University of Alberta

Solar-driven photoreactivation of microorganisms: Impacts of light source and water matrix

by

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ABSTRACT

The inactivation fluence of a target wastewater plant in Alberta, Canada was determined in the laboratory and was found to be close to that reported by the target wastewater plant. The effect of inactivation fluence, effective reactivation fluence, temperature, and river water on the percent photoreactivation of total coliforms was investigated under both indoor and outdoor conditions. A concept, namely the 'effective reactivation fluence', based on weighting the spectral fluence rate by the action spectrum for photoreactivation, is introduced. Higher inactivation fluence and effective reactivation fluence decreased the percent photoreactivation of total coliforms, while higher temperature increased it. Also, the percent photoreactivation of total coliforms decreased on increasing the percent river water. In addition, the effect of various covers on the percent photoreactivation of total coliforms was studied representing higher percent photoreactivation of total coliforms by cutting off the UV-B portion of sunlight.

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Table of Contents

List of Tables

List of Figures

CHAPTER 1
INTRODUCTION

1.1. Photoreactivation Processes in Wastewater

Ultraviolet (UV) treatment has been used increasingly in recent years in many water and wastewater treatment plants as an alternative disinfection method to chlorination. This is based on several advantages, such as high disinfection efficiency with most viruses, bacteria and protozoa, no unidentified toxic disinfection byproducts (DBPs) and ease of operation (Hallmich and Gehr, 2010; Guo *et al*., 2008; Shang *et al*., 2009). Exposure to a sufficient inactivation fluence[1] results in pathogen inactivation, which results in damage to the nucleic acids of the microorganism as a result of thymine–thymine dimer formation. This prevents DNA replication and thus stops pathogen reproduction (Hallmich and Gehr, 2010; Guo *et al*., 2008; Shang *et al*., 2009; Suß *et al*., 2009). However, microorganisms possess the ability to repair the DNA damage caused by UV exposure by two mechanisms including light-dependent (photoreactivation) and light-independent (dark repair) mechanisms (Hallmich and Gehr, 2010; Guo *et al.*, 2008; Shang *et* al., 2009; Suß *et al*., 2009; Bohrerova and Linden, 2007).

Photoreactivation is a process by which light in the wavelength range of 310–480 nm is utilized by microorganisms to repair damaged DNA (Guo *et al*., 2008;

[1] Fluence is also called 'UV dose'; however, in this thesis, the term 'fluence' will be used exclusively. Also the modifiers 'inactivation' or 'photoreactivation' will be applied depending on mode of action of the fluence.

Shang *et al*., 2009; Suß *et al*., 2009; Bohrerova and Linden, 2007). This issue has gained importance because the number of microorganisms can increase as a result of photoreactivation in a few hours after treatment, representing a potential disadvantage for the application of UV disinfection (Hallmich and Gehr, 2010; Guo *et al*., 2008).

Photoreactivation should not play a significant role in a drinking water disinfection plant, where light exposure cannot occur in the distribution system as the water reaches the consumers. However, photoreactivation has an important impact on wastewater disinfection, where the discharged water can potentially be exposed to sunlight immediately after the UV treatment. Also photoreactivation occurs in various waterborne bacterial species and strains at different rates and extents (Bohrerova and Linden, 2007). Therefore, to control the quality of receiving waters and to reduce a possible human health risk, waterborne pathogen deposition and distribution in the environment via hydrological connections should be studied (Suß *et al*., 2009). The investigation of subsequent bacterial repair after UV treatment in wastewater treatment plants is also necessary (Bohrerova and Linden, 2007).

1.2. Photoreactivation Mechanism

The mechanism of direct repair is based on the covalent modification of the DNA, rather than removal or replacement of bases or nucleotides. During UV exposure, UV light is absorbed by DNA, resulting in the formation of covalent bonds between neighbouring pyrimidine nucleotides in the DNA chain, which form

mutagenic lesions, including cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidine 6-4 photoproducts (6-4 PP). This process inhibits DNA replication and leads to the cell death (Maclean *et al*., 2008). Photoreactivation is the most characterized kind of direct repair in which an enzyme called *photolyase* absorbs near ultraviolet and visible light to initiate an enzymatic reaction to break the cyclobutane and 6-4 PP rings joining the pyrimidines. This process repairs the pyrimidine dimers in DNA by using light energy according to the classical Michaelis–Menten reaction scheme as follows (Sancar and Sancar, 1988; Yasbin, 2002; Sancar, 2000):

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P} \qquad (1-1)$$

The first step of the reaction is formation of an enzyme-substrate (ES) complex, which is not a rapid step (rate constant k_1), indicating difficult diffusion of the enzyme into a damaged cell. However, the dissociation rate of ES is slow (rate constant k_2), but photolysis has a rapid rate (rate constant k_3) that is greater than rate constant k_2 even at a very low light intensity. Also, the conversion rate of the ES complex to dissociated dimers depends on the light intensity (Sancar, 2000). Electrostatic contact of *photolyase* with two phosphates in the DNA backbone limits its ionic interactions with DNA. So the selectivity of *photolyase* depends on minimum electrostatic interaction with phosphates in the DNA backbone to increase enzyme diffusion in the pathway of ES complex formation (Sancar and Sancar, 1988). Also, Sutherland *et al.* (1973) found that the results of electrophoresis indicated that the enzyme activity of *E. coli* is optimum in the pH range of 5.5 to 8.5 and reaches its maximum at 7.2. This may be because the

E. coli colony forming ability decreases in buffers of pH 10 and 4 (Musarrat and Ahmad, 1987)

After scanning DNA lesions, photolyases bind to them, followed by photon absorption and the initiation of photoreactivation (Maclean *et al.*, 2008). The enzyme activation wavelengths for photoreactivation depend on its source and its chromophore cofactors. An important chromophore involved in this process is the reduced form of flavin adenine dinucleotide ($FADH_2$), which forms $FADH^-$ following photon absorption. Subsequent electron donation from the chromophore to pyrimidine dimers results in the formation of a pyrimidine dimer anion. This anion is unstable and splits to pyrimidine monomers with regeneration of $FADH_2$ (Sancar and Sancar, 1988; Malhotra *et al*., 1992; Brazard *et al*., 2010).

All photolyases have chromophore cofactors, including $FADH_2$ and either deazaflavin or folate enzymes, which have the same role in absorbing photons and transferring energy to $FADH_2$. However, it was revealed that damaged DNA can be repaired more rapidly by organisms that have deazaflavin enzymes than those containing folate enzymes. This is because deazaflavin enzymes can absorb more photons in solar spectrum (λ = 430–450 nm) than folate enzymes (λ = 350–400 nm). Also, they have higher molar absorption coefficients and better performance in transferring energy compared to the folate enzymes (Malhotra *et al*., 1992). However, $FADH_2$ can absorb light directly and initiate photolysis (Sancar, 2000).

1.3. Types of Microorganisms Used for Photoreactivation Experiments

Generally, measurements of indicator bacteria using traditional plate count techniques are used to monitor the efficacy of wastewater disinfection processes (Suß *et al*., 2009; Hallmich and Gehr, 2010). In most cases *E. coli* or some other pure cultured microorganisms has been used as an indicator for comparisons between low-pressure (LP) and medium pressure (MP) lamps or for comparison between percent photoreactivation of various microorganisms. For example, Oguma *et al*. (2004) compared the photoreactivation of pure cultured *Legionella pneumophila* with that for pure cultured *E. coli* following exposure to both LP and MP UV lamps. In addition, Suß *et al*. (2009) compared the degree of photoreactivation of pure cultured *Pseudomonas aeruginosa* and *Enterococcus faecium* after exposure to LP UV lamp. Also, Bohrerova and Linden (2006) examined photoreactivation in other pure cultured bacteria, such as *Mycobacterium terrae*, following exposure to both LP and MP UV lamps.

Other than pure cultured bacteria, some studies have been carried out on wastewater containing mixed microorganisms, in which the photoreactivation of fecal coliforms under both sunlight and artificial lights was investigated (Martin and Gehr, 2007; Hallmich and Gehr, 2010). Also, photoreactivation of total coliforms, fecal coliforms, and *Streptococcus faecalis* in the samples collected from unfiltered secondary effluent after exposure to LP UV lamps with various inactivation fluences was modeled (Sanz *et al*., 2007). In addition, the effect of temperature, visible light and type of UV lamps on the degree of

photoreactivation of *E. coli* and enterococci from disinfected wastewater was investigated (Locas *et al*., 2008). Moreover, the degree of photoreactivation of total coliforms in municipal wastewater samples was compared with that for pure cultured *E. coli* following exposure to both LP and MP UV lamps (Guo *et al*., 2008). Furthermore, photoreactivation of fecal coliforms, total coliforms, and *E. coli* as indicator bacteria from secondary effluent of a wastewater treatment plant was investigated after exposure to different inactivation fluences by applying LP lamp (Yoon *et al*., 2007).

Photoreactivation of some protozoa, such as purified *Cryptosporidium parvum* under both LP and MP UV lamps, and purified *Giardia lamblia* under LP UV lamp were also investigated in other research studies (Zimmer *et al.*, 2003; Shin *et al.*, 2001; Oguma *et al*., 2001; Li *et al.*, 2008).

1.4. Methods Used for Controlling Photoreactivation

One principal method to control photoreactivation is applying sufficiently high inactivation fluences because they cause irreversible damage in the microorganism's cell, such that the damage cannot be repaired by photoreactivation (Locas *et al*., 2008; Guo *et al*. 2008). Several researchers have investigated this issue and have reported reduction of photoreactivation in *E. coli*, total coliforms and fecal coliforms with increasing the inactivation fluence following exposure to LP or MP UV lamp (Guo *et al*., 2008; Yoon *et al*., 2007; Locas *et al*., 2008).

Another proposed principal method to control photoreactivation is switching from a low-pressure (LP) UV system to a medium pressure (MP) UV system. However, the advantages of using a MP UV lamp over a LP lamp in terms of photoreactivation are still in doubt (Guo *et al.*, 2008). For example, Zimmer and Slawson (2002) studied the effect of the inactivation fluence and also LP and MP lamps on photoreactivation of *E. coli.* They found that *E. coli* did not undergo any photoreactivation following exposure to the MP UV lamp at inactivation fluences more than 3 mJ/cm^2. However, *E. coli* showed photorepair following exposure to the low-pressure UV source, at inactivation fluences up to 10 mJ/cm^2. In addition, Locas *et al.* (2008) reported that the percent photoreactivation of *E. coli* was higher after exposure to a LP UV lamp as compared to a MP UV lamp. Hu *et al*. (2005) and Oguma *et al*. (2002) obtained the same results with *E. coli*. On the other hand, Bohrerova and Linden (2006) showed that a MP UV lamp was not more efficient than a LP UV lamp in reducing the photoreactivation of *Mycobacterium terrae*, even at a high inactivation fluence of about 20 mJ/cm^2. Oguma *et al*. (2004) also reported that *L. pneumophila* underwent the same degree of photoreactivation after exposure to both LP and MP UV lamps. In addition, Zimmer *et al*. (2003) studied the photoreactivation of *Cryptosporidium parvum* following exposure to both LP and MP UV lamps by applying inactivation fluences from 1 to 3 mJ/cm^2. They found that no detectable photoreactivation occurred after exposure to LP or MP UV lamp. Finally, Guo *et al.* (2008) stated that the reason for these contradictions between the results of photoreactivation after exposure to either LP or MP UV lamps might arise from an incorrect method

of determining the germicidal UV dose by MP lamps. The log inactivation versus germicidal inactivation fluence for LP and MP lamps should be statistically the same. However, based on the standard collimated beam test proposed by Bolton and Linden (2003), using a radiometer detector that has significant sensitivity at wavelengths greater than 300 nm can cause germicidal inactivation fluence to be overestimated for MP lamp. So, Guo *et al.* (2008) used an additional correction factor of 0.778 to correct the wavelength dependence of the sensor for MP lamp. By applying proper correction, MP UV lamp did not show any advantage over LP lamp in terms of photoreactivation.

1.5. Effective Factors for Photoreactivation

The principal factors that influence photoreactivation are the irradiance and duration of the visible light exposure, temperature, and the inactivation fluence (Kashimada *et al*., 1996; Oguma *et al*., 2002; Zimmer and Slawson, 2002; Salcedo *et al*., 2007; Hu and Quek, 2008; Locas *et al*., 2008). In addition, research shows that photoreactivation is affected by wavelength (Herndl *et al*., 1997; Sinton *et al*., 1994; Curtis *et al*., 1992; Arana *et al*., 1992; Sancar and Sancar, 1988; Tosa and Hirata, 1999; Sinha and Häder, 2002; Bohrerova and Linden, 2007).

1.6. Research Objectives

Photoreactivation is gaining more attention because it can reduce the efficiency of UV disinfection of wastewater several hours after treatment (Hallmich and Gehr, 2010; Guo *et al*., 2008; Shang *et al*., 2009; Suß *et al*., 2009; Bohrerova and

Linden, 2007). In addition, the focus of many research activities on the single species has caused a considerable lack in knowledge about complex natural communities of microorganisms and their response to UV treatment (Suß *et al*., 2009). Also, most of the experiments related to photoreactivation have been carried out under laboratory conditions (Guo *et al*., 2008). However, laboratory cultures cannot reflect the conditions in receiving waters with the effect of sunlight and nutrients because sunlight usually has much higher irradiance than sunlight lamps (Tosa and Hirata, 1999). Therefore, this study focuses on an investigation of the potential photoreactivation and the trends of the photoreactivation phenomena of microorganisms in the wastewater effluent from a municipal plant in Alberta after MP ultraviolet disinfection. Also, this research will address the effect of photoreactivation under sunlight and real outdoor conditions on real wastewater containing mixed microorganisms.

A concept has been introduced, namely the 'effective reactivation fluence', (ERF), which is the integral of the spectral fluence rate weighted by the reactivation action and multiplied by the exposure time in seconds.

The findings of this study are environmentally important because effluents of most wastewater companies are offloaded to river waters and there is a concern to know what happens to the UV-treated bacteria after their exposure to sunlight.

1.6.1. Laboratory determination of the inactivation fluence

Research studies have shown that severe alteration is induced on bacteria by using high inactivation fluences which inhibit their reactivation, while low inactivation

fluences provide good conditions for photoreactivation (Sanz *et al*., 2007). Therefore, the first objective of this study is to develop a method for laboratory determination of the applied inactivation fluence at the target wastewater plant. Total coliforms were selected as the measuring bacteria to plot an inactivation curve. The MP lamp was also used for this purpose because the same lamp type was applied in the target wastewater plant. The laboratory determined inactivation fluences were then compared with the inactivation fluences reported at the wastewater plant.

1.6.2. Determination of percent photoreactivation in the lab

After the above initial assessment, the percent photoreactivation of total coliforms in the samples with different compositions of wastewater and river water was determined in the lab by a sunlight lamp. From this, the effect of inactivation fluence, temperature, light intensity, exposure time, and river water on photoreactivation can be quantified.

1.6.3. Determination of percent photoreactivation in the real outdoor conditions

Outdoor experiments were carried out under sunlight and with the same compositions as indoor experiments. This attempt was made to compare the lab conditions with outdoor conditions, and also to investigate the effect of some factors including light intensity, temperature, exposure time, and river water on percent photoreactivation.

1.7. Terminology

All the terms and concepts used in this thesis are defined as follows.

Irradiance (W m^{-2}): Total radiant power incident from all upward directions on an infinitesimal element with surface area of d*S* divided by d*S* (Bolton, 2010).

Integrated irradiance: spectral irradiance integrated over a defined wavelength.

Fluence rate (W m^{-2}): Total radiant power incident from all directions through an infinitesimally small sphere with cross section of d*A*, divided by d*A* (Bolton, 2010).

Fluence (J m^{-2}): Total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere with cross section of d*A*, divided by d*A*. It is important to mention that if the 'fluence rate' is constant over time, the 'fluence' is given by the fluence rate times the exposure time in seconds (Bolton, 2010).

Inactivation fluence (J m^{-2} or mJ cm^{-2}): radiant energy incident on micro-organisms in the germicidal range during a certain exposure time to inactivate them.

Reactivation fluence (kJ m^{-2} or J cm^{-2}): radiant energy incident on microorganisms over wavelength range of 300–500 nm during a certain exposure time to reactivate them after inactivation.

Effective reactivation fluence (kJ m^{-2} or J cm^{-2}) (ERF): the integral of the spectral fluence rate weighted by the reactivation action and multiplied by the exposure time in seconds.

Lux (lx): a measure of the light intensity. One lux is equal to one lumen per square meter. The lumen is a measure of the total amount of visible light emitted by a source and differs from power (radiant flux). Luminous flux is the varying sensitivity of the human eye to different wavelengths of light, while radiant flux reflects the total power of all light emitted, independent of the eye's ability to perceive it.

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CHAPTER 2
EXPERIMENTAL METHODS AND DESIGN

2.1. Introduction

The methods and materials used in this thesis, including the kind of wastewater samples, UV source and apparatus, culturing microorganisms, and evaluation of photoreactivation data, are described in this chapter.

2.2. Wastewater Samples

The wastewater samples used in the experiments were collected from the influent and effluent of the UV unit at a municipal wastewater treatment plant in Alberta, Canada, where the effluent is discharged directly to a river.

2.3. UV source and apparatus

UV exposure tests were carried out employing a collimated beam apparatus (Figure 2-1), using the standard protocol as described by Bolton and Linden (2003), with the additional correction suggested by Guo *et al*. (2008). The collimated beam apparatus was manufactured by Calgon Carbon Corp. (Model No. ps1-1-120). A 1 KW medium pressure (Calgon Carbon, Pittsburgh, PA, USA) lamp was used for generating a quasi parallel beam of UV light.

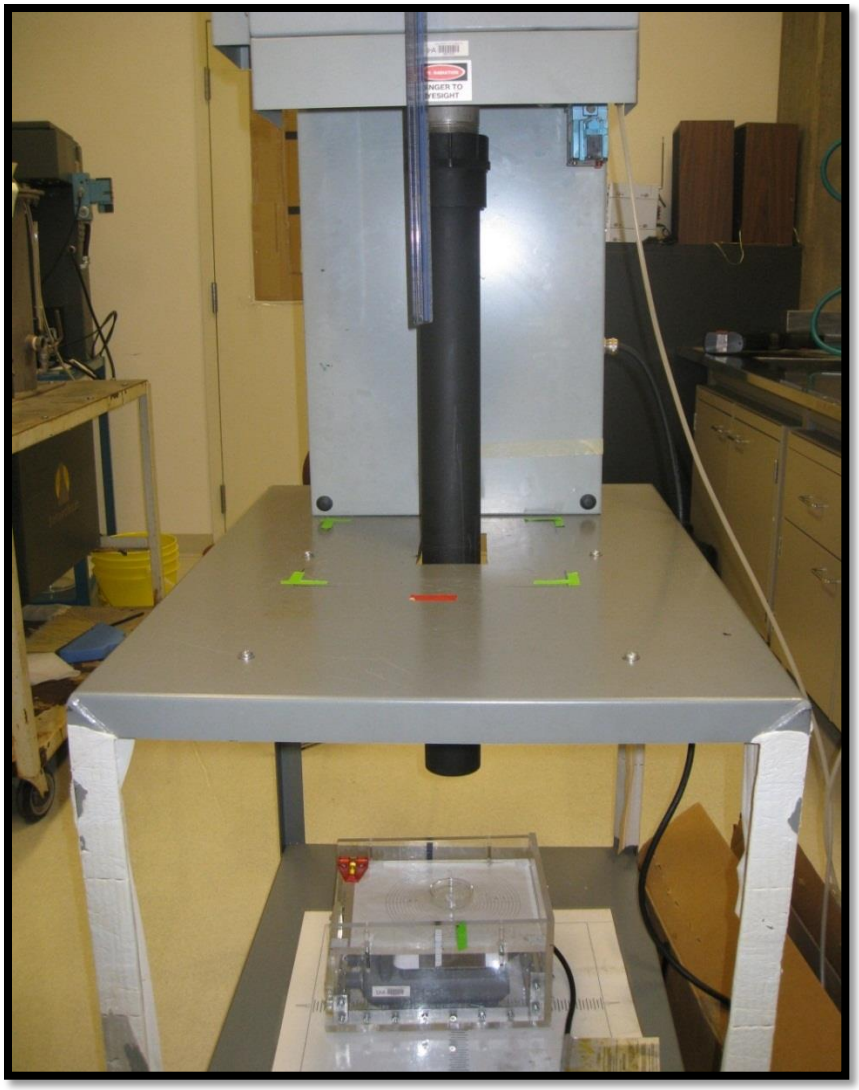

Figure 2-1: Diagram of the UV Collimated beam apparatus

Because of the high irradiance of the MP lamp that reduced exposure time for a 5 mJ/cm^2 inactivation fluence to less than 30 s even at the longest distance, a filter with a mesh size of 0.43 mm was also put into the UV beam to reduce the irradiance and hence increase the exposure time. The irradiance incident on the water surface for each sample was measured by a radiometer (International Light Inc. Model IL 1400A) along with a detector (International Light Inc. Model

SED240). The absolute irradiance of sunlight was measured by the spectroradiometer (JAZ-A, Ocean Optics Inc.) with the software program SpectraSuite. For the indoor photoreactivation experiments, a sunlight lamp (20 W, F20T12, Philips, USA) was used as the light source.

2.4. Culturing and counting microorganisms

Following exposure to the sunlight lamp, or natural sunlight, proper dilutions were carried out to have the counts around10-100 per plate. Then each water sample was filtered through a membrane (0.45 μm, Millipore, USA). Next the membrane was put on MF-Endo agar in triplicate, and incubated at 37 ºC for 24 h to culture the total coliforms. A standard count technique was applied for all samples. Only counts in the range 10–100 per plate were considered, and the number of bacteria was calculated as CFU/mL.

2.5. Quantitative evaluation of photoreactivation

After counting microorganisms, the effect of dark reactivation and photoreactivation was evaluated by computing the percent photoreactivation, defined by Lindenauer and Darby (1994) as follows:

$$\text{Percent photoreactivation } (\%) = \frac{N_p - N}{N_0 - N} \times 100 \qquad (2-2)$$

where, N_p = cell number in the photoreactivated sample (CFU/mL), N = immediate survival cell count after UV disinfection (CFU/mL), and N_0 = cell number before UV disinfection (CFU/mL).

After calculating the total percent reactivation, the percent dark reactivation was subtracted from it for each sample to determine the net photoreactivation.

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CHAPTER 3
BACKGROUND STUDIES OF ULTRAVIOLET INACTIVATION AND PHOTOREACTIVATION OF A WASTEWATER EFFLUENT

3.1. Introduction

The application of a high inactivation fluence produces lesions in the microorganism cell structure that are too numerous to be repaired, while application of a low inactivation fluence facilitates conditions for photoreactivation (Sanz *et al*., 2007; Locas *et al*., 2008). Hijnen *et al.* (2006) and Harris *et al.* (1987) reported that higher inactivation fluences are required for inactivation of bacteria under conditions when photoreactivation occurs. Tosa and Hirata (1999) stated that the inactivation of fecal coliforms with photoreactivation occurring requires an inactivation fluence that is 4.4 times more than that without the possibility of photoreactivation (i.e., no post-inactivation exposure to visible or near UV light). Also, Hoyer (1998) showed that a 4 log reduction of *E. coli* caused by a 10 mJ/cm^2 inactivation fluence is completely reversed with photoreactivation, and the inactivation fluence for a safe 99.99% inactivation of *E. coli* is about 30 mJ/cm^2. Also, he found that the required inactivation fluence for 4 log reduction of pathogens, facultative pathogens and indicator germs with photoreactivation present is 18–34 mJ/cm^2, which is 2–4 times the fluence required without the presence of photoreactivation.

There are some contradictions about the fluence required for the UV inactivation of bacteria with and without the possibility of photoreactivation. Oguma *et al.* (2001) showed that the fluences required for 90, 99, and 99.9% inactivation of *E. coli* without photoreactivation were 2, 4, and 6 mJ/cm^2, respectively. However, Harris *et al.* (1987) showed that the fluence required for 90 and 99.9% inactivation of *E. coli* with the presence of photoreactivation was about 7 and 10 mJ/cm^2, respectively. Also, Kashimada *et al.* (1996) found that the fluence required for 90% inactivation of fecal coliforms with the presence of photoreactivation was about 24 mJ/cm^2. However, Guo *et al*. (2008) and Yoon *et al*. (2007) showed that exposure to an inactivation fluence higher than 15 mJ/cm^2 almost totally inhibits the photoreactivation of bacteria in wastewater. Comparing these research studies, it is necessary to determine the inactivation fluence in the laboratory in order to estimate the percent photoreactivation.

The application of sufficiently high inactivation fluences is one of the principal methods available to control photoreactivation. Hu and Quek (2008) showed that thymine dimer repair rates decreased significantly as the inactivation fluence increased. Guo *et al*. (2008) investigated the effect of inactivation fluence on photoreactivation of municipal wastewater samples and pure cultured *E. coli*. They found that both *E. coli* and total coliforms in the wastewater underwent photoreactivation after both LP and MP exposure, but using inactivation fluences higher than 15 mJ/cm^2 reduced significantly the percent photoreactivation. Also, Yoon *et al*. (2007) reported that the number of total coliforms, fecal coliforms and *E. coli* bacteria increased after exposure to a LP UV lamp by applying an

inactivation fluence about 6 mJ/cm^2 because of photoreactivation, but they did not detect any photoreactivation after applying a high inactivation fluence (about 16 mJ/cm^2). Similarly, Locas *et al*. (2008) and Lindenauer and Darby (1994) showed that photoreactivation of *E. coli* in wastewater samples decreased with increasing inactivation fluences. Sanz *et al.* (2007) reported the same results for total coliforms and fecal coliforms and stated that high inactivation fluences cause severe damage to bacteria that cannot be repaired. Harris *et al.* (1987), Hu *et al.* (2005) and Oguma *et al.* (2002) showed similar results for pure cultured *E. coli*. Oguma *et al.* (2002) also showed that high inactivation fluences could break DNA into shorter chains. Therefore, considering these research studies, investigating the photoreactivation trend of bacteria after applying various inactivation fluences is an important issue.

Irradiance and reactivation fluence also have major effects on the photo-reactivation process. When investigating the effect of irradiance on photoreactivation of *E. coli*, Locas *et al.* (2008) reported an increase by 7 times in the number of bacteria at 5600 lx in 6 h compared to a dark control. The increase was about 4 times at 1600 lx. However, based on another research study, a high average photoreactivation irradiance has lethal effects on bacteria (Bohrerova and Linden, 2007). Bohrerova and Linden (2007) investigated the effect of irradiance on the photoreactivation of *E. coli* under sunlight and several indoor lamps. They found that during the exposure time the average sunlight irradiance was at least 10 times higher than that for the indoor lamps. Consequently, the photoreactivation fluence after 15 min of sunlight exposure was the same as that after the whole

exposure time under the sunlight lamps. Also, the time-based photorepair rate after the first 15 min of sunlight exposure was faster than that under the sunlight lamps. However, the photorepair rate decreased after 15 min of sunlight exposure. They found that the amount of UV–A (315–400) portion of sunlight was three times higher compared to the indoor lamps. So, they concluded that the higher amount of the UV–A portion, the higher the average irradiance and consequently the higher photoreactivation fluence of sunlight after the exposure time had lethal effects on bacteria and reduced the rate of the photorepair process. Hence, the inactivation and reactivation effects of sunlight should be considered at the same time to obtain the exact effect of irradiance and reactivation fluence on the photoreactivation process.

Temperature is another important factor for photoreactivation. The temperature effect on photoreactivation has been investigated in some research studies (Chan and Killick, 1995; Locas *et al*., 2008; Salcedo *et al.*, 2007). It was concluded that higher temperatures increased significantly the percent photoreactivation. For example, Locas *et al*. (2008) showed that photoreactivation in *E. coli* increased by 5 times at 25 °C compared to 4 °C over 6 h. Salcedo *et al*. (2007) also investigated the photoreactivation of total coliforms and fecal coliforms in the temperature range 5–30 °C by measuring the bacterial survival ratio every 5 °C. They showed that both photoreactivation and dark repair of total coliforms and fecal coliforms increased with increasing the temperature. They also proposed an exponential model for the dependence of kinetic parameters on temperature. In addition, they mentioned that the bacterial reactivation extent increased at higher

temperatures because of a temperature shock, which initiates the reactivation process.

Quek and Hu (2008) showed that photoreactivation in *E. coli* in the temperature range 23–37 °C was higher than that at too high (50 °C) or too low (4 °C) temperatures after an exposure of four hours. This is because this range is close to the bacterial growth temperature. They stated that the bacterial cells become inactive at too low and too high temperatures. Based on these research studies, it is important to investigate the effect of temperature on the photoreactivation of bacteria in the wastewater effluents after UV disinfection.

3.2. Objectives

Considering the importance of the inactivation fluence on photoreactivation, the first objective of this chapter is to determine in the laboratory the applied inactivation fluence of the target wastewater plant that can influence the photoreactivation of bacteria in the effluent under sunlight lamp or real outdoor conditions.

The effect of inactivation fluences on photoreactivation of total coliforms in the samples collected from a municipal plant in Alberta was then investigated. In most cases, the percent reactivation has been plotted against the ERF, which properly takes account of the photon absorption that initiates the reactivation process.

Next the effect of reactivation fluence on photoreactivation of total coliforms was investigated under indoor and outdoor conditions by applying various filters to change the integrated irradiance and consequently the reactivation fluence rate.

Finally, the effect of temperature on photoreactivation was investigated. In spite of the fact that the effect of temperature on photoreactivation and dark repair has been studied in several research studies, the impact of indoor and outdoor conditions on this issue has not been investigated. Hence, in this chapter, the effect of temperature on the photoreactivation of bacteria in the target wastewater plant effluent under indoor conditions was compared with that under outdoor conditions. Five temperatures, namely 5, 10, 15, 20 and 25 °C, were applied to photoreactivation and dark repair experiments over four hours.

3.3. Materials and Methods

The kind of wastewater samples and UV source and apparatus were described in Chapter 2, sections 2.2. and 2.3.

3.3.1. UV exposures

Total coliforms were selected as the representative bacteria (Guo *et al*. 2008). The MP lamp was used for this purpose because the same lamp type was applied in the target wastewater plant. A LP lamp also was used in the laboratory to determine the inactivation fluence to investigate any possible difference between the LP and MP UV systems. For the designed UV exposure time, water samples (25 mL) contained in Petri dishes (diameter: 60 mm) were put under the collimating tube

and gently stirred. The inactivation fluences (UV doses) were applied by changing the exposure times, while the irradiance was fixed at 0.14 mW/cm^2 (corrected by the sensor factor) and 0.24 mW/cm^2 throughout the experiment for the MP and LP UV lamps, respectively. All experiments were carried out at room temperature (20 ± 1 ºC).

The effect of the inactivation fluences on photoreactivation was investigated following application of various inactivation fluences by a MP UV lamp on water samples (section 3.3.2.1). In the determination of the laboratory inactivation fluence (UV dose), proper dilutions were carried out following UV exposure to have the counts around10-100 per plate. Then the microorganisms were cultured and counted based on the methods in section 2.4 and 2.5.

3.3.2. Photoreactivation experiments

Total coliforms were used as the representative bacteria for all the following experiments. All water samples were transferred to Pyrex® dishes (200 mL) and then covered with Saran Wrap® to avoid sample evaporation.

3.3.2.1. Effect of inactivation fluence

Following application of various inactivation fluences (5, 10, 15 and 20 mJ/cm^2), water samples were transferred to Pyrex® dishes and were put into a water bath. The water bath temperature was controlled by periodically adding ice pieces and kept at 20 ± 1°C. Dark controls were employed with a black plastic cover over each control sample. A sunlight lamp was positioned 10 cm above the samples.

Water samples were collected from the dishes every hour for up to 4 h. Also, a sample was collected in the first half hour.

3.3.2.2. Effect of photoreactivation fluence

Effluent samples were collected from the target Wastewater Plant. The inactivation fluence (as indicated on the plant computer) was about 23 mJ/cm^2. To compare the effect of a sunlight lamp with natural sunlight, the experiments were carried out under both indoor and outdoor conditions. The effluent samples were transferred to Pyrex® dishes. After that, three metal filters (0.63, 0.42, and 0.25 mm) were put on the top of the Pyrex® dishes to compare the effect of various filters on the percent photoreactivation of total coliforms in the effluent. Also, various numbers (2, 3 and 4) of filters (mesh size of 0.25 mm) were applied on the top of the other effluent samples to decrease the reactivation fluence as much as possible. The dishes were put into a water bath. The water bath temperature was controlled by ice pieces and kept at 20 ± 1°C. A dark control was put under a black plastic. For the outdoor experiments, water samples were collected from the dishes every hour for up to 4 h. Also, a sample was collected in the first half hour. For the indoor experiments, a sunlight lamp was positioned 5 cm above the samples. Because of low irradiance of the sunlight lamp compared to sunlight, exposure time under sunlight lamp was extended to 10 h and water samples were collected from the dishes every 2 h. A water sample was also collected in the first hour (this was considered as 0.5 h).

3.3.2.3. Effect of temperature

The effluent temperatures vary between 10 to 20 °C in the target wastewater plant. So, five temperatures (5, 10, 15, 20 and 25 ºC) were used to compare the effect of temperature on the percent photoreactivation of total coliforms in the effluent. According to the computer system at the UV unit in the target wastewater plant, the inactivation fluence was about 23 mJ/cm^2. To compare the effect of a sunlight lamp with natural sunlight, the experiments were carried out under both indoor and outdoor conditions. The effluent samples were transferred to Pyrex® dishes and were put into a water bath, whose temperature was controlled by adding ice pieces as necessary. Dark controls were employed with a black plastic cover over each control sample. For the indoor experiments, a sunlight lamp was positioned 10 cm above the samples. To adjust the sunlight lamp ERF to the level of natural sunlight ERF, two filters with a mesh size of 0.25 mm were used. The natural sunlight integrated effective irradiance was 4.8 mW/cm^2. So, two filters with a mesh size of 0.25 mm were put on top of each Pyrex® dish to reduce the integrated effective irradiance of sunlight to 0.42 mW/cm^2. Also, because of the lower integrated effective irradiance of the sunlight lamp (0.21 mW/cm^2) than that for natural sunlight even by using two filters (0.42 mW/cm^2), the exposure time under the sunlight lamp was extended to 8 h instead of 4 h. By this way, the sunlight lamp ERF in 8 h (6.2 J/cm^2) was almost adjusted to the level of natural sunlight ERF in 4 h (6.1 J/cm^2). Considering the larger exposure time under the sunlight lamp, water samples were collected from the dishes every 2 h for up to 8 h. A water sample was also collected in the first hour

(this was considered as 0.5 h). For the outdoor conditions water samples were collected from the dishes every hour for up to 4 h.

3.3.3. Culturing and counting microorganisms and quantitative evaluation of photoreactivation

After collecting samples in the photoreactivation experiments, the microorganisms were cultured and counted based on section 2.4 in Chapter 2. Then the percent photoreactivation was calculated based on section 2.5 in Chapter 2.

3.4. Results and Discussion

3.4.1. Laboratory determination of the applied inactivation fluence (UV dose)

The inactivation curve of total coliforms by MP and LP UV lamps is shown in Figure 3-1 (See raw data in Table A-1 of Appendix A). As shown in Figure 3-1, the log reduction increased linearly as the inactivation fluence increases. However, it levelled off at the end of the curve probably because of the shielding effect of the particles (Crittenden *et al*., 2005). In addition, the curve for the MP lamp fell on top of the curve for the LP lamp, which indicates that there is no difference in inactivation trends caused by either a LP or MP UV lamp. This was also found by Guo *et al*. (2008).

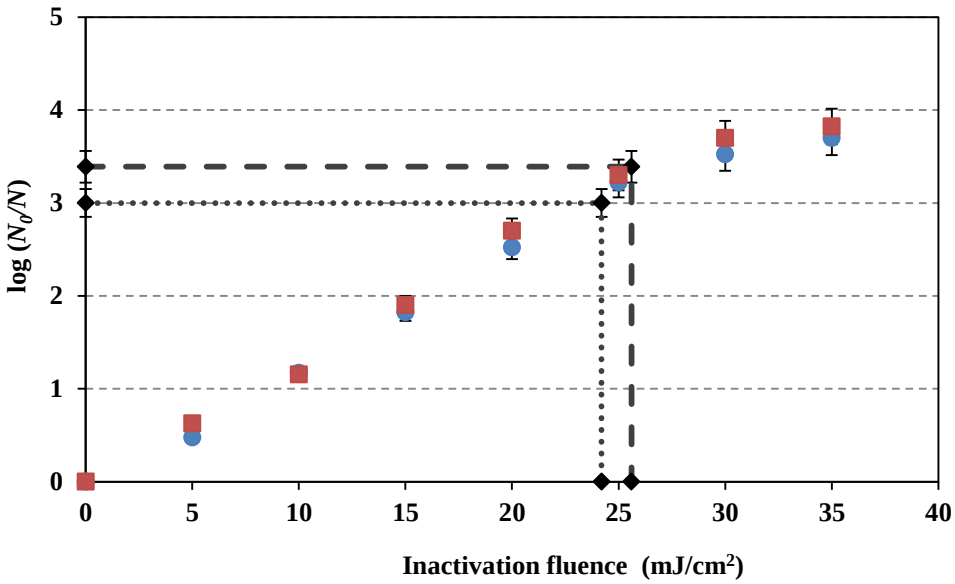

Figure 3-1: Log reduction of total coliforms in the wastewater after MP and LP UV exposure. • MP, ■ LP, inactivation fluence determined in the lab (dashed line), inactivation fluence reported by the target wastewater plant (dotted line)

Each experiment was repeated three times for each lamp by collecting water samples from the target wastewater plant on three different days. All results were evaluated statistically. The differences were not significant at $p > 0.05$ as determined by ANOVA. The average inactivation fluence reported by the target wastewater plant on three days was 24.2 mJ/cm^2 and they determined a log reduction of 3. However, based on laboratory experiments in this study and the ratio of influent to effluent counts, the log reduction for the wastewater treatment plant was 3.4 at which the inactivation fluence is 25.5 mJ/cm^2 according to Figure 3-1 for both the LP and MP UV systems (See the raw data in Tables A-2 of Appendix A). So, the indication of inactivation fluence at the target wastewater plant is reasonably accurate. The most probable reason for the various ratios of the inactivation fluences on three days could be a variation of the percent

transmittance of the wastewater from day to day; this influences the amount of UV received by the bacteria in the UV reactors.

3.4.2. Effect of inactivation fluence on the level of photoreactivation

After applying various inactivation fluences (5 to 10 mJ/cm^2) using a MP lamp, the net percent photoreactivation for total coliforms under a sunlight lamp is shown in Figure 3-2 (See the raw data in Tables A-3 and A-4 of Appendix A). The percent photoreactivation for total coliforms decreased with increasing the inactivation fluence as reported in other papers. This is because using high inactivation fluences causes damage in the microorganism's cell structure, such that photoreactivation is inhibited (Guo *et al*., 2008; Yoon *et al*., 2007; Locas *et al*., 2008).

The percent photoreactivation of total coliforms, after applying an inactivation fluence of 5 mJ/cm^2 and 4 h of exposure to the sunlight lamp, was about 16%, while it was reduced to less than 2% by applying a 20 mJ/cm^2 inactivation fluence. This indicates that the percent photoreactivation can be reduced significantly by applying inactivation fluences as high as 20 mJ/cm^2. This issue is important for the wastewater treatment plants to regulate the inactivation fluence in order to prevent a high percent photoreactivation. Another important issue is that, there is a plateau region at the end of the photoreactivation curves after applying various inactivation fluences. This may arise from the nutrients in the effluent, which can help the bacteria to grow. Suß *et al*. (2009) observed a similar trend and stated that at this stage repaired bacterial cells recover their cultivability.

Other research shows that photoreactivation is influenced by exposure time. Zimmer and Slawson (2002) stated that each *E. coli* organism has only 20 *photolyase* enzymes and only 5 dimers can be repaired by each enzyme per min. So, the repair mechanism is dependent on the frequency of *photolyase* attachment to the dimers (Oguma *et al*., 2004). Lindenauer and Darby (1994) reported that photoreactivation reached a maximum with increasing exposure time, after which no further increase in photoreactivation occurred. This is because all the recoverable dimers could be repaired after a certain exposure time. Locas *et al*. (2008) also showed that the photoreactivation degree increased with increasing exposure times.

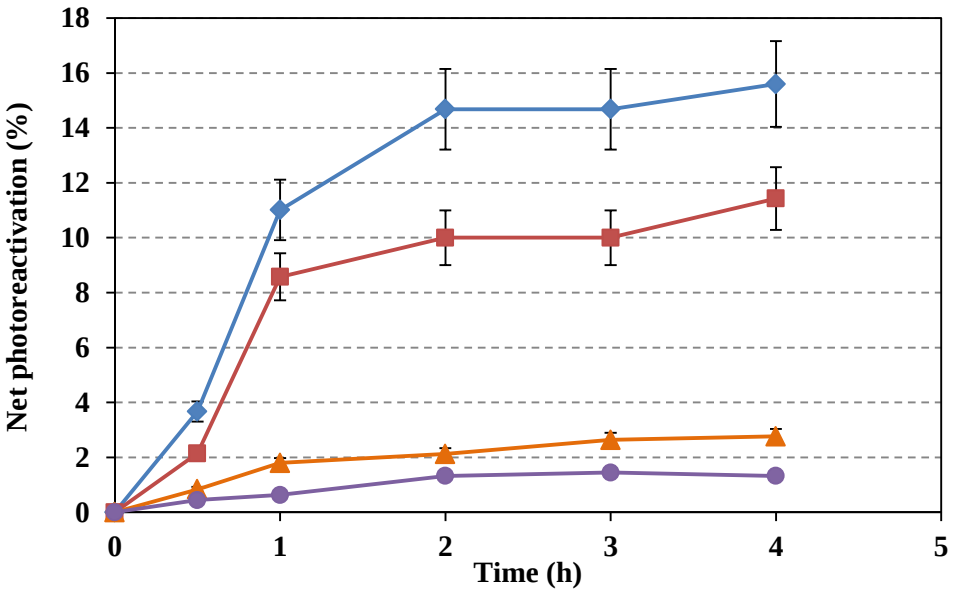

Figure 3-2: Net photoreactivation of total coliforms under a sunlight lamp after applying various inactivation fluences using a MP UV lamp. ♦ 5 mJ/cm^2 ; ■ 10 mJ/cm^2; ▲15 mJ/cm^2, ● 20 mJ/cm^2

3.4.3. Effective photoreactivation fluence

Figure 3-3 shows the net percent photoreactivation versus time under sunlight and the sunlight lamp (See the raw data in Tables A-5 and A-7 of Appendix A). It can be seen that there was an increase and then a decrease in the number of bacteria under outdoor conditions. The increase arises from the effect of photoreactivation, and the decrease results from the impact of extended sunlight exposure. Yoon *et al*. (2007) found that the UV portion of sunlight can cause inactivation after a long exposure time to sunlight. By comparing the percent photoreactivation under sunlight and the sunlight lamp, it can be seen that the percent photoreactivation decreased versus time under sunlight, while it increased under the sunlight lamp. This may result from the lack of UV–B portion in the spectrum of the sunlight lamp and also the lower fluence of the sunlight lamp compared to sunlight (Bohrerova and Linden, 2007; Herndle *et al*., 1997).

Bohrerova and Linden (2007) first introduced the concept of effective photoreactivation fluence to evaluate the photoreactivation results. So, to make the results of photoreactivation experiments independent of light source, a concept, namely the ‘effective reactivation fluence’ based on weighting the spectral fluence rate by the action spectrum for photoreactivation, is introduced and all the photoreactivation results were evaluated based on it. This issue will be introduced and discussed as follows.

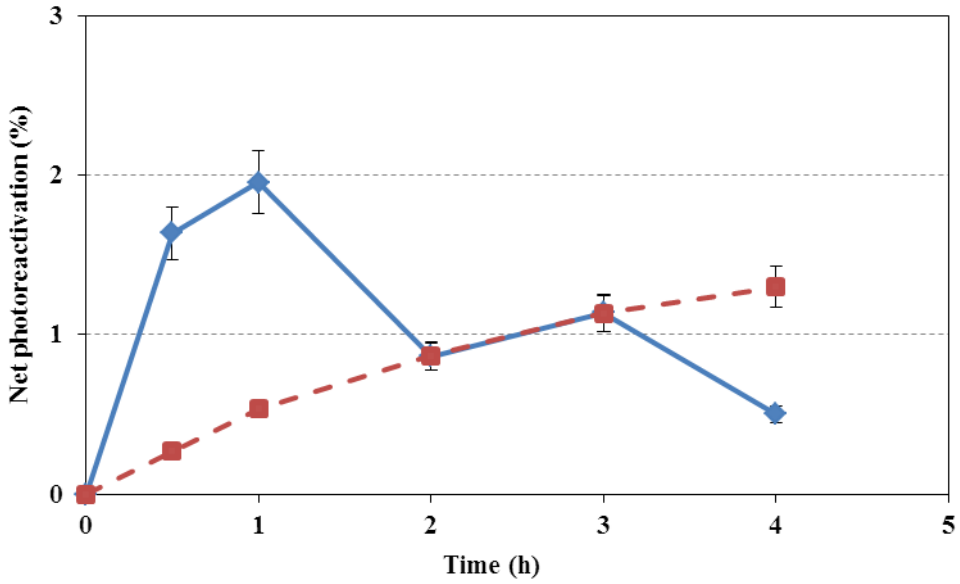

Figure 3-3: Net photoreactivation of total coliforms under natural sunlight (♦) and the sunlight lamp (■) after applying 23 mJ/cm^2 inactivation fluence as determined by the target wastewater plant

The action spectrum reflects the relative biological or chemical photoresponse per number of incident photons versus wavelength (Bolton, 2010). Kelner (1956) used the following relationship to determine relative absorption coefficients at different wavelengths.

$$\frac{ß_1}{ß_2} = \frac{i_2 t_2 \lambda_2}{i_1 t_1 \lambda_1} \qquad (3-1)$$

Where ß is the absorption coefficient at wavelength λ, i is the irradiance of reactivating light, and t is the length of the reactivation period necessary to produce a given degree of photoreactivation. This equation indicates the relative number of quanta which are required for the same degree of photoreactivation when two wavelengths are compared. Kelner (1956) assigned a value of one to the activity at 365 nm to compare two species because the same amount of energy at 365 nm is required to produce the same degree of photoreactivation. He plotted

the action curve for *E. coli* as shown in Figure 3-4 based on equation 3-1 assuming the activity at 365 nm to be equal to one. It can be seen that after a sharp peak near 375 nm, the *E. coli* activity decreases with increasing wavelength. Also, the region after 500 nm is inactive with low photoactivity, which is less than 3 percent compared to the activity at 365 nm. Later, research showed that *E. coli* has maximum activity around 380 nm (Sancar and Sancar, 1984; Eker *et al.*, 1987; Sancar *et al.*, 1987). Takao *et al*. (1989) used the same method of Kelner (1956) to plot the action spectra of photoreactivation for *E. coli* as shown in Figure 3-5 by assuming the activity at 385 nm to be equal to one.

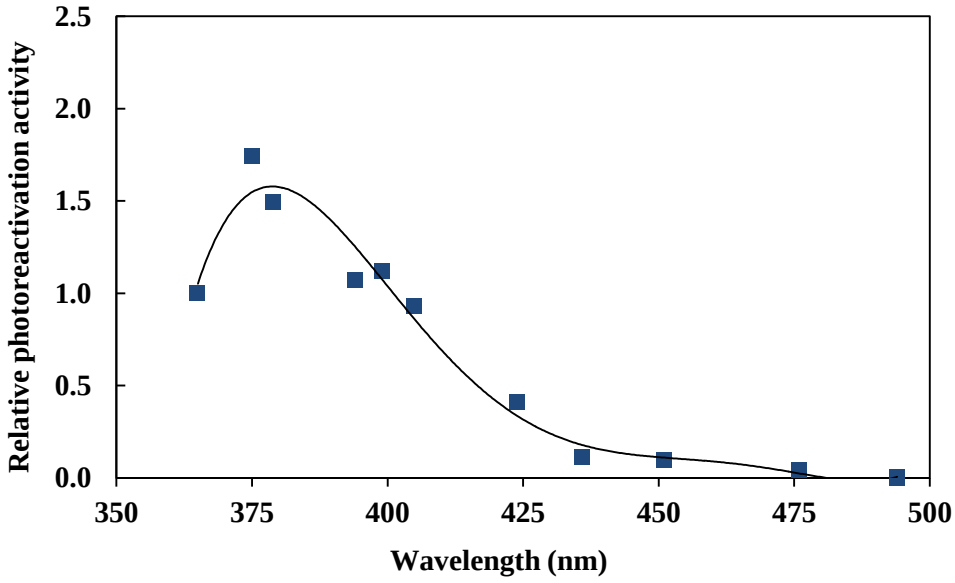

Figure 3-4: Action spectra for photoreactivation of *E. coli* (plotted from the data presented in Kelner (1956))

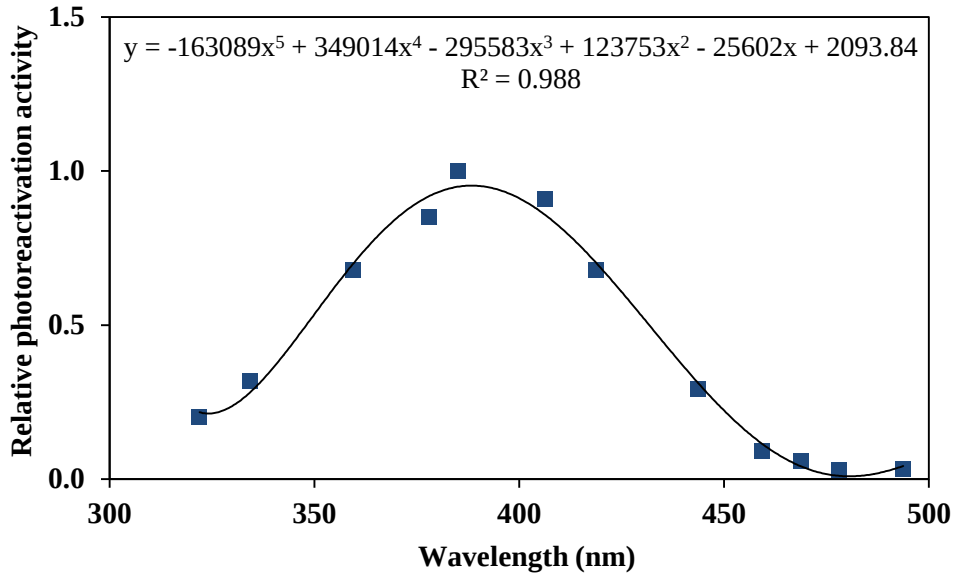

Figure 3-5: Action spectra for photoreactivation of *E. coli*; points (■) plotted from the data presented in Takao et al. (1989); the fitting line (see equation) is based x = wavelength divided by 1000.

In this research study, the data presented by Takao *et al*. (1989) was used to plot action spectrum of *E. coli*. Figure 3-6 and 3-7 show the absolute irradiance and effective spectral irradiance (ESI) versus wavelength for the indoor and outdoor conditions. To determine the ESI, average spectral irradiance (SI) values were multiplied by the average action spectrum factor (AS) values in each band. The SI values were measured by a spectroradiometer and the AS values were estimated by the data presented in the research study for *E. coli* by Takao *et al*. (1989). The sum of the ESI values over the wavelength range of 310–480 nm gives the total effective irradiance (EI). Based on Figure 3-6 and Figure 3-7, the EI values in the wavelength range of 310–480 nm were 2.8 and 33.3 mW/cm^2 for the sunlight lamp and natural sunlight, respectively.

The integrated effective reactivation irradiance can be estimated by integrating the ESI values in each band and summing over all the wavelengths. The effective reactivation fluence (ERF) can be determined by multiplying the ESI by time in seconds. In this research study, the ERF was used to characterize the photoreactivation process because it properly takes account of the effectiveness of each wavelength band in the photoreactivation process. So, to display the trend of the photoreactivation of bacteria in a wastewater effluent under various conditions, all figures in this thesis have been plotted versus the ERF.

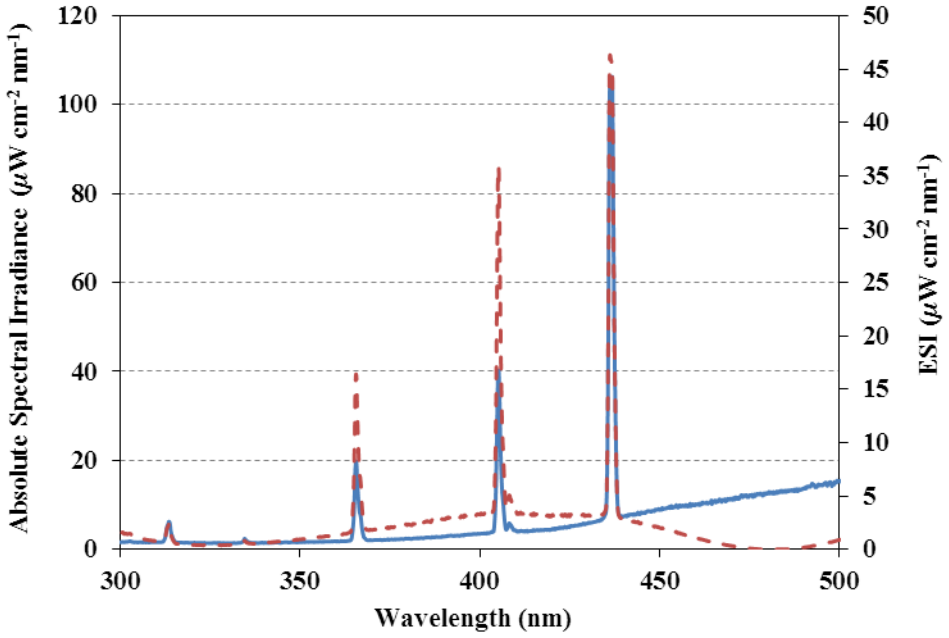

Figure 3-6: Absolute irradiance (solid line) and effective spectral irradiance (ESI) (dashed line) under indoor conditions.

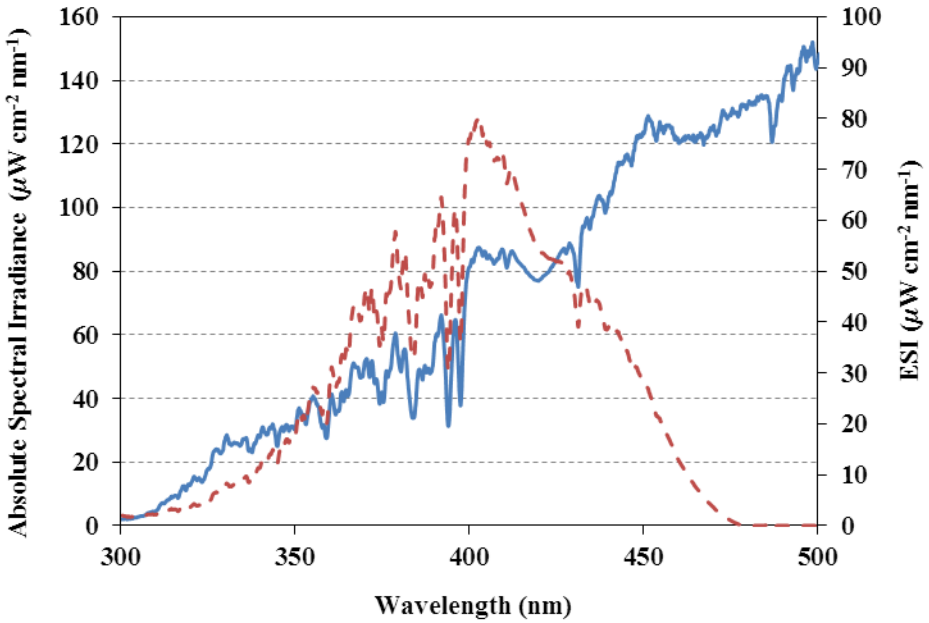

Figure 3-7: Absolute spectral irradiance (solid line) and effective spectral irradiance (ESI) (dashed line) under outdoor conditions.

Three filters with various mesh sizes (0.63, 0.42, and 0.25 mm) were used to investigate the effect of reactivation fluence rate on photoreactivation. First, the absolute irradiance of the solar light was measured by a spectroradiometer. Then various filters were put over the spectroradiometer detector, and the absolute irradiance was measured for each of them. The transmittance, integrated effective spectral irradiance, and ERF of the light by using each filter under indoor and outdoor conditions are given in Table 3-1 (See Appendix B for raw data and the method of calculating ERF). The transmittance is the ratio of the integrated effective spectral irradiance of the light by using each filter to the integrated effective spectral irradiance of the light without any filter multiplied by 100. It can be seen that the transmittance, integrated effective spectral irradiance, and

ERF decreased with decreasing the filter mesh size and increasing the number of filters under both indoor and outdoor conditions.

The net percent photoreactivation of total coliforms by using various filters under indoor and outdoor conditions is presented in Figure 3-8 versus the ERF (See raw data in Tables A-5 to A-8 of Appendix A). Based on Figure 3-8, it can be seen that the percent photoreactivation increased with decreasing ERF values by using various filters under both indoor and outdoor conditions. Also, there was a plateau region in each photoreactivation curve which shrank with decreasing ERF values by using various filters under both indoor and outdoor conditions.

As shown in Figure 3-8a, the percent photoreactivation under solar light was the lowest among the other samples. This arises from the high solar fluence rate, which causes bacterial inactivation (Bohrerova and Linden, 2007; Yoon *et al*. 2007). In addition, the percent photoreactivation increased by using the filter with mesh size of 0.63 mm followed by the filters with mesh size of 0.42 and 0.25 mm under outdoor conditions. This arises from the decreasing ERF with decreasing the filter mesh size based on Table 3-1. It was also reported in other papers that the percent photoreactivation increases with decreasing weighted photo-reactivation fluence (Bohrerova and Linden, 2007). Also, based on Figure 3-8a, there is a plateau region in the photoreactivation curve of the sample without any filter which shrank by using the filter with mesh size of 0.63 mm followed by the filters with mesh size of 0.42 and 0.25 mm under outdoor conditions. Because the photoreactivation fluence of natural sunlight was too much, even when using one filter with a mesh size of 0.25 mm compared to the sunlight lamp, various

numbers of filters with a mesh size of 0.25 mm were also used. As shown in Figure 3-8a, not only the inactivation effect of sunlight was offset by using three such filters, but also there was an increase in the percent photoreactivation by using such four filters. This indicates that the percent photoreactivation increases significantly with decreasing ERF to 1 J/cm^2 by using four filters (0.25 mm mesh size) (Table 3-1). Bohrerova and Linden (2007) also reported this issue by using several sunlight lamps with various emission spectra. However, this experiment shows that it is also possible to reduce the reactivation fluence of natural sunlight to the level of the sunlight lamps and achieve the same results by using four filters (mesh size of 0.25 mm). Also, based on Figure 3-8a, the plateau region shrank by using three filters (0.25 mm mesh size) followed by four such filters under outdoor conditions.

Table 3-1: Effect of various filters on transmittance, ERF and integrated ESI after 4 h exposure

Filter mesh size (mm)	Number of filters	Transmittance (%)	Outdoor		Indoor	
			Integrated ESI (mW/cm^2)	ERF after 4 h exposure (J/cm^2)	Integrated ESI (mW/cm^2)	ERF after 4 h exposure (J/cm^2)
-	0	100.0	5.2	73.7	0.46	6.6
0.63	1	40.0	2.0	29.1	0.18	2.6
0.42	1	31.6	1.6	22.7	0.14	2.0
0.25	1	26.6	1.3	18.9	0.12	1.7
	2	11.8	0.64	9.2	0.06	0.86
	3	2.9	0.15	2.2	0.01	0.20
	4	0.95	0.05	0.75	0.005	0.07

Another important issue is that there was an increase and then a decrease in the number of bacteria by using various filters with mesh sizes of 0.63, 0.42, 0.25 mm, and also two filters with mesh size of 0.25 mm under outdoor conditions (Figure 3-8a). The increase undoubtedly arises from the effect of photo-reactivation, and the decrease arises from the impact of extended sunlight exposure. Research shows that the deep UV portion of sunlight can cause inactivation after a long exposure time to sunlight (Yoon *et al*. 2007). Moreover, there was another increase after the decrease. This may result from the effect of nutrients which can improve bacterial growth, or the ability of bacteria to recover their cultivability (Suß *et al.* 2009). Another effective factor on this issue could be exposure time because photolyase should frequently attach to the dimmers for the repair process (Oguma *et al*., 2004). However, the last decrease in the number of bacteria could result from the lack of nutrients or the high ERF arising from the long exposure time to sunlight (Suß *et al*. 2009; Bohrerova and Linden, 2007). Bosshard *et al.* (2010) also showed that UV–A light fluence more than 300 kJ/m^2 (30 J/cm^2) can cause inactivation in *E. coli* cell because of the loss of membrane potential, glucose uptake activity and culturability of the cells.

The results of indoor experiments versus the ERF (Figure 3-8b) showed that the plateau region shrank by decreasing the filter mesh size and increasing the number of filters (0.25 mm mesh size). By comparing the percent photoreactivation under sunlight and the sunlight lamp (compare Figure 3-8a with Figure 3-8b), it can be seen that the percent photoreactivation decreased versus the ERF after 4 h of solar radiation, while it increased under the sunlight lamp.

This may result from the lack of a UV–B portion in the spectrum of the sunlight lamp and also the low ERF of the indoor sunlight lamp compared to sunlight (Bohrerova and Linden, 2007; Herndle *et al*., 1997). Based on Figure 3-8, the percent photoreactivation of bacteria was lower under indoor conditions compared to the outdoor conditions. Bohrerova and Linden (2007) also observed various levels of photoreactivation under several sunlight lamps and sunlight with the same level of weighted photoreactivation fluence. In addition, Bosshard *et al.* (2009) showed that the effect of UV-A (320–400 nm) on inactivating *E. coli* cells is slightly more under sunlight lamp compared to sunlight. As mentioned in section 3.3.2.2, because of the low irradiance of the sunlight lamp compared to that of natural sunlight, the exposure time was extended to 10 h under the sunlight lamp compared to 4 h under sunlight. As mentioned in Chapter 1, the photoreactivation process has a rapid rate. So, *photolyase* enzymes probably lose their activity during a long exposure time, which leads to a lower bacterial percent photoreactivation under the sunlight lamp.

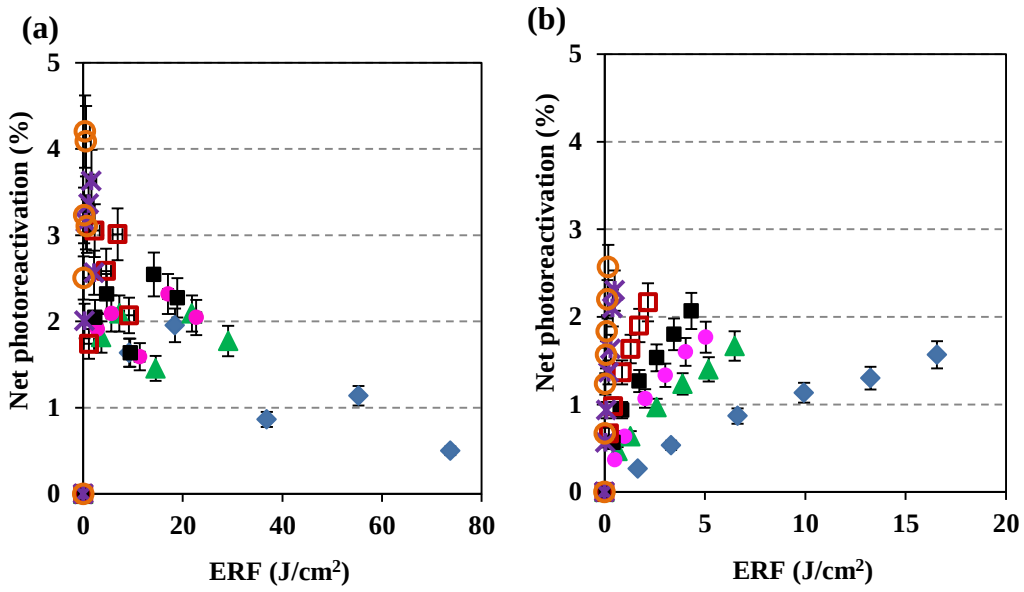

Figure 3-8: Effect of using various filters on the net photoreactivation of total coliforms based on the ERF under natural sunlight (a) and the sunlight lamp (b) after applying 23 mJ/cm^2 inactivation fluence as determined by the target wastewater plant. ♦ full light; ▲ 1 filter (0.63 mm mesh size); • 1 filter (0.42 mm mesh size); ■ 1 filter (0.25 mm mesh size); □ 2 filters (0.25 mm mesh size); * 3 filters (0.25 mm mesh size); O 4 filters (0.25 mm mesh size)

Figure 3-9 shows the results of all indoor and outdoor experiments. It can be seen that the reactivation of bacteria occurred before 20 J/cm^2 ERF and inactivation occurred after that. Bohrerova and Linden (2007) observed the same trend for photoreactivation of pure cultured *E. coli* ATCC 11229 after applying 10 mJ/cm^2 inactivation fluence under several sunlight lamps and sunlight as shown in Figure 3-10. However, the inactivation started after 2 J/cm^2 ERF for *E. coli* ATCC 11229. This may arise from the lack of nutrients in pure cultured sample of *E. coli*, while higher nutrients value in the wastewater effluent sample can help bacteria to grow under higher ERF values. Also, as shown in Figure 3-9, the percent photoreactivation is less than 5% after applying a 23 mJ/cm^2 inactivation

fluence under both indoor and outdoor conditions. Because the 23 mJ/cm^2 inactivation fluence is close to the average inactivation fluence for the target wastewater plant determined in this study (25.5 mJ/cm^2), these results show a low level of photoreactivation for the target wastewater plant. Also, the percent photoreactivation was less than 1 percent for *E. coli* ATCC 11229 after applying a 10 mJ/cm^2 inactivation fluence based on Figure 3-10. This result is different from that of Bohrerova and Linden (2007) because they used a different strain of *E. Coli,* which was inactivated by applying a 10 mJ/cm^2 inactivation fluence and showed a very low level of photoreactivation.

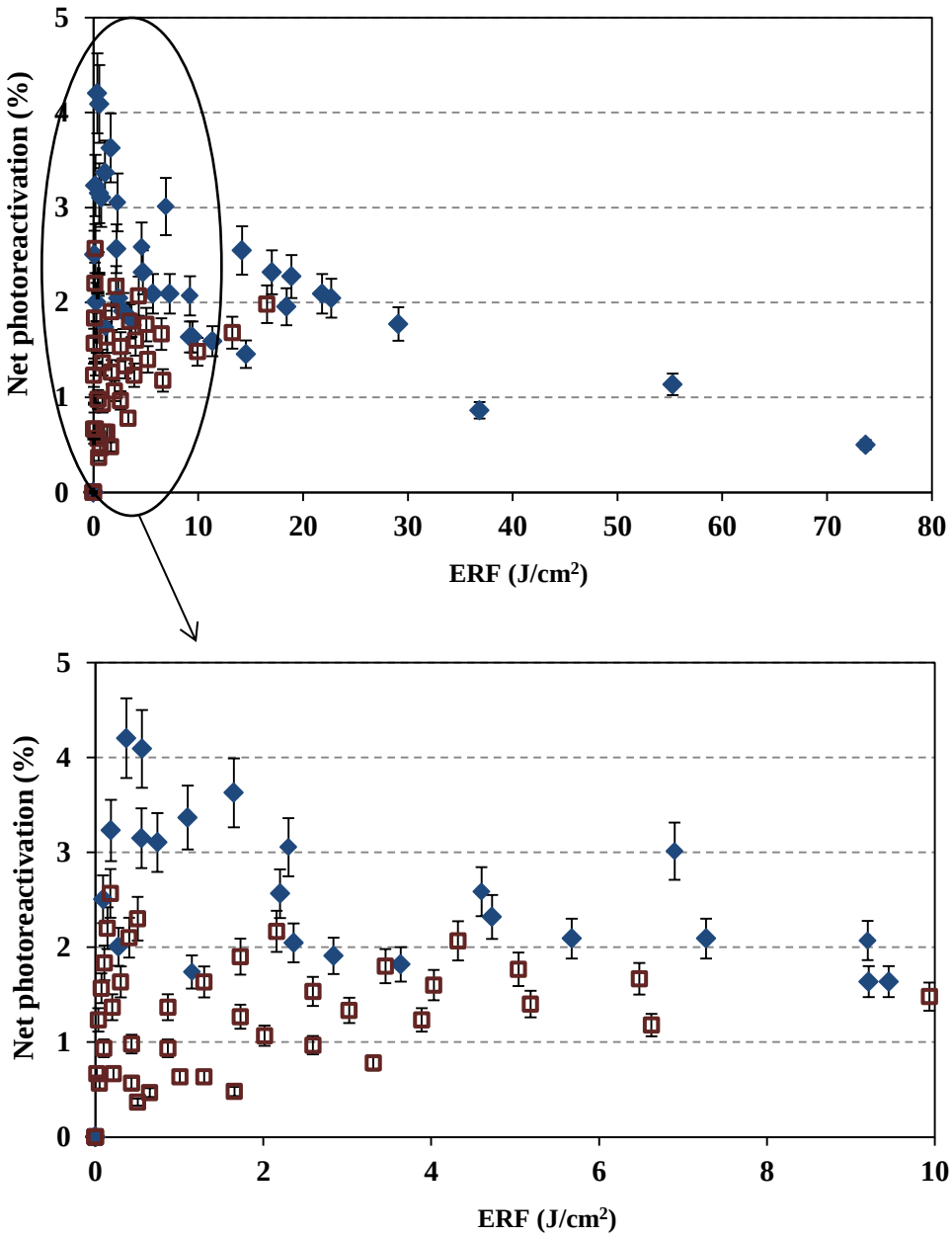

Figure 3-9: Effect of using various filters on the net photoreactivation of total coliforms based on the ERF under natural sunlight (♦) and the sunlight lamp (□) after applying 23 mJ/cm^2 inactivation fluence as determined by the target wastewater plant.

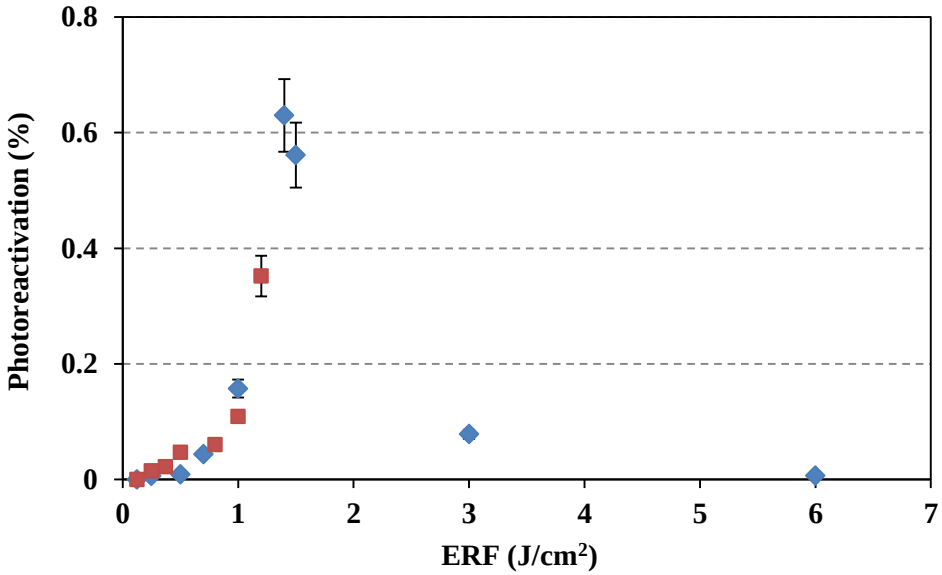

Figure 3-10: Photoreactivation of *E. coli* ATCC 11229 under the sunlight lamp (■) and sunlight (♦) (plotted from the data presented in Bohrerova and Linden (2007))

3.4.4. Effect of temperature

The results for the percent dark reactivation and net photoreactivation of total coliforms in the effluent of the target wastewater plant at five temperatures under indoor and outdoor conditions versus the ERF are presented in Figure 3-11 (See raw data in Tables A-9 to A-12 of Appendix A). It can be seen that the net photoreactivation and dark repair of total coliforms increases with increasing temperature under both outdoor and indoor conditions. This arises from the temperature effect on the reactivation process (Salcedo *et al.* 2007). This characteristic was also reported in other research studies (Chan and Killick, 1995; Locas *et al.*, 2008; Salcedo *et al.*, 2007). Both photoreactivation and dark reactivation increased significantly after 15 °C. Also, comparing Figure 3-8 with Figure 3-11, it can be seen that the percent photoreactivation at 20 ºC is lower at the same ERF in Figure 3-11 compared to Figure 3-8. It could be because of

different wastewater quality (turbidity and nutrients) or different bacterial growth rate in different samples (Berney *et al.,* 2006). Another important issue is that the net percent photoreactivation of total coliforms was found to be higher under outdoor conditions than that under indoor conditions. Based on the reciprocity-law, the results should only be a function of the total radiant energy (fluence), and be independent of irradiance and time (Zetterberg, 1964). However, Peak and Peak (1982) showed that this assumption is incorrect for *E. coli* cells exposed to monochromatic UV–A light at a wavelength of 365 nm. Also, based on the second low of photochemistry, the total amount of photoreaction should be directly proportional to the product of the absorbed photon flow and the time of illumination (Bolton, 2010). However, the results of this study at various temperatures showed that the second low of photochemistry and the reciprocity-law are not followed for the photoreactivation experiments in the wavelength range of 300–500 nm.

The integrated effective irradiance under indoor conditions was 0.21 mW/cm^2 compared to that under outdoor conditions that was about 0.42 mW/cm^2. So, the exposure time under indoor conditions was doubled to achieve the same ERF values under indoor and outdoor conditions. However, the percent photoreactivation was twice under outdoor conditions compared to the indoor conditions at various temperatures. So, the reciprocity-law and the second low of photochemistry cannot be applicable for the photoreactivation. As mentioned in the first chapter of this study, during photoreactivation process an enzyme called *photolyase* absorbs near ultraviolet and visible light to initiate an enzymatic

reaction for repairing the pyrimidine dimers in DNA by using light energy according to the classical Michaelis–Menten reaction (Sancar, 2000). This reaction has a rapid rate. This means that however *photolyase* needs an adequate time to absorb light and initiate the repair reaction, it loses its activity by extending exposure time to high values. Hence, the results of photoreactivation experiments under indoor conditions with higher exposure time were lower than that under outdoor experiments with lower exposure time in the same range of ERF. Other possible reasons for the higher percent photoreactivation of bacteria under outdoor conditions compared to indoor conditions could be some inaccuracy in the action spectrum used for calculating the ERF values and the effect of dark processes or other photochemical processes that are involved at high light irradiance and could influence the photoreactivation results.

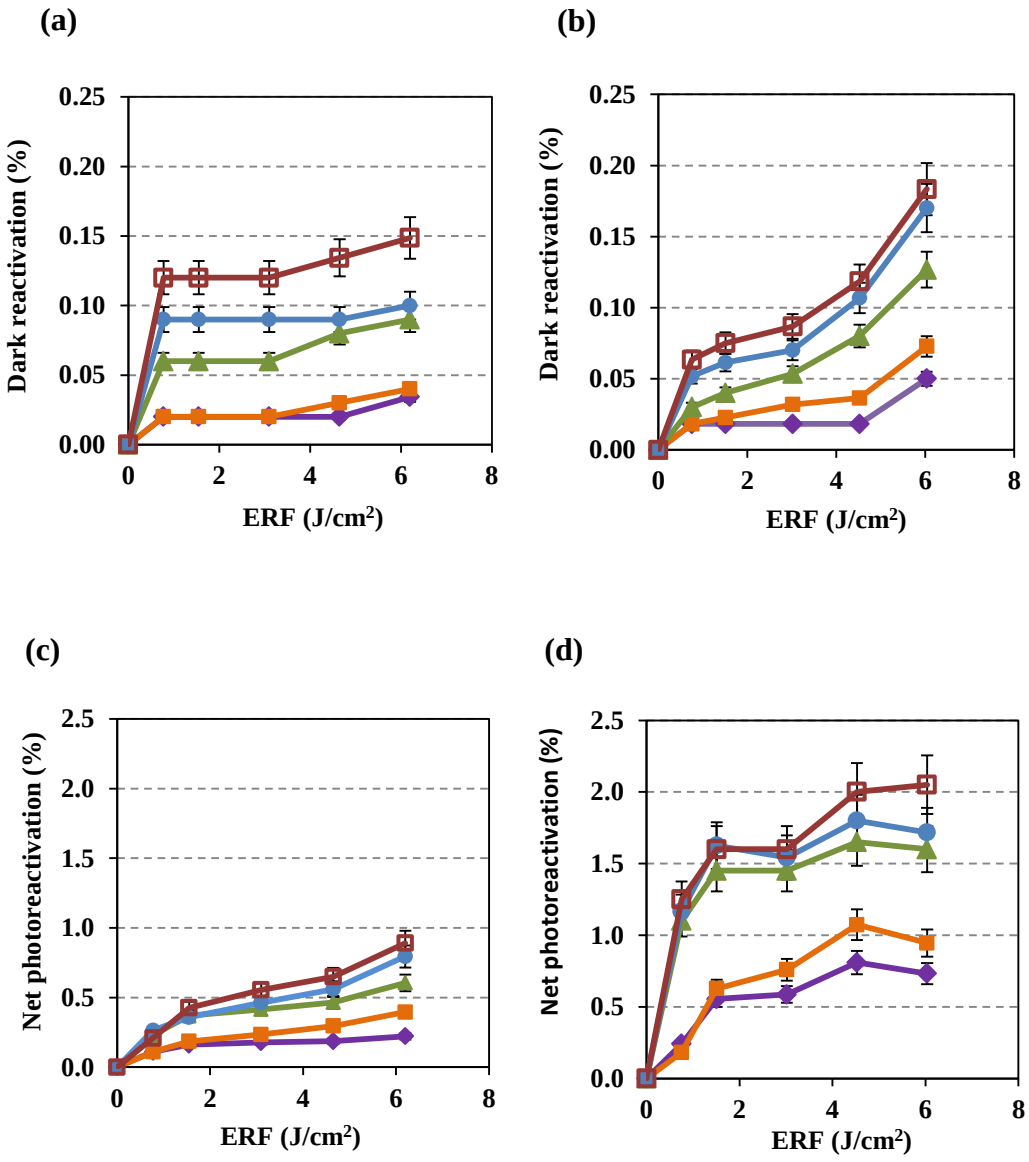

Figure 3-11: Dark reactivation (a,b) and net photoreactivation (c,d) of total coliforms at various temperatures under a sunlight lamp (a,b) and under natural sunlight (c,d) after applying a 23 mJ/cm^2 inactivation fluence by the target wastewater plant. ♦ 5 ºC; ■ 10 ºC; ▲ 15 ºC ; ● 20 ºC; □ 25 ºC

3.5. Conclusions

These experiments indicated that the inactivation fluence determined in the laboratory was essentially the same as that reported in the target wastewater plant, which was about 25 mJ/cm^2. Also, the effect of inactivation fluence, ERF, and temperature on the percent photoreactivation was assessed. This study demonstrated that the percent photoreactivation decreases as the inactivation fluence increases. This results from the damage in the microorganism cells caused by high inactivation fluence, which is too extensive to be repaired during the photoreactivation process. Furthermore, the results suggest that the percent photoreactivation increases with increasing temperature because high temperatures enhance the reactivation process. However, the percent photoreactivation was lower under indoor conditions than that under outdoor conditions. Also, the effect of various filters on integrated irradiance and transmission factor of sunlight proved that the percent photoreactivation of bacteria increases with decreasing ERF under both indoor and outdoor conditions.

These results provide the fundamentals for designing conditions and parameters for later studies, which focused on determination of the percent photoreactivation of bacteria in a mixture of effluent and river water in the following chapters.

3.6. References

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CHAPTER 4
EFFECT OF VARIOUS COVERS ON PHOTOREACTIVATION

4.1. Introduction

In most papers, the wavelength range of about 310–480 nm has been reported as the wavelengths that activate photoreactivation; however, there are some contradictions about the exact inactivation and photoreactivating wavelengths (Liltved and Landfald, 2000). Herndl *et al*. (1997) found that UV-A (315–400) causes photorepair as soon as the effect of UV-B (280–315) stress on bacteria is released. In addition, Sinton *et al.* (1994) found that the effect of UV-B portion of sunlight on the inactivation of *E. coli* is twice that of UV-A. In another study, Sinha and Häder (2002) stated that small amounts of UV-B can induce adverse impacts on living systems. In addition, Yoon *et al*. (2007) stated that the effect of natural sunlight on bacteria is more inactivation rather than reactivation. However, Sancar and Sancar (1988) mentioned that the most effective wavelength range for the photorestoration of dimers in *E. coli* is 365–400 nm. Also, Tosa and Hirata (1999) stated that light at a wavelength of about 360 nm has the most important effect on photoreactivation of *E. coli*. In another study, Sinton *et al.* (1994) investigated the effect of optical filters on inactivation of fecal coliforms in wastewater samples. They used various optical filters including polyester, glass, acrylic, and polycarbonate, which could cut off wavelengths below 318, 337, 342,

and 396, respectively. They found that the inactivation decreased for the filters that have more spectral cut off compared to the others. The lowest level of inactivation was observed for polycarbonate followed by acrylic, glass, and polyester. In another research study, Herndl *et al*. (1997) showed that bacterial activity declined by 20 to 42% after exposure to both artificial and solar UV-B radiation. However, bacterial activity increased after exposure to UV-A following UV-B exposure. So, comparing these research studies, it would be important to block the UV-B portion of sunlight and investigate its effect on the percent photoreactivation of bacteria.

4.2. Objectives

Considering the effect of various wavelengths on photoreactivation, the principal objective of this chapter was to cut off the UV-B portion of the light by using a polyethylene terephthalate (PET) bottle and investigate the effect of this issue on the photoreactivation of bacteria under both indoor and outdoor conditions. The results were also compared with the results of using a Pyrex® lid and Saran Wrap®, which can transmit all wavelengths above 300 nm. In addition, three mesh filters with a size of 0.25 mm were used to investigate the effect of reducing the reactivation fluence rate and various covers simultaneously.

4.3. Materials and Methods

The kind of wastewater samples and UV source and apparatus were described in Chapter 2, sections 2.2 and 2.3. The transmission spectra of the filters were

determined by scanning the filter materials from 300 to 500 nm compared to an air reference by using a UV spectrophotometer (Varian Cary 50 Bio).

4.3.1. UV exposures

For the designed UV exposure time, water samples (25 mL) contained in Petri dishes (diameter: 60 mm) were put under the collimating tube and gently stirred. An inactivation fluence of 10 mJ/cm^2 was applied by adjusting exposure time to a precalculated value, while the irradiance was fixed at 0.14 mW/cm^2 (corrected by the sensor factor) throughout the experiments. All experiments were carried out at room temperature (20 ± 1 ºC).

4.3.2. Photoreactivation experiments

Total coliforms were used as the representative bacteria. Following application of the 10 mJ/cm^2 inactivation fluence, the water samples were transferred to containers. Three types of containers including PET bottles, Pyrex® dishes with a Pyrex® lid, and a Pyrex® dish covered with Saran Wrap® were used to compare the effect of various covers on the percent photoreactivation of total coliforms. Also, three mesh filters with a size of 0.25 mm were applied on the top of the samples covered with the three covers (PET, Pyrex®, and Saran Wrap®) to investigate the effect of reducing the reactivation fluence and various covers at the same time. The containers were put in a water bath. The water bath temperature was controlled by ice pieces and kept at 20 ± 1°C. Dark controls were employed with a black plastic cover over each control sample. The experiments were carried out under both indoor and outdoor conditions. For indoor conditions, a sunlight

lamp was positioned 10 cm above the samples. Water samples were collected from the dishes every hour for up to 4 h. Also, a sample was collected in the first half hour.

4.3.3. Culturing and counting microorganisms and quantitative evaluation of photoreactivation

After collecting samples in the photoreactivation experiments, the microorganisms were cultured and counted based on section 2.4 in Chapter 2. Then the percent photoreactivation was calculated based on section 2.5 in Chapter 2.

4.4. Results and Discussion

The transmission spectra of a Pyrex® lid, a PET bottle and Saran Wrap® are presented in Figure 4-1. It can be seen that the PET bottle blocks the wavelengths below 320 nm, but the Pyrex® lid and Saran Wrap® can transmit almost all wavelengths of the light above 300 nm.

The net percent photoreactivation of total coliforms after applying an inactivation fluence of 10 mJ/cm^2 by using various covers with and without three filters with mesh size of 0.25 mm versus the ERF under indoor and outdoor conditions is shown in Figure 4-2 (See raw data in Tables A-13 to A-16 of Appendix A). Based on Figure 4-2a,b, the percent photoreactivation of total coliforms increased by using the PET bottle with or without three mesh filters under outdoor conditions. This arises from the effect of the PET bottle on blocking the wavelength range of 300–320 nm, which includes the UV-B portion of natural sunlight which has an

inactivation effect on bacteria. However, based on Figure 4-2c,d, the differences in the percent photoreactivation of bacteria by using various covers with or without three mesh filters under indoor conditions were not significant ($p > 0.05$ as determined by ANOVA). This results from the lack of the wavelengths below 365 nm in the sunlight lamp spectrum. So using the PET bottle did not affect the percent photoreactivation of bacteria under indoor conditions. In addition, comparing Figure 4-2a,c with Figure 4-2b,d, it can be seen that the percent photoreactivation increased by using 3 mesh filters over each cover under both indoor and outdoor conditions because the filters reduced the reactivation fluence rate and ERF of the light (Bohrerova and Linden, 2007). Another important issue is that the percent photoreactivation decreased gradually after 4 h under outdoor conditions (Figure 4-2a,b) because of high sunlight fluence rate, which could have inactivation effects on bacteria especially at longer exposure times (Yoon *et al*., 2007; Bohrerova and Linden, 2007; Herndle *et al*., 1997). In comparing Fig 4-2c and d, it is clear that the photoreactivation reaches a plateau at an ERF of 0.01 J/cm^2 or less. Fig. 4-2c shows that the plateau extends up to at least 4 J/cm^2. Also, based on Figure 4-2a, the percent photoreactivation of all covers is almost the same after 4 h solar radiation. It is important for application of PET bottles for water disinfection under sunlight because this research study shows that the effect of PET bottle on bacteria at long exposure time is similar to the effect of other covers and it can be used for water disinfection at long exposure time.

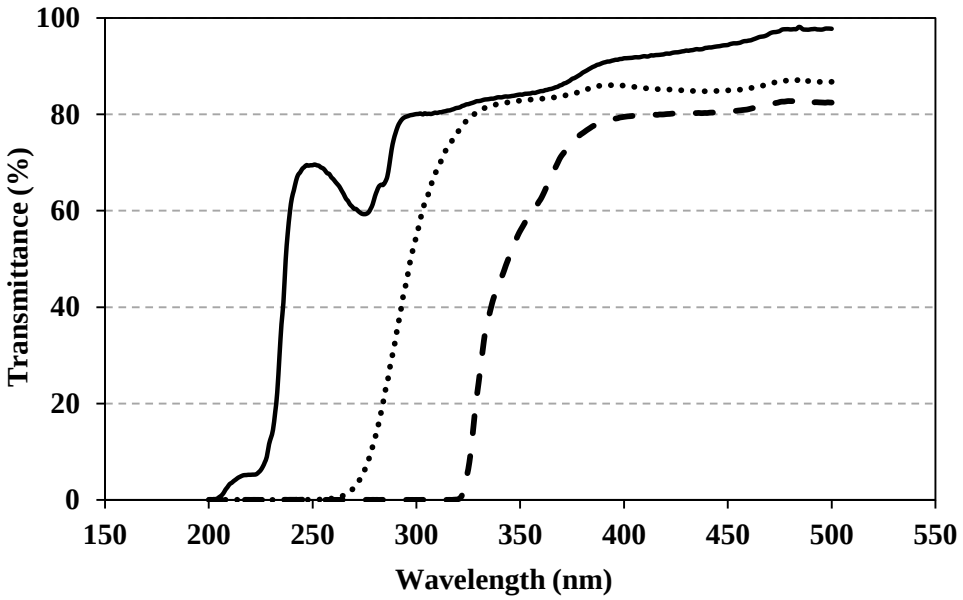

Figure 4-1: Transmittance spectra of a Pyrex® lid (dotted line), a PET bottle (dashed line) and Saran Wrap® (solid line).

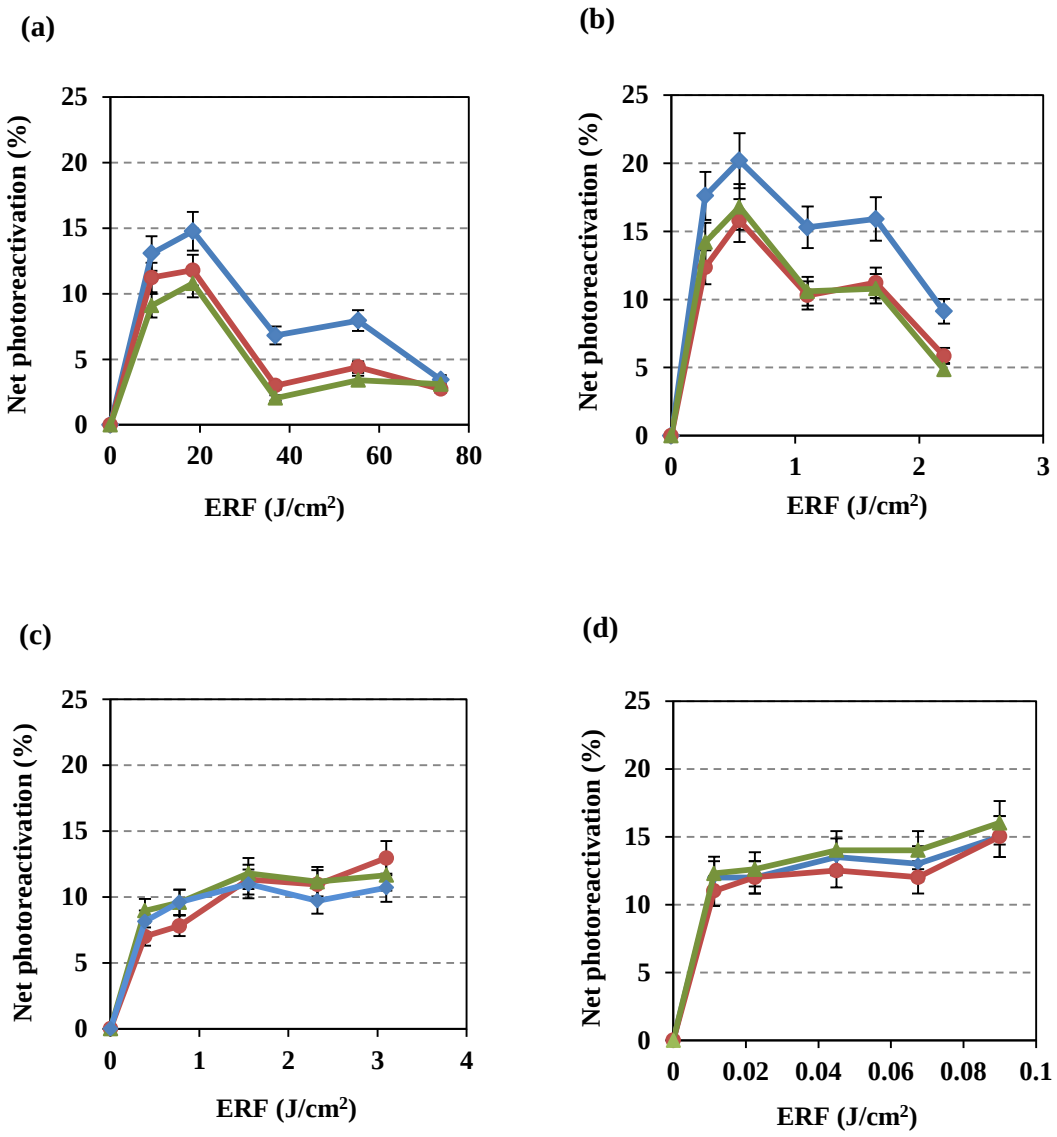

Figure 4-2: Net photoreactivation of total coliforms under natural sunlight (a,b) and under a sunlight lamp (c,d) after applying a 10 mJ/cm^2 inactivation fluence and various covers, (a, c) no mesh filter, (b, d) with three filters (0.25 mm mesh size). ♦ PET bottle; ● Pyrex® lid; ▲ Saran Wrap®

4.5. Conclusions

These experiments demonstrated that the UV-B portion of natural sunlight has an important effect on inactivation of bacteria because the percent photoreactivation

of bacteria increased by using a PET bottle, which blocked this portion of the light. However, the percent photoreactivation of bacteria did not change under indoor conditions by using the PET bottle because sunlight lamp spectrum does not include UV-B portion of the light. Also, these experiments assessed the simultaneous effect of various covers and 3 mesh filters on the percent photoreactivation of bacteria. The results suggested that with the presence of any kind of covers, using the filters increased the percent photoreactivation of bacteria under both indoor and outdoor conditions because of the effect of the filters on reducing the reactivation fluence rate and the ERF.

4.6. References

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CHAPTER 5
PHOTOREACTIVATION OF TOTAL COLIFORMS IN A MIXTURE OF EFFLUENT AND RIVER WATER

5.1. Introduction

Since effluents of most wastewater treatment plants are offloaded to water bodies where UV-treated bacteria are exposed to sunlight, it is important to investigate the effect of the water bodies and their nutrients on photoreactivation of bacteria. However, there are few research studies about this issue. Liltved and Landfald, (2000) investigated the effect of sunlight exposure on photoreactivation of UV-irradiated bacteria and inactivation of non-irradiated bacteria in river water. They found that sunlight has an inactivation effect on non-irradiated bacteria. Also, the level of inactivation of bacteria was increased by adding humic substances to the river water because humic substances caused indirect damage to the bacteria by combination with oxygen. In addition, they showed that sunlight induced a rapid photoreactivation to the UV-irradiated bacteria in the river water during 20 min, but sunlight inactivation effect overtook after that.

In another study, Arana *et al*. (1992) added pure culture *E. coli* to the filtered river water and investigated the effect of visible light of a sunlight lamp on bacteria. They found that however the number of bacteria remained constant under dark conditions, it decreased after a long exposure (24 h) to a sunlight lamp. Also, they showed that the number of bacteria remained constant under the sunlight lamp by

adding catalase that blocked formation of peroxides because hydrogen peroxide generated photochemically, could reduce the cultivability of *E. coli* under the sunlight lamp.

Sinton *et al*., (1994) investigated the effect of a mixture of effluent and seawater on the inactivation of fecal coliforms. They showed that inactivation of bacteria occurred under both dark and sunlight conditions because of osmotic stress under saline conditions. However, the inactivation under sunlight conditions was more than that under dark conditions because of cell wall damage from sunlight exposure.

Herndle *et al.* (1997) also investigated the effect of UV-A and UV-B portions of solar radiation on bacterial activity in filtered seawater. They found that the UV-B portion of sunlight decreased bacterial activity but exposure to UV-A following UV-B could improve bacterial activity. Also, they stated that the UV-B caused photolytic cleavage of dissolved organic matters (DOM) which were taken up by bacteria in the surface layers of the water. However, the inactivation effect of the UV-B in the surface layers reduced bacterial activity. On the other hand, because of the light attenuation by DOM, only UV-A was available for bacteria in the deeper layers. Also, wind-induced turbulence mixed the upper layers of the water column and transferred cleaved DOM into deeper layers. These speculated that these factors caused more bacterial activity in the deeper layers.

5.2. Objectives

Considering offloading wastewater effluents to the water bodies and the effect of various water bodies and their nutrients on photoreactivation of bacteria, the principal objective of this chapter was to investigate the effect of a mixture of spiked filtered river water and effluent on the photoreactivation of bacteria under both indoor and outdoor conditions.

5.3. Materials and Methods

The kind of wastewater samples and UV source and apparatus were described in Chapter 2, section 2.2 and 2.3. Also, river water samples were collected from the North Saskatchewan River.

5.3.1. Methods, reagents and apparatus used for determination of sample characteristics

All experiments were carried out according to the standard methods for the Examination of Water and Wastewater (APHA *et al.*, 2005). Samples alkalinity and bicarbonate concentrations were measured based on titration method (APHA *et al.*, 2005) by using an alkalinity meter (Mettler Toledo, DL53, Switzerland). Samples pH was measured by a pH meter (Accumet AR50, Fisher Scientific). The sample colour was measured based on the platinum-cobalt method (APHA *et al.*, 2005) by adjusting the samples pH to 7.6. A color standard stock (APHA 500, Ricca Chemical Company, USA) was also used for stock solution preparations. The ammonia concentration was determined based on the ammonia selective electrode method using known addition (APHA *et al.*, 2005) by an ammonia pre-

assembled body with a membrane (FSSP9774987, Thermo Scientific, USA) attached to the pH meter probe. Ammonium chloride (Fisher Scientific, certified A.C.S.) was also used for preparation of ammonia stock solution. The concentration of phosphorus was determined based on vanadomolybdo-phosphoric acid colorimetric method (APHA *et al.*, 2005) by using ammonium vanadate-molybdate reagent. In addition, for separation of bacteria from the effluent samples, a centrifuge instrument (Eppendorf centrifuge 5810R, Brinkmann instruments Inc., USA) was used. Also, prior to measuring the absorbance and dissolved organic carbon (DOC) concentrations, the samples were filtered through a pre-rinsed filter (0.45 μm, Millipore, USA) to remove particles and suspended organic matter. A UV spectrophotometer (Varian Cary 50 Bio) was used for measuring the absorbance, and an Apollo 9000 TOC Combustion Analyzer (FOLIO Instruments Inc.) was used for measuring the DOC concentrations. The turbidity of the mixtures was measured by an Orbeco-Hellige 965 Digital Nephelometric Turbidimeter (Orbeco Analytical Systems Inc., Sarasota, FL, USA).

5.3.2. UV exposures

For the designed UV exposure time, water samples (25 mL) contained in Petri dishes (diameter: 60 mm) were put under the collimating tube and gently stirred. An inactivation fluence of 10 mJ/cm^2 was applied by adjusting the exposure time to a precalculated value, while the irradiance was fixed at 0.14 mW/cm^2 (corrected by the sensor factor) throughout the experiments. All experiments were carried out at room temperature (20 ± 1 ºC).

5.3.3. Photoreactivation experiments

To compare the effect of a sunlight lamp with natural sunlight, the experiments were carried out under both indoor and outdoor conditions. Total coliforms were used as the representative bacteria. River water was filtered through a membrane (0.45 μm, Millipore, USA) to remove river water bacteria. Following application of a 10 mJ/cm^2 inactivation fluence, effluent bacteria cells were separated by centrifugation at 10000 RPM for 45 min. Then, filtered river water samples were spiked with the separated cells to the final concentration as the effluent samples. After that, 6 samples including effluent, spiked filtered river water, and 4 compositions of 20, 40, 60, and 80% effluent mixed with the spiked filtered river water were prepared. Water samples were transferred to Pyrex® dishes and then covered with Saran Wrap® to avoid sample evaporation. Then, the dishes were put in a water bath. The water bath temperature was controlled by ice pieces and kept at 20 ± 1°C. Dark controls were employed with a black plastic cover over each control sample. To adjust the sunlight lamp ERF to the level of natural sunlight ERF, two filters with a mesh size of 0.25 mm were used. The natural sunlight integrated effective irradiance was 4.8 mW/cm^2. So, two filters with a mesh size of 0.25 mm were put on top of each Pyrex® dish to reduce the integrated effective irradiance of sunlight to 0.42 mW/cm^2. Also, because of the lower integrated effective irradiance of the sunlight lamp (0.21 mW/cm^2) than that for natural sunlight even by using two filters (0.42 mW/cm^2), the exposure time under the sunlight lamp was extended to 8 h instead of 4 h. By this way, the sunlight lamp ERF in 8 h (6.2 J/cm^2) was almost adjusted to the level of natural sunlight ERF in

4 h (6.1 J/cm^2). Considering the larger exposure time under the sunlight lamp, water samples were collected from the dishes every 2 h for up to 8 h. A water sample was also collected in the first hour (this was considered as 0.5 h). For the outdoor conditions water samples were collected from the dishes every hour for up to 4 h.

5.3.4. Culturing and counting microorganisms

After collecting samples in the photoreactivation experiments, the microorganisms were cultured and counted based on section 2.4 in Chapter 2.

To confirm the presence of *E. coli* bacteria in total coliforms colonies, the standard test method (APHA *et al.*, 2005, method 8074) was used. Based on this method, membrane filters with total coliforms colonies transferred to MUG agar and incubated for 4 h at 35 ± 0.5 °C. Then a flash light UV lamp with long wavelength light at 366 nm was used to recognize *E. coli* colonies grown in the total coliforms colonies.

5.3.5. Quantitative evaluation of photoreactivation

After counting the microorganisms, the percent photoreactivation was calculated based on section 2.5 in Chapter 2.

5.4. Results and Discussion

The net percent photoreactivation of total coliforms versus the ERF in the mixtures of the effluent and spiked filtered river water after applying a 10 mJ/cm^2 inactivation fluence under both indoor and outdoor conditions is shown in

Figure 5-1 (See raw data in Tables A-17 to A-20 of Appendix A). It can be seen that the percent photoreactivation of the mixtures decreased by increasing the percentage of spiked filtered river water under both indoor and outdoor conditions. This arises from lower level of nutrients in the spiked filtered river water; this will be discussed in the following section. In addition, the samples including spiked filtered river water are more transparent than the effluent sample because of lower nutrients, colour and turbidity values (Tables 5-1 and 5-2). The dilution effect of the spiked filtered river water on the mixtures could intensify the reverse effect of the ERF on bacteria by making the samples more transparent and transferring more light to the bacteria under both indoor and outdoor conditions. As was mentioned in previous chapters, the percent photoreactivation of the bacteria decreases with increasing ERF (Bohrerova, and Linden, 2007). Also, by comparing Figure 5-1a with Figure 5-1b, it can be seen that the percent photoreactivation of the effluent decreased more significantly and sharply with increasing spiked filtered river water content under outdoor conditions compared to indoor conditions. This is because the dilution effects of spiked filtered river not only increased the reverse effect of ERF under outdoor conditions but also intensified the inactivation effect of UV-B portion of sunlight on bacteria. So, sunlight penetration and consequently inactivation effect of UV-B portion of sunlight on bacteria increased in these samples which decreased the percent photoreactivation of bacteria (Arana *et al.*, 1992; Herndle *et al.*, 1997).

The percent photoreactivation of the mixtures under the sunlight lamp was less than that under sunlight, which discussed in Chapter 3 in section 3.4.4.

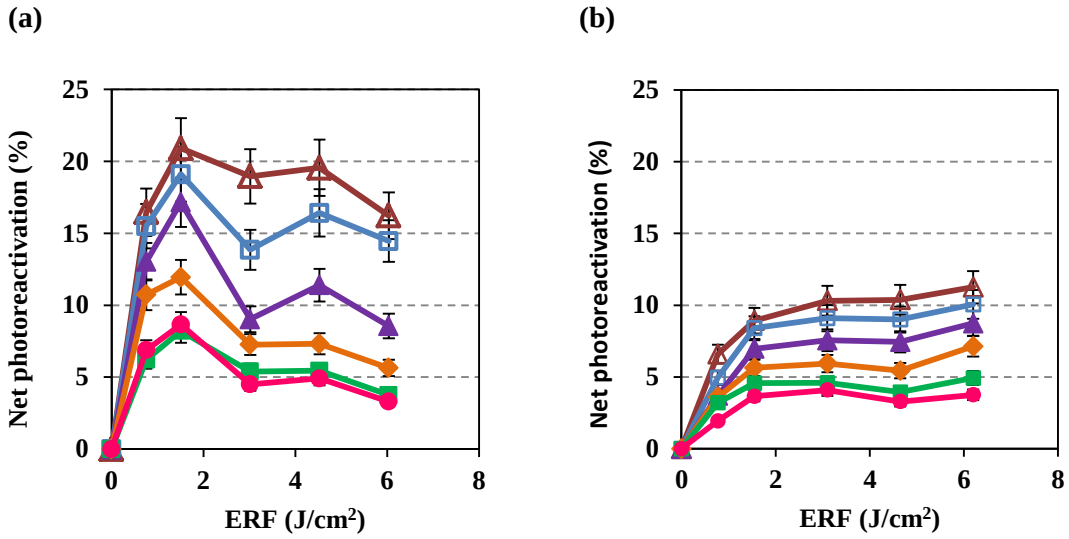

Figure 5-1: Net photoreactivation of total coliforms under natural sunlight (a) and under a sunlight lamp (b) after applying a 10 mJ/cm^2 inactivation fluence. ● 100% spiked filtered river water; ■ 80% spiked filtered river water + 20% effluent; ♦ 60% spiked filtered river water + 40% effluent; ▲ 40% spiked filtered river water + 60% effluent; □ 20% spiked filtered river water + 80% effluent; Δ 100% effluent.

5.4.1. Effect of nutrients on the percent photoreactivation of the mixtures

The characteristics of all mixtures including spiked filtered river water were measured based on the standard methods for the Examination of Water and Wastewater (APHA *et al.*, 2005) and presented in Table 5-1 and Table 5-2.

Based on the *E. coli* confirmation test method, all of the total coliforms bacteria presented in the samples contained *E. coli*. The principal *E. coli* bacterial cell requirements are carbon, phosphorus, nitrogen, and calcium (Murray *et al.*, 2003; Cano and Colome, 1986). So, the concentration of dissolved organic carbon, orthophosphates, ammonia and alkalinity as calcium carbonate were measured. Other parameters, such as UV absorption coefficient at 254 nm

(UV_{254}), turbidity, colour, and pH were also measured to compare the mixtures including spiked filtered river water with the effluent and spiked filtered river water samples. The UV_{254} and the DOC concentrations of the mixtures are presented in Table 5-1. Based on the data collected from a number of water sources and references, there is a good correlation between the UV_{254} and the DOC concentrations, and the UV_{254} increases with increasing DOC concentration (Zuo and Jones, 1997; Liu and Fitzpatrick, 2010; Liu, 2010). According to Table 5-1, it can be seen that the UV_{254} and the DOC concentrations of the mixtures decreased with increasing percent spiked filtered river water. This issue could be one of the reasons for decreasing the percent photoreactivation of the mixture with increasing spiked filtered river water.

Another important issue is the effect of specific UV absorbance (SUVA), which is the ratio of UV_{254} to DOC concentrations. The SUVA value indicates aromatic carbon content and hydrophobicity of water samples (Bazrafshan *et al*., 2012). As shown in Table 5-1, the SUVA values were almost constant for the mixtures including spiked filtered river water. Research shows that bacteria prefer non-aromatic carbon sources (Park *et al*., 2010). So, the mixtures including spiked filtered river water not only have lower DOC content, but also they have higher aromatic carbon content. This would be another reason for the lower percent photoreactivation of bacteria in the mixtures including spiked filtered river water.

Ammonia and orthophosphates concentrations were also measured and are presented in Table 5-2. *E. coli* cells require them for proteins and nucleic acid synthesis and cell replications (Cano and Colome, 1986). *E. coli* cells use

phosphorus for survival mechanisms, such as transport of nutrients into the cell, biofilm formation, and motility. The minimum required concentration of phosphorus for *E. coli* cells is 5 μg/L (Juhna *et al*., 2007). Research shows that the survival of culturable *E. coli* in the water samples will be prolonged by higher phosphorus concentrations (Juhna *et al*., 2007). In addition, *E. coli* prefers ammonia as a nitrogen source to synthesise its own amino acids (Yuan *et al*., 2009). Yuan *et al*. (2009) showed that using 2 mM (36 mg/L) ammonium limited *E. coli* cell growth while using 10 mM (180 mg/L) of ammonium restore cell growth rate. Based on Table 5-1, it can be seen that the ammonia and orthophosphate concentrations of the mixtures decreased with increasing percent spiked filtered river water. So, another reason for decreasing the percent photoreactivation of the samples with increasing the percent spiked filtered river water is lack of ammonia and phosphorus concentrations that *E. coli* cells need for the cell growth and replication.

The alkalinity values of the mixtures that were principally caused by bicarbonate were also presented in Table 5-2. Based on Table 5-2, the alkalinity values and bicarbonate concentrations of the samples decreased with increasing spiked filtered river water. Research shows that *E. coli* cells need low quantities of calcium (Murray *et al*., 2003). However, calcium concentration higher than 120 mg/L causes inhibition of cellular metabolism because of an accumulation of minerals (Huang and Pinder, 1995). The highest amount of $CaCO_3$ in the mixtures is about 295 mg/L, which corresponds to 118 mg/L of calcium. Also, based on Table 5-2, bicarbonate concentration in all samples is less than 6 mM. Arthurs *et*

al. (2001) stated that bicarbonate is a relatively nonreactive monovalent ion, and concentrations up to 180 mM do not have a serious impact on *E. coli* viability.

The color and turbidity values of the samples were also measured and are presented in Table 5-2. The color and turbidity values of spiked filtered river water were less than those for the effluent. Also, the color and turbidity values of the mixtures decreased with increasing spiked filtered river water. This arises from lower nutrient levels in spiked filtered river water.

Finally, the pH values of the samples were also measured and are presented in Table 5-2. It can be seen that the pH of all samples is between 7 and 8. Research shows that *E. coli* photoreactivating enzymes are active in pH range of 5.5 to 8.5, with an optimum at 7.2 (Sutherland *et al.*, 1973).

Table 5-1: Characteristics of effluent, river water, and mixtures of effluent with spiked filtered river water

Sample ID	DOC (mg/L)	UV_{254} (cm^{-1})	SUVA (L/mg.m)	Orthophosphates (mg/L)	Ammonia concentration (mg/L)
Effluent	17.7 ± 0.2	0.25 ± 0.02	1.40	1.5 ± 0.1	0.11 ± 0.01
Spiked filtered river water	15.5 ± 0.2	0.22 ± 0.02	1.42	1.0 ± 0.1	0.04 ± 0.01
Filtered river water	14.7 ± 0.2	0.21 ± 0.02	1.46	0.8 ± 0.1	0.03 ± 0.01
80% Effluent + 20% Spiked filtered river water	17.2 ± 0.2	0.24 ± 0.02	1.40	1.4 ± 0.1	0.10 ± 0.01
60% Effluent + 40% Spiked filtered river water	16.7 ± 0.2	0.23 ± 0.02	1.40	1.3 ± 0.1	0.06 ± 0.01
40% Effluent + 60% Spiked filtered river water	16.3 ± 0.2	0.23 ± 0.02	1.40	1.1 ± 0.1	0.04 ± 0.01
20% Effluent + 80% Spiked filtered river water	15.6 ± 0.2	0.22 ± 0.02	1.42	1.0 ± 0.1	0.03 ± 0.01

Table 5-2: Characteristics of effluent, river water, and mixtures of effluent with spiked filtered river water

Sample ID	Alkalinity (mg $CaCO_3$/L)	Bicarbonate concentration (mM)	Color (CU)	Turbidity (NTU)	pH
Effluent	294.5 ± 0.2	5.4 ± 0.2	51.5 ± 0.1	10.1 ± 0.1	7.4 ± 0.1
Spiked filtered river water	246.0 ± 0.2	4.6 ± 0.2	36.5 ± 0.1	9.6 ± 0.1	7.9 ± 0.1
Filtered river water	217.0 ± 0.2	4.2 ± 0.2	31.5 ± 0.1	9.3 ± 0.1	7.9 ± 0.1
80% Effluent + 20% Spiked filtered river water	285.1 ± 0.2	5.3 ± 0.2	48.0 ± 0.1	10.0 ± 0.1	7.5 ± 0.1
60% Effluent + 40% Spiked filtered river water	273.7 ± 0.2	5.2 ± 0.2	44.5 ± 0.1	9.9 ± 0.1	7.5 ± 0.1
40% Effluent + 60% Spiked filtered river water	257.5 ± 0.2	5.0 ± 0.2	41.0 ± 0.1	9.8 ± 0.1	7.6 ± 0.1
20% Effluent + 80% Spiked filtered river water	248.0 ± 0.2	4.8 ± 0.2	37.5 ± 0.1	9.6 ± 0.1	7.7 ± 0.1

5.5. Conclusions

These experiments demonstrated that the percent photoreactivation of the bacteria in the effluent samples decreased with increasing proportions of spiked filtered river water under both indoor and outdoor conditions because of their lower nutrient values, such as DOC, phosphorus and ammonia, compared to the effluent. Another reason for the lower percent photoreactivation of bacteria in the mixtures including spiked filtered river water under outdoor conditions is that river water increased the dilution factor, the reverse effect of the ERF, and the inactivation effect of the UV-B portion of sunlight on bacteria by transferring more sunlight to the samples. Also, dilution effect of the river water increased the reverse effect of the ERF on the mixtures and decreased the percent photoreactivation of the bacteria under indoor conditions.

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CHAPTER 6
GENERAL CONCLUSIONS

6.1. General overview

As mentioned in previous chapters, the inactivation fluence has important effect on the photoreactivation of bacteria. Higher inactivation fluences are required for inactivation of bacteria under conditions when there is the possibility of photoreactivation (Hijnen *et al.,* 2006; Harris *et al.,* 1987; Tosa and Hirata, 1999; Hoyer, 1998). So, the laboratory inactivation fluence for the target wastewater plant was determined to estimate the percent photoreactivation.

One of the principal methods that can be used to control photoreactivation is the application of sufficiently high inactivation fluences (Hu and Quek, 2008; Guo *et al*., 2008; Yoon *et al*., 2007; Locas *et al*., 2008). Thus, the effect of various inactivation fluences (5 to 20 mJ/cm^2) on the percent photoreactivation of bacteria was investigated.

Bohrerova and Linden (2007) showed that the higher average irradiance and consequently the higher weighted photoreactivation fluence of sunlight (more than 2 J/cm^2) during exposure time reduced the percent photoreactivation of bacteria by comparing the effect of sunlight and several indoor lamps on the photoreactivation. Hence, the effect of reducing reactivation fluence rate of sunlight and a sunlight lamp by using filters with various mesh sizes was studied and compared. Also, a concept, namely the 'effective reactivation fluence' (ERF)

based on weighting the spectral fluence rate by the action spectrum for photoreactivation, was introduced. Then, the effect of various filters and conditions on photoreactivation of bacteria was investigated and compared based on ERF.

Research shows that higher temperatures increase significantly the percent photoreactivation of bacteria (Locas *et al*., 2008; Salcedo *et al*., 2007). Therefore, the effect of various temperatures (5 to 25 ºC) on the photoreactivation of bacteria under indoor and outdoor conditions was investigated and compared.

Herndl *et al*. (1997) showed that bacterial activity declined after exposure to solar UV-B radiation. Sinton *et al.* (1994) also showed that bacterial inactivation under sunlight radiation decreased by using filters which have more spectral cut off compared to the others. So, the effect of blocking UV-B portion of sunlight by using a PET bottle on the percent photoreactivation of bacteria was studied and compared with other covers including a Pyrex® lid and Saran Wrap®. The results were also compared with the indoor conditions.

Liltved and Landfald (2000), Arana *et al*. (1992), Sinton *et al*. (1994), and Herndle *et al.* (1997) observed bacterial inactivation under sunlight or a sunlight lamp in various water bodies including river water or seawater. Considering these research studies and the fact that effluents of most wastewater treatment plants are offloaded to the water bodies after UV disinfection, the effect of mixtures of river water and effluent on the photoreactivation of bacteria under both indoor and outdoor conditions was investigated.

6.2. Summary of findings of this work

A general summary of the whole research is given below based on the experimental results and the conclusions of previous chapters.

6.2.1. Laboratory determination of the inactivation fluence

The inactivation curve of total coliforms in the influent of the target wastewater plant was plotted by using MP and LP UV lamps on various days and the laboratory inactivation fluence of the target wastewater plant was determined by using the curve and the ratio of the influent to effluent counts. The laboratory inactivation fluence for the target wastewater plant was found to be 25.5 mJ/cm^2 producing a 3.4 log reduction. However, the average inactivation fluence reported by the target wastewater plant was 23 mJ/cm^2 producing a 3 log reduction. The inactivation fluence determined in the laboratory was almost the same as that reported by the target wastewater plant.

6.2.2. Effect of inactivation fluence on the level of photoreactivation

The changes in the percent photoreactivation of bacteria after applying various inactivation fluences namely 5, 10, 15, and 20 mJ/cm^2 by a MP UV lamp were obtained. It was demonstrated that for total coliforms, the percent photo-reactivation decreased as the inactivation fluence increased.

6.2.3. Effect of effective reactivation fluence

The effect of reactivation fluence and ERF on photoreactivation of total coliforms was studied by applying various filters to change the reactivation fluence rate and

ERF under both indoor and outdoor conditions. The results demonstrated that the percent photoreactivation increased as the integrated irradiance, and consequently the reactivation fluence rate and ERF decreased during 4 h of exposure to sunlight by using various filters.

6.2.4. Effect of temperature

The effect of five temperatures (5, 10, 15, 20 and 25 ºC) on the percent photoreactivation of bacteria was investigated. These experiments demonstrated that the percent photoreactivation of bacteria increased with increasing temperature under both indoor and outdoor conditions. However, the percent photoreactivation was greater under outdoor conditions compared to the indoor conditions.

6.2.5. Effect of various covers

The effect of three types of covers including a PET bottle, a Pyrex® lid, and Saran Wrap® on the percent photoreactivation of total coliforms was investigated. Also, three mesh filters with a size of 0.25 mm were applied on the top of the samples covered with the three covers (PET, Pyrex®, and Saran Wrap®) to investigate the effect of reducing the reactivation fluence rate and various covers simultaneously. The percent photoreactivation of total coliforms increased under both indoor and outdoor conditions by using three filters over each cover. Also, using the PET bottle with or without three mesh filters increased the percent photoreactivation of total coliforms under outdoor conditions. However, using the PET bottle did not influence the level of photoreactivation of bacteria under indoor conditions.

6.2.6. Effect of river water

The effect of mixtures of spiked filtered river water and effluent with various compositions including 20, 40, 60, and 80% of spiked filtered river water on the percent photoreactivation of bacteria under both indoor and outdoor conditions was investigated. The results demonstrated that the percent photoreactivation of total coliforms decreased with increasing the percent river water under both indoor and outdoor conditions.

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APPENDIX A

Raw data (CFU Counts) for calculating log inactivation, percent photoreactivation, and dark reactivation of bacteria

Table A-1: Raw data for calculating log inactivation by using either a low-pressure (LP) or a medium-pressure (MP) ultraviolet lamps (Chapter 3, section 3.4.1)

Inactivation Fluence (mJ/cm^2)	CFU/mL for MP lamp			CFU/mL for LP lamp		
	#1	#2	#3	#1	#2	#3
0	1000	1000	1000	1000	1000	1000
5	310	360	340	220	250	240
10	70	60	70	70	70	70
15	15	15	14	15	10	13
20	3	3	3	2	3	1
25	0.6	0.6	0.5	0.5	0.6	0.4
30	0.3	0.3	0.3	0.1	0.3	0.2
35	0.2	0.2	0.2	0.2	0.1	0.2

Table A-2: Inactivation fluence determined in the lab and at the target wastewater plant on three days (Chapter 3, section 3.4.1)

Inactivation fluence reported by the target wastewater plant (mJ/cm^2)	Inactivation fluence determined in the lab (mJ/cm^2)	Ratio
29.7	26.4	1.13
22.6	25.5	0.89
20.2	24.5	0.82
Average: 24.2	25.5	0.95

Table A-3: Raw data (CFU/mL) for investigating the effect of inactivation fluence (5, 10 mJ/cm^2) on photoreactivation, $N_{influent}$ = 800 (Chapter 3, section 3.4.2)

Time (h)	Inactivation fluence											
	5 mJ/cm^2						10 mJ/cm^2					
	Light			Dark			Light			Dark		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	230	290	250	230	290	250	70	50	60	70	50	60
0.5	270	270	300	260	260	260	90	70	80	60	70	60
1	340	300	320	260	260	260	140	130	130	80	60	70
2	430	270	340	250	280	270	140	150	140	70	70	70
3	360	340	340	260	270	270	150	150	140	80	70	70
4	330	370	350	250	280	270	150	160	170	80	70	70

Table A-4: Raw data (CFU/mL) for investigating the effect of inactivation fluence on photoreactivation (15, 20 mJ/cm^2), $N_{influent}$ = 800 (Chapter 3, section 3.4.2)

Time (h)	Inactivation fluence											
	15 mJ/cm^2						20 mJ/cm^2					
	Light			Dark			Light			Dark		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	16	8	11	16	8	11	2	2	1	2	2	1
0.5	23	17	20	13	14	14	6	6	5	2	2	2
1	36	25	29	15	16	16	7	8	8	3	2	3
2	36	38	38	20	22	20	18	20	18	8	8	8
3	40	40	40	20	19	20	18	23	19	8	9	8
4	44	43	44	23	22	20	21	19	20	9	10	10

Table A-5: Raw data (CFU/mL) for investigating the effect of filters with various mesh sizes (0.63, 0.42, and 0.25 mm) on photoreactivation under outdoor conditions, $N_{influent}$ = 1100, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.3)

Time (h)	Mesh size														
	Without filter			0.63 mm			0.42 mm			0.25 mm			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
0.5	20	22	21	24	22	23	26	22	24	26	26	25	3	3	3
1	26	26	25	27	27	27	27	27	27	27	33	29	4	4	4
2	14	16	14	22	20	21	23	23	22	22	24	23	5	5	5
3	19	19	18	31	27	29	32	32	31	32	36	34	6	6	6
4	15	15	15	27	31	29	32	32	32	37	33	34	9	10	10

Table A-6: Raw data (CFU/mL) for investigating the effect of various number of filters with a mesh size of 0.25 mm on photoreactivation under outdoor conditions, $N_{influent}$ = 1100, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.3)

Time (h)	Number of filters with a mesh size of 0.25											
	2			3			4			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	2	2	2	2	2	2	2	2	2	2	2	2
0.5	22	21	21	25	26	24	31	30	30	2	2	3
1	35	36	36	38	39	37	39	38	38	2	2	3
2	30	31	31	41	40	42	50	49	49	4	3	2
3	37	37	38	44	43	43	50	49	49	4	5	3
4	27	27	26	32	31	31	39	38	38	5	3	6

Table A-7: Raw data (CFU/mL) for investigating the effect of the filters with various mesh sizes (0.63, 0.42, and 0.25 mm) on photoreactivation under indoor conditions, $N_{influent}$ = 1100, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.3)

Time (h)	Mesh size														
	Without filter			0.63 mm			0.42 mm			0.25 mm			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
0.5	7	7	8	10	9	10	8	9	9	11	12	10	2	2	3
1	11	11	10	12	12	11	13	13	13	15	15	15	2	3	3
2	16	15	14	16	16	16	16	17	17	19	18	19	3	3	4
3	18	17	18	21	19	20	21	20	21	23	23	23	4	3	4
4	21	21	21	22	22	22	25	23	24	26	27	27	4	4	4
5	24	25	25	25	24	26	26	27	27	29	30	28	4	4	5

Table A-8: Raw data (CFU/mL) for investigating the effect of various number of filters with a mesh size of 0.25 mm on photoreactivation under indoor conditions, $N_{influent}$ = 1100, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.3)

Time (h)	Number of filters with a mesh size of 0.25											
	2			3			4			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	2	2	2	2	2	2	2	2	2	2	2	2
0.5	10	9	10	11	11	12	12	13	13	2	2	3
1	13	13	13	15	15	14	18	18	18	2	3	3
2	17	18	18	22	20	21	23	22	23	3	3	4
3	25	23	24	24	24	24	26	25	25	4	3	4
4	26	27	27	27	28	28	29	29	28	4	4	4
5	29	28	29	30	31	30	32	33	33	4	4	5

Table A-9: Raw data (CFU/mL) for investigating the effect of temperature (5, 10 °C) on photoreactivation under outdoor conditions, $N_{influent}$ = 800, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.4)

Time (h)	Temperature											
	5 °C						10 °C					
	Light			Dark			Light			Dark		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.5	2	2	3	0.4	0.4	0.3	2	3	1	0.3	0.5	0.3
1	5	4	5	0.4	0.4	0.3	7	6	5	0.3	0.4	0.5
2	5	6	4	0.4	0.4	0.3	9	6	7	0.4	0.5	0.4
3	7	6	8	0.4	0.4	0.3	9	9	9	0.5	0.6	0.4
4	6	7	7	0.6	0.6	0.6	9	8	8	0.9	0.7	0.7

Table A-10: Raw data for investigating the effect of temperature (5, 10 °C) on photoreactivation under indoor conditions, $N_{influent}$ = 800, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.4)

Time (h)	Temperature											
	5 °C						10 °C					
	Light			Dark			Light			Dark		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.5	2	1	1	1	0.1	0.1	1	1	2	0	1	0
1	2	2	1	1	0.1	0.1	2	1	2	1	0	0
2	2	2	2	1	0.1	0.1	2	3	2	1	0	0
3	2	2	2	1	0.1	0.1	2	3	3	1	0.2	0.2
4	2	2	3	0.4	0.5	0.5	3	3	4	1	0.2	0.4

Table A-11: Raw data for investigating the effect of temperature (15, 20, 25 °C) on photoreactivation under outdoor conditions, $N_{influent}$ = 800, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.4)

Time (h)	Temperature																	
	15 °C						20 °C						25 °C					
	Light			Dark			Light			Dark			Light			Dark		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.5	9	10	9	1	0	0	9	9	9	1	0	0	10	11	10	1	0	0
1	13	12	12	0	1	1	13	13	14	1	1	0	13	13	15	1	1	0
2	14	12	11	1	1	0	12	14	12	1	1	0	13	14	15	0	2	1
3	15	14	14	1	1	1	14	16	16	1	1	1	19	17	17	1	1	2
4	15	14	14	1	2	1	17	17	19	2	1	2	18	17	20	1	2	2

Table A-12: Raw data for investigating the effect of temperature (15, 20, 25 °C) on photoreactivation under indoor conditions, $N_{influent}$ = 800, inactivation fluence = 23 mJ/cm^2 (Chapter 3, section 3.4.4)

Time (h)	Temperature																	
	15 °C						20 °C						25 °C					
	Light			Dark			Light			Dark			Light			Dark		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.5	3	3	2	1	1	0.2	2	3	3	1	1	0.8	3	3	3	1	1	1
1	4	4	3	1	1	0.2	5	4	4	1	1	0.8	4	4	4	1	1	1
2	4	4	4	1	1	0.2	5	6	5	1	1	0.8	4	5	5	1	1	1
3	4	5	7	1	1	0.5	5	6	7	1	1	0.8	5	6	6	2	1	1
4	6	6	6	1	1	0.9	8	8	9	1	1	1	8	8	7	2	1	1

Table A-13: Raw data (CFU/mL) for investigating the effect of various covers (Pyrex® lid, PET bottle, and Saran Wrap®) on photoreactivation under outdoor conditions, $N_{influent}$ = 1000, inactivation fluence = 10 mJ/cm^2 (Chapter 4, section 4.4)

Time (h)	Saran Wrap®			Pyrex®			PET bottle			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	70	70	70	70	70	70	70	70	70	70	70	70
0.5	160	160	170	170	190	190	200	200	200	80	80	80
1	190	180	180	200	190	190	230	210	220	80	80	90
2	120	110	100	110	120	120	140	160	160	90	90	90
3	120	140	120	130	140	130	170	180	150	100	90	90
4	130	120	120	120	120	120	130	130	120	100	90	90

Table A-14: Raw data (CFU/mL) for investigating the effect of various covers (Pyrex® lid, PET bottle, and Saran Wrap®) and 3 filters with mesh size of 0.25 mm on photoreactivation simultaneously under outdoor conditions, $N_{influent}$ = 1000, inactivation fluence = 10 mJ/cm^2 (Chapter 4, section 4.4)

Time (h)	Saran Wrap®			Pyrex® lid			PET bottle			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	70	70	70	70	70	70	70	70	70	70	70	70
0.5	210	210	220	190	190	200	240	250	240	80	80	80
1	250	240	230	240	220	230	260	270	280	80	80	90
2	200	190	180	190	190	180	230	230	240	90	90	90
3	190	200	190	200	200	190	250	230	240	100	90	90
4	140	130	140	150	140	150	170	180	180	100	90	90

Table A-15: Raw data (CFU/mL) for investigating the effect of various covers (Pyrex® lid, PET bottle, and Saran Wrap®) on photoreactivation under indoor conditions, $N_{influent}$ = 1000, inactivation fluence = 10 mJ/cm^2 (Chapter 4, section 4.4)

Time (h)	Saran Wrap®			Pyrex® lid			PET bottle			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	70	70	70	70	70	70	70	70	70	70	70	70
0.5	190	190	190	150	150	170	150	140	140	80	80	80
1	210	200	190	170	180	170	140	160	170	80	80	90
2	220	230	230	190	190	190	190	200	200	90	90	90
3	230	220	220	190	180	180	190	200	190	100	90	90
4	220	230	240	200	190	190	220	210	210	100	90	90

Table A-16: Raw data (CFU/mL) for investigating the effect of various covers (Pyrex® lid, PET bottle, and Saran Wrap®) and 3 filters with mesh size of 0.25 mm on photoreactivation simultaneously under indoor conditions, $N_{influent}$ = 1000, inactivation fluence = 10 mJ/cm^2 (Chapter 4, section 4.4)

Time (h)	Saran Wrap®			Pyrex® lid			PET bottle			Dark control		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	70	70	70	70	70	70	70	70	70	70	70	70
0.5	190	180	210	190	190	200	180	190	180	80	80	80
1	200	200	200	190	200	200	190	200	200	80	80	90
2	220	230	210	220	220	210	210	200	210	90	90	90
3	230	230	210	210	210	220	210	210	200	100	90	90
4	240	250	240	230	240	230	230	240	230	100	90	90

Table A-17: Raw data (CFU/mL) for investigating the effect of mixtures of spiked river water and effluent with various compositions (20, 40, 60, and 80%) on photoreactivation under outdoor conditions, $N_{influent}$ = 1000, inactivation fluence = 10 mJ/cm^2 (Chapter 5, section 5.4)

Time (h)	Effluent			20% river water + 80% effluent			40% river water + 60% effluent			60% river water + 40% effluent			80% river water + 20% effluent			River water		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
0.5	220	230	220	220	210	220	190	190	180	160	170	170	130	120	120	120	130	130
1	280	260	280	250	260	250	240	220	230	170	180	190	140	140	140	150	140	140
2	250	260	260	200	210	210	150	160	170	140	140	140	120	110	120	110	110	110
3	270	280	270	250	230	240	190	180	190	140	150	150	120	130	120	120	120	110
4	240	240	250	230	230	230	170	170	170	130	130	150	110	110	120	110	100	100

Table A-18: Raw data (CFU/mL) for dark control of mixtures of spiked river water and effluent with various compositions (20, 40, 60, and 80%) on photoreactivation under outdoor conditions, $N_{influent}$ = 1000, inactivation fluence = 10 mJ/cm^2 (Chapter 5, section 5.4)

Time (h)	Effluent			20% river water + 80% effluent			40% river water + 60% effluent			60% river water + 40% effluent			80% river water + 20% effluent			River water		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
0.5	70	70	70	70	70	70	60	60	70	60	70	70	60	60	70	60	60	70
1	80	70	80	70	70	80	70	70	70	70	70	70	60	60	70	60	60	70
2	90	80	70	70	80	80	70	70	80	70	70	70	60	70	70	60	70	70
3	80	90	90	80	80	90	80	80	80	70	80	80	70	70	70	70	70	70
4	80	90	100	90	90	100	90	90	90	80	90	80	70	80	80	70	70	80

Table A-19: Raw data (CFU/mL) for investigating the effect of mixtures of spiked river water and effluent with various compositions (20, 40, 60, and 80%) on photoreactivation under indoor conditions, $N_{influent}$ = 800, inactivation fluence = 10 mJ/cm^2 (Chapter 5, section 5.4)

Time (h)	Effluent			20% river water + 80% effluent			40% river water + 60% effluent			60% river water + 40% effluent			80% river water + 20% effluent			River water		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
0.5	100	110	110	90	100	100	90	80	80	80	90	80	80	80	70	70	70	70
1	130	140	120	120	130	130	110	110	120	100	100	90	90	90	80	80	80	80
2	140	150	140	120	140	140	110	120	120	100	110	100	90	90	90	90	80	80
3	150	150	150	70	80	80	120	130	120	100	110	100	90	90	90	80	80	80
4	160	160	160	150	150	150	130	140	130	130	120	110	90	110	100	100	80	80

Table A-20: Raw data (CFU/mL) for dark control of mixtures of spiked river water and effluent with various compositions (20, 40, 60, and 80%) on photoreactivation under indoor conditions, $N_{influent}$ = 800, inactivation fluence = 10 mJ/cm^2 (Chapter 5, section 5.4)

Time (h)	Effluent			20% river water + 80% effluent			40% river water + 60% effluent			60% river water + 40% effluent			80% river water + 20% effluent			River water		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
0	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
0.5	60	50	60	60	60	60	60	60	50	60	60	50	50	50	60	50	50	60
1	60	60	70	70	60	60	60	60	60	60	60	50	60	50	50	50	50	60
2	60	70	70	70	60	60	60	60	60	60	60	60	50	60	60	50	50	60
3	70	70	70	70	70	60	70	60	70	70	60	60	60	60	60	50	60	60
4	80	80	70	70	70	80	70	70	60	70	60	70	70	60	60	60	60	60

APPENDIX B

Raw data for calculating the ERF by using the spectral irradiance (SI) and the action spectrum (AS)

Method of calculating ERF:

Based on Table B-1, to determine the ESI, average spectral irradiance (SI) values were multiplied by the average action spectrum factor (AS) values in each band. The SI values were measured by a spectroradiometer and the AS values were estimated by the data presented in a research study for *E. coli* by Takao *et al*. (1989). The integrated effective reactivation irradiance can be estimated by integrating the ESI values in each band and summing over all the wavelengths. The effective reactivation fluence (ERF) can be determined by multiplying the ESI by time in seconds.

Sample calculation:

Based on Table B-1, for outdoor experiment without any filter the integrated effective spectral irradiance (ESI) is 5.2 (mW/cm^2), so the ERF after 4 hours would be as follows:

$(5.2 \times 4 \times 3600)/1000 \sim 73.7$ (J/cm^2)

Table B-1: Raw data for calculating integrated (ESI) (Chapter 3, section 3.4.3)

λ (nm)	SI (μW/cm^2/nm)	AS	ESI (μW/cm^2/nm)	Integ.(ESI)
299	1.9	1.05	1.99	2.09
300	2.0	0.98	1.94	1.98
301	2.1	0.91	1.88	1.91
302	2.2	0.85	1.83	1.86
303	2.4	0.79	1.93	1.88
304	2.8	0.73	2.04	1.98
305	3.2	0.68	2.15	2.10
306	3.5	0.63	2.20	2.18
307	4.0	0.59	2.34	2.27
308	4.6	0.55	2.55	2.44
309	5.0	0.51	2.58	2.56
310	5.2	0.48	2.51	2.54
311	7.0	0.45	3.11	2.81
312	7.7	0.42	3.24	3.17
313	8.4	0.39	3.29	3.26
314	9.2	0.37	3.39	3.34
315	9.6	0.35	3.37	3.38
316	9.4	0.33	3.14	3.25
317	10.9	0.32	3.47	3.30
318	12.2	0.30	3.70	3.59
319	12.4	0.29	3.65	3.68
320	13.5	0.28	3.83	3.74
321	14.7	0.28	4.08	3.96
322	14.3	0.27	3.91	3.99
323	13.8	0.27	3.73	3.82
324	15.0	0.27	4.03	3.88
325	17.3	0.27	4.63	4.33
326	20.4	0.27	5.52	5.07
327	22.6	0.27	6.18	5.85
328	22.0	0.28	6.12	6.15
329	22.7	0.28	6.43	6.28
330	25.8	0.29	7.51	6.97
331	24.7	0.30	7.38	7.44
332	24.2	0.31	7.46	7.42
333	24.4	0.32	7.76	7.61

λ (nm)	SI (μW/cm^2/nm)	AS	ESI (μW/cm^2/nm)	Integ.(ESI)
334	23.8	0.33	7.84	7.80
335	25.2	0.34	8.60	8.22
336	24.3	0.35	8.59	8.59
337	22.1	0.37	8.12	8.35
338	23.6	0.38	9.00	8.56
339	25.6	0.40	10.13	9.56
340	27.1	0.41	11.12	10.62
341	27.9	0.43	11.87	11.49
342	27.2	0.44	12.04	11.96
343	28.8	0.46	13.22	12.63
344	27.1	0.48	12.89	13.06
345	25.0	0.49	12.32	12.60
346	28.2	0.51	14.38	13.35
347	29.0	0.53	15.27	14.82
348	28.4	0.54	15.45	15.36
349	28.6	0.56	16.06	15.76
350	29.8	0.58	17.27	16.66
351	33.3	0.60	19.88	18.57
352	32.0	0.61	19.64	19.76
353	30.8	0.63	19.46	19.55
354	35.4	0.65	22.95	21.20
355	37.3	0.67	24.82	23.88
356	35.5	0.68	24.23	24.53
357	31.4	0.70	21.96	23.10
358	28.5	0.71	20.38	21.17
359	27.3	0.73	19.99	20.18
360	36.5	0.75	27.26	23.62
361	34.5	0.76	26.28	26.77
362	33.3	0.78	25.87	26.07
363	37.5	0.79	29.67	27.77
364	38.0	0.81	30.64	30.15
365	38.8	0.82	31.76	31.20
366	44.8	0.83	37.29	34.52
367	46.6	0.85	39.40	38.34
368	45.1	0.86	38.72	39.06
369	43.6	0.87	37.89	38.30
370	47.6	0.88	41.88	39.88
371	45.2	0.89	40.25	41.06

λ (nm)	SI (μW/cm^2/nm)	AS	ESI (μW/cm^2/nm)	Integ.(ESI)
372	46.2	0.90	41.63	40.94
373	42.0	0.91	38.23	39.93
374	37.4	0.92	34.40	36.31
375	37.4	0.93	34.74	34.57
376	43.2	0.94	40.40	37.57
377	45.2	0.94	42.63	41.52
378	52.8	0.95	50.10	46.37
379	52.8	0.96	50.48	50.29
380	46.0	0.96	44.20	47.34
381	50.0	0.97	48.30	46.25
382	45.2	0.97	43.79	46.04
383	34.4	0.97	33.50	38.64
384	32.6	0.98	31.79	32.64
385	42.6	0.98	41.69	36.74
386	43.6	0.98	42.71	42.20
387	44.8	0.98	43.88	43.29
388	44.8	0.98	43.89	43.88
389	45.0	0.98	44.12	44.00
390	52.6	0.98	51.53	47.83
391	57.0	0.98	55.69	53.61
392	58.4	0.97	56.95	56.32
393	43.9	0.97	42.69	49.82
394	31.5	0.97	30.51	36.60
395	50.3	0.96	48.46	39.49
396	57.2	0.96	54.91	51.69
397	39.9	0.95	38.07	46.49
398	50.7	0.95	48.04	43.06
399	70.3	0.94	66.21	57.13
400	76.8	0.94	71.81	69.01
401	79.2	0.93	73.45	72.63
402	82.1	0.92	75.51	74.48
403	82.7	0.91	75.36	75.43
404	81.4	0.90	73.42	74.39
405	80.4	0.89	71.73	72.58
406	80.0	0.88	70.59	71.16
407	78.7	0.87	68.57	69.58
408	80.2	0.86	69.00	68.79

λ (nm)	SI (μW/cm^2/nm)	AS	ESI (μW/cm^2/nm)	Integ.(ESI)
409	83.3	0.85	70.76	69.88
410	80.6	0.84	67.49	69.13
411	79.3	0.83	65.44	66.47
412	83.1	0.81	67.56	66.50
413	82.0	0.80	65.56	66.56
414	81.1	0.79	63.79	64.68
415	80.8	0.77	62.48	63.14
416	80.1	0.76	60.83	61.66
417	79.1	0.74	58.93	59.88
418	77.9	0.73	56.90	57.92
419	77.2	0.72	55.23	56.07
420	77.2	0.70	54.05	54.64
421	78.2	0.69	53.56	53.80
422	79.1	0.67	52.99	53.27
423	79.3	0.65	51.83	52.41
424	80.8	0.64	51.54	51.69
425	82.1	0.62	51.10	51.32
426	82.8	0.61	50.17	50.63
427	83.3	0.59	49.08	49.63
428	83.3	0.57	47.72	48.40
429	84.0	0.56	46.75	47.24
430	78.7	0.54	42.51	44.63
431	70.9	0.52	37.14	39.82
432	85.0	0.51	43.10	40.12
433	90.6	0.49	44.45	43.77
434	90.5	0.47	42.89	43.67
435	91.7	0.46	41.93	42.41
436	96.5	0.44	42.55	42.24
437	99.1	0.42	42.07	42.31
438	97.3	0.41	39.71	40.89
439	94.0	0.39	36.85	38.28
440	98.2	0.38	36.95	36.90
441	100.3	0.36	36.13	36.54
442	105.7	0.34	36.40	36.26
443	108.2	0.33	35.58	35.99
444	108.4	0.31	33.99	34.79
445	110.1	0.30	32.85	33.42

λ (nm)	SI (μW/cm^2/nm)	AS	ESI (μW/cm^2/nm)	Integ.(ESI)
446	107.8	0.28	30.54	31.70
447	110.2	0.27	29.60	30.07
448	115.3	0.25	29.32	29.46
449	115.8	0.24	27.80	28.56
450	117.5	0.23	26.58	27.19
451	121.7	0.21	25.88	26.23
452	120.8	0.20	24.09	24.98
453	116.4	0.19	21.72	22.90
454	117.3	0.17	20.41	21.07
455	119.0	0.16	19.25	19.83
456	119.3	0.15	17.89	18.57
457	120.2	0.14	16.65	17.27
458	119.1	0.13	15.18	15.92
459	116.6	0.12	13.62	14.40
460	115.9	0.11	12.36	12.99
461	116.5	0.10	11.28	11.82
462	117.2	0.09	10.25	10.76
463	117.7	0.08	9.24	9.74
464	116.7	0.07	8.18	8.71
465	116.3	0.06	7.22	7.70
466	117.5	0.05	6.40	6.81
467	115.8	0.05	5.49	5.94
468	116.7	0.04	4.76	5.12
469	118.6	0.03	4.10	4.43
470	118.7	0.03	3.44	3.77
471	117.6	0.02	2.80	3.12
472	121.3	0.02	2.31	2.55
473	123.6	0.01	1.83	2.07
474	122.7	0.01	1.36	1.60
475	124.0	0.01	0.97	1.17
476	123.6	0.01	0.63	0.80
477	123.0	0.00	0.34	0.48
478	125.9	0.00	0.12	0.23
479	125.3	0.00	0.00	0.06
480	126.2	0.00	0.00	0.00
481	126.0	0.00	0.00	0.00
482	127.1	0.00	0.00	0.00
483	127.4	0.00	0.00	0.00

λ (nm)	SI (μW/cm^2/nm)	AS	ESI (μW/cm^2/nm)	Integ.(ESI)
484	127.6	0.00	0.00	0.00
485	127.3	0.00	0.10	0.05
486	121.3	0.00	0.30	0.20
487	112.6	0.00	0.51	0.40
488	120.1	0.01	0.84	0.67
489	125.0	0.01	1.22	1.03
490	127.7	0.01	1.65	1.43
491	132.6	0.02	2.17	1.91
492	131.8	0.02	2.66	2.41
493	127.2	0.02	3.08	2.87
494	130.4	0.03	3.73	3.40
495	133.2	0.03	4.42	4.07
496	134.8	0.04	5.12	4.77
497	133.7	0.04	5.74	5.43
498	135.3	0.05	6.50	6.12
499	130.4	0.05	6.96	6.73
500	131.9	0.06	7.75	7.35