coagulated river water; coagulated process water. Each of sample categories is described in detail below.

MQ Water (Particle-free)

Dose-response of spiked *E. coli* in MQ water (Milli-Q UV Plus, 0.22 μm MilliPak-40 Ultra Pure Water system, MilliPore Corp.) was determined using UV irradiation and chlorine as a control. The MQ water samples were prepared as described below.

A 1 mL aliquot of the *E. coli* suspension was spiked into a 1000 mL flask containing 500 mL MQ water, which had a pH adjusted to 7.0 using a pH 7.0 buffer solution (VWR). The 500 mL suspension was continuously shaken at 100 rpm (Model M49235, Thermolyne, USA) for one hour.

Wastewater Particles

Fecal particles were obtained from the secondary effluent of the Waterloo Wastewater Treatment Plant (Waterloo WWTP) in the Regional Municipality of Waterloo. The Waterloo WWTP has a design capacity of approximately 73 million liter per day (MLD) and currently treats domestic wastewater at a flow rate of 37 MLD.

The WWTP process units include a bar screen, vortex grit remover (future), primary clarifier, aeration tank (surface aerator), secondary clarifier, and chlorination. The plant currently adds ferrous chloride ($FeCl_2$) as coagulant for phosphorus removal in the return activated sludge at the inlet of the aeration tank.

The sampling point was in the effluent well from the secondary clarifier prior to the chlorine-dosing well. Hence, there was no chlorine residual in the samples. The secondary effluent samples were collected in a sterile 10 L polypropylene container and transported on ice to the laboratory.

A filtered secondary effluent sample was obtained by filtering a 500 mL sample using a 0.45-μm membrane filter (GN-6, VWR). 1 mL aliquots of the *E. coli* suspension were spiked into two 1000 mL flasks containing either 500 mL filtered or 500 mL unfiltered samples. Both flasks were continuously shaken at 100 rpm (Model M49235, Thermolyne, USA) for one hour.

Surface Water Particles

River sediments or particles were obtained from samples of raw surface water in the Grand River Watershed (GRCA, 2004). The sampling point was the municipal intake location of the Mannheim Water Treatment Plant in the Regional Municipality of Waterloo.

During the present study, the Natural Sciences and Engineering Research Council (NSERC) Chair in drinking water treatment at the University of Waterloo has been undertaking another research project (slow-sand filtration) with MS Filter Inc. at the same location. The established pilot treatment system included an influent tube that delivered raw water pumped from the adjacent Grand River. River water samples were collected in a sterile 10 L polypropylene container and transported on ice to the laboratory.

The preparation procedure to obtain filtered and unfiltered river water samples was the same as that for secondary effluent samples.

Coagulated Surface Water Particles

A standard jar tester (Phipps & Bird Inc., VA) was used to determine the coagulant dose for optimum floc formation. Alum stock solution was used as coagulant and was added to river water samples to form floc particles. The 1000 mg/L stock solution was prepared by dissolving 0.3 g alum ($AlK(SO_4)_2 \cdot 12H_2O$, J. T. Baker, NJ) in 300 mL MQ water. The jar test was processed according to the laboratory procedures prepared for the EnvE 101 course (Environmental Engineering Concepts 2) at the University of Waterloo.

First, 10, 12.5, 15, 17.5, and 20 mL of 1000 mg/L alum stock solution were added to five 2000 mL beakers (VWR) containing 500 mL river water sample to produce the selected alum

dosages of 20, 25, 30, 35, 40 mg/L, respectively. Second, each sample was rapidly mixed for 30 seconds at 100 rpm for coagulation, stirred for 15 minutes at 30 rpm for flocculation, and allowed to stand for 15 minutes for static settling. Third, turbidity of the supernatants was measured (Model 2100P, Hach) in each beaker after settling.

Based on the results of the jar test, an optimum alum dosage of 30 mg/L (Section 5.1.3) was selected and applied to form the floc particles as follows.

First, a 1 mL aliquot of the *E. coli* suspension was spiked into a beaker containing 500 mL river water sample, followed by adding 15 mL alum stock solution. Second, the resulting solution was mixed for 30 seconds at 100 rpm for coagulation, and then for 15 minutes at 30 rpm for flocculation. Third, the formed suspensions with floc particles were transferred to petri dishes (Section 3.2.8) for UV inactivation.

The paddles in the jar tester were sterilized by 85% ethanol (VWR) before use. Note that the filtration was not necessary because filtered river water samples ("particle-free") were already available and the contact time was not applicable. The freshly formed floc particles were visible and fragile immediately following flocculation and they inevitably broke down to tiny invisible floc particles when transferred for UV irradiation.

Coagulated Process Water Particles

Process water was obtained from the flocculation tank of the Mannheim Water Treatment Plant in the Regional Municipality of Waterloo. The plant is a conventional treatment plant that treats surface water from the Grand River. It has a design capacity of 72 MLD and the process consists of pre-ozonation, coagulation, flocculation, sedimentation, filtration, and disinfection (UV and chlorine). Poly-aluminum chloride (PAC) was used for coagulation and flocculation at the time of sampling.

The sampling point was the upflow weir prior to the flocculation tank after the PAC was dosed. The overflow of the weir was collected in a sterile 1 L sample bottle as coagulated process water and transported on ice to the laboratory.

The sample arrived in the laboratory approximately 10 to 15 minutes after it was collected on site. The expected process of flocculation was simulated to form floc particles immediately upon arrival. First, a 1 mL aliquot of the *E. coli* suspension was spiked into a beaker containing 500 mL process water sample. Second, the solution was mixed for approximately 15 to 45 minutes (based on the formation of visible floc) at 30 rpm for flocculation using a standard jar tester (Phipps & Bird Inc., VA). Third, the formed suspensions with floc particles were transferred to petri dishes (Section 3.2.8) for UV inactivation.

Once again, the paddles in the jar tester were sterilized by 85% ethanol before use. The freshly formed floc particles were visible and fragile immediately following flocculation and they inevitably broke down to tiny invisible floc particles when transferred for UV irradiation and particle analysis.

Runoff Water Particles (Planned)

Soil particles were planned to obtain from samples of runoff water in the Grand River during the related events (e.g. precipitation and stormwater). However, the appropriate sampling occasion has not occurred when the runoff water samples were expected.

3.2.7 Water Quality Parameters

The water quality parameters that were measured included pH, DOC, turbidity, TSS, and UVA (UVT). pH was measured using a pH meter (Model 420A, Orion Research Inc.). DOC was measured by a TOC analyzer (Model 1010, OI Analytical, TX) in mg/L as described in Standard Method 5310B. Turbidity was measured by a portable turbidity meter (Model 2100P, Hach) in NTU. TSS was measured in mg/L as described in Standard Method 2540D.

In water samples, UVA is the product of the UVA coefficient (cm^{-1}) and the path length (cm). UVA coefficients were measured in cm^{-1} for the samples before exposure to UV irradiation (detail in Section 3.2.8). Chlorine demand was measured prior to chlorination as described in Standard Method 2350B (detail in Section 3.2.9).

3.2.8 UV Irradiation

UV Source

A UV bench-scale collimated beam apparatus (Calgon Carbon Corp.) was used to irradiate the samples in the present study. The apparatus was equipped with a replaceable LP (12 W) or MP (1 kW) mercury UV lamp, which was enclosed in a metal cabinet. Two replaceable polyvinyl chloride collimating tubes (0.39 m and 0.93 m) were connected with and located beneath the cabinet. The shorter tube was used for the higher dose range, while the longer tube was used for the lower dose range. The tubes were darkened on the inside and collimated a beam of UV light to irradiate the sample. Ideally, the irradiation was expected to be nearly uniform and perpendicular over the sample surface.

The sample to be irradiated was placed below the center of the collimating tubes on a magnetic stir plate. A schematic of the apparatus setup is shown in Figure C.1 (UVDGM, 2003) and a picture of the actual apparatus that was used in this study is shown in Figure C.2.

Sample Irradiation

The circular beam of UV light was about 60 mm in diameter. A 20 mL aliquot of the prepared samples was pipetted into a 52 mm plastic petri dish (VWR), forming a water path length of 9.4 mm (i.e. the depth of the sample in the petri dish).

The petri dish was placed under the center of the collimating tube on a stir plate and the sample was thoroughly mixed with a 12.7×3.175 mm stir bar (FisherBrand, VWR) prior to irradiation. After removing the lid, the sample was exposed to LP or MP UV irradiation while being constantly stirred. The stir rate was carefully set so that there was no vortex forming on the sample surface. A new petri dish was used for every exposure.

UV exposure times were calculated using a spreadsheet (Bolton and Linden, 2003) to deliver the designated UV doses. The delivered UV doses were 5, 10, 15, 25, and 40 mJ/cm^2 for LP irradiation and 1, 3, 5, 10, 15, 25, and 40 mJ/cm^2 for MP irradiation (1 and 3 mJ/cm^2 were selected after *E. coli* was found to be sensitive to MP irradiation). Generally speaking, UV doses of 5, 10, and 25 mJ/cm^2 were selected because a possible linear log inactivation was expected at lower doses; UV doses of 25 and 40 mJ/cm^2 were selected because the UV inactivation efficacy could be evaluated at the current regulation standards. Because a minimum exposure time of 30 seconds was required (Bolton and Linden, 2003), the minimum MP dose of 1 mJ/cm^2 was applicable in the laboratory. The irradiation was duplicated for every dose in either LP or MP UV experiments.

Zimmer (2002) and Sommer *et al.* (2000) reported that there was limited or no dark repair of *E. coli* following exposure to LP UV irradiation at the selected doses. In addition, no dark and photo repair were observed at the same doses with respect to MP UV irradiation

(Zimmer, 2002). Hence, all samples were irradiated in the dark at room temperature and post-irradiated samples were stored in the dark before analysis.

After irradiation, *E. coli* were enumerated as described in Section 3.2.3 to determine the post-irradiated survival concentration. Each sample was enumerated in duplicate series (i.e. two aliquots from one petri dish).

LP UV Dose Determination

The correction factors include water factor, petri factor, reflection factor and divergence factor as Equation 3.1 (Bolton and Linden, 2003).

$E_{avg} = E_0 \times Petri\ factor \times Reflection\ factor \times Divergence\ factor \times Water\ factor$ Equation 3.1

E_{avg}—average intensity for the sample suspension after all corrections in mW/cm^2

E_0—UV incident irradiance at the center of the suspension's surface in mW/cm^2, which is measured by a calibrated radiometer (Model 1L 1700, International Light) with a SED 240 UV detector at 254 nm.

Petri factor—corrects for the non-uniform distribution of UV light across the surface area of the sample to be irradiated in the container (e.g. 52 mm petri dishes in this study). Practically, a longer collimating tube will have a greater petri factor and provide a more uniform distribution over the sample surface.

Reflection factor—corrects for the reflection of UV light at the air-water interface. Regardless of the type of lamp (LP or MP) being used, the value of reflectance, R, at the air-water interface is 0.025 estimated by Fresnel's Law based on the refraction index of 1.0 and 1.372 for air and water respectively. Hence, the reflection factor is a constant of 0.975 (1-0.025=0.975).

Divergence factor—corrects for the UV light that strays outside of the petri dish before passing the entire depth. It is calculated based on the proportion of the depth of sample suspension to the distance from lamp centerline to suspension surface. Practically, a longer collimating tube will have a greater divergence factor and a better collimated beam of UV light.

Water factor—corrects for the UVA of the sample suspension. The UVA coefficient in cm^{-1} of each sample was measured by a 1 cm quartz cell in a UV spectrophotometer (Hewlett-Packard 8453 UV-Visible Spectrophotometer, Canada) at 254 nm prior to irradiation. A lower sample UVA will result in a greater water factor.

This proposed protocol for the LP UV bench-scale test was also included in the Ultraviolet Disinfection Guidance Manual (UVDGM, USEPA 2003). UVDGM consolidated the concepts of biodosimetry and RED to validate full-scale UV reactors for drinking water applications. Based on the protocol, the mathematic equation for LP UV dose calculation was developed in the bench-scale test. The parameters in Equation 3.2 are respectively related to the corresponding term factors in Equation 3.1.

$$D = E_{avg} \times t = E_0 \times P_f \times (1-R) \times \frac{L}{d+L} \times \frac{1-10^{ad}}{ad \ln(10)} \times t \qquad \text{Equation 3.2}$$

Where

D = UV dose in mJ/cm^2

P_f = Petri Factor

R = Reflectance at the air-water interface

L = Distance from lamp centerline to suspension surface in cm

d = Depth of the suspension in cm

a = UV absorption coefficient (Base 10) of the suspension at 254 nm in cm^{-1}

t = Exposure time in seconds

MP UV Dose Determination

MP UV dose determination is more complex than that for LP UV due to the polychromatic emission of MP UV light. In addition to the factors used to calculate the LP UV dose, additional factors, such as the radiometer sensor factor and the germicidal factor, are necessary to determine the average intensity for MP UV light (Cabaj *et al.*, 2001; Bolton and Linden, 2003). The UV irradiance (E_0) is also measured by the calibrated radiometer at 254 nm. Determination of the petri factor, reflection factor, and divergence factor are the same as that for LP light.

$$E_{avg} = E_0 \times \text{Petri factor} \times \text{Reflection factor} \times \text{Divergence factor} \times \text{Water factor} \times \text{Sensor factor} \times \text{Germicidal factor} \qquad \text{Equation 3.3}$$

Water factor (adjusted)—corrects for the UVA of the sample suspension. The UVA coefficient in cm^{-1} of each sample was measured prior to irradiation using the spectrophotometer at 5 nm increments in the germicidal wavelength from 200 to 299 nm.

Radiometer sensor factor—corrects for the wavelength bias of the radiometer based on the emission spectrum of MP UV light. The manufacturer (International Light) provided this factor of 1.206.

Germicidal factor—corrects for the germicidal effectiveness of the selected wavelength (200~299 nm) compared to 254 nm by weighting the polychromatic irradiation, which is generally represented by the DNA absorbance spectrum (Section 2.3.2).

Calculation Spreadsheets

A series of calculation spreadsheets, in Microsoft Excel, programmed by Dr. Bolton, are accessible on the web site of the International Ultraviolet Association (IUVA, 2004). There were four titled categories available at the time of this study:

- UV Dose for Low Pressure Lamps-Deep (to be used with a low pressure lamp where the vertical path length in the dish is >20 mm)
- UV Dose for Low Pressure Lamps-Shallow (to be used with a low pressure lamp where the vertical path length in the dish is <20 mm)
- UV Dose for Medium Pressure Lamps-Deep (to be used with a medium pressure lamp where the vertical path length in the dish is >20 mm)
- UV Dose for Medium Pressure Lamps-Shallow (to be used with a medium pressure lamp where the vertical path length in the dish is <20 mm)

Since the actual path length (i.e. the depth of sample suspension to be irradiated) was 9.4 mm in this study, the ‘UV Dose for Low Pressure Lamps-Shallow’ and ‘UV Dose for Medium Pressure Lamps-Shallow’ calculation spreadsheet were employed in the LP and MP UV bench-scale experiments, respectively.

3.2.9 Chlorination

Stock Solutions and Standardization

Two standard stock solutions, chlorine (Cl_2) and sodium thiosulfate ($Na_2S_2O_3$), were used in the chlorination experiments.

A standard chorine stock solution was prepared by diluting 1 mL 5.25% commercial bleach (52,500 mg/L, sodium hypochlorite, NaOCl) into 210 mL MQ water. The resulting stock solution (250 mg/L) was stored in a 300 mL glass-stoppered bottle (VWR) in the dark. The concentration of actually used solutions was standardized each time prior to use as described in Standard Method 2350B and 4500-Cl.D (APHA *et al.*, 1998).

A standard sodium thiosulfate solution was prepared by dissolving 0.75 g sodium thiosulfate ($Na_2S_2O_3$, Fisher Scientific) into 250 mL MQ water. The 0.3% stock solution was stored in a glass-stoppered bottle in the dark. The solution was standardized every time before use as described in Standard Method 4500-Cl.B (APHA *et al.*, 1998).

Batch Reaction

Five 40-mL glass vials (VWR) were selected as batch reactors for five incremental chlorine dosages due to the limited sample volume and convenience of operation. A 25 mL aliquot of the prepared samples (Section 3.3.1) was pipetted into each vial and then pH was adjusted to 7.0 by adding 0.2 mL pH 7.0 buffer solution (VWR).

A corresponding amount of the chlorine stock solution was added to each vial in series after chlorination was completed in the previous vial for five selected dosages. During every 1-minute reaction time, the capped vial was turned upside down five times and then continuously stirred with a 12.7×3.175 mm stir bar on a magnetic stir plate. At the end of every 1-minute reaction time, 0.2 mL $Na_2S_2O_3$ stock solution (0.3%) was immediately spiked into the vial and the vial was then capped and turned upside down five times to mix thoroughly to neutralize the residual chlorine and stop the reaction.

After chlorination, *E. coli* were enumerated as described in Section 3.2.3 to determine the post survival concentration. Each sample was enumerated in duplicate series (i.e. two aliquots from one vial).

CT Applications

Based on the C×T concept (Section 3.1), the concentration of chlorine residual should be used to calculate the C×T values. However, the residual concentrations could not be measured because chlorine was neutralized to stop the reaction at 1-minute contact time in the reactor. Conversely, the residual concentration was calculated by the difference between the initial concentration and the pre-measured chlorine demand of the samples as Equation 3.4 in mg/L.

Initial Concentration = Chlorine Demand + Residual Concentration Equation 3.4

Analogous to the UV experiments, the chlorine dose-response of *E. coli* in MQ water was first determined as a control. Note that the MQ water samples (Section 3.2.6) had no chlorine demand, which implies that the residual concentration equals the initial concentration. A series dose of 0.01, 0.02, 0.03, 0.05, and 0.1 mg·minutes/L was selected after several preliminary experiments. Because the contact time was fixed at 1 minute, the corresponding initial chlorine concentrations were 0.01, 0.02, 0.03, 0.05, and 0.1 mg/L, respectively.

Coagulated river water samples were used in the chlorination experiments because the shielding effects were observed in the LP UV experiments (Section 5.1.3). Chlorine demand of the coagulated samples were determined at pH 7 at 4^{o}C for 1 minute by the amperometric titrator (Wallace & Tiernan Inc.) as described in Standard Method 4500-Cl.D (APHA *et al.*, 1998). Therefore the required initial concentration of chlorine was calculated according to Equation 3.4.

3.2.10 Particle Analysis

Particle analysis was performed using a dynamic particle analyzer (DPA 4100, Brightwell Technologies Inc., 2004). Because the DPA 4100 is limited to reliable analysis for particle sizes less than 400 μm, pre-filtration of the prepared samples was necessary to prevent plugging of the instrument.

When the samples were prepared and ready for exposure to UV irradiation, part of the samples (200 mL) were pre-filtered using a 230-μm stainless steel screen (SS-8F-K4-230, Nupro Company, OH). The filtrates were transferred to an accessory flask and analyzed using the DPA 4100 as described in the procedure manual (Brightwell Technologies Inc., 2004).

3.2.11 Statistical Analysis

The average (mean value), standard deviation (SD), and relative standard deviation (RSD) are shown with data in the tables and figures (SD as the Y error bars) where applicable.

A pair of data was compared for statistical significance using t-tests (with a 95% confidence level). A set of data was compared for statistical significance using least significant difference (LSD) and Bonferroni t-tests for multiple comparisons (with a 99% confidence level). Analysis of variance (ANOVA table) and 95% confidence intervals were calculated where appropriate.

All the statistical methods are in accordance with the course notes of CHE 622 in the department of Chemical Engineering at the University of Waterloo (Duever, 2002). The 'Analysis ToolPak' in Microsoft Excel was used in the data analysis calculations.

Chapter 4

Preliminary Results

4.1 Objectives

Before applying UV irradiation and chlorine to the prepared samples, three preliminary experiments were performed to determine: whether it is possible to incubate and sustain the required concentration of environmental total coliforms in the laboratory; whether it is possible to separate the particle-associated bacteria by settling; and whether it is possible to simulate the association (e.g. attachment or enmeshment) between bacteria and particles under laboratory conditions.

4.2 Total Coliforms

According to the recommendation of UV bench-scale experiments (NWRI/AWWARF, 2001) and the UV dose-response table of microorganisms (Wright and Sakamoto, 2001), the minimum initial concentration of coliform bacteria to be irradiated is approximately 6 log (10^6 cells/mL) at the designated dose of 40 mJ/cm^2. Natural total coliforms were enumerated at less than 3 log (Table 4.1) so that an increase of more than 3 log was required in the laboratory.

To increase the concentration of total coliforms, the creek water was first incubated and then inoculated with the prepared nutrients, and incubated again. The prepared nutrients included

filtered creek water, groundwater, a cocktail of biodegradable organic matter (BOM), and dechlorinated tap water. The growth of coliform bacteria is depicted as the trend of the log concentration of total coliforms in water samples, as shown in Table 4.1 and Figure 4.1.

Table 4.1 Laboratory incubation of total coliforms

Total coliforms concentration [log (cells/mL)]					
Date	**Incubation time (hour)**	**0**	**24**	**48**	**72**
Trial 1 on Dec. 2003	Creek water	2.40	4.04	4.27	
	BOM			4.27	4.82
	Creek filtrate			4.27	5.26
Trial 2 on Jan. 2004	Creek water	1.73	2.15		
	Groundwater		2.15	0.00	
	Groundwater with BOM		2.15	0.00	
Trial 3 on Feb. 2004	Creek water	2.60	4.43		
	Groundwater		4.43	4.15	4.31
	Dechlorinated tap water		4.43	4.65	4.54
Statistical analysis	Average	2.24	3.54	4.34	4.73
	Standard deviation	0.46	1.22		
	Relative standard deviation	20%	34%		

In Table 4.1, the shaded areas represent observed log concentrations of total coliforms at different time intervals (0, 24, 48, and 72 hours). All horizontal bars formed by the connected shaded areas represent the processes of incubation; the three vertical bars formed by the

connected shaded areas (across 'Creek water') represent the processes of inoculation from creek water to different nutrients, respectively. For example, in trial 2, creek water was first incubated for 24 hours (0 to 24 hours) and then inoculated to either groundwater or groundwater supplemented with BOM. The resulting samples were thereafter incubated for another 24 hours (24 to 48 hours).

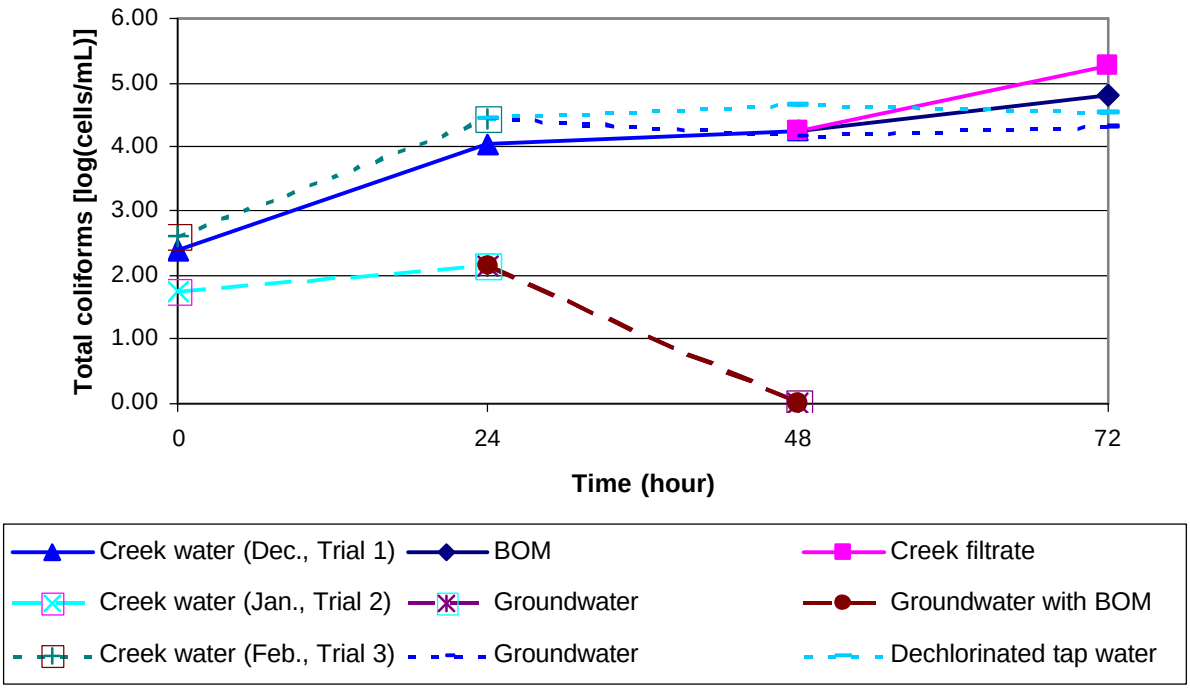


Figure 4.1 Laboratory incubation of total coliforms

The initial concentration of total coliforms from unamended Laurel Creek water had an average value of 2.24 log for sampling performed during three months (Dec. 2003, Jan. 2004,

and Feb. 2004). After a 24-hour incubation without any supplements, the concentration of total coliforms increased to an average of 3.54 log in the creek water. In trial 1, the creek water was incubated up to 48 hours without any supplements. The observed increase between 24 and 48 hours was only 0.23 log. Therefore, in the trials 2 and 3, creek water was first incubated for 24 hours only.

In Trial 2, all the total coliforms died off after a 48-hour incubation. One of the possible reasons was that coliform bacteria were shocked due to the acute change of living environment. In trial 1, a maximum concentration of 5.26 log was observed in the creek filtrate after a 72-hour incubation, which was still below the minimum requirement of 6 log (10^6 cells/mL) concentration.

Considering the environmental variables and time constraints, further experiments on total coliforms were discontinued. Instead, a laboratory grown *E. coli* ATCC 11229 (*E. coli*) was eventually selected as an indicator organism for the subsequent experiments. Note that *E. coli* is well established to grow and maintain the required concentrations in the laboratory (Section 3.2.2).

4.3 Settling of Bacteria with Particles

Since reliable methods for bacteria extraction are site specific and complex, there is no widely accepted procedure for quantifying particle-associated bacteria (Section 2.4.1).

Alternatively, a helpful approach is to separate potential particle-associated bacteria from the dispersed ones and then enumerate them. It was hypothesized that the settling velocity of particles would increase significantly if the particle size would increase due to the association between bacteria and particles (Stoke's Law).

4.3.1 Settling of Bacteria Only

Initially, the settling characteristic of bacteria was investigated under laboratory conditions. The settling pattern of *E. coli* is depicted by the change in log concentration of *E. coli* in the supernatant of the samples (Section 3.2.4, A), as shown in Table 4.2 and Figure 4.2. A control sample was continuously shaken and analyzed in parallel to account for the growth of *E. coli* during the settling time.

Table 4.2 Settling pattern of *E. coli* only

***E. coli* Concentration [log (cells/mL)]**					
Settling time (hour)	**0**	**2**	**6**	**12**	**24**
Continuous shaking (control)	6.53	6.45	6.65	7.21	7.28
Supernatant after settling	6.53	6.51	6.69	7.15	7.18

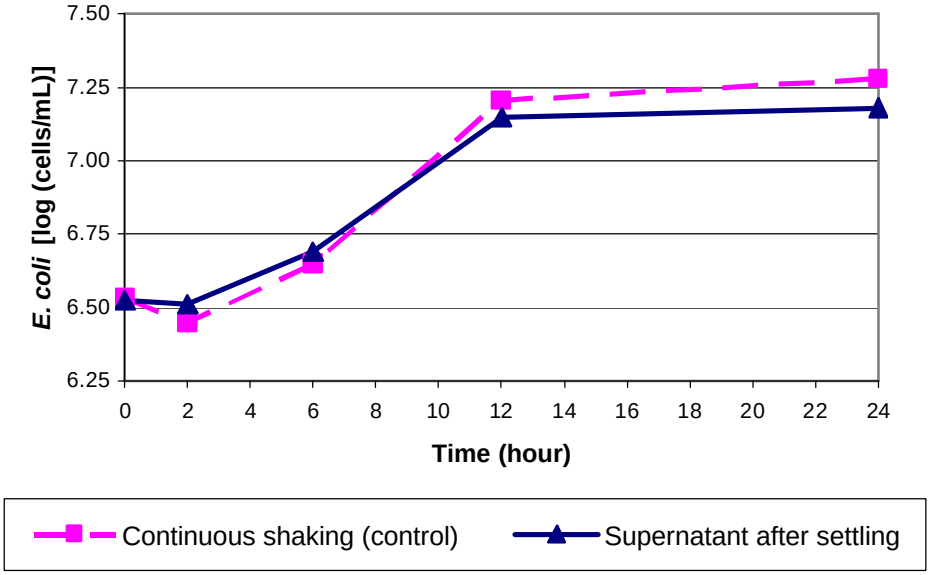


Figure 4.2 Settling pattern of *E. coli* only

A typical growth trend occurred in both the supernatant of the settled samples and the suspension of the shaken samples (as a control). It included a lag phase for the first 2 hours, a log phase from 2 to 12 hours, and a stationary phase in the following 12 hours. Therefore, *E. coli* does not seem to settle out over a 24-hour settling duration.

4.3.2 Settling of Particles Only

The settling pattern of kaolin particles (Section 3.2.4, B) is depicted as the settled fraction of total suspended solids (TSS) at specified time intervals, as shown in Table 4.3 and Figure 4.3. Standard deviations are shown as bars on the data points in the figure. The settled fraction of TSS is calculated as Equation 4.1.

$$\text{Settled fraction of TSS} = \frac{\text{Initial TSS} - \text{TSS of supernatant}}{\text{Initial TSS}} \times 100\% \qquad \text{Equation 4.1}$$

Table 4.3 Settling pattern of kaolin particles

Settled fraction of TSS (%)					
Settling time (hour)	**0**	**2**	**6**	**12**	**24**
Average	0%	48%	80%	87%	93%
Standard deviation	0%	1%	3%	3%	2%

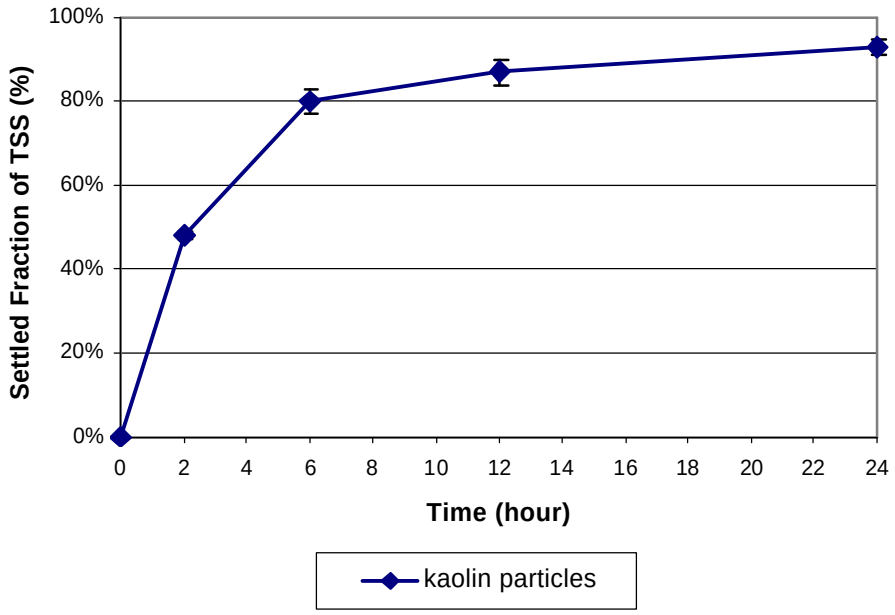


Figure 4.3 Settling pattern of kaolin particles

The initial TSS concentrations of the samples were 68, 85, and 90 mg/L and kaolin particle size ranged from 0.1 to 4 μm. An average of 80% of initial TSS was observed to settle out during the first 6 hours. Hence, settling kaolin particles was effective.

4.3.3 Settling of Bacteria with Particles

The settling pattern of kaolin particles with *E. coli* (Section 3.2.4, C) is depicted as the settled fraction of total suspended solids (TSS) at time intervals of 2, 6, and 10 hours, as shown in Table 4.4 and Figure 4.4. Calculation of the settled fraction of TSS is the same as shown in Equation 4.1. The initial concentration of TSS was lowered to 25 mg/L, which is closer to the natural TSS level of surface water sources (Montgomery, 1985). The contact times for potential association between kaolin particles and *E. coli* were 0.5, 2.0, 4.0, and 8.0 hours before settling.

Table 4.4 Settling pattern of kaolin particles with *E. coli*

Settled fraction of TSS (%)				
Settling time (hour)	**0**	**2**	**6**	**10**
Kaolin only (control)	0%	24%	48%	62%
0.5 hour contact time	0%	14%	43%	62%
2.0 hour contact time	0%	18%	55%	64%
4.0 hour contact time	0%	22%	65%	65%
8.0 hour contact time	0%	32%	52%	68%

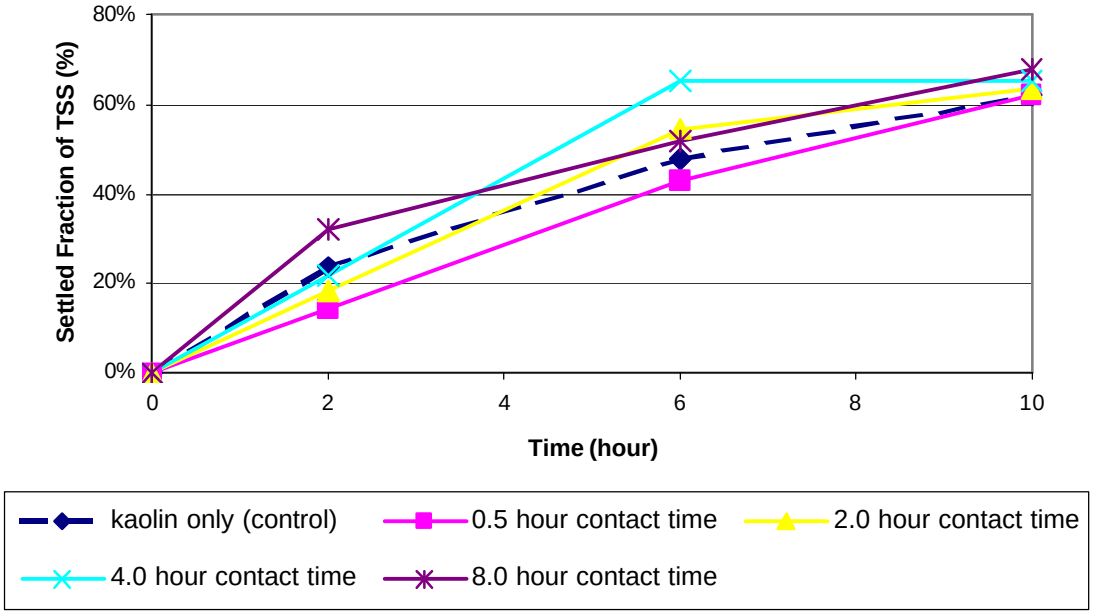


Figure 4.4 Settling pattern of kaolin particles with *E. coli*

Neither the presence of *E. coli* nor the contact times showed a remarkable impact on the settling pattern of kaolin particles. One of the possible reasons is that kaolin particles and *E. coli* did not interact with each other during the 8-hour contact time under laboratory conditions.

4.4 Attachment of Bacteria with Particles

No standard procedure for quantifying the particle-associated bacteria currently exists because reliable methods for bacteria extraction are site specific and complex (Camper *et al.*, 1985a; McDaniel and Capone, 1985). Conversely, a reverse approach is to simulate the association between particles and bacteria under laboratory conditions.

It was hypothesized that the number of the bacterial colonies when kaolin was present could decrease significantly because a group of clumped particles and bacteria produce one colony on the plate (Petri *et al.*, 2000). Consequently, the apparent concentration as enumerated using plate counts of *E. coli* was expected to decrease after the contact time.

4.4.1 Attachment of *E. coli* with Kaolin Particles

Potential attachment is depicted as the change in the log concentration of *E. coli* in samples (Section 3.2.5, A), as shown in Table 4.5 and Figure 4.5. A control sample containing only *E. coli* was analyzed in parallel to account for the growth of *E. coli* during the 3.5-hour contact time.

Table 4.5 Attachment of *E. coli* with kaolin particles

***E. coli* Concentration [log (cells/mL)]**				
Settling time (hour)	**0**	**0.5**	**1.5**	**3.5**
E. coli in MQ water (control)	6.00	5.95	5.89	6.00
E. coli with kaolin particles	6.00	5.96	5.98	5.88

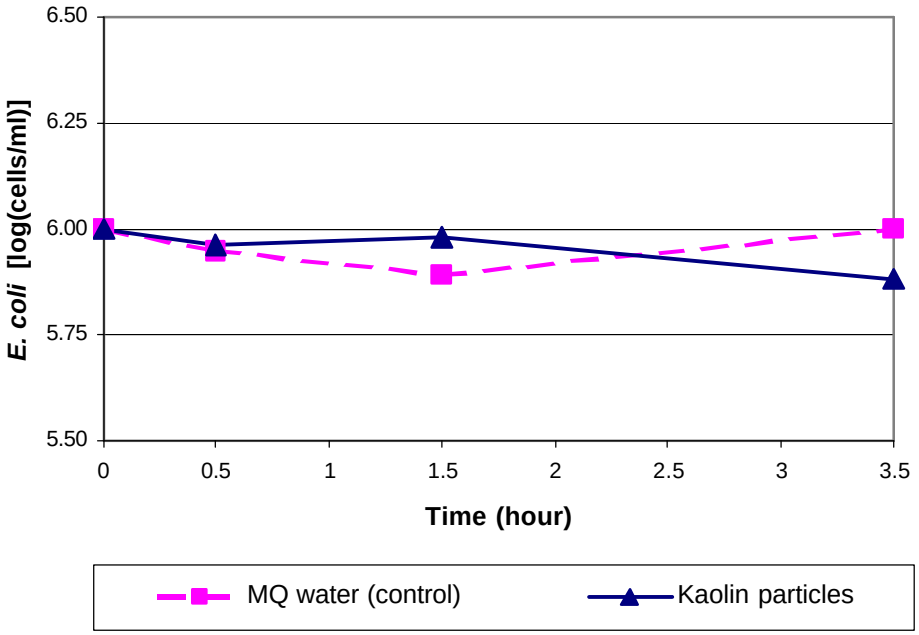


Figure 4.5 Attachment of *E. coli* with kaolin particles

During a short 3.5-hour contact time, there was no remarkable change in the log concentrations of *E. coli* in either sample (*E. coli* only and *E. coli* with kaolin particles). Two possible reasons are that (1) the size of kaolin particles ranges from 0.1 to 4 μm, which is suspected to be too small for attachment compared to the size of *E. coli* (about 1 μm); (2) the contact time is not long enough for adequate interaction between kaolin particles and *E. coli*.

4.4.2 Attachment of *E. coli* with River Water Particles

A secondary attachment experiment was performed between river water samples and *E. coli* (Section 3.2.5, B) with a contact time up to 24 hours. The general water quality parameters of river water samples are listed in Table 4.6.

The potential attachment is also depicted as the change in the log concentration of *E. coli* in the samples, as shown in Table 4.7 and Figure 4.6. A control sample containing *E. coli* in the 0.45-μm river water filtrate was analyzed in parallel to account for the growth of *E. coli* during the 24-hour contact time.

Table 4.6 General quality parameters of river water (Attachment)

Water Quality Parameters		
Parameter	**Value**	**Remark**
pH	7.82	
Temperature	4^{o}C	
Turbidity	15.4 NTU	
TSS	14 mg/L	
DOC	6.06 mg/L	0.45-μm filtration

Table 4.7 Attachment of *E. coli* with river water particles

***E. coli* Concentration [log (cells/mL)]**				
Settling time (hour)	**0**	**6**	**12**	**24**
E. coli with river water filtrate (control)	5.83	5.95	6.90	7.03
E. coli with river water particles	5.83	5.66	6.70	7.00

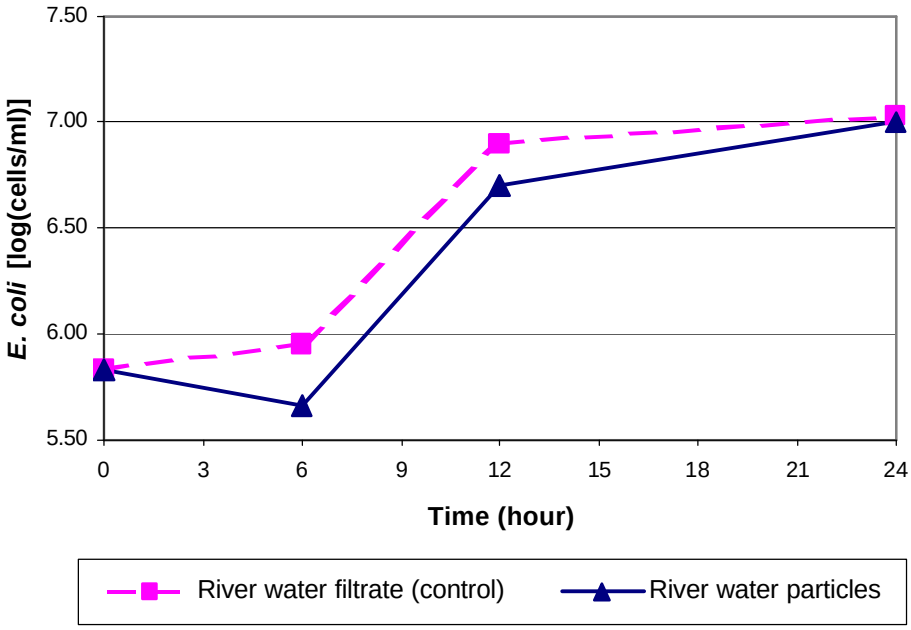


Figure 4.6 Attachment of *E. coli* with river water particles

E. coli in both samples showed typical growth curves. No significant drop in the *E. coli* concentrations was observed, even after a 24-hour contact time between *E. coli* and river water particles. This could be explained in two ways: (1) the association between bacteria and particles occurred within a small portion of total *E. coli*, therefore the dispersed *E. coli* were dominant in the samples; (2) the mechanisms of association, such as attachment or occlusion, are negligible under laboratory conditions.

Since both bacteria and particles are possibly negatively charged in a natural aqueous environment, they may act like colloidal particulate matter without any electrostatic

attraction. Thus, it is difficult to attach *E. coli* to particles successfully under laboratory conditions unless a coagulant is introduced for destabilization.

4.5 Summary

To determine suitable target indicator bacteria for spiking into the samples, the first set of preliminary experiments (Section 4.2) has been performed to incubate and maintain the environmental coliform bacteria in the simulated natural nutrients. The results showed that, after the 72-hour incubation in the laboratory, the maximum observed concentration of natural total coliforms was 5.26 log, which was still below the requirement. Eventually, laboratory grown *E. coli* was used instead to continue and complete the subsequent experiments in this study.

Since there is no commonly accepted method to quantify particle-associated microorganisms (Section 2.4.1), the other two sets of preliminary experiments (Section 4.3 and 4.4) were conducted to separate and simulate the potential particle-associated bacteria under laboratory conditions through settling and attachment. The results showed that it was unfeasible to simulate the potential mechanism of association between particles (kaolin or from river water) and bacteria through the processes of “settling” or “attachment” in the laboratory. Whether the potential association occurred was still unknown.

Although effective methods for quantifying the particle-associated bacteria were not determined in this thesis, the potential influence of particles on UV inactivation could still be investigated in terms of UV dose-response of the target bacteria from an engineering point of view.

Chapter 5

UV Inactivation and Chlorination

This chapter includes the main experimental results of this study. The first three sections present the dose-response of *E. coli* following low-pressure (LP) UV irradiation, medium-pressure (MP) UV irradiation, and chlorination with respect to different particle sources. The fourth section integrates the experimental results with the comprehensive particle analysis. Further discussion is highlighted in the final section.

Once again, the terms “UV dose” and “delivered UV dose”, both of them are used interchangeably to indicate the UV dose delivered to the target microorganisms in the bench-scale collimated beam apparatus in this study. Note that the particulates, as well as the soluble UVA substance, were already taken into account in UV dose determination in terms of the correction factors (Section 3.2.8, Bolton and Linden, 2003).

5.1 Dose-response by Low-pressure UV Irradiation

The influence of particles on LP UV inactivation was investigated with respect to three particle sources including secondary effluent from the wastewater treatment plant, surface water from the Grand River, and process water from the drinking water treatment plant.

The dose-response of *E. coli* in MQ water (Section 3.2.6) was determined as a control. The dose-response both before and after 0.45-μm filtration was compared for each of the particulate sources (secondary effluent and river water). River water samples were also coagulated using alum in the laboratory and coagulated process water was collected on site in the plant. The dose-response of floc-associated *E. coli* after coagulation was also determined and compared.

The initial concentration of spiked *E. coli* in all samples is approximately 10^6 cells/mL before irradiation. The dose-response of *E. coli* in each experiment is shown as the log inactivation (Equation 2.1, Section 2.2.5) in this thesis.

5.1.1 *E. coli* in MQ Water

UV dose-response of *E. coli* in MQ water is shown in Table 5.1 and Figure 5.1. Standard deviations (SD) are depicted as bars on the data points. As expected, the higher the delivered dose, the higher the observed log inactivation. The dose-response curve shows a nearly linear correlation at lower doses of 5, 10, and 15 mJ/cm^2 and then levels out to a "shoulder" region at higher doses of 25 and 40 mJ/cm^2. The inactivation rates were 1.5 log at 5 mJ/cm^2 and 4.4 log at 10 mJ/cm^2, which were consistent with those of Zimmer (2002).

Table 5.1 Dose-response of *E. coli* in MQ water (LP)

Dose-response of *E. coli* in MQ water (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	1.58	0.41	5
10	4.56	0.10	5
15	5.20	0.10	3
25	5.50	0.29	5
40	5.73	0.43	5

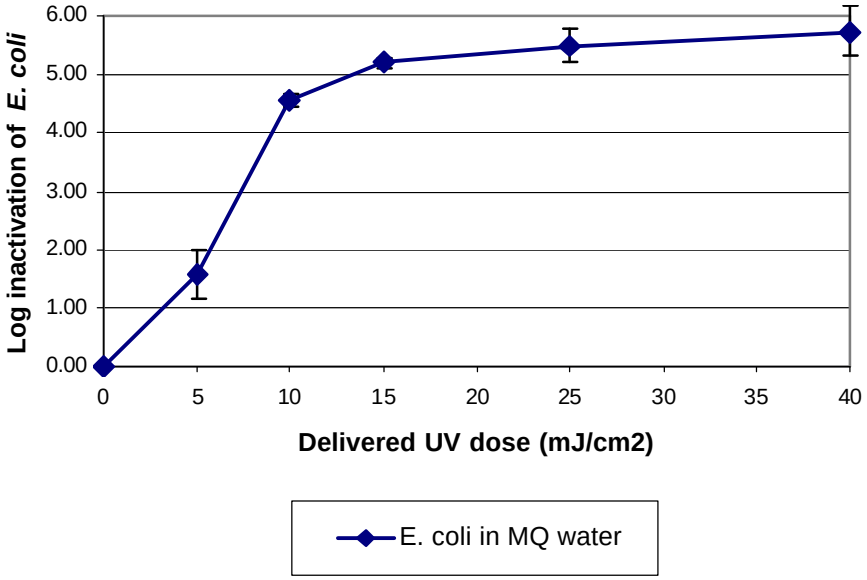


Figure 5.1 Dose-response of *E. coli* in MQ water (LP)

Although more than 5 log inactivation was achieved at 15 mJ/cm^2, there was still some survival of spiked *E. coli* after the regulated standard of 40 mJ/cm^2. Considering the concentration of spiked *E. coli* was quite high (~10^6 cells/mL), it is possible that some bacteria clumped together so that the inner portion was able to escape irradiation even though the outer portion was fully exposed to irradiation.

It is as difficult to quantify the clumped bacteria as to quantify the particle-associated bacteria. Because the concentration of spiked *E. coli* was stable in these experiments, no further experiment was carried out to distinguish the impact of bacteria clumping itself.

5.1.2 Particulate Source of Secondary Effluent

In past studies, the impact of particulate matter on UV inactivation of coliform bacteria was first explored using secondary effluent of wastewater treatment facilities (Section 2.4.2). Initially, secondary effluent from the Waterloo Wastewater Treatment Plant was evaluated. The general water quality parameters of secondary effluent samples are listed in Table 5.2.

Table 5.2 General quality parameters of secondary effluent (UV)

Water Quality Parameters (4 batches of samples)		
Parameter	**Value**	**Remark**
pH	7.73~7.91	
Temperature	4^{o}C	
Turbidity	3.13~4.8 NTU	
TSS	4.2~5.3 mg/L	
DOC	12.96~14.21mg/L	0.45-μm filtration
UVA before filtration	0.3023~0.3334 cm^{-1}	UVT=46.4~49.9 %
UVA of 0.45-μm filtrate	0.2092~0.2247 cm^{-1}	UVT=59.6~61.8 %

Secondary effluent samples were shown to have a relatively high background *E. coli* concentration of 10^4~10^5 cells/mL. Since the concentration of spiked *E. coli* was approximately 10^6 cells/mL, the background *E. coli* could be possibly significant after a 1- or 2-log inactivation of spiked *E. coli*. Therefore, UV dose-response of the background *E. coli* in unfiltered secondary effluent samples (Table 5.3 and Figure 5.2) was determined to compare with that of spiked *E. coli* (laboratory grown).

Table 5.3 Dose-response of background *E. coli* in unfiltered secondary effluent (LP)

Dose-response of background *E. coli* in unfiltered secondary effluent (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	2.74	N/A	1
10	3.99	N/A	1
15	4.80	N/A	1
25	5.04	N/A	1
40	5.17	N/A	1

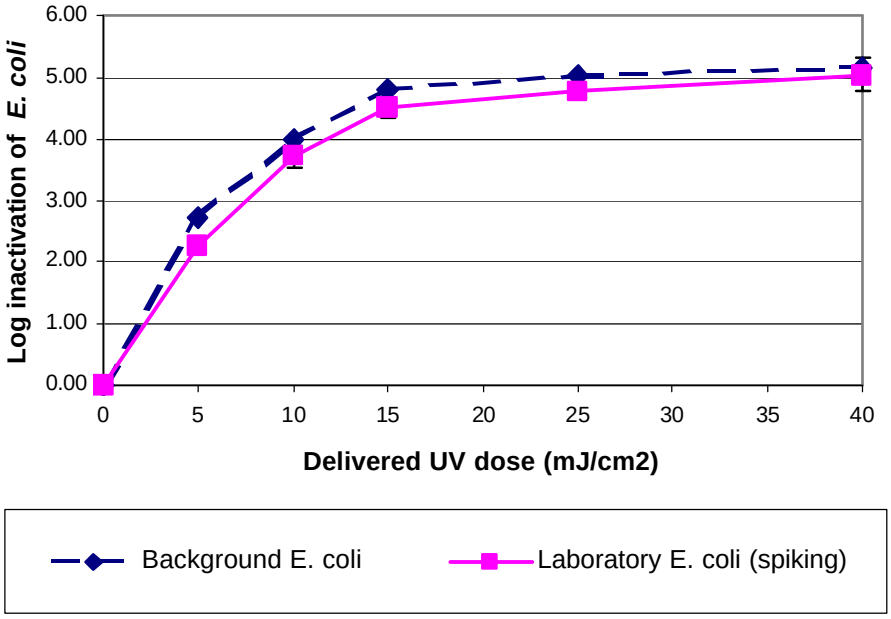


Figure 5.2 Dose-response of background *E. coli* in unfiltered secondary effluent (LP)

In Figure 5.2, the UV dose-response of spiked *E. coli* in unfiltered secondary effluent samples (Table 5.5) is also included for comparison. The UV dose-response of the background *E. coli* and spiked *E. coli* had no statistical difference at a 95% confidence level (α=5%). Because UV inactivation rates of both *E. coli* are similar, the influence of the background *E. coli* is not a concern regarding the initial concentration of spiked *E. coli* (~10^6 cells/mL). Hence, in the subsequent experiments, the term "*E. coli*" or "spiked *E. coli*" both refer to the laboratory grown *E. coli* for spiking unless it is labeled as "background *E. coli*".

UV dose-response of spiked *E. coli* was determined in both filtered secondary effluent (0.45-μm membrane filtration) and unfiltered secondary effluent samples (Table 5.4, 5.5 and Figure 5.3). The dose-response of *E. coli* in MQ water is also included for comparison.

Table 5.4 Dose-response of *E. coli* in filtered secondary effluent (LP)

Dose-response of *E. coli* in filtered secondary effluent (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	1.95	0.42	3
10	4.84	0.25	3
15	5.81	0.46	3
25	5.97	0.12	3
40	6.00	0.28	3

Table 5.5 Dose-response of *E. coli* in unfiltered secondary effluent (LP)

Dose-response of *E. coli* in unfiltered secondary effluent (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	2.26	0.12	3
10	3.72	0.18	3
15	4.50	0.16	3
25	4.76	0.08	3
40	5.04	0.26	3

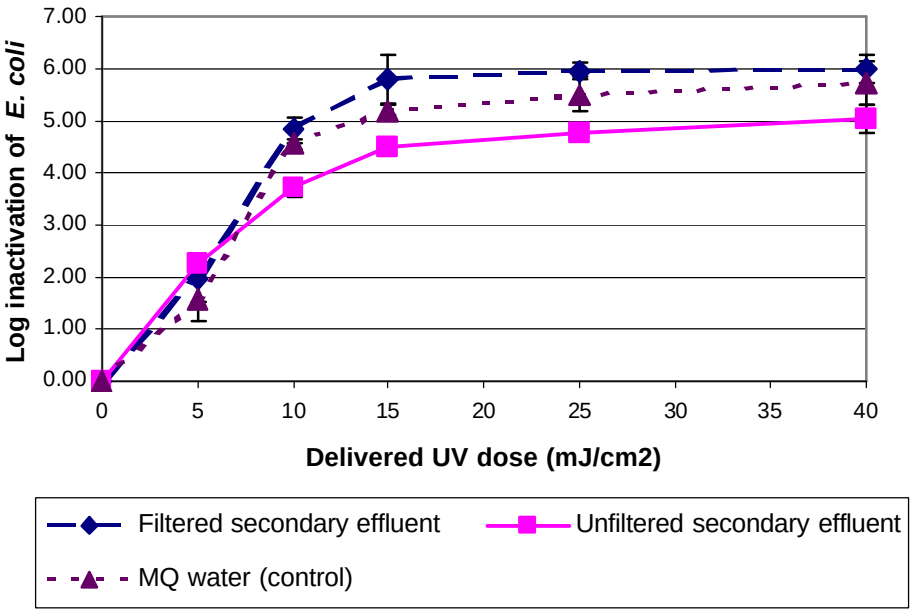


Figure 5.3 Dose-response of *E. coli* in secondary effluent (LP)

All three curves exhibit an approximate 2 log inactivation at 5 mJ/cm^2 without any statistical difference (least significant difference, LSD=1.05). It is inferred that 99% of spiked *E. coli* in secondary effluent samples were capable of being inactivated in the dispersed state. However, significant reduction of UV inactivation was shown at higher doses (= 10 mJ/cm^2), when a corresponding higher log inactivation was expected. At the higher doses, significant differences of the log inactivation between filtered and unfiltered secondary effluent (LSD=0.56~0.87) are 1.12, 1.31, 1.21 for 10, 15, 25 mJ/cm^2, respectively. The maximum inactivation at 40 mJ/cm^2 is 6 log for filtered secondary effluent and 5.04 log for unfiltered secondary effluent (LSD=1.01). It is concluded that less than 1% of *E. coli* were rendered more resistant to UV inactivation due to the presence of fecal particles in the secondary effluent. These observations agree with those of previous research (Qualls *et al.*, 1985; Loge *et al.*, 1996, 1999).

5.1.3 Particulate Source of River Water

Further studies were considered necessary to investigate particulate sources other than sewage for drinking water applications. For this reason, surface water of the Grand River was sampled from the intake location of the Mannheim Water Treatment Plant. The general water quality parameters of river water are listed in Table 5.6. Note that UVA of river water samples were measured both before and after 0.45-μm filtration. The particulate contribution to total UVA was calculated to be 12%~36%, increasing when the turbidity or TSS increased.

The first step was conducted to compare the dose-response of spiked *E. coli* in either filtered or unfiltered river water. The second step was to undertake coagulation using alum to simulate the floc-associated *E. coli*, and its dose-response was determined and compared.

Table 5.6 General quality parameters of river water (UV)

Water Quality Parameters (5 batches of samples)		
Parameter	**Value**	**Remark**
pH	7.63~8.35	
Temperature	4^{o}C	
Turbidity	12.0~32.4 NTU	
TSS	11.8~34.4 mg/L	
DOC	6.06~6.86 mg/L	0.45-μm filtration
UVA before filtration	0.2355~0.3421 cm^{-1}	UVT=45.5~58.1 %
UVA of 0.45-μm filtrate	0.2054~0.2199 cm^{-1}	UVT=60.3~62.3 %

The concentrations of background *E. coli* were negligible in river water samples, generally less than 1 cell/mL (22~89 CFU/100mL); therefore, the background effect was not a concern after the laboratory grown *E. coli* were spiked into the samples.

Prior to the introduction of alum, a standard jar test was carried out to determine the optimal dosage for floc formation. Based on the results (Table 5.7), a dosage of 30 mg/L was chosen in the range of turbidity from 12.0 to 32.4 NTU.

Table 5.7 Jar test for river water coagulation

Turbidity of the supernatant after coagulation and settling					
Jar #	**1**	**2**	**3**	**4**	**5**
Alum dosage (mg/L)	20	25	30	35	40
Turbidity (NTU)	4.01	2.56	1.89	1.69	1.45

Finally, the dose-response of *E. coli* was determined for filtered, unfiltered, and coagulated river water (Table 5.8, 5.9, 5.10; Figure 5.4), and the dose-response of *E. coli* in MQ water is also included for comparison.

Table 5.8 Dose-response of *E. coli* in filtered river water (LP)

Dose-response of *E. coli* in filtered river water (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	1.54	0.31	3
10	4.65	0.35	3
15	5.40	0.41	3
25	5.66	0.38	3
40	6.15	0.61	3

Table 5.9 Dose-response of *E. coli* in unfiltered river water (LP)

Dose-response of *E. coli* in unfiltered river water (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	1.97	0.41	3
10	4.37	0.35	3
15	5.18	0.38	3
25	5.29	0.33	3
40	5.61	0.28	3

Table 5.10 Dose-response of *E. coli* in coagulated river water (LP)

Dose-response of *E. coli* in coagulated river water (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	2.06	0.41	3
10	3.34	0.33	3
15	3.63	0.14	3
25	4.60	0.29	3
40	4.82	0.27	3

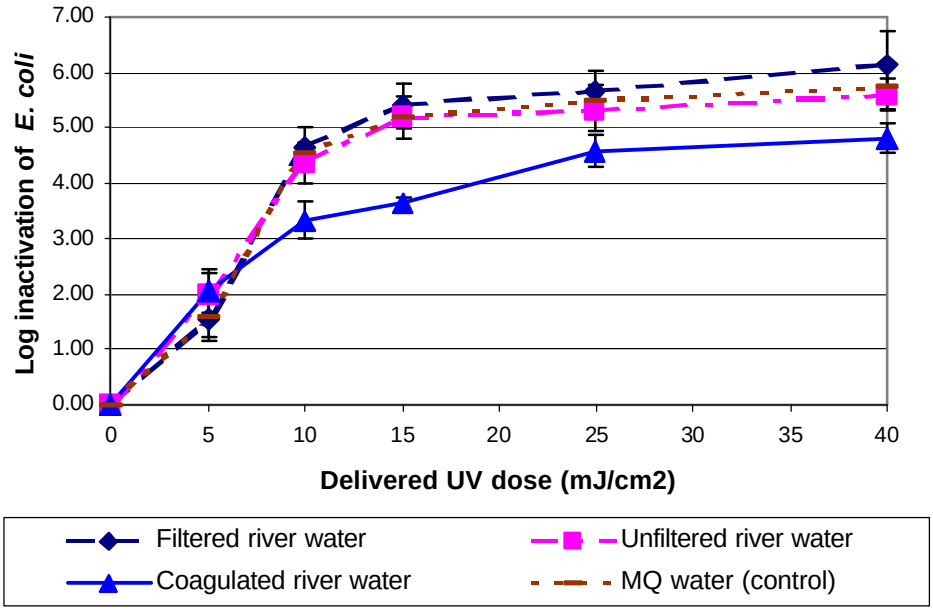


Figure 5.4 Dose-response of *E. coli* in river water (LP)

Unlike secondary effluent samples, the presence of surface water particles appears to have little or no impact on the dose-response of spiked *E. coli*. The three dose-response curves for MQ water, filtered river water, and unfiltered river water are not statistically different (LSD=0.80~1.15). However, a shielding effect was observed in coagulated river water samples similar to in secondary effluent samples (Section 5.1.2).

All four curves showed approximately 2 log inactivation (i.e. 99% removal) at 5 mJ/cm^2 without any statistical difference (LSD=1.06). It is inferred that 99% of *E. coli* in river water samples were capable of being inactivated in the dispersed state, even in coagulated river

water samples. On the other hand, lower log inactivations were observed when high log inactivations were expected at increased doses for coagulated river water samples. At higher doses, the significant differences (LSD=0.80~1.15) of the log inactivation between coagulated river water and the other samples are 1.03~1.31, 1.55~1.77, 0.69~1.06, and 0.79~1.33 for 10, 15, 25, 40 mJ/cm^2, respectively. The maximum inactivations are approximately 5 log for coagulated river water and 6 log for the remaining samples at 40 mJ/cm^2.

It is therefore concluded that the presence of surface water particles has essentially no influence on UV dose-response of spiked *E. coli* if they are appropriately accounted for in UV dose determination. Less than 1% of *E. coli* were rendered more resistant to UV inactivation when the floc-associated *E. coli* were introduced after the process of coagulation. However, this could be important in achieving desired log removals.

5.1.4 Particulate Source of Process Water

Based on the significant finding of floc-associated *E. coli* introduced after coagulation and flocculation (Section 5.1.3) in the laboratory, additional studies were considered crucial to investigate floc particles in a full-scale drinking water treatment facility in terms of particulate impact on UV inactivation. Hence, coagulated water was sampled from the flocculation tank of the Mannheim Water Treatment Plant. The general water quality parameters of the coagualted water are listed in Table 5.11.

Table 5.11 General quality parameters of coagulated process water (UV)

Water Quality Parameters (2 batches of samples)		
Parameter	**Value**	**Remark**
pH	7.75~8.11	
Temperature		Not measured
Turbidity	5.26~16.8 NTU	
TSS	6.19~15.3 mg/L	
DOC		Not measured
UVA	0.3050~0.3769 cm^{-1}	UVT=42.0~49.5 %

The dose-response of spiked *E. coli* in coagulated process water was first determined (Table 5.12) and then compared with that for the filtered and unfiltered river water (Section 5.1.3) in Figure 5.5. Once again, the dose-response of *E. coli* in MQ water is also included for comparison.

Table 5.12 Dose-response of *E. coli* in coagulated process water (LP)

Dose-response of *E. coli* in coagulated process water (LP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
5	1.73	0.13	3
10	3.21	0.45	3
15	4.07	0.26	3
25	4.71	0.34	3
40	4.96	0.29	3

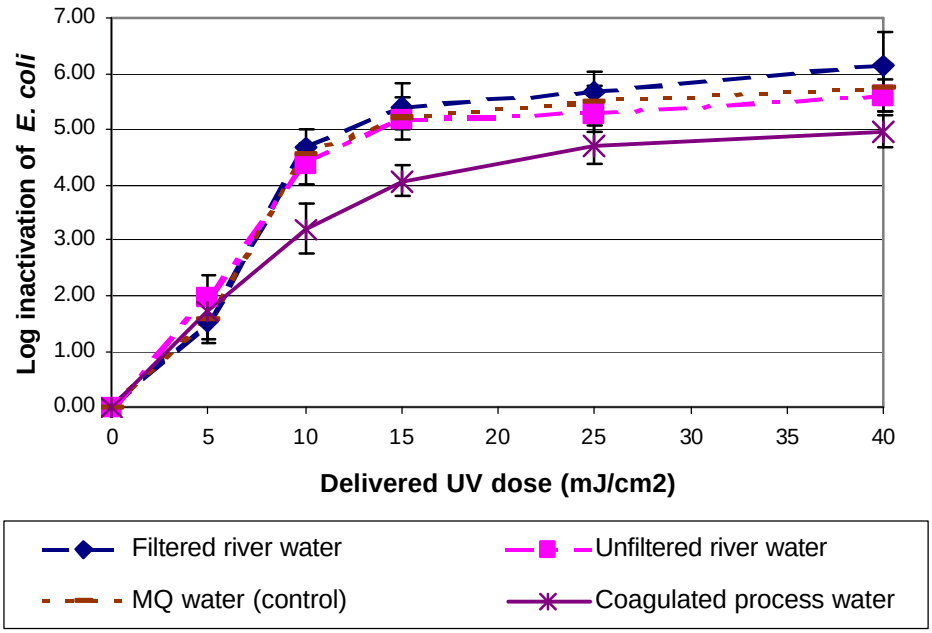


Figure 5.5 Dose-response of *E. coli* in coagulated process water (LP)

At 5 mJ/cm^2, all four curves showed approximately 2 log inactivation without any statistical difference (LSD=0.92). Conversely, lower log inactivations were observed when high log inactivations were expected at increased doses for coagulated process water samples. At higher doses, the significant differences (LSD=0.86~1.16) of the log inactivation between coagulated process water and the other samples are 1.16~1.44, 1.11~1.33, 0.58~0.95, and 0.65~1.19 for 10, 15, 25, 40 mJ/cm^2, respectively. The maximum inactivations are approximately 5 log for coagulated process water and 6 log for the remaining samples at 40 mJ/cm^2.

Therefore, a shielding effect was observed in coagulated process water samples similar to in coagulated river water samples (Section 5.1.3). In fact, a statistical analysis showed the UV dose-response had no difference (95% confidence level) at all doses between coagulated river water and coagulated process water (Figure 5.6).

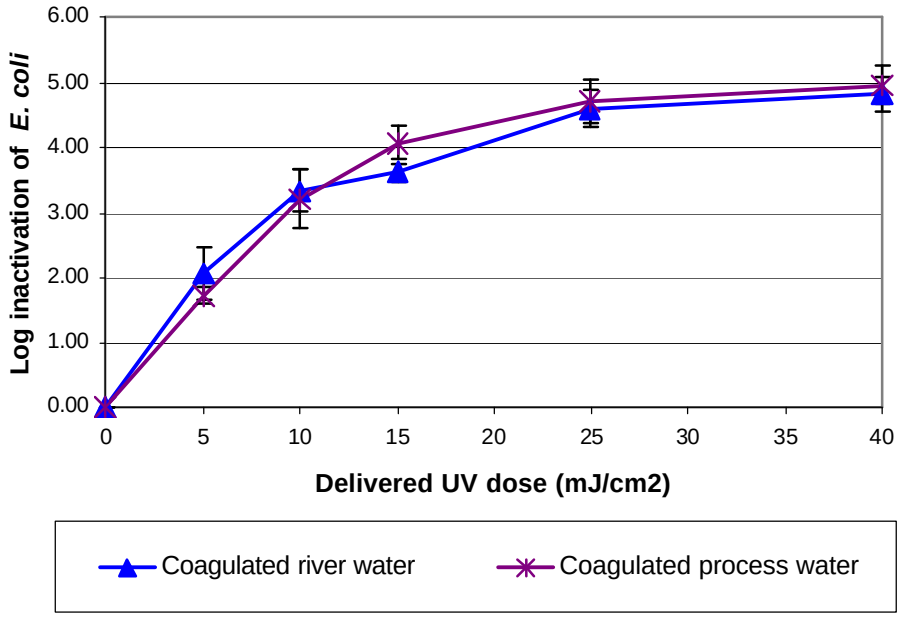


Figure 5.6 Dose-response of *E. coli* in coagulated river and process water (LP)

In conclusion, the floc-associated *E. coli* were rendered more resistant to UV inactivation as long as floc particles were introduced after the processes of coagulation and flocculation in either the laboratory or the water treatment facilities. This implies that poor removal of the

floc particles present prior to disinfection may impair the efficacy of UV inactivation downstream in water treatment facilities.

5.2 Dose-response by Medium-pressure UV Irradiation

It is well known that MP UV light has much higher intensity with polychromatic wavelengths (Section 2.2.1) than LP UV light. From the LP experiments, shielding effects were observed in both unfiltered secondary effluent and coagulated river water samples. Another issue of concern is whether MP UV light could effectively inactivate spiked *E. coli* in unfiltered secondary effluent and coagulated river water.

Analogous to the LP experiments, the dose-response of spiked *E. coli* in MQ water was first determined as a control. The dose-response of spiked *E. coli* in unfiltered secondary effluent or coagulated river water was compared to that for MQ water. Note that there was no filtration process involved in MP experiments because based on the LP experiments, it was reasonable to assume the dose-response of spiked *E. coli* in the filtrates would be the same as in MQ water.

Once again, the initial concentration of spiked *E. coli* was approximately 10^6 cells/mL prior to irradiation and the dose-response in every experiment was also shown as the log inactivation.

5.2.1 *E. coli* in MQ Water

The dose-response of *E. coli* in MQ water is shown in Table 5.13 and Figure 5.7; the dose-response subjected to LP UV irradiation is also included for comparison. Standard deviations are shown as bars for each data point.

Table 5.13 Dose-response of *E. coli* in MQ water (MP)

Dose-response of *E. coli* in MQ water (MP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
1	2.03	0.15	3
3	4.19	0.24	3
5	4.24	0.12	3
10	4.46	0.23	2
15	4.58	0.18	2
25	4.56	0.14	2
40	5.39	0.12	3

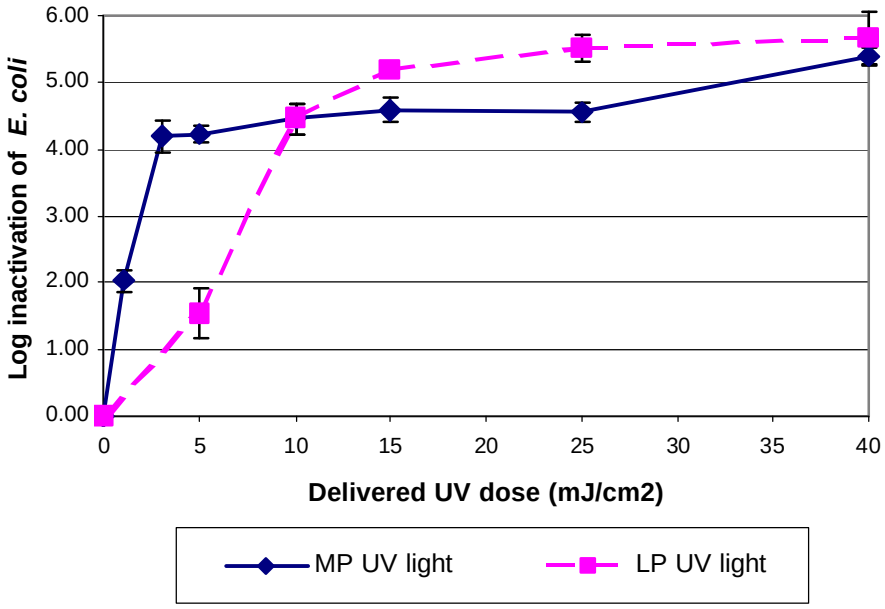


Figure 5.7 Dose-response of *E. coli* in MQ water (MP)

MP UV light was comparatively effective for inactivating *E. coli*; more than 4 log inactivation was achieved at 3 mJ/cm^2 compared with nearly 10 mJ/cm^2 of LP UV light required to obtain the same efficacy. However, the log inactivation did not increase with an increased UV dose. In fact, the log inactivations of 4.24, 4.46, 4.58, and 4.56 log observed at 5, 10, 15, and 25 mJ/cm^2 had no statistical difference (95% confidence level or α=5%). Comparatively, Zimmer (2002) observed log inactivations of 5.2, 5.3 and 5.7 log at 5, 8, and 10 mJ/cm^2 respectively following MP UV irradiation. Because the initial concentration of *E. coli* was 10^6~10^8 cells/mL in Zimmer (2002) and nearly 10^6 cells/mL in this study, these experimental results are still comparable if the log inactivations are relative to the initial concentrations.

Although over 5 log inactivation was achieved at 40 mJ/cm^2, there was still some survival of spiked *E. coli* remaining. As with the LP experiments, it is likely that some bacteria clumped together and thus were shielded from irradiation. Another interesting observation is that the log inactivations following LP UV irradiation are greater than those of MP UV irradiation for doses of 15 and 25 mJ/cm^2.

5.2.2 Particulate Source of Secondary Effluent and River Water

As with the LP experiments, the particulate impacts on the dose-response of *E. coli* following MP UV irradiation were investigated for unfiltered secondary effluent and coagulated river water samples. The sampling procedures were the same as the LP experiments and the general water quality parameters of secondary effluent and river water samples were the same as listed in Section 5.1.

The results for the two samples are shown in Tables 5.14, 5.15 and Figure 5.8. The dose-response of *E. coli* in MQ water is also included for comparison (Figure 5.7).

Table 5.14 Dose-response of *E. coli* in unfiltered secondary effluent (MP)

Dose-response of *E. coli* in unfiltered secondary effluent (MP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
1	2.02	0.05	5
3	3.01	0.17	5
5	4.29	0.21	4
10	4.25	N/A	1
15	4.54	N/A	1
25	4.65	0.11	2
40	5.19	0.09	2

Table 5.15 Dose-response of *E. coli* in coagulated river water (MP)

Dose-response of *E. coli* in coagulated river water (MP)			
Delivered UV Dose (mJ/cm^2)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
1	2.12	0.21	3
3	3.07	0.14	3
5	4.20	0.18	3
10	4.48	0.08	2
15	4.33	0.15	2
25	4.69	0.17	2
40	5.57	0.31	3

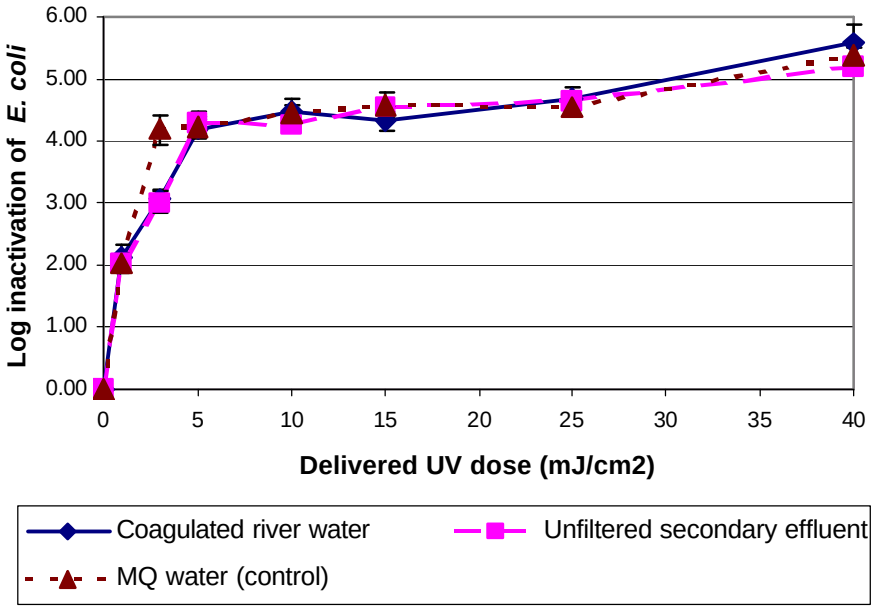


Figure 5.8 *E. coli* in coagulated river water and unfiltered secondary effluent (MP)

With all samples, an approximately 4.5 log inactivation observed at 5, 10, 15, and 25 mJ/cm^2 had no statistical difference (α=5%). Therefore, MP UV light was able to effectively inactivate the *E. coli* in both unfiltered secondary effluent and coagulated river water samples and all three curves showed a similar trend for doses greater than 5 mJ/cm^2. One possible explanation is that *E. coli* is highly sensitive to MP UV irradiation.

Since the *E. coli* were too easily to be inactivated at doses greater than 5 mJ/cm^2, two lower doses of 1 and 3 mJ/cm^2 were applied to explore the potential impact of particles on MP UV irradiation. Figure 5.9 provides a closer examination of the dose-response at 1, 3, and 5

mJ/cm^2 for all three samples. All of them demonstrated approximately 2 log inactivation at 1 mJ/cm^2 with no statistical difference (LSD=0.47). It is therefore inferred that 99% of *E. coli* were capable of being inactivated at the dispersed state in all samples. Only at 3 mJ/cm^2, the significant differences (LSD=0.58) of the log inactivation are 1.18 and 1.12 for unfiltered secondary effluent and coagulated river water respectively.

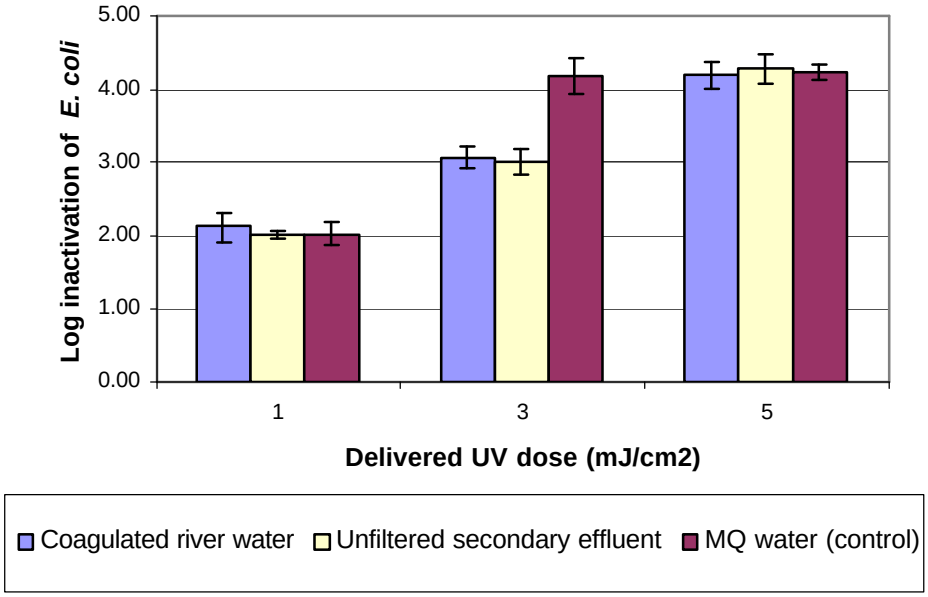


Figure 5.9 Dose-response of *E. coli* in coagulated river water and unfiltered secondary effluent at 1, 3, and 5 mJ/cm^2 (MP)

It is concluded that because of its higher intensity and output MP UV light can successfully inactivate the *E. coli* associated with the particles in secondary effluent or coagulated river

water samples. Nevertheless, these biosolid- or floc-associated *E. coli* (less than 1%) were more resistant to a lower dose of 3 mJ/cm^2 for MP UV irradiation.

5.3 Dose-response by Chlorine

In studies conducted by other researchers (Section 2.4.2), particle-associated microorganisms have been found to be more resistant to chlorine disinfection than dispersed cells. Another goal of this research was to compare the effectiveness of chlorination versus UV irradiation on inactivation of *E. coli* under the influence of particles.

As with the experimental design for LP and MP UV inactivation, the dose-response of *E. coli* in MQ water by chlorine was determined first as a control; the chlorine dose-response of *E. coli* in coagulated river water samples was then investigated. Note that the dose of chlorine is interpreted in terms of CT values in mg·minutes/L instead of UV dose in mJ/cm^2.

5.3.1 *E. coli* in MQ Water

The chlorine dose-response of *E. coli* in MQ water is shown in Table 5.16 and Figure 5.10. *E. coli* was quite sensitive to free chlorine, a C×T value of 0.03 mg·minutes/L for 3.62 log inactivation at pH 7.0 at 4^{o}C. Comparatively, Kaneko (1998) reported that the C×T value for 4 log inactivation of *E. coli* O157:H7 and K12 was 0.067-0.071 mg·minutes/L at pH 7.2 at 30^{o}C without any presence of turbidity.

Table 5.16 Dose-response of *E. coli* in MQ water (Chlorine)

Dose-response of *E. coli* in MQ water (Chlorine)			
Free chlorine CT (mg·minutes/L)	**Log inactivation**	**SD**	**Number of Trials**
0	0.00	N/A	N/A
0.01	1.00	0.12	3
0.02	2.59	0.38	3
0.03	3.62	0.17	2
0.05	4.80	0.33	3
0.1	5.73	0.14	3

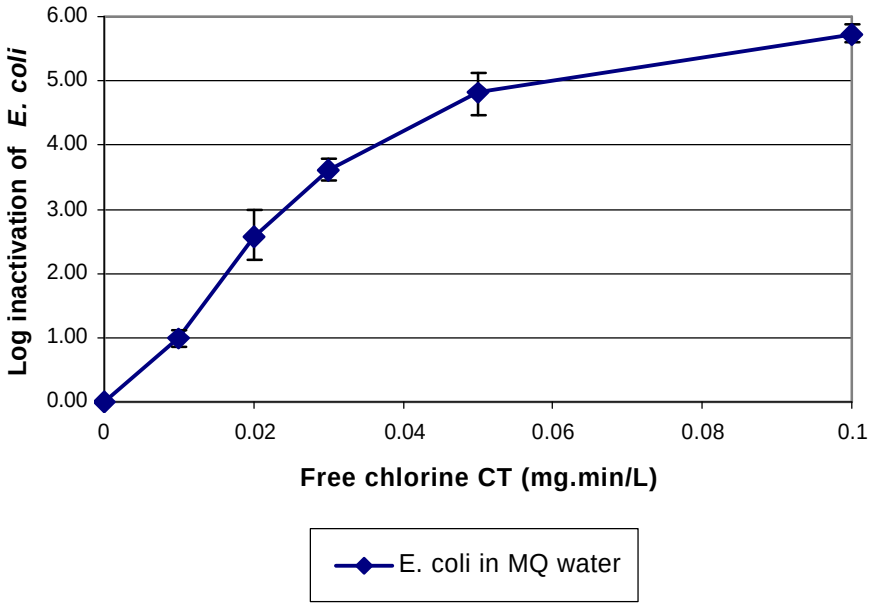


Figure 5.10 Dose-response of *E. coli* in MQ water (Chlorine)

5.3.2 *E. coli* in Coagulated River Water

Since chlorine demand of MQ water is zero, the residual concentration is equal the initial concentration (Equation 3.4). On the other hand, for coagulated river water samples, the initial concentrations (namely the required dosages of the chlorine stock solution, Section 3.2.9) were estimated by the sum of pre-measured chlorine demand and the designated residual concentration (Equation 3.4).

General water quality parameters of river water samples are listed in Table 5.17 and the chlorine demand of coagulated river water samples was 1.53 mg/L. Based on the results of *E. coli* in MQ water, the required initial concentrations were then estimated to be 1.54, 1.55, 1.56, 1.58, and 1.63 mg/L for chlorination of coagulated river water samples. However, after several preliminary experiments, it was found that no spiked *E. coli* (initial concentration of 1.66×10^5 cells/mL) were detected after adding the 0.5 mg/L chlorine (initial concentration) for 1 minute at pH 7.0 at 4^oC.

Table 5.17 General quality parameters of river water (Chlorine)

Water Quality Parameters		
Parameter	**Measure**	**Remarks**
pH	7.63	
Temperature	4^{o}C	
Turbidity	26.5 NTU	
TSS	21.6 mg/L	
DOC	6.73 mg/L	0.45-μm filtration
Chlorine demand	1.53 mg/L	

Practically, it was difficult to accurately measure the 0.01-mg/L difference of chlorine among the required initial concentrations of 1.54, 1.55, 1.56, and 1.58 mg/L in the laboratory. Hence, this strain of *E. coli* was too sensitive to chlorine for this study. Chlorine likely reacted with chlorine-consuming substances and inactivated spiked *E. coli* simultaneously in a short time. In conclusion, it is impractical to estimate the appropriate initial concentration of chlorine based on chlorine demand and residual concentration in the laboratory. Therefore, experiments involving chlorination was discontinued in this study.

5.4 Particle Analysis

In order to further evaluate the emerging shielding effects in the previous experimental results, potential change of particle size distribution and the related images were investigated for four samples as below (Section 3.2.6).

A. Unfiltered secondary effluent samples before and after spiking *E. coli*

B. Unfiltered river water samples before and after spiking *E. coli*

C. Coagulated river water samples before and after coagulation

D. Coagulated process water samples before and after flocculation

PSD is interpreted as the percentage of total counts. Note that there are two boundaries in the particle counts, 2 μm and 10 μm. The particle analyzer used (DPA 4100, Brightwell Technologies Inc., Ottawa) has a lower cutoff of particle size at 2 μm; the higher level was set at 10 μm because of the so-called critical particle size (CPS, Section 2.4.3). The given particle size represents an average for the increment range (e.g. 3.5 μm represents the range from 3 to 4 μm).

5.4.1 Secondary Effluent Particles

Typical PSD of secondary effluent samples before and after spiking *E. coli* are shown in Table 5.18 and Figure 5.11. PSD in both samples had no remarkable change. 62% and 67%

of the particles were less than 3 μm before and after spiking, respectively. There were also 14% and 16% of particles greater than 10 μm present in the samples.

Table 5.18 PSD of secondary effluent samples before and after spiking *E. coli* (DPA)

PSD of secondary effluent particles as % of total # (DPA)			
Particle diameter (μm)	**Before**	**After**	**Number of Trials**
≤ 2	39%	48%	3
2.5	23%	19%	3
3.5	5%	4%	3
4.5	5%	4%	3
5.5	3%	2%	3
6.5	3%	2%	3
7.5	3%	2%	3
8.5	3%	1%	3
9.5	2%	1%	3
≥ 10	14%	16%	3
Mean	8.8 μm	10.6 μm	3
SD	12.8 μm	13.4 μm	3
Total #/mL	110,369	100,457	3

Two typical images of the suspension before and after spiking *E. coli* are shown in Figure 5.12. The suspended particles are visually amorphous floc (i.e. particles associated with one another). Based on the two images, no noticeable change was observed as a result of spiking.

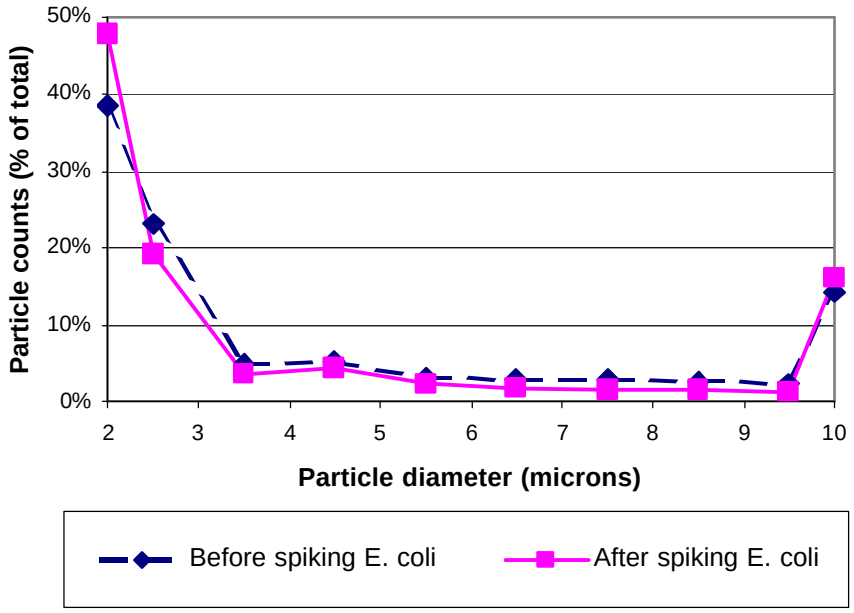


Figure 5.11 PSD of secondary effluent samples before and after spiking *E. coli* (DPA)

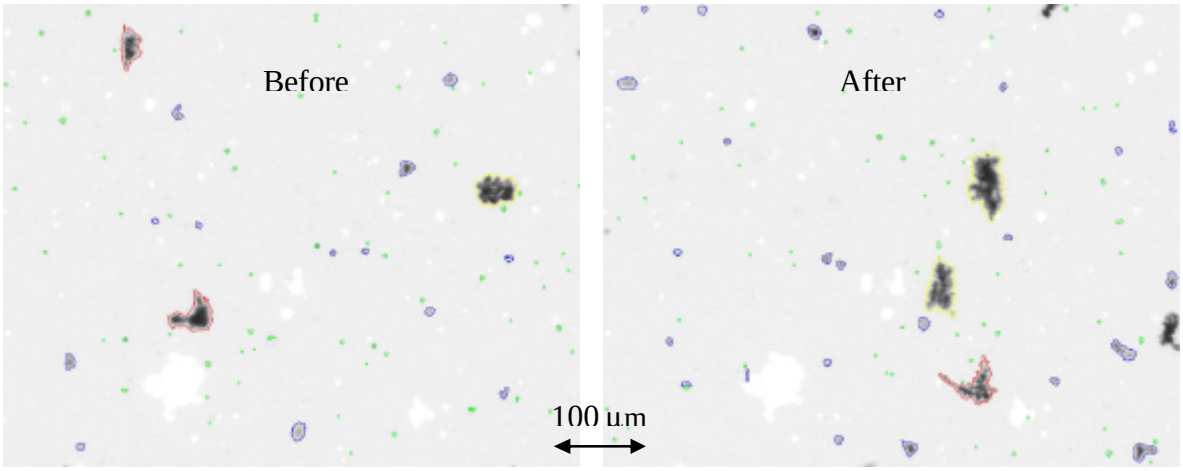


Figure 5.12 Images of secondary effluent particles before and after spiking *E. coli* (DPA)

Both the LP and MP experiments showed that the presence of biosolids significantly affected UV inactivation of spiked *E. coli* in secondary effluent samples. However, the association between spiked *E. coli* and secondary effluent particles could not be explained by the PSD of these particles.

5.4.2 River Water Particles

Typical PSD of river water samples before and after spiking *E. coli* are shown in Table 5.19 and Figure 5.13. PSD of the samples are similar in every tier of particle size.

Table 5.19 PSD of river water samples before and after spiking *E. coli* (DPA)

PSD of river water particles as % of total # (DPA)			
Particle diameter (μm)	**Before**	**After**	**Number of Trials**
≤ 2	41%	41%	3
2.5	33%	34%	3
3.5	6%	6%	3
4.5	6%	6%	3
5.5	3%	3%	3
6.5	2%	2%	3
7.5	2%	2%	3
8.5	1%	1%	3
9.5	1%	1%	3
≥ 10	5%	4%	3
Mean	4.7 μm	4.3 μm	3
SD	4.2 μm	3.8 μm	3
Total #/mL	100,000	88,735	3

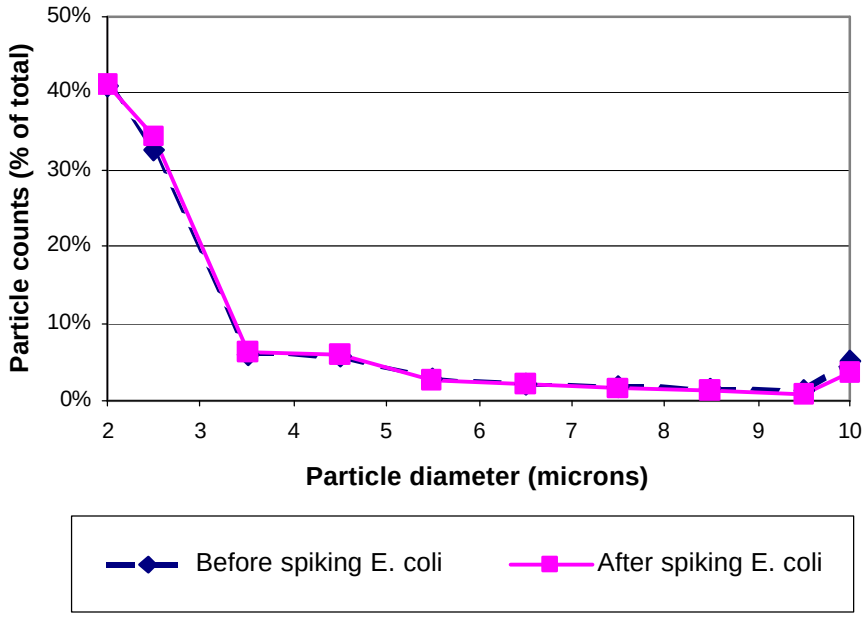


Figure 5.13 PSD of river water samples before and after spiking *E. coli* (DPA)

Particles less than 3 μm were dominant before and after spiking (74% and 75%). Particles greater than 10 μm contributed a smaller percentage than those in secondary effluent samples (5% and 4%).

Two typical images of the suspension before and after spiking *E. coli* are shown in Figure 5.14. The suspended particles seem more dense and compact than those in the images of secondary effluent samples.

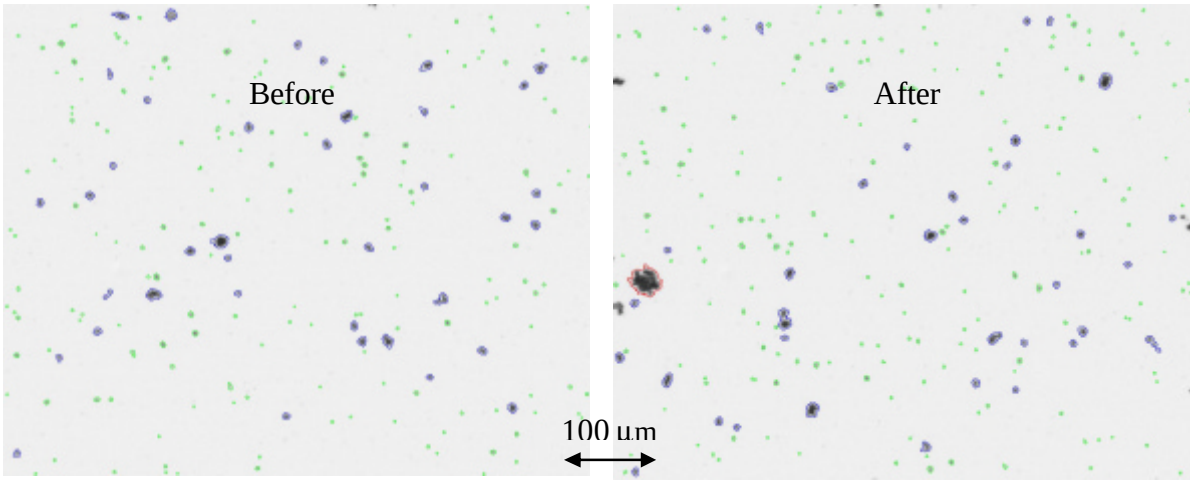


Figure 5.14 Images of river water particles before and after spiking *E. coli* (DPA)

5.4.3 Coagulated River Water Particles

Typical PSD of river water samples before and after coagulation are shown in Table 5.20 and Figure 5.15. As expected, the process of coagulation tremendously changed the PSD of river water samples.

After coagulation, the percentage of particles less than 3 µm decreased from 56% to 31%, while the percentage of particles greater than 10 µm increased from 12% to 55%. A more than 3-fold increase was observed for the mean particle size. Conversely, the total number of particles decreased more than 14-fold, which implies the enmeshment of particles with one another.

The previous results showed that the floc-associated *E. coli* resulting from coagulation were more resistant to UV inactivation than spiked *E. coli* in filtered and unfiltered river water samples. The remarkable changes of PSD support the idea that the floc-associated *E. coli* were formed through the aggregation of particles and *E. coli* in the process of coagulation. This enmeshment shielded the floc-associated *E. coli* from UV inactivation.

Table 5.20 PSD of river water samples before and after coagulation (DPA)

PSD of river water as % of total # (DPA)			
Particle diameter (㎜)	**Before**	**After**	**Number of Trials**
≤ 2	26%	21%	3
2.5	30%	10%	3
3.5	8%	2%	3
4.5	8%	3%	3
5.5	4%	2%	3
6.5	4%	1%	3
7.5	3%	2%	3
8.5	3%	2%	3
9.5	2%	2%	3
≥ 10	12%	55%	3
Mean	6.3 μm	21.8 μm	3
SD	6.2 μm	16.4 μm	3
Total #/mL	231,202	16,129	3

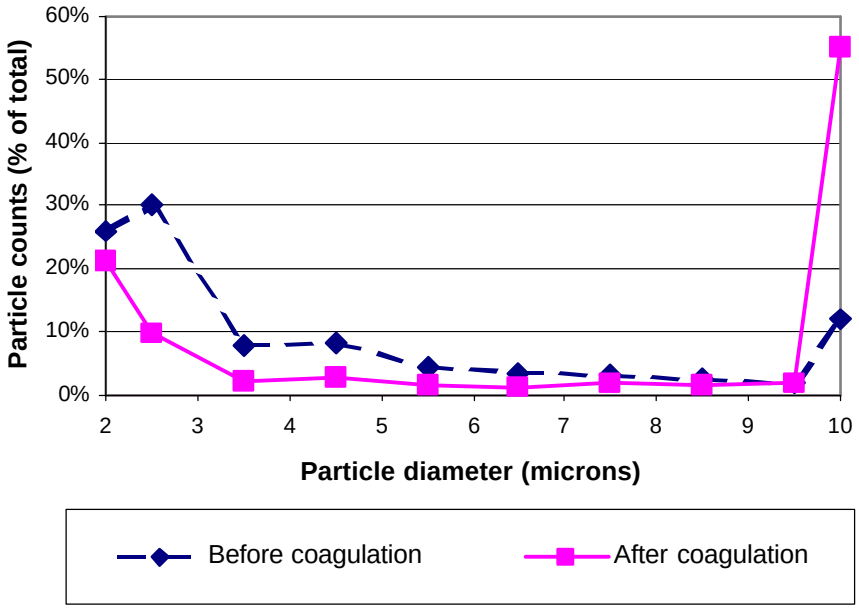


Figure 5.15 PSD of river water samples before and after coagulation (DPA)

Two images typical for the suspension are shown in Figure 5.16. As expected, a remarkable change is seen in terms of the size, density, and composition of the suspended particles. Many visible floc and particulate aggregates were present after the process of coagulation, as expected.

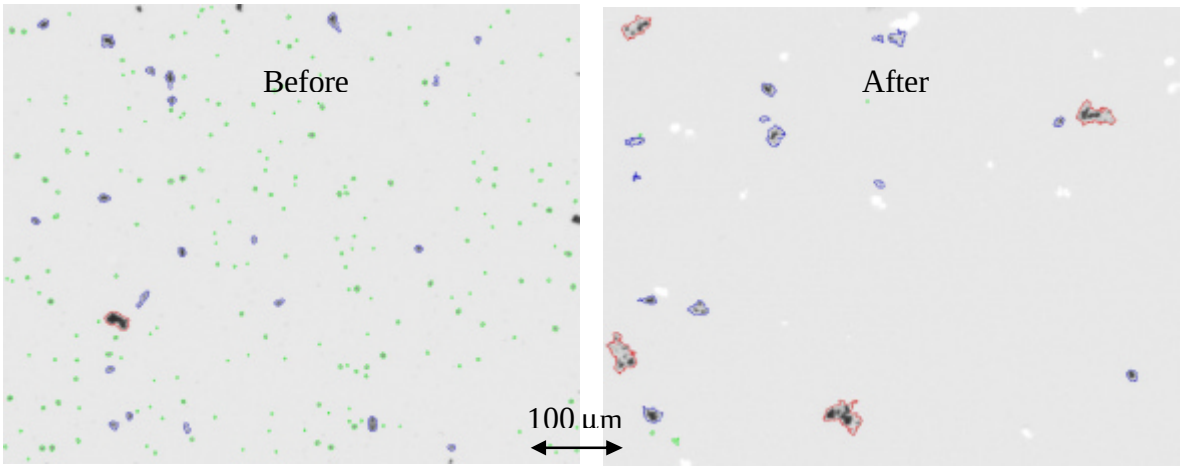


Figure 5.16 Images of river water particles before and after coagulation (DPA)

5.4.4 Coagulated Process Water Particles

Typical PSD of process water samples before and after flocculation are shown in Table 5.21 and Figure 5.17. The process of flocculation greatly changed the PSD of process water samples as would be expected.

After flocculation, the percentage of particles less than 3 µm decreased from 56% to 39%, while the percentage of particles greater than 10 µm increased from 21% to 50%. A more than 3-fold increase was observed for the mean particle size. Conversely, the total number of particles decreased more than one third, which implies the enmeshment of particles with one another.

Table 5.21 PSD of process water samples before and after flocculation (DPA)

PSD of process water as % of total # (DPA)			
Particle diameter (㎜)	**Before**	**After**	**Number of Trials**
≤ 2	36%	29%	3
2.5	20%	10%	3
3.5	6%	3%	3
4.5	6%	3%	3
5.5	3%	1%	3
6.5	3%	1%	3
7.5	3%	1%	3
8.5	2%	1%	3
9.5	2%	1%	3
≥ 10	21%	50%	3
Mean	10.5 μm	33.7 μm	3
SD	22.5 μm	39.6 μm	3
Total #/mL	18,943	11,699	3

The changes of PSD support the idea that the floc-associated *E. coli* were formed through coagulation and flocculation in coagulated process water similar to in coagulated river water. These enmeshments shielded the floc-associated *E. coli* from UV inactivation in same way.

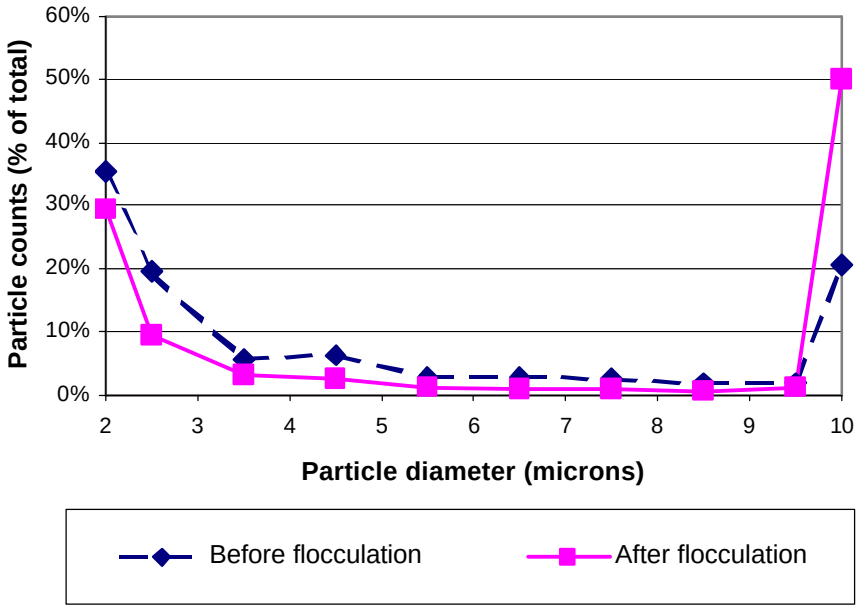


Figure 5.17 PSD of process water samples before and after flocculation (DPA)

Two images typical for the suspension before and after the simulated process of flocculation (Section 3.2.6) are shown in Figure 5.18. Many visible floc and particulate aggregates were present even before the flocculation. This could be explained by the preliminary formation of floc particles during the sample transportation after the coagulant (PAC) was dosed. After deliberate flocculation, much larger floc particles were present in the suspension, which implied the aggregation of small floc particles through the process.

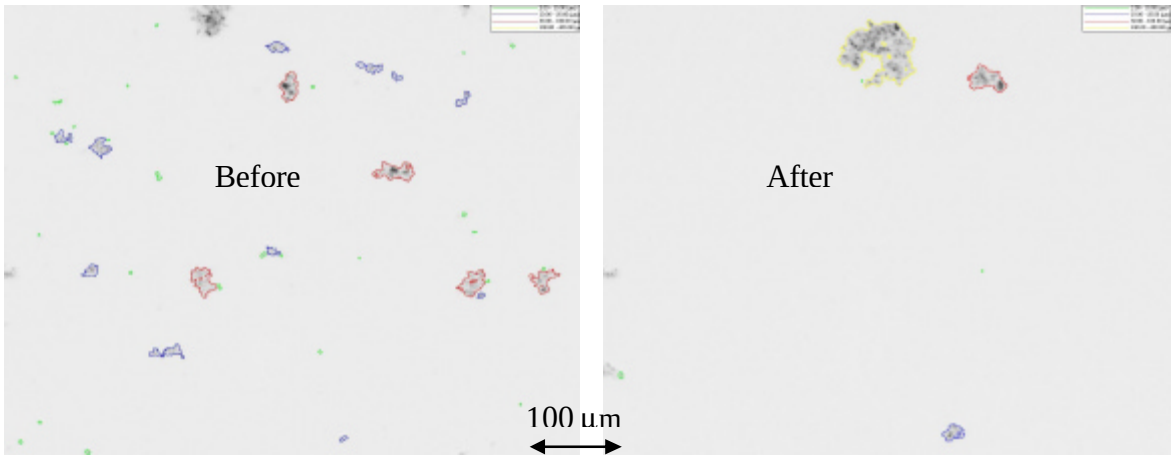


Figure 5.18 Images of process water particles before and after flocculation (DPA)

5.5 Discussion

UV dose determination was essential to this research, but unlike the widely used chemical disinfectant chlorine, there is no measurable residual of UV irradiation. The concept of CT values is not applicable for UV inactivation, which is part of the reason why it is more complex to regulate UV inactivation.

During the time of this research, the principles presented by Bolton and Linden (2003) were believed to be the most accurate calculation methods available to date; they were included in the most up-to-date UVDGM (2003) as an accepted method for UV dose determination in bench-scale UV experiments. The corresponding calculation spreadsheets were attained from the IUVA website.

It is suspected that particles may shield pathogens from disinfection; however, such phenomena were not consistently observed during this study. For instance, a turbidity of 3.13~4.8 NTU had a significant impact on UV inactivation of spiked *E. coli* in the secondary effluent, while a turbidity of 12.0~32.4 NTU had no influence on UV inactivation of spiked *E. coli* in the river water. The significant finding of floc particles through coagulation agreed with that of Templeton *et al.* (2003), who found that MS2 bacteriophage enmeshed in coagulated clay particles (kaolin) have escaped UV inactivation within a drinking water application. Thus, the shielding effects might depend on the type of particles and the nature of the association between microorganisms and particles (Hoff, 1978). Further research regarding the interaction between particles and microorganisms is necessary for a better understanding. Even until recently, there was no universally accepted method to quantify or differentiate the microorganisms associated with particles. Commonly used procedures, such as extraction, are quite site-specific and depend on a lot of variables (Camper *et al.*, 1985a; McDaniel and Capone, 1985).

There was no extraction trial of microorganisms from particles in these experiments because of a lack of reliable apparatus in the laboratory. Instead, other experiments, such as “settling” and “attachment”, were tried to simulate the association between particles and bacteria. Unfortunately, it proved difficult to facilitate interactions between particles and bacteria under the laboratory conditions. Possibly because of the electrostatic repulsion between negatively charged particles and microorganisms (Montgomery, 1985), over the short contact time, particles and microorganisms remained stable unless highly positively charged

surfaces, such as coagulant, were introduced or unless sweep coagulation conditions were created.

Particle removal is one of the main treatment goals for surface water as particles are ubiquitous in surface waters. The issue of particle association with target pathogens is a topic of concern, challenging chlorination and UV inactivation. The impact is of particular importance to UV inactivation because it relies on the physical propagation of UV light in water, as opposed to the diffusion mechanism of chlorination. The shielding effect was previously found to greatly hinder the UV inactivation of coliform bacteria in secondary effluent of wastewater treatment facilities. Analogous research on other particulate sources is necessary for UV application in drinking water treatment systems. The experimental results for coagulated water samples indicated that floc particles had a deleterious impact on UV inactivation as long as they were introduced after the processes of coagulation and flocculation in either the laboratory or the water treatment plant.

As a launch point for this research, wastewater particles (mostly fecal particles) from secondary effluent were investigated to confirm the previous results. Thereafter, for the context of drinking water, two particulate sources of interest were selected, including surface water and coagulated surface water. Together, the sources represent the range of water quality typically seen in drinking water treatment. Note that the turbidity of 12.0~32.4 NTU for the surface water samples in this study was greater than 5 NTU, which was set as the

filtration avoidance criteria in the SWTR (40 CFR 141.71, USEPA, 1979), while the turbidity was generally below 5 NTU in secondary effluents.

Since the quantity of microorganisms of interest is generally limited in natural drinking water sources, it is difficult to directly investigate the "natural" particle-associated microorganisms. However, there are cases in which water samples may contain considerable numbers of particles and bacteria, such as samples of runoff or stormwater after a heavy rain event. Spiking or seeding target microorganisms seems necessary for the purposes of the laboratory experiments. One concern is how much the spiking procedure represents the "natural" association between particles and microorganisms. Nevertheless, the seeding experiments provided a critical reference for further studies.

Both LP UV and MP UV irradiation can effectively inactivate the dispersed *E. coli*; however, when *E. coli* clump together or associate with particles, a regulated standard dose of 40 mJ/cm^2 cannot completely inactivate the *E. coli*. Although the *E. coli* resistant to UV inactivation are only a minor portion of the total count (less than 1%), they will be a decisive factor regarding if high levels of log inactivation are required to meet stringent regulations.

In secondary effluents, the suspended biosolids (3.13~4.8 NTU) could shield both existing and spiked *E. coli* from UV inactivation. There are two possible factors that might contribute to this phenomenon. One is that a coagulant was still present as a result of the prior treatment

process (such as ferrous chloride, $FeCl_2$, which is used for phosphorus removal, Section 3.2.6). The other is that there is a significant level of surfactant present in the domestic wastewater. The surfactant concentration is typically below 0.1 mg/L in environmental water, but ranges from 1 to 20 mg/L in raw domestic wastewater (APHA *et al.*, 1998).

Strictly speaking, this strain of *E. coli* (*E. coli* ATCC 11229) is not ideal for the MP and chlorination experiments due to its sensitivity to both MP UV light and chlorine. A more resistant target microorganism is better for future study.

Comprehensive particle analysis is a direct and crucial approach for evaluating the particulate impact on the disinfection processes. Nevertheless, further steps for quantifying the particle-associated microorganisms were beyond the scope of this thesis and thus were not carried out.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

1. In river water samples, the floc-associated *E. coli* resulting from coagulation had a significant influence on the inactivation results. An average significant difference of the log inactivation was 1.25 for each LP dose of 10, 15, and 25 mJ/cm^2 when comparing filtered or unfiltered river water to coagulated river water samples. A significant difference of 1.12 log inactivation was seen at a MP dose of 3 mJ/cm^2 when comparing coagulated river water samples to MQ water.

2. In process water samples, the floc-associated *E. coli* proved to have a significant influence on UV inactivation. An average significant difference of the log inactivation was 1.10 for each LP dose of 10, 15, and 25 mJ/cm^2 when comparing coagulated process water samples to filtered or unfiltered river water samples. There was no statistical difference between coagulated process water in the water treatment facility and coagulated river water in the laboratory in terms of floc particulate impact on UV inactivation.

3. In river water samples, the presence of surface water particles had no influence on UV inactivation of spiked *E. coli*. No significant difference was observed when comparing filtered river water (particle-free) to unfiltered river water (particle-containing, 12.0~32.4 NTU) samples at all LP doses.

4. In secondary effluent samples, the suspended biosolids were confirmed to have a significant impact on UV inactivation of both existing and spiked *E. coli*. An average significant difference of the log inactivation was 1.21 for each LP dose of 10, 15, and 25 mJ/cm^2 when comparing filtered secondary effluent (particle-free) to unfiltered secondary effluent (particle-containing, 3.13~4.8 NTU) samples. A significant difference of 1.18 log inactivation was seen at a MP dose of 3 mJ/cm^2 when comparing unfiltered secondary effluent samples to MQ water. LP UV dose-response of the existing *E. coli* and the spiked laboratory grown *E. coli* had no statistical difference.

5. As was expected, particle analysis showed a tremendous change of PSD after coagulation of river water samples. A remarkable difference between the floc (biologically or chemically induced) and the dispersed particles was visually evident.

6. In all samples, both LP UV and MP UV could effectively inactive the spiked laboratory grown *E. coli* (~ 10^6 cells/mL); the universal 2 log inactivation was achieved at LP dose of 5 mJ/cm^2 or MP dose of 1 mJ/cm^2. Generally, the greater the delivered UV doses, the higher the log inactivation. With one exception, greater than 5 log inactivation showed similar results at the regulated dose of 40 mJ/cm^2 for both LP UV and MP UV; however, there was always some survival of *E. coli* following exposure to UV irradiation. Complete kill did not occur at the tested doses.

7. In MQ water, greater than 4 log inactivation was observed at a LP UV dose of 10 mJ/cm^2 or MP UV dose of 3 mJ/cm^2, respectively. The log inactivations were not significantly different (α=5%) at MP doses of 5, 10, 15, and 25 mJ/cm^2.

8. A sustainable source of natural coliform bacteria was difficult to maintain under laboratory conditions. It was impractical to simulate potential mechanisms of association (e.g. “adsorption” or “absorption”) between particles and bacteria through the “settling” and “attachment” in the laboratory without induced coagulation and flocculation.

9. The *E. coli* strain used was too sensitive to carry on the experiments of chlorination under the laboratory conditions investigated. In coagulated river water samples, there was no detectable *E. coli* remaining after 0.5 mg/L chlorine (initial concentration) for 1 minute at pH 7.0 at 4^{o}C despite a chlorine demand level of 1.53 mg/L. Considering the survival of *E. coli* in the UV experiments, chlorine seems more effective for this organism than both LP and MP UV irradiation at the acceptable doses.

6.2 Recommendations

Although many researchers commonly used the procedures for extracting microorganisms from particles (Section 2.4.1), a quick way to find and enumerate particle-associated microorganisms does not currently exist. There is great research potential for focusing on the interactions between particles and microorganisms. Some fundamental questions, such as how they associate together and what is the viable status of microorganisms within particles, need to be answered before fully understanding the consequences of the interaction on disinfection.

In “unfiltered” drinking water sources in North America, the concentration of microorganisms of interest is usually limited because of extensive watershed protection and

water quality monitoring. Based on the results of the seeded experiments in this research, particle-associated issues do not seem to be an urgent concern for disinfection in those systems. However, precautions should be taken when there is potential high concentration of microorganisms occurring in the increased stormwater runoff and other related events.

The chemically induced floc through coagulation and flocculation has the capability of shielding pathogens from UV inactivation. The observations are reasonably transferable to the mechanism of coagulation and flocculation in nature; the formed floc likely enmeshes microorganisms. The results imply a potential risk of floc particles (with associated more resistant organisms) before disinfection. Several events could cause the deterioration of water quality (e.g. particle break-through during settling and filtration) before disinfection, such as transient upset of influent quality, unstable performance of upstream settling, and poor filtration, etc. Therefore, the formed floc particles should be carefully removed prior to disinfection.

Future research should address naturally isolated coliform bacteria or other candidate microorganisms, such as viruses. They are generally smaller than bacteria, which make them more likely to be embedded in floc under appropriate water system conditions.

Another possible application of this study is to incorporate the impact of particle association into the proposed validation procedure for full-scale UV reactors (UVDGM, 2003). Petri *et*

al. (2000) concluded that coagulant, which is either naturally present (e.g. reduced iron in ground water) or added purposely, would interfere with the results of UV reactor validation. Based on the setup of this study, it is recommended that some preliminary experiments be performed to determine how to deal with any unexpected effects caused by particles.

In agreement with other researchers (e.g. Dietrich *et al.*, 2003), further studies are necessary to explore the penetration mechanism of UV irradiation and chlorine diffusion targeting particle-associated microorganisms.

Appendix A

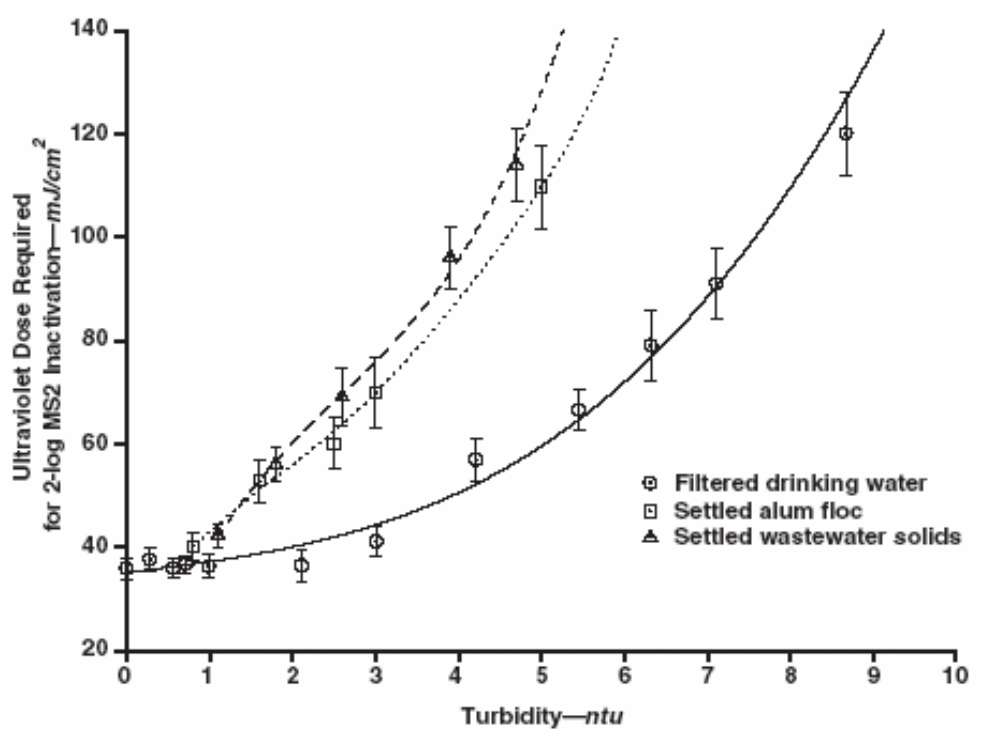


Figure A.1 Particulate impact of settled alum floc and settled wastewater solids on UV inactivation of MS2 bacteriophage (Malley, 2000)

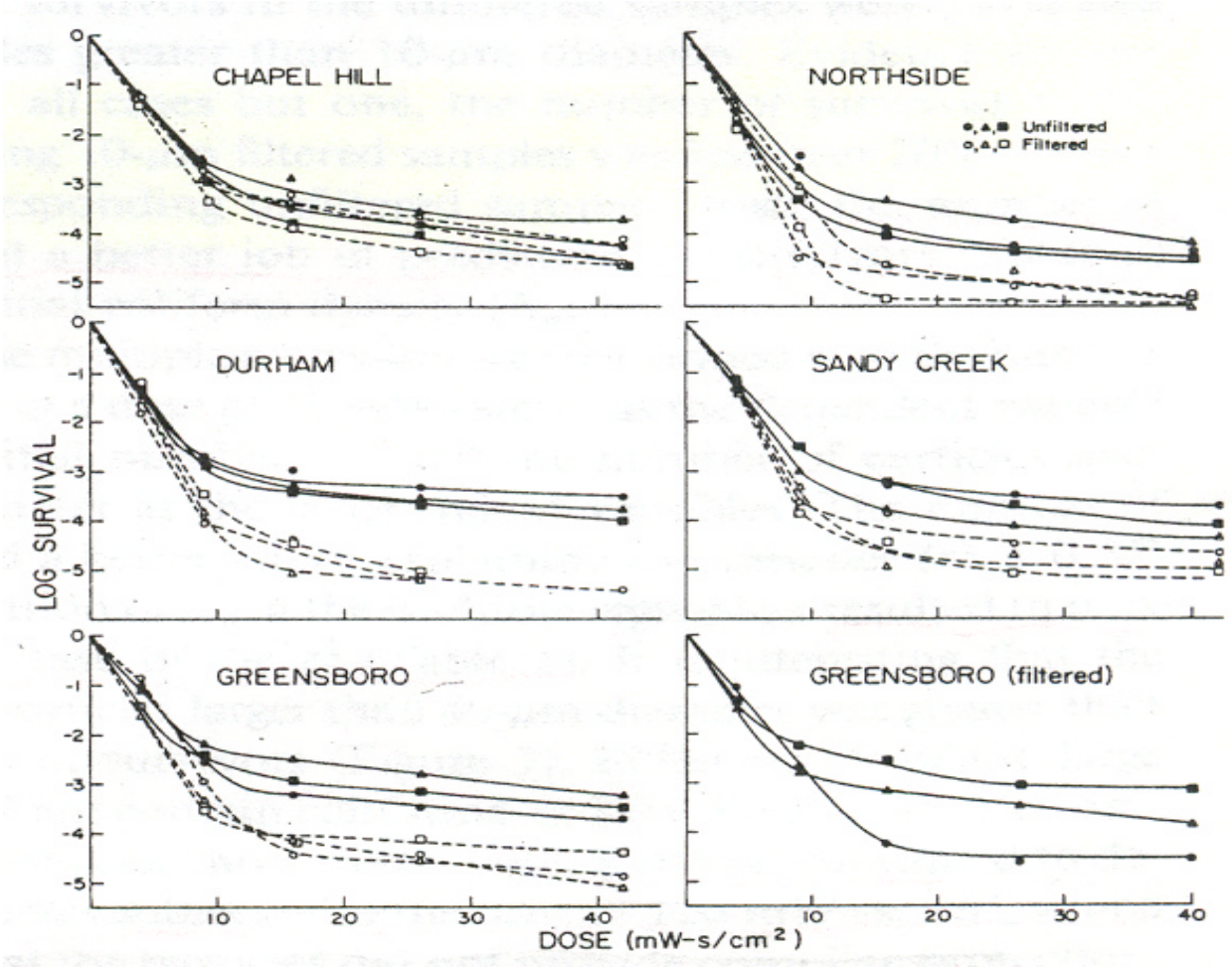


Figure A.2 Comparison of UV log survival between unfiltered (solid lines) and filtered samples (10 mm filtrate, broken lines) from secondary effluent in six wastewater treatment plants (Qualls *et al.*, 1985)

Appendix B

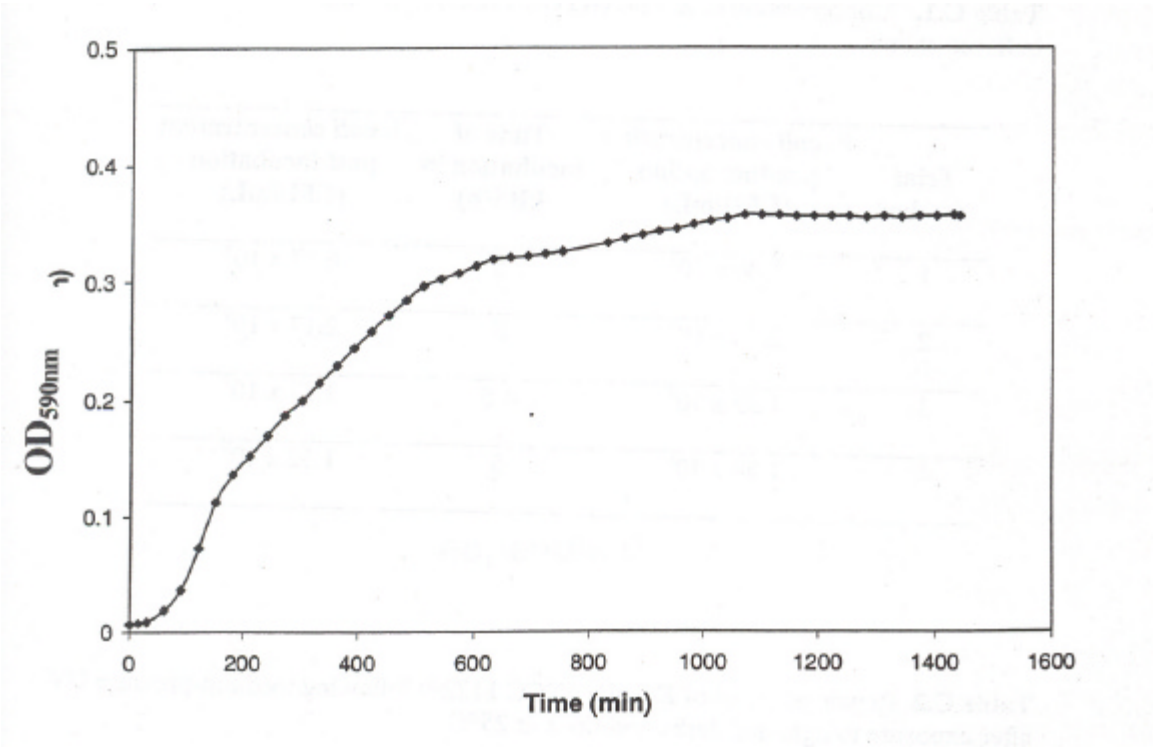


Figure B.1 Growth curve of *E. coli* in nutrient broth over a 24-hour incubation at 37°C (Zimmer, 2002)

Appendix C

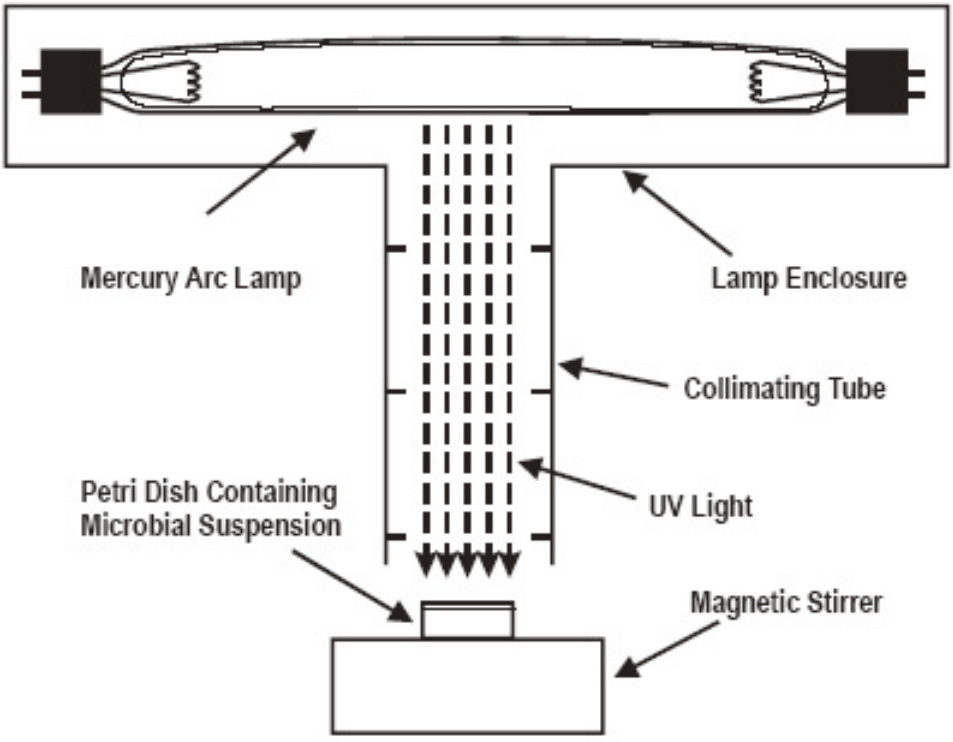


Figure C.1 Schematic of UV bench-scale collimated beam apparatus (courtesy of UVDGM, 2003)

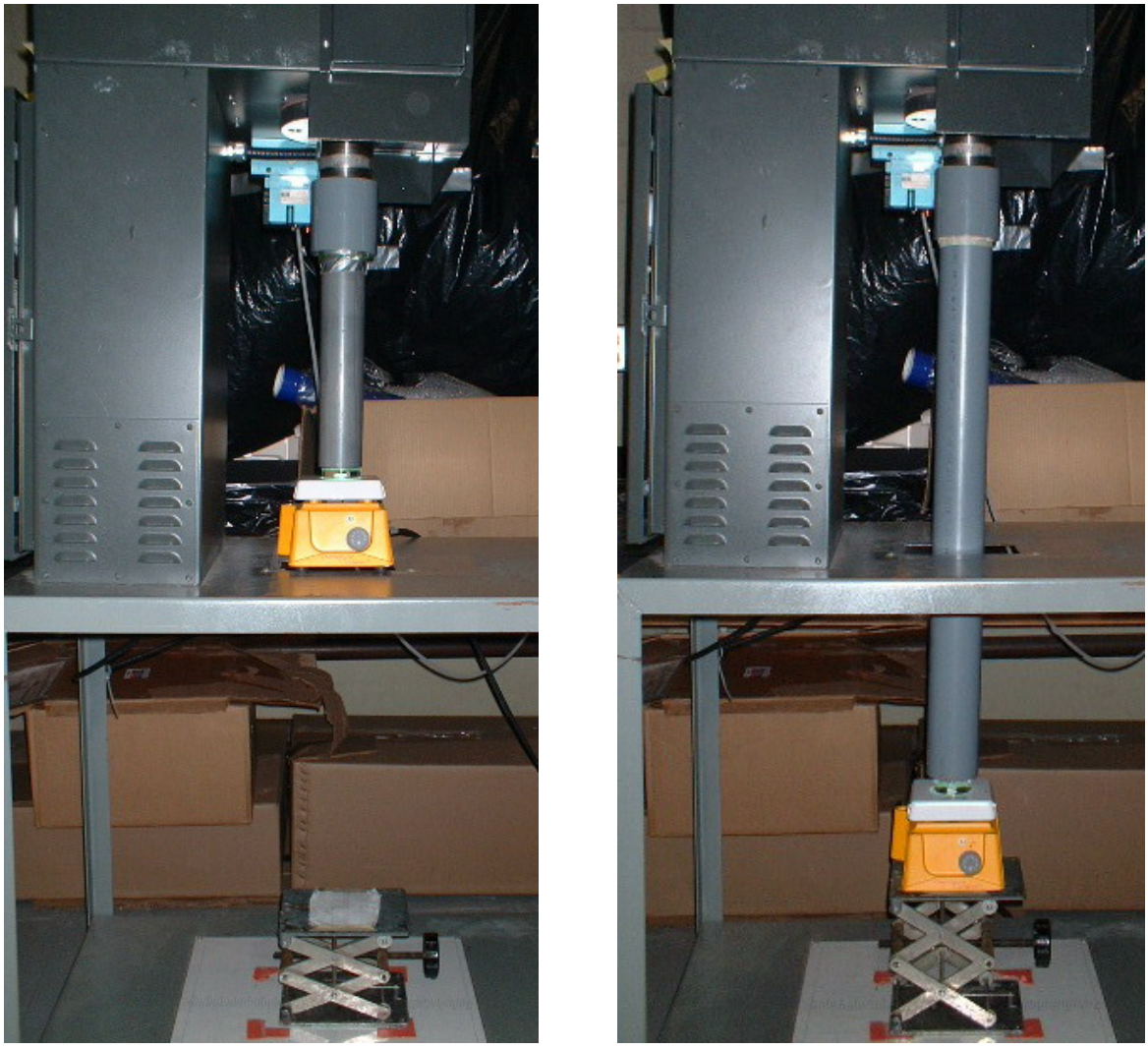


Figure C.2 LP or MP UV Bench-scale collimated beam apparatus (left: a shorter beam; right: a longer beam)

References

Anderson WB. Personal communication. August 2004.

APHA, AWWA, and WEF. Standard methods for the examination of water and wastewater. Washington 1998 (20th ed.)

Babich H, Stotzky G. Reduction in inactivation rates of bacteriophages by clay minerals in lake water. Water Res 1980;14:185-187

Bakken LR. Separation and purification of bacteria from soil. Appl Environ Microbiol 1985;49:1482-1487

Bales RC, Hinkle SR, Kroeger TW, Stocking K. Bacteriophage adsorption during transport through porous media: chemical perturbations and reversibility. Environ Sci Technol 1991;25:2088-2095

Batch LF, Schulz CR, Linden KG. Evaluating water quality effects on UV disinfection of MS2 coliphage. JAWWA 2004;96(7):75-87

Berman D, Rice EW, Hoff JC. Inactivation of particle-associated coliforms by chlorine and monochloramine. Appl Environ Microbiol 1988;54:507-512

Bitton G, Henis Y, Lahav N. Effect of several clay minerals and humic acid on the survival of Klebsiella aerogenes exposed to ultraviolet irradiation. Appl. Microbiol 1972;23:870

Bitton G. Introduction to environmental virology. Wiley-Interscience, NY 1980;326

Black JG. Microbiology: Principles and Explorations 4th ed. Prentice Hall Inc. Upper Saddle River, New Jersey 1999.

Bolton JR. Ultraviolet application handbook. Bolton Photosciences Inc. 1999.

Bolton JR, Linden KG. Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. J Environ Eng—ASCE 2003;129:3:209

Borst M, Selvakumar A. Particle-associated microorganisms in stormwater runoff. Wat Res 2003;37:215-223

Brightwell Technologies Inc. www.brightwell.ca accessed the website on Oct. 12, 2004

Bukhari Z, Hargy TM, Bolton JR, Dussert B, Clancy JL. Medium-pressure UV for oocyst inactivation. JAWWA 1999;91:86-94

Cabaj A, Sommer R, Pribil W, Haider T. What means “dose” in UV-disinfection with medium pressure lamps. Ozone Science & Engineering 2001;23:239-244.

Camper AK, LeChevallier MW, Broadaway SC, McFeters GA. Bacteria associated with granular activated carbon particles in drinking water. Appl Environ Microbiol 1986;52:434-438

Camper AK, LeChevallier MW, Broadaway SC, McFeters GA. Evaluation of procedures to desorb bacteria from granular activated carbon. Journal of microbiol methods 1985a;3:187-198

Camper AK, LeChevallier MW, Broadaway SC, McFeters GA. Growth and persistence of pathogens on granular activated carbon filters. Appl Environ Microbiol 1985b;50:1378-1382

Carins WL. Comparing disinfection by ultraviolet light and chlorination—the implications of mechanism for practice. Planning, design, and operations of effluent disinfection systems specialty conference series. WEF, Whippany, NJ 1993;555

Chang JCH, Osoff SF, Lobe DC, Dorfman MH, Dumais CM, Qualls RG, Johnson JD. UV inactivation of pathogenic and indicator microorganisms. Appl Environ Microbiol 1985;49(6):1361-1365.

Christensen J, Linden KG. How particles affect UV light in the UV disinfection of unfiltered drinking water. JAWWA 2003:95(4):179-189

Christensen J, Linden KG. New findings regarding the impacts of suspended particles on UV disinfection of drinking water. Proceeding of the AWWA annual conference. New Orleans, LA 2002.

Christensen J, Linden KG. Ultraviolet disinfection of unfiltered drinking water: particle impacts. Proceeding of the IUVA 1st international congress. Washington DC 2001.

Clancy JL, Bukhari Z, Hargy TM, Bolton JR, Dussert BW, Marshall MM. Using UV to inactivate *Cryptosporidium.* JAWWA 2000;92:9:97-104

Clancy JL, Hargy TM, Marshall MM, Dyksen JE. UV light inactivation of *Cryptosporidium oocysts.* JAWWA 1998;90:9:92-102

Craik SA . The impact of turbidity on *Cryptosporidium* and *Giardia* inactivation by ultraviolet light. Proc. AWWA WQTC. Seattle 2002.

Dietrich JP, Basagaoglu H, Loge FJ, Ginn TR. Preliminary assessment of transport processes influencing the penetration of chlorine into wastewater particles and the subsequent inactivation of particle-associated organisms. Water Res 2003;37:139-149

Droppo IG, Ongley ED. Flocculation of suspended sediment in rivers of southeastern Canada. Water Res 1994;28(8):1799-1809

Duever TA. Statistics in engineering, course notes for ChE 622. Department of Chemical Engineering, University of Waterloo 2002.

Edzwald JK. Coagulation-sedimentation-filtration processes for removing organic substances from drinking water. Control of organic substances in water and wastewater. USEPA, EPA 600/8-83-001, 1983;2663p

Elley WN, Schleyer MH. Comparison of homogenization and ultrasonication as techniques in extracting attached sedimentary bacteria. Marine ecology-progress series 1984;15:247-250

Emerick RW, Loge FJ, Ginn T, Darby JL. Modeling the inactivation of coliform bacteria associated with particles. Water Environ Res 2000;72:432-438

Emerick RW, Loge FJ, Thompson D, Darby JL. Factors influencing ultraviolet disinfection performance—Part II - association of coliform bacteria with wastewater particles. Water Environ Res 1999;71:1178-1187

Gagnon GA, Huck PM. Removal of easily biodegradable organic compounds by drinking water biofilms: analysis of kinetics and mass transfer. Wat Res 2001;35(10):2554-2564

Gagnon GA, Slawson RM, Huck PM. Effect of easily biodegradable organic compounds on bacterial growth in a bench-scale drinking water distribution system. Can J Civ Eng 2000;27:412-420

Geesey GG, Costerson JW. Microbiology of a Northern River: bacterial distribution and relationship to suspended sediments and organic carbon. Can J Microbiol 1979;25:1058

Grand River Conservation Authority (GRCA). Laurel Creek Conservation Area http://www.grandriver.ca/index.cfm (accessed on Jul. 26, 2004)

Harris DG, Adams VD, Sorensen DL, Curtis MS. Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of bacteria. Wat Res 1987;21(6):687-692

Harris DH. The influence of photoreactivation and water quality on ultraviolet disinfection of secondary municipal wastewater. JWPCF 1987;59(8):781

Hejkal TW, Wellings FM, Lewis AL, Larock PA. Distribution of viruses associated with particles in wastewater. Appl and Environ Microbiol 1981;41(3):628-634

Hejkal TW, Wellings FM, Larock PA, Lewis AL. Survival of Poliovirus Within Organic Solids During Chlorination. Applied and Environ Microbiol 1979;38(1):114-118.

Herson DS, Mcgonigle B, Payer MA, Baker KH. Attachment as a factor in the protection of enterobacter cloacae from chlorination. Appl and Environ Microbiol 1990;56:3822-3829

Hoff JC. The relationship of turbidity to disinfection of potable water—Evaluation of the microbiology standards for drinking water. USEPA 1978;103:(EPA-570/9-78-OOL).

Hoyer O. Testing performance and monitoring of UV systems for drinking water disinfection. Wat Suppl. 1998;16(1-2):424-429

Huck PM. Course note of CivE 670: Physical-chemical processes for water and wastewater treatment. Winter 2003

International Ultraviolet Association (IUVA) web link: http://www.iuva.org/MemberZone/index.htm (accessed on Sept. 25, 2004)

Jagger J. Introduction to research in ultraviolet photobiology. Englewood Cliffs, NJ: Prentice Hall, Inc. 1967.

Jolis D, Lam C, Pitt P. Particle effects on ultraviolet disinfection of coliform bacteria in recycled water. Water Environ Res 2001;73:233-236

Kaneko M. Chlorination of pathogenic *E. coli* O157. Wat Sci Tech 1998;38(12):141-144

Kavanaugh MC, Tate CH, Trussell RR, Treweek G. Use of particle size distribution measurements for selection and control of solid/liquid separation processes. Particulates in Water. Advances in chemistry series, 189. American Chemical Society. Washington DC, 1980.

Knudson GB. Photoreactivation of UV-irradiated Legionella Pneumoplila and other Legionella species. App and Environ Microbiol 1985;49(4):975-980.

LeChevallier MW, Babcock TM, Lee RG. Examination and characterization of distribution system biofilms. Applied and Environ Microbiol 1987;53:2714-2724

LeChevallier MW, Cawthon CD, Lee RG. Factors promoting survival of bacteria in chlorinated water supplies. Applied and Environ Microbiol 1988a;54:649

LeChevallier MW, Cawthon CD, Lee RG. Inactivation of biofilm bacteria. Applied and Environ Microbiol 1988b;54:2492

LeChevallier MW, Evans TM, Seidler RJ. Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. Applied and Environ Microbiol 1981;42(1):159-167.

LeChevallier MW, Hassenauer TS, Camper AK, McFeters GA. Disinfection of bacteria attached to granular activated carbon. Applied and Environ Microbiol 1984;48:918-923

Liltved H, Cripps SJ. Removal of particle-associated bacteria by prefiltration and ultraviolet irradiation. Aquacult Res 1999;30:445--450

Linden KG, Darby JL. Ultraviolet disinfection of marginal effluents: determining ultraviolet absorbance and subsequent estimation of ultraviolet intensity. Wat Environ Res 1998;70(2):214

Linden KG, Shin GA, Sobsey MD. Comparative effectiveness of UV wavelengths for the inactivation of Cryptosporidium parvum oocysts in water. Water Sci Tech 2001;43(12):171-174

Linden KG, Batch L, Schulz C. UV Disinfection of filtered water supplies: water quality impacts on MS2 dose-response curves. Proceedings of the AWWA annual conference, New Orleans, LA. June 2002

Liu W, Andrews SA, Bolton JR, Linden KG, Sharpless C, Stefan M. Comparison of DBP formation from different UV technologies at bench scale. IWA World Water Congress, Melbourne, Australia 2002

Loge FJ, Emerick RW, Ginn TR, Darby JL. Association of coliform bacteria with wastewater particles: impact of operational parameters of the activated sludge process. Water Res 2002;36(1):41-48

Loge FJ, Emerick RW, Health M, Jacangelo J, Tchobanoglous G, Darby JL. Ultraviolet disinfection of secondary effluents: Prediction of performance and design. Water Environ Res 1996;68:900-916

Loge FJ, Emerick RW, Thompson DE, Darby JL. Factors influencing UV disinfection performance—part I - light penetration into wastewater particles. Water Environ Res 1999;71:377-381

Loge FJ, Emerick RW, Thompson DE, Nelson DC, Darby JL. Development of a fluorescent 16s rRNA oligonucleotide probe specific to the family enterobacteriaceae. Water Environ Res 1999;71:75-83

Malley JPJr. Engineering of UV disinfection systems for drinking water. IUVA News 2000;2(3):8

McCoy WF, Olson BH. Analysis of the microbiological particulates in municipal drinking water scanning electron microscopy/X-ray energy spectroscopy. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. B Hyg. Umweilthyg. Krankenhaushyg. Arbeitshyg. Praev. Med. 1987;183:511-529

McCoy WF, Olson BH. Relationship among turbidity, particle counts and bacteriological quality within water distribution lines. Wat Res 1986;20(8):1023-1029

McDaniel JA, Capone DG. A comparison of procedures for the separation of aquatic bacteria from sediments for subsequent direct enumeration. J of microbial methods 1985;3:291-302

Meulemans CCE. The basic principles of UV-sterilization of water. In: Ozone + Ultraviolet Water Treatment, Aquatec Amsterdam, Paris: International Ozone Association 1986:B.1.1-B.1.13.

Montgomery JM. Water treatment principles and design. NY, Wiley, 1985.

Neis U, Tiehm A. Particle size analysis in primary and secondary wastewater effluents. Wat Sci Tech 1997;36(4):151-158

NWRI (National Water Research Institute) and AWWARF (American Water Works Association Research Foundation). Ultraviolet disinfection guidelines for drinking water and water reuse. Fountain Valley, CA 2000

Oliver BG, Cosgrove EG. The disinfection of sewage treatment plant effluents using ultraviolet light. Canadian J of Chem Eng 1975;53:170-174

Oppenheimer J, Gillogly T, Stolarik G, Ward R. Comparing the efficiency of low and medium pressure UV light for inactivating *Giardia muris* and *Cryptosporidium parvum* in waters with low and high levels of turbidity. Proceedings of AWWA annual conference, New Orleans LA 2002.

Ormeci B, Linden KG. Comparison of UV and chlorine inactivation of particle and non-particle associated coliform. Wat Sci Technol: Wat Supply 2002;2(5-6):403-410

Park CH, Huck PM. A conceptual model for cryptosporidium transport in watersheds. Water Qual Res J Canada 2003;38(1):77-113

Parker JA, Darby JL. Particle-associated coliform in secondary effluents: shielding from ultraviolet light disinfection. Water Environ. Res 1995;67:1065-1075

Passantino L, Malley JPJr, Knudson M, Ward R, Kim J. Effect of low turbidity and algae on UV disinfection performance. JAWWA 2004;96(6):128-137

Pernitsky DJ, Finch GR, Huck PM. Recovery of attached bacteria from GAC fines and implications for disinfection efficacy. Water Res 1997;31(3):385-390

Pernitsky DJ. Disinfection kinetics of biologically treated drinking water and the impact of released GAC fines on disinfection performance. Master's thesis 1993; University of Alberta

Petri MP, Fang G, Malley JP, Moran DC, Wright H. Groundwater UV disinfection: challenges and solutions. Proceeding of the WQTC, AWWA 2000.

Prescott LM, Harley JP, Klein DA. Microbiology 2nd ed. Wm. C. Brown Communications, Inc. Dubuque, IA 1993

Qualls RG, Flynn MP, Johnson JD. The role of suspended particles in ultraviolet disinfection. J Water Pollu Control Fed 1983;55:1280-1285

Qualls RG, Johnson JD. Bioassay and dose measurement in UV disinfection. Appl and Environ Microbiol 1983;45:872-877

Qualls RG, Johnson JD. Modeling and efficiency of ultraviolet disinfection systems. Water Res 1985;19:1039-1046

Qualls RG, Ossoff SF, Chang JCH, Dorfman MH, Dumais CM, Lobe DC, Johnson JD. Factors controlling sensitivity in ultraviolet disinfection of secondary effluents. J Water Pollu Control Fed 1985;57:1006-1011

Ramsay AJ. Extraction of bacteria from soil: efficiency of shaking or ultrasonication as indicated by direct counts and autoradiography. Soil boil biochem 1984;16:475-481

Ridgway HF, Olson BH. Chlorine resistance patterns of bacteria from two drinking water distribution systems. Appl and Environ Microbiol 1982;44:972-987

Ridgway HF, Olson BH. Scanning electron microscope evidence for bacterial colonization of a drinking-water distribution system. Appl and Environ Microbiol 1981;41:274-287

Roper MM, Marshall KC. Effects of salinity on sedimentation and of particulates on survivial of bacteria in estuarine habitats. Geomicrobiol J 1979;1:103

Scheible OK, Bassel CD. Ultroviolet disinfection in a secondary wastewater treatment plant effluent. EPA-600/S2-81-152, USEPA 1981

Schillinger JE, Gannon JJ. Bacterial adsorption and suspended particles in urban stormwater. JWPCF 1985;57:384-389

Schoenen D, Kolch A. Photoreactivation of *E. coli* depending on light intensity after UV irradiation. Zbl. Hyg. 1992;192:565-570

Severin BF, Suidan MT, Engelbrecht RS. Effects of temperature on ultraviolet light disinfection. Environ Sci Tech 1983;17(12):717-721.

Severin BF, Suidan MT, Rittmann BE, Engelbrecht RS. Inactivation kinetics in a flow-through UV reactor. JWPCF 1984;56:164.

Shin GA, Linden KG, Arrowood MJ, Sobsey MD. Low-pressure inactivation and DNA repair potential of Cryptosporidium parvum oocysts. Appl Environ Microbial 2001;67(7):3029-3032

Snowball MR, Hornsey IS. Purification of water supplies using ultraviolet light. Developments in food microbiology, edited by Robinson RK. NY: ElsevierApplied Science 1988:171-191.

Sobsey MD. Inactivation of health-related microorganisms in water by disinfection processes. Wat Sci Technol 1989;21(3):179-195

Sobsey MD, Fuji T, Hall RM. Inactivation of cell-associated and dispersed hepatitis A virus in water. JAWWA 1991;November:64-67

Sommer R, Lhotsky M, Haider T, Cabaj A. UV inactivation, liquid-holding recovery, and photoreactivation of *Escherichia coli* O157 and other pathogenic *Escherichia coli* strains in water. J. Food Prot 2000;63(8):1015-1020

Stagg CH, Wallis C, Ward CH. Inactivation of clay-associated bacteriophage MS-2 by chlorine. Appl and Environ Microbiol 1977;33(2):385-391

Stewart MH, Wolfe RL, Means EG. Assessment of the bacteriological activity associated with granular activated carbon treatment of drinking water. Appl and Environ Microbiol 1990;56:3822-3829

Taghipour F. Ultraviolet and ionizing radiation for microorganism inactivation. Wat Res 2004;38:3940-3948

Templeton M, Andrews RC, Hofmann R, Whitby GE. UV inactivation of floc-associated MS-2 coliphage. Proceeding of OMWA/OWWA conference, Hamilton 2003.

Tree JA, Adams MR, Lees DN. Chlorination of indicator bacteria and viruses in primary sewage effluent. Appl and Environ Microbiol 2003;69(4):2038-2043

Tree JA, Adams MR, Lees DN. Virus inactivation during disinfection of wastewater by chlorination and UV irradiation and the efficacy of F+ bacteriophage as a 'viral indicator'. Wat Sci Technol 1997;35(11-12):227-232

Urfer D, Huck PM. A study of the impacts of periodic ozone residuals on biologically active filters. Ozone Sci Eng 1999;22:77-97

USEPA. Code of Federal Regulations and additional material related to Title 40: Protection of Environment 1979. http://www.epa.gov/epahome/cfr40.htm (accessed on Jun. 29, 2004)

USEPA. Ultraviolet Disinfection Guidance Manual (UVDGM): Proposal draft. Ground Water and Drinking Water, Washington 2003.

Ward RW, DeGrave GM. Residual toxicity of several disinfectants in domestic wastewater. JWPCF 1978;50:46

Whitby GE, Palmateer G. The effect of UV transmission, suspended solids, and photoreactivation on microorganisms in wastewater treated with UV light. Wat Sci Tech 1993;37(3-4):379

Whitby GE, Scheible OK. The history of UV and wastewater. IUVA News 2004;6(3):15-26

Womba P, Bellmy W, Malley J, Douglas C. UV disinfection and disinfection byproduct characteristics of an unfiltered water supply, Project 2747. AWWARF periodic report. Denver CO 2002.

Wright HB, Sakamoto G. UV dose required to achieve incremental log inactivation of bacteria, viruses, and protozoa. Trojan Tech Inc. revision of Sept. 12, 2001

Zimmer JL. Inactivation and potential repair of selected waterborne pathogens exposed to ultraviolet radiation. Master's thesis, University of Waterloo 2002.

Zimmer JL, Slawson RM. Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low- pressure UV sources used in drinking water. App and Environ Microbiol 2002;68(7):3293-3299.

Zimmer JL, Slawson RM, Huck PM. Inactivation and potential repair of Cryptosporidium parvum following low- and medium-pressure ultraviolet irradiation. Wat Res 2003;37(14):3517-3523.