Study of the Inactivation of Microorganisms Using UV-LEDs

by

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Abstract

Application of ultraviolet (UV) radiation for water treatment has been increasing steadily in the past two decades. Further, significant improvements in semiconductor technology have made ultraviolet light emitting diodes (UV-LEDs) a viable alternative to conventional UV sources for water treatment. However, utilizing UV-LEDs for water disinfection comes with challenges related to their radiation measurements due to their specific structure, operation, and radiation pattern. Without a standardized measurement method, the efficacy of this new radiation source on the inactivation of waterborne microorganisms could not be determined accurately.

In this study, in order to determine the fluence delivered to a microorganism’s solution, first, a method was developed to properly operate, control, and measure the output of the UV-LEDs. It was found that, not only the operational conditions affect the UV-LEDs output, but also the measurement techniques were critical in obtaining accurate results. Then, the radiation distribution was simulated. The radiation model was validated by two common measurement techniques, chemical actinometry and radiometry. Using the validated model, common radiation modeling presumptions such as the point source assumption and symmetry assumption for radiation profile of UV-LEDs were evaluated. Subsequently the radiation model and the operational method were implemented to develop a protocol for fluence determination of UV-LED systems. In this protocol, the average fluence was estimated by measuring the irradiance at a few points for a collimated and uniform radiation on a petri dish surface containing microorganism solution. Finally, the developed fluence determination protocol was tested in different setups to evaluate the radiation distribution and its effect on microbial inactivation.
kinetics measurements. A novel setup was presented for UV-LED kinetics studies; further, the inactivation kinetics of a common waterborne microorganism, E. coli, was measured. This study includes a fundamental holistic insight for fluence determination of UV-LED systems. The developed protocols for UV-LED operation and fluence determination studies help researchers to perform reliable UV-LED inactivation studies and obtain precise kinetics data.
Lay Summary

Nearly 800 million people do not have access to safe drinking water around the world and 3.4 million die every year due to water related illnesses caused by poor sanitation and insufficient disinfection. Ultraviolet (UV) disinfection is an effective technology for the inactivation of harmful microorganisms in water and since it does not involve addition of any chemicals, e.g., chlorine, it is of great interest for industrial application. Significant improvements in semiconductor technology have made ultraviolet light emitting diodes (UV-LEDs) a viable alternative to conventional UV sources for water treatment. However, utilizing UV-LEDs for water disinfection comes with challenges related to their radiation measurements due to their specific structure, operation, and radiation pattern and conventional radiation measurement methods cannot be used for UV-LED systems. This study focused on understanding these challenges and developing a method to study the inactivation of waterborne microorganisms using UV-LEDs.
Preface

I, Ataollah Kheyrandish, was the principal author of this thesis. All the literature review, project definition, experiment design, and experiments conduction were done solely by me under the supervision of Dr. Mohseni and Dr. Taghipour as the principal investigators for this project. The followings are the list of publications from this project in academic journals.

A version of chapter 4 was published in “Water Research” and “International UV association” journal:


A version of chapter 5 was published in “Journal of Photochemistry and Photobiology A: Chemistry”:


A version of chapter 6 was submitted to a journal and it is under revision:

A version of chapter 4 was presented in the following conference as a podium presentation:

• Kheyrandish, A.; Taghipour, F.; Mohseni, M. UV LED characterization and modeling: The first steps to study UV-LED inactivation of microorganisms in water. International Ultraviolet Association world conference, 2016, Vancouver, BC, Canada.

A version of chapter 5 was presented in the following conference as a podium presentation:

• Kheyrandish, A.; Taghipour, F.; Mohseni, M. UV-LED Characterization and modeling: the first steps to study UV-LED inactivation of microorganisms in water. 65th Canadian Chemical Engineering Conference, 2015, Calgary, AB, Canada.
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<th>Description</th>
<th>Unit</th>
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<tr>
<td>A</td>
<td>Area</td>
<td>cm(^2)</td>
</tr>
<tr>
<td>c</td>
<td>speed of light in vacuum</td>
<td>m.sec(^{-1})</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
<td>unitless</td>
</tr>
<tr>
<td>D</td>
<td>Distance between solution surface and the UV-LED</td>
<td>cm</td>
</tr>
<tr>
<td>E</td>
<td>Irradiance</td>
<td>mW.cm(^{-2})</td>
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<tr>
<td>E(_0)</td>
<td>Fluence rate</td>
<td>mW.cm(^{-2})</td>
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<tr>
<td>F(_0)</td>
<td>Fluence</td>
<td>mJ.cm(^{-2})</td>
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<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
<td>nm</td>
</tr>
<tr>
<td>h</td>
<td>Planck’s constant</td>
<td>J.s</td>
</tr>
<tr>
<td>N</td>
<td>Number of colonies</td>
<td>unitless</td>
</tr>
<tr>
<td>P</td>
<td>Radiant power</td>
<td>mW</td>
</tr>
<tr>
<td>r</td>
<td>radius</td>
<td>cm</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
<td>sec</td>
</tr>
<tr>
<td>v</td>
<td>Volume</td>
<td>cm(^3)</td>
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### Greek Symbols

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<tr>
<td>(\alpha)</td>
<td>Absorption coefficient</td>
<td>cm(^{-1})</td>
</tr>
<tr>
<td>(\phi)</td>
<td>Quantum yield</td>
<td>Einstein·mol(^{-1})</td>
</tr>
<tr>
<td>(\varphi)</td>
<td>Azimuthal angle</td>
<td>Degree</td>
</tr>
<tr>
<td>(\varepsilon)</td>
<td>Molar absorption coefficient</td>
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<tr>
<td>(\lambda)</td>
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<tr>
<td>(\theta)</td>
<td>Polar angle</td>
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### List of Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AOP</td>
<td>Advanced Oxidation Process</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>CF</td>
<td>Collimation factor</td>
</tr>
<tr>
<td>CFD</td>
<td>Computational fluid dynamics</td>
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<td>CFU</td>
<td>Colony forming unit</td>
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<td>DBP</td>
<td>Disinfection by-product</td>
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<td>Divergent factor</td>
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<td>Escherichia coli</td>
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<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>HAA</td>
<td>Haloacetic acid</td>
</tr>
<tr>
<td>IUVA</td>
<td>International Ultraviolet Association</td>
</tr>
<tr>
<td>LB broth</td>
<td>Lysogeny broth</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural organic matter</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCB</td>
<td>Printed circuit board</td>
</tr>
<tr>
<td>PF</td>
<td>Petri factor</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td>RED</td>
<td>Reduction equivalent dose</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RRP</td>
<td>Relative radiation profile</td>
</tr>
<tr>
<td>SET</td>
<td>Sensor Electronics Technology</td>
</tr>
<tr>
<td>SPD</td>
<td>Spectral power distribution</td>
</tr>
<tr>
<td>SWTR</td>
<td>Surface water treatment rule</td>
</tr>
<tr>
<td>TEC</td>
<td>Thermoelectric cooler</td>
</tr>
<tr>
<td>THM</td>
<td>Trihalomethane</td>
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<td>University of British Columbia</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environment Program</td>
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<tr>
<td>USEPA</td>
<td>The United States Environmental Protection Agency</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>WF</td>
<td>Water factor</td>
</tr>
<tr>
<td>WPE</td>
<td>Wall plug efficiency</td>
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To God,

My Parents,

and

My beloved Zahra
Chapter 1: Introduction

Access to safe drinking water is a basic human right. However, over 844 million people lack access to a clean or improved water source [1]. Approximately more than 3.4 million people, mostly children, die each year from water, sanitation, and hygiene-related diseases [2]. Around 30% of these deaths are caused by diarrhea, which is often the result of consuming non-treated, contaminated water. In order to reduce the pathogenic microorganisms in water source, a variety of chemical or physical treatment processes may be used. While microorganism reduction happens throughout the entirety of the water treatment process, the main reduction takes place in the last stage called disinfection [3].

1.1 Water disinfection

Water disinfection involves killing or inactivating pathogens using chemical or physical processes. Chemical processes include chlorination, ozonation, etc. and physical processes include filtration, ultraviolet (UV), etc. Depends on the water quality, water plants use a combination of these methods, rather than a single disinfection method. The most common disinfection methods are chlorination and ozonation which have some drawbacks. Although chlorination is effective against bacteria and viruses, it is ineffective against Cryptosporidium oocysts and Giardia [4], [5]. Moreover, chlorine may produce disinfection by-products (DBPs) in the presence of natural organic matter (NOM). The most studied by-products are trihalomethane (THM) and haloacetic acids (HAAs), but almost 70% of the potential DBPs are still unknown [6]. Unlike chlorination, ozonation is not a pH dependent process and it is an
effective method for Cryptosporidium oocysts removal. However, similarity to chlorination, DBPs like bromate, ketone, and formaldehyde formation have been reported for ozonation[7]. Other alternative methods are available for water disinfection which are applicable for emergency or special conditions. These disinfection methods include heat, extreme PH, permanganate, electron beam irradiation, etc. The details of each disinfection methods are available in the literature (e.g., [8]). In the subsequent section, UV disinfection method which has some advantages over chlorination and ozonation is presented.

1.2 UV disinfection

UV radiation has been used for water disinfection for more than a century. UV radiation’s disinfection ability was first discovered in 1877 [9] by testing the inactivation of bacteria under sunlight. The microbial inactivation of UV radiation was first in the city of Marseilles, which utilized a Westinghouse Cooper Hewitt mercury lamp in fused quartz to disinfect water in a continuous reactor, with a flow rate of 36 m$^3$/h [3]. Disinfection water treatment plants using UV radiation were installed in many places between 1916 and 1936, but due to the high electricity and maintenance costs, these facilities were abandoned. At that time, it was believed that chlorine was more effective to remove waterborne microorganisms, and due to the lower operational cost, chlorination become the favored disinfection method. The discovery of disinfection by products from chlorine disinfection and their harmful effect have shifted the interest toward UV, which has no known DBP. The application of UV radiation increased, especially for wastewater systems. In the late 1990s, inactivation of chlorine resistant protozoa was reported using medium pressure mercury UV lamps [10]–[12]. This advantage, along with
the small footprint, shorter process time, no need for chemical handling and storage, and no known toxic by-products, made UV disinfection an attractive alternative to chlorination. Today, many municipal drinking water plants are utilizing UV radiation for disinfecting water, including New York (USA), Vancouver (Canada), San Francisco (USA), and Paris (France).

1.2.1 Ultraviolet radiation

UV radiation is part of the electromagnetic spectrum with wavelengths from 100–400 nm. As illustrated in Figure 1-1, the UV spectrum is between visible light and X-ray. In most literature, UV radiation was addressed as “UV light” which is not an accurate term since the term “light” refers to the visible part of the electromagnetic spectrum. UV radiation is categorized in four different sub-groups; Vacuum UV with radiation from 100–200 nm, UVC with radiation from 200–285 nm, UVB with radiation from 285–315 nm, and UVA with radiation from 315 nm to visible light. UV radiation emits in different wavelengths in packages of energy called photons. The photons at shorter wavelengths have more energy. The energy of a photon at each wavelength can be related to the wavelength, with the following equation:

\[ u = \frac{hc}{\lambda} \]  

**Equation 1-1**

where

- \( u \) = energy of a photon (J)
- \( c \) = speed of light in vacuum (m/s)
- \( h \) = Planck’s constant (J.s)
- \( \lambda \) = radiation wavelength (m)
Figure 1.1. UV radiation sub-categories in electromagnetic spectrum

The term ‘vacuum UV’ indicates that this radiation can be transferred only under vacuum or absence of oxygen, since the photons in this region can be absorbed by air (mostly O\textsubscript{2}). The application of vacuum UV is not of interest for water disinfection because of the high absorption of water at these wavelengths.

The UVC spectrum is the most effective range to disinfect water due to the high absorption of microorganisms at these wavelengths. The absorption spectra of different microorganisms are different from each other, but the absorption peak wavelength always happens between 200 nm and 300 nm.

The radiation with longer wavelengths (>300 nm) cannot lead to any disinfection on its own. However, UVB and UVA might cause water disinfection in photocatalytic processes, in which longer wavelengths, absorbed by proper catalysts, lead to an oxidant production. The produced oxidants, such as hydroxyl radicals, oxidize the microorganism component and disinfect water.
1.2.2 UV radiation sources

Several UV radiation sources are available for water disinfection, including low-pressure electric discharge lamps, medium-to-high-pressure electric discharge lamps, flash lamps, lasers, micro-plasma, and light emitting diodes (LEDs). In electric discharge lamps, a gas is usually enclosed in a quartz tube, with two electrodes on the sides and a heating filament on one side. The filament helps to generate enough electrons to start the current between the electrodes. Since the electric resistance decreases by increasing the current, a current limiting driver called a ballast, is needed to regulate current. Low-pressure electric gas discharge lamps emit radiation in a very narrow spectrum (almost a line). The wavelength of this line is related to the lamp’s enclosed gas. The most common low-pressure electric gas discharge lamps for water disinfection are low-pressure mercury lamp which emits radiation at 253.7 nm (Figure 1-2). Unlike low-pressure gas discharge lamps with monochromatic radiation spectrum, medium to high-pressure gas discharge lamps emit radiation in a wide spectrum with many peak wavelengths, called the polychromatic radiation spectrum. In these lamps, by increasing the gas pressure inside the lamp tube, more radiation is reabsorbed by the gas and leads to emitting radiation with different peak wavelengths (Figure 1-2). With relatively high-pressure continuous spectrum can be achieved, such as in deuterium lamps which are being used in spectrophotometers as radiation sources for the ultraviolet region.
Low pressure and medium pressure mercury lamps are the most common conventional UV sources for water disinfection. Low-pressure mercury lamps have more germicidal efficiency compared to medium pressure lamps. This is due to more than 85 % output at 253.7 nm which is naturally close to the absorption peak wavelength of the waterborne microorganisms (~265 nm). Medium pressure lamps, on the other hand, have relatively more radiant power, which in some cases can deliver more UVC radiation compared to low-pressure lamps. Depending on the quality of water, reactor design, and operational conditions, one may choose one lamp over another. For example, in systems with low flow rate where the transmittance of water is high, low-pressure mercury lamps are suggested, while for high flow rates and more diverse water quality, medium pressure lamps are suggested. Table 1-1 presents a comparison between the characteristics of low pressure and medium pressure mercury lamps.
Flash lamps include xenon lamps, where xenon gas is ionized by a pulse, generated by utilizing a capacitor. The emitted energy is related to the capacitance of the capacitor and operational voltage. The use of these lamps is not as common as gas discharge lamps for water treatment applications [14], [15].

Micro-plasma is another form of gas discharge lamps, with flat shape electrodes and gas trapped in between. These new UV radiation sources are relatively cheap, mercury-free, and have a high lifetime (>50,000 hours). The amount of heat generated is quite low, which makes them not require any heat sink. Their ability to turn on/off instantly makes them a good option for point-of-use systems [16], [17]. The application of these UV sources for water disinfection is under study and very few published studies are available in the literature, though.

The characteristics and application of UV-LEDs for water disinfection, along with a comprehensive comparison between UV-LEDs and conventional mercury lamps are presented in the following section.
### Table 1-1. A comparison between low pressure and medium pressure mercury lamps [8], [18]

<table>
<thead>
<tr>
<th></th>
<th>Low Pressure</th>
<th>Medium Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Power consumption (W)</strong></td>
<td>40-100</td>
<td>1000-10000</td>
</tr>
<tr>
<td><strong>Germicidal output (%)</strong></td>
<td>30-40</td>
<td>10-15</td>
</tr>
<tr>
<td><strong>HG vapour pressure (kPa)</strong></td>
<td>0.0009</td>
<td>40-4000</td>
</tr>
<tr>
<td><strong>Lamp life (hr)</strong></td>
<td>8000-10000</td>
<td>4000-8000</td>
</tr>
<tr>
<td><strong>Lamp output at the end of life time (%)</strong></td>
<td>75-80</td>
<td>75-80</td>
</tr>
<tr>
<td><strong>Operational Temperature (°C)</strong></td>
<td>40</td>
<td>800</td>
</tr>
<tr>
<td><strong>Warm up time (min)</strong></td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

#### 1.2.3 Ultraviolet light emitting diodes

The ultraviolet light emitting diodes (UV-LEDs) are solid state, semiconductor based UV radiation sources. The first LED was discovered in 1961 by James R. Biard, who was trying to make an X-band GaAs diode but accidentally got infra-red radiation [19]. Eventually, red and yellow LEDs were discovered in 1962 and 1972, respectively. Shuji Nakamura discovered the bright blue LED using gallium nitride and, in parallel with Isamu Akasaki and Hiroshi Amano, was awarded the Nobel Prize in physics - 2014. The first commercial UV-LED was developed by NITRIDE Semiconductors Co. in April 2000, opening a new chapter in UV radiation sources and applications [20]. Since then, most studies are focused on increasing the efficiency and output power of UV-LEDs.

UV-LED consist of two semiconductors, with one of them having excess electrons, called N-junction, and the other one having holes that can accept electron, called P-junction. When an electrical potential difference is applied to the sides of an LED, the holes and electrons accumulate around the surface between these two junctions; as the voltage increases the density
of holes and electrons increases (Figure 1-3). At a specific voltage, electrons travel from conduction band to valence band, causing energy to release as quanta of radiation which are called photons. The amount of energy released is related to the band gap energy of the semiconductors (Figure 1-3). Based on the semiconductor’s material, the wavelength of the emitted radiation can be different (diamond 235 nm, Boron nitrate 215 nm, Aluminum nitrate 210 nm, Aluminum gallium nitrate and Aluminum gallium Indium nitrate 210 nm) [21], [22].

![Figure 1-3. Schematic view of a UV-LED and energy level of the electrons inside the UV-LED[23]](image)

UV-LEDs have a broad application in various fields such as sensors, analytical equipment, sterilization, 3D printing, tanning, and water disinfection. Their advantages over conventional mercury lamps make them a potential alternative for UV water disinfection. Some of these advantages are as follows:
- Environment: Unlike conventional UV radiation sources for water disinfection, there is no mercury involved in the UV-LED structure. Considering the United Nations Environment Program (UNEP) extensively restricts the production of mercury through the “Minamata Convention on Mercury” agreement, adopted in 2013 by UNEP, UV-LEDs are potentially a good alternative to UV mercury lamps [24].

- Size: UV-LEDs are as small as one or two millimeters and more flexible reactor designs are possible compared to the mercury lamps which are more than a couple centimeters in size.

- Robustness: Conventional mercury lamps consist of trapped pressured gas in a quartz tube which is bulky and fragile. On the other hand, UV-LEDs are not fragile and can be used in more severe conditions.

- Operational voltage: The relatively lower operating voltage of UV-LEDs, and the merit of operating with DC electricity (e.g., batteries), make them a good option for places with limited or no access to the electricity network.

- Warm-up time: The output of mercury lamps is not consistent in the first 20 minutes of operation, while UV-LEDs’ warm-up time is significantly shorter. As a result, UV-LEDs can be implemented for point-of-use water disinfection, where the UV source turns on/off more frequently. The lifetime of some mercury lamps is affected by on/off cycles, while UV-LEDs can be turned on and off without any degradation in their output [25].

- Wavelength: Conventional mercury lamp spectrum cannot be changed. The radiation spectrum is monochromatic for low-pressure mercury lamp, and polychromatic for medium pressure
mercury lamps, while UV-LEDs’ spectrum can be selected using different semiconductors. Moreover, although the output wavelength of low-pressure mercury lamps is in the germicidal region, it is not at the peak wavelength of the pathogen absorption spectrum. The absorption peak wavelength of DNA is between 265 nm and 270 nm [26], and the absorption peak wavelength of proteins is 280 nm [27]. UV-LED offers the possibility of disinfecting at an optimal wavelength, rather than disinfecting at 253.7 nm with low-pressure mercury lamps or the polychromatic radiation of medium pressure mercury lamps.

Lifetime and efficiency of UV-LEDs have been reported to be around 100,000 hours and 75% [28]–[30], respectively. However, these numbers are related to LEDs in the visible region, and not UV-LEDs. The UV-LED’s lifetime and efficiency are much less than those for conventional lamps [31]–[34]. Wall plug efficiency (WPE) of UVC LEDs is around 2–3 % [35]. Their lifetime depends on the manufacturer, material, and output wavelength. The maximum lifetime for UVC LEDs was reported by Sensor Electronics Technology (SET) where they reached a lifetime of more than 10000 hours [36]. A study in 2015 predicted WPE of 5 % and radiant power of 40 mW by 2020, for 265 nm UV-LEDs, even though 90 mW radiant power for 265 nm UV-LED was reported, recently [37]. Considering the major improvements in visible LED, higher output and WPE are expected for UV-LED in upcoming years.

1.3 Fundamental key terms in photochemistry

In this section the most common terms in measuring and reporting radiation intensity are presented. Some terms may have different definitions in different textbooks; radiation measurement terminology used in this study was obtained from “Ultraviolet Applications
Handbook” [38]. Some of the terms are presented here due to their importance and to increased clarity of the text.

**Radiant Power (P):** The radiant power of a lamp is the total emitted radiation energy of the lamp in all directions in one second. Radiant power is usually represented in watts (W).

**Irradiance (E):** Irradiance is the total radiation power delivered to an element of surface containing the point under consideration from upward directions divided by the area of the flat surface (mW.cm\(^{-2}\)).

**Fluence rate (E\(_{0}\)):** Fluence rate represents all radiation power delivered to an infinitely small sphere from all directions divided by the cross-sectional area of the sphere (mW.cm\(^{-2}\)).

**Fluence (F\(_{0}\)):** Fluence (UV dose) represents the energy of all the incident radiation from all directions to an infinitely small sphere divided by the cross-sectional area of the sphere (mJ.cm\(^{-2}\)).

### 1.4 Research rationale

Despite the improvement in UV-LEDs performance and their increases in their application for water disinfection, no fundamental study is available in the open literature to consider the sensitivity of UV-LEDs to operational conditions. Comprehensive studies are available for other UV radiation sources, like mercury lamps, but measuring, monitoring, and maintaining UV-LED’s output for water disinfection application is missing from the literature. Therefore, it is of great interest to investigate the effect of operational condition and to evaluate the accuracy of
equipment on UV-LED output measurement. Moreover, UV-LEDs are emerging UV radiation sources for water disinfection applications. Due to their characteristics and differences with conventional mercury lamps, accurate UV dose measurement in microbial inactivation tests for UV-LEDs may not be possible using conventional equipment. Developing a method to measure the UV dose in UV-LED systems is of great interest in the study of inactivation kinetics, and eventually for design UV-LED reactors more accurately.

1.5 Thesis layout

This dissertation includes a summary of the methods, experiments, and results of four years of research at the University of British Columbia (UBC). All the data are presented in eight chapters, including a more basic introductory chapter for the readers not familiar with the field. The structure of the other chapters is as follow:

**CHAPTER 2** provides a literature review of the published studies on UV-LED for water disinfection. Different methods for inactivation kinetics studies of waterborne microorganisms are reviewed. The knowledge gap is highlighted and research objectives are stated based on the knowledge gap. Moreover, the research significance is articulated in this chapter.

**CHAPTER 3** includes a detailed description of methods, fabricated setups, and analytical techniques used in this research.

**CHAPTER 4** includes the investigation into the impact of the variety of parameters on UV-LEDs. It presents a comprehensive protocol to characterize UV-LEDs and properly operate them.
**Chapter 5** contributes to developing a model to estimate the radiation distribution of UV-LEDs, and to assess the accuracy of some simplifying assumptions for UV-LED radiation sources.

**Chapter 6** shows the feasibility of using conventional fluence determination protocols for UV-LED systems. A novel protocol for fluence determination is presented in this chapter.

**Chapter 7** provides the kinetics studies, utilizing the proposed protocol for inactivating a target waterborne microorganism. Different setups are explored in this chapter, to get an enhanced radiation distribution inside a water sample.

**Chapter 8** presents the overall conclusion of this work. Some recommendations for future work are also presented.
Chapter 2: Literature Review

2.1 Ultraviolet disinfection

UV disinfection, as described earlier, is a physical water disinfection method with relatively low capital and maintenance costs. UV radiation does not change the quality of water, since no chemical is added for the purpose of disinfection. The disinfection process with the UV radiation needs less resident time, so the UV reactors are smaller in size than the chemical disinfection systems. Moreover, UV is effective at inactivation some waterborne microorganisms resistant to chlorination. Bolton et al. [10] studied the inactivation of Cryptosporidium, which is resistant to chlorination, and showed that this oocyst can be inactivated by 4 logs with a UV dose as low as 41 mJ.cm$^{-2}$, with a medium pressure mercury lamp. Some other studies, in parallel, reported the reduction of Cryptosporidium and Giardia using UV radiation [12], [39]–[41]. These studies resulted in acceptance of UV disinfection process by regulatory agencies (e.g., US Environmental Protection Agency) for the disinfection of surface water supplies. Since then, more studies were performed on the reduction of bacteria, viruses, and parasites. Haji Malayeri et al. [42] gathered a list of waterborne microorganisms and the required UV dose for various log reductions. The replication of that study will be avoided here.

2.2 UV-LED disinfection

Ultraviolet light emitting diodes (UV-LEDs) are a promising source of UV radiation that can emit radiation from 210 nm up to visible light [43]. The number of published studies using UV-LED for water disinfection is limited [44], but due to their special advantages to conventional
mercury lamps, such as tunable output wavelength, they are increasingly receiving the attention of the researchers for use in new UV reactors.

2.2.1 **Wavelength effect on UV disinfection**

UV radiation causes disinfection with different mechanisms based on the spectral absorption of the target microorganism and the UV source spectral radiation. Radiation with wavelength longer than 300 nm is not so effective to inactivate microorganisms directly. The radiation longer than 300 nm can be absorbed by the protein and produce Reactive Oxygen Species (ROS), such as hydroxyl radicals. ROSs can destroy the cell membrane and eventually disinfect water. The water disinfection through this process needs higher energy (longer time) compared to that with shorter wavelengths [45]. Usually, to disinfect water with longer wavelengths, another chemical like a catalyst or hydrogen peroxide is needed. In the presence of these chemicals, more ROSs are formed and subsequently it results in the inactivation of waterborne microorganisms more efficiently. Microbial inactivation using shorter wavelengths (200–300 nm) are more efficient due to the higher absorption of UV radiation by the microorganisms’ DNA/RNA or protein (Figure 2-1). UV radiation with these wavelengths has a germicidal effect and it can result in microbial inactivation, directly. That is why the UV spectrum with the wavelengths of 200-300 nm is called germicidal range. The absorbed UV radiation by the DNA/RNA in this spectrum results in the production of cyclobutane pyrimidine dimers and 6-4 photoproducts (6-4 PPs) which cause microbial inactivation [46]. Details of the inactivation mechanisms associated with different microorganisms are described elsewhere and are beyond the scope of this chapter [8], [18]. The effect of wavelength on UV inactivation of waterborne microorganisms has been
investigated widely in the literature. For example, Rauth [47] investigated the effect of wavelength (260–302 nm) on plaque-forming (inactivation) of bacteriophage MS2. Medium pressure mercury lamp, equipped with a monochromator, was implemented to narrow the wavelength’s spectrum and to study the effect of wavelength [48]. The results showed a peak inactivation wavelength at around 265 nm which is close to the peak absorption wavelength of DNA. Similar studies with higher wavelengths’ resolution and with wider spectrum have also been conducted by other researchers such as Mamane-Gravetz [49] and Pirnie [50]. Recently, Beck et al. [51] investigated the effect of wavelength on a variety of microorganisms including bacteriophage MS2 with a precise monochromatic UV radiation source—a tunable laser. Their results showed a local maximum at 259 nm for spectral sensitivity of MS2 [51]. For the shorter wavelengths (toward 200 nm), more inactivation was achieved. As explained earlier, although the absorption of the microorganisms is increased for shorter wavelengths and more inactivation is expected, these wavelengths are not effective for water disinfection since the absorption of the water molecules is very high at these wavelengths.
Unlike conventional mercury lamps, which have a limited variety of output radiation spectra, UV-LEDs are available with many output wavelengths. The radiation spectrum of UV-LEDs is narrow and usually is represented by the full width at half maximum (FWHM), which is the width of the spectrum at a wavelength in which the radiation intensity is half of the radiation intensity of the peak wavelength. The variety of UV-LEDs available in the market opened an opportunity to investigate the effect of wavelength’s tailoring. In other words, UV-LEDs could be used for water disinfection more efficiently. For each microorganism, a UV-LED with a peak wavelength matching the inactivation peak wavelength of that microorganism, can be used to utilize the radiation more effectively. Research on the disinfection efficiency of UV-LEDs has produce conflicting results. Chatterley et al. [53] used a 265 nm UV-LED to study the inactivation of E. coli in comparison with a conventional low-pressure mercury lamp in a bench.
scale collimated beam apparatus. As expected, better results were reported for UV-LED since the absorption peak wavelength of E. coli is around 265 nm [54]. In a similar study, Sholtes et al. [55] compared the E. coli inactivation using low-pressure mercury lampas well as with 260 nm UV-LED. The results showed the same inactivation kinetics for both UV sources. Another study, conducted by a research group at the University of Tokyo, Japan, measured the performance of 265 nm UV-LED for E. coli inactivation [56]. This study, however, showed low-pressure mercury lamp to provide better inactivation kinetics for E. coli. Moreover, a comparison between inactivation kinetics of the same microorganism with the same UV-LED showed different rate constants. Studying the inactivation kinetics of bacteriophage MS2 with a 255 nm UV-LED showed rate constants of 0.078 and 0.038 (cm².mJ⁻¹) in two separate studies [57], [58]. As can be noted from these few examples, various studies conducted by different research groups have led to different conclusion, on the potential benefits of UV-LEDs. Unfortunately, the exact knowledge behind such discrepancies is not known and such fundamental information is missing in the literature.

2.2.2 Selecting target waterborne microorganisms

One of the key parameters affecting the design of a UV reactor is knowledge of the inactivation kinetics of the target waterborne microorganisms. There are numerous microorganisms such as bacteria, viruses, and parasites present in a water source. Studying the inactivation of all these microorganisms is not practical and feasible economically. Instead, some indicator microorganisms from each group have been studied to represent each group. A microorganism needs to have some special characteristics to indicate a group of pathogens. An indicator
microorganism should be present whenever the target pathogen is available, the resistance of the indicator should be the same as the target pathogens, and the indicator should be able to be quantified with common bioassays [52].

E. coli, a gram-negative, non-spore-forming, aerobic, and facultative anaerobic bacteria, for example, has been used as an indicator for a group of bacteria called coliform bacteria. E. coli has many strains that are categorized into two main groups, pathogenic and non-pathogenic strains. The non-pathogenic E. coli has been widely used in the literature to validate UV reactors as it was suggested by USEPA [50]. A review of the kinetics of some strains of E. coli with UV-LEDs and mercury lamps was published, recently [42].

There are also some microorganisms’ surrogates that have been used for water disinfection. Surrogates are usually more resistant to UV radiation and they are easier/safer to be studied. For example, coliphage MS2 and Bacillus Subtilis have been used widely in North America and Europe, respectively, to validate UV reactors. A summary of the kinetics studies on these surrogates has been published recently [42].

2.3 UV source characterization

For each new radiation source, a standardized technique is required, to measure its output power and other specifications, and to obtain accurate and consistent results for microbiological and photochemical experiments. In the case of mercury UV lamps, a protocol for measuring the lamp output was developed by studying the role of different parameters on the lamp’s output [59]. Further, a more practical protocol was suggested [60], which was tested with a round robin
method by different industry participants [61]. This protocol was then adopted by the International Ultraviolet Association (IUVA).

For UV-LEDs, there has been no comprehensive study to investigate the effect of operational condition on the UV-LED performance. In addition, improper operation of UV-LEDs has been reported in the literature. For example, Nelson et al. [62] have used seven 265 nm UV-LEDs to disinfect water from total *coli*form and *E. coli*. The results showed 2.5 log inactivation for *E. coli* after 50 minutes. The UV-LEDs were connected in series to each other, a resistor, and alkaline battery to control the current and voltage of the circuit. The ohmic resistance of the resistors was calculated based on the Ohm's law. However, applying the Ohm's law for UV-LEDs has to be considered with caution since the resistance of the UV-LEDs changes with the UV-LED’s temperature. As the temperature of the UV-LED increases, the resistance of the UV-LED decreases and with a constant voltage power supply, such as an alkaline battery, more currents flow through the UV-LED and in some cases it might burn the UV-LED. UV-LEDs are current driven UV sources. Thus, a constant current driver is needed to operate them rather than a constant voltage driver, such as batteries.

Another result of improper operation of UV-LEDs can be observed in the UV-LEDs output changes. Wurtele et al. [26] studied the inactivation kinetics of *B. subtilis* spores with 269 and 282 nm UV-LEDs, and reported better inactivation at 269 nm UV-LED. The authors have monitored the radiant power of one UV-LED over time, separately, in parallel with the experiments as a reference. Almost 30% reduction of radiant power was reported for 265 nm UV-LED in the first 40 hrs of operation, while the reported time by the manufacturer for 30%
reduction in radiant power is more than 1000 hrs. The huge difference between the manufacturer and the measured times might be related to the improper operation of the UV-LED. For example, it is known that driving a UV-LED with excessive current will rapidly degrade the UV-LED.

A part of contradictory/unexpected published data on inactivation of the waterborne microorganisms using UV-LEDs might be related to not considering the behaviour of the UV-LEDs under different operational conditions. For example, in a study involving the effect of combination of two wavelengths (265 nm and 310 nm) in order to inactivate E. coli, ten UV-LEDs from each wavelength were places at the center of a tubular flow-through reactor [56]. E. coli was inactivated with a rate constant of 0.30 and 0.00 cm².mJ⁻¹ for 265 nm and 310 nm UV-LEDs, respectively, when the UV-LEDs of each wavelength were operated separately. Operating these UV-LEDs simultaneously resulted in inactivation kinetics with a rate constant of 0.13 cm².mJ⁻¹ which are lower than that obtained by 265 nm alone. Since the photoreactivation was controlled in this study, the lower inactivation for the combination of wavelengths might be related to the effect of the temperature on the output of 265 nm UV-LED which was not considered in this study. Such inconsistent results highlight the need for a standard method to operate and characterize UV-LEDs output.

2.4 UV-LED source modeling

While a number of optical fluence models exists for mercury lamps [63]–[65], few attempts have been made towards the development of optical models for UV-LEDs. In some UV-LEDs’ models, the optical output was estimated by modeling the radiation from the inside of the UV-LED’s package (the package which includes the UV-LED die), considering the reflection from
the package, the refraction from the lens, and absorption of the UV-LEDs’ package [66]–[68]. While valuable, these models that provide radiation distribution inside the UV-LED’s package do not have much applicability for the design of photoreactors for water disinfection. Moreover, measuring the radiation distribution inside the package in order to validate the model is challenging.

Some studies assumed UV-LEDs as Lambertian radiation sources [69], whereas others simplified the UV-LEDs’ radiation profiles by taking up a uniform radiation distribution on a spatial spherical cap with an angle calculated from viewing angle of the UV-LED [58], [70]. In fact, UV-LEDs with different radiation profiles might have the same viewing angles (Figure 2-2). It means that modeling UV-LEDs by using just the viewing angle is not possible. Several researchers used curve fitting to fit different equations on the factory reported UV-LED’s radiation profile [70]–[72]. This approach is essential for individual UV-LEDs, but unlike mercury lamps, UV-LEDs’ radiation profiles are diverse, making it difficult to model them with a single fitted equation. The other approach to model the radiation distribution of the UV-LEDs could be to predict the radiation distribution based on the measured radiation profile of the UV-LEDs. This is because, for water treatment applications, the radiation distribution delivered to the water solution is more important than the UV-LED radiation profile.
Measuring the near-field radiation distribution of the UV-LEDs for designing UV-LED reactors for water treatment applications is challenging. The average fluence rate is a key data for determining the inactivation kinetics of a waterborne microorganism [13]. To determine the average fluence rate, the radiation distribution has to be determined. Since there is no physical method available to specifically measure the near-field radiation distribution of a UV-LED, a validated optical model is needed to predict the radiation distribution at near fields.

2.5 UV disinfection kinetics study

In order to study the inactivation kinetics of waterborne microorganisms, the microorganism’s solution is usually introduced to the UV radiation in a petri dish in a bench scale setup. To determine the UV response of different microorganisms in a petri dish, different approaches have been taken in the literature. In some studies, the UV response is reported as a function of nominal or measured radiant power of the radiation source [73]. Since the output power of UV...
lamps are a function of operational condition and the delivered radiation to the microorganism is dependent on the setup configuration, the results of these studies have to be considered with caution. In some other studies, the UV response is presented as a function of exposure time [62], [74]–[76]. However, the most accurate method to measure and report the UV inactivation kinetics of a microorganism is the determination of average fluence introduced to the solution and target microorganism.

Collimated beam apparatus was established to facilitate the determination of the average fluence for the UV mercury lamps and it has been adopted by the IUVA and the US-EPA as a standard protocol [13], [50]. In this method, to obtain uniform radiation distribution inside a sample solution, the sample needs to be positioned at least 20 cm away from the UV lamp, and the “petri factor” is used to evaluate the uniformity of incident radiation across the surface of the fluid sample. At this distance, the incident radiation to the surface of the solution was found collimated (uni–directional) which means the fluence rate could be estimated by measuring irradiance and the fluence distribution in the fluid sample could be calculated with a simple application of Beer's law[64].

The conventional collimated beam approach is not applicable to UV-LED systems. UV-LEDs have various radiation profiles [77] and a relatively low radiant power. At 20 cm, the incident radiation to the sample solution is not strong enough to obtain meaningful inactivation at a reasonable time period, particularly for the UV-resistant microorganisms. Thus, closer distances have been tried in the literature. At closer distances, the incident radiation might not be collimated due to the UV-LED’s radiation profile (note, UV-LEDs usually have a wide viewing
angle of over 100°), which would prevent simple calculation of the intensity distribution in the fluid sample. Furthermore, the non-normal incidence would increase the amount of reflection from the fluid surface. While there have been several efforts in the literature for fluence determination of UV-LEDs [57], [58], [62], [78], [79], there is no systematic approach taking into account the specific characteristics of UV-LEDs such as radiation profiles, spectral power distribution (SPD), and operational conditions.

2.6 Knowledge gaps and research questions

Despite the growing number of UV-LED studies in a variety of applications, including water disinfection, no standard method is available considering the characteristics of these UV sources. UV-LED manufacturers are claiming different characteristics such as radiant power for the new fabricated UV-LEDs which must be evaluated. Moreover, aging of the UV-LEDs affects their output and the extent of this effect, must be evaluated. In fact, unlike mercury lamps, no systematic approach has been taken to characterize UV-LEDs before utilizing them, which leads to the first research question of the current study:

**QUESTION 1:** Is the conventional protocol of the mercury lamps appropriate to characterize UV-LEDs output and efficiency? If not, could any possible standard method be developed to characterize UV-LEDs?

The hypothesis is that the conventional method for characterizing mercury lamp cannot be implemented to characterize UV-LEDs due to the specific specification of UV-LEDs, such as radiation profile and specific geometry. Unlike mercury lamps, UV-LEDs come with different
radiation profiles and spectral power distributions. A method must be developed to measure these specifications.

In addition to the measurement of UV-LEDs specification, the impact of the operational conditions is not available in the open literature. Parameters like medium and case temperature of the UV-LEDs, and electrical forward current can potentially affect the output of UV-LEDs. It is important to mention that in almost all the UV-LED studies for water disinfection, characterization of the UV-LED and the impact of operational conditions on the UV-LED output and efficiency were not considered.

UV-LEDs kinetics studies usually take place in a petri dish as a batch reactor with a bench scale setup. Since UV-LEDs come in a variety of radiation profiles, different UV radiation distributions are achieved on the petri dish surface. Modeling the radiation distribution of the UV-LED leads to estimating fluence rate and irradiance distribution in front of the UV-LED. As described in section 2.4, the few UV-LEDs modeling efforts available have inaccurate assumptions. Therefore, it is significantly valuable to model both irradiance and fluence rate of the UV-LEDs output and evaluate the model physically. Based on this knowledge gap, the second research question is stated below.

**QUESTION 2:** Is modeling the output of UV-LEDs helpful to determine the radiation distribution on the surface of the petri dish for kinetics studies? How would the radiation profile of UV-LEDs affect measured radiation intensity (both irradiance and fluence rate)?
It is hypothesized that the radiation distribution of a collection of UV-LEDs can be modeled by using the relative radiation pattern of each UV-LED. This model can predict the irradiance and fluence rate in the three-dimensional field in front of the UV-LED. Then, the simplified assumption of the other models can be evaluated through this model.

As described in section 2.5, methodology for determination of the UV dose delivered to the target microorganism from UV-LEDs for water disinfection is not well established. There is a comprehensive protocol available for determining UV dose for mercury lamps. Although a great number of studies have used this protocol for UV-LEDs, there is no holistic study available to evaluate the applicability of this protocol for UV-LED systems. It is of a great interest to determine UV dose in UV-LED systems, without which the reported kinetics results and reactor designs will not be accurate. Based on this knowledge gap, the third research question is stated below.

**QUESTION 3:** How do the different UV-LED specifications (compared to those of mercury lamp) affect the applicability of mercury lamps UV dose determination protocol for UV-LED systems? Is it possible to develop a protocol to determine UV dose of the UV-LED systems?

It is expected that by revising the correction factors in mercury lamp protocol, by defining new correction factors, and by studying the radiation distribution of different UV-LEDs inside the petri dish solution, a holistic protocol can developed and presented for UV-LED systems.

Given the increasing interest in the inactivation kinetics of indicator microorganisms in order to validate the UV reactors, and lack of standard protocol to measure UV dose for UV-LED
systems, it is very valuable to study the inactivation kinetics of a microorganism with the developed protocol in the previous section. Based on this need and potential challenges, the fourth research question is stated below.

**QUESTION 4:** How is the measured inactivation kinetics of a waterborne microorganism affected by the UV radiation distribution on the surface of the petri dish? What would a proper setup look like for performing kinetics studies and implementing the aforementioned protocol?

The hypothesis is that the protocol might not be properly implemented in conventional bench scale setup due to the low radiant power of UV-LEDs. It is expected to get more average irradiance/fluence rate on the petri dish surface for kinetics studies with different setup designs. Optical devices like reflectors and collimating lenses along with highly reflective material columns are among the potential options to deliver greater average irradiance from a single UV-LED to the petri dish surface. The radiation distribution on the petri dish surface and in the fluid sample caused by these optical devices are different and can affect the calculation of UV dose for microbial inactivation tests.

### 2.7 Scope and objectives

UV dose determination for UV-LED systems is of great interest for the purpose of designing efficient UV reactors. The UV dose determination highly depends on proper operation of the UV-LED and considering the UV-LED characterization and setup configuration. Thus, the overall objective of this research is to determine the UV dose for UV-LED systems in order to perform microbial kinetics studies. More specifically, this study aim to design a comprehensive
set of experiments to investigate the parameters contributing to average UV dose determination from different UV-LEDs. This overall goal is achieved through set of specific tasks conducted under the following sub-objectives:

- Study and compare different UV-LEDs with different specifications available in the market and develop a standard method to operate and characterize them in order to obtain consistent and more reliable output from UV-LEDs. This can be done through holistic investigation of the characteristics of the UV-LEDs, and investigation of the parameters affecting the UV-LEDs output.

- Investigate the impact of UV-LED characterization on the delivered irradiance to the target microbial sample by a physically validated optical model. The model includes both irradiance and fluence rate estimation in the three-dimensional field in front of the UV-LED.

- Study, investigate, and predict the impact of radiation distribution on the surface of a petri dish which filled with target water sample for microbial inactivation kinetics studies by considering the water absorption, incident radiation trajectories, and radiation distribution inside the water sample to develop a protocol for UV dose determination.

- Implement the developed UV dose determination protocol to measure the inactivation kinetics of a target microorganism and investigate different setup effect on the measured inactivation kinetics.
Chapter 3: Experimental Setups and Procedures

The experimental methodologies for achieving the stated objectives are presented in this chapter. Any specific procedure or experiment of each objective will be explained in separate chapters.

3.1 Printed circuit board design and preparation

Printed circuit boards (PCB) for UV-LED mounting was designed using Eagle PCB CAD software for the UV-LEDs which came without PCB. To enhance the thermal management, two-layer PCB with many through-hole pads was used. Through-hole pads consist of copper on both (top and bottom) sides of the board and help to conduct the UV-LED’s heat more efficiently. The designed PCB was fabricated by ITEAD intelligent systems CO.

To mount the UV-LED on the PCB, solder paste (solder paste no clean 63/37 35GM, Kester solder) and reflow oven (AS-5001, SMT max) was used following the suggested procedure by the UV-LED’s manufacturer.

3.2 UV-LED characterization setup

A setup was designed and fabricated to measure UV-LEDs specifications, such as SPD, radiant power, and radiation profile. Also, controlling and monitoring the temperature and the electrical current were included as the setup features (Figure 3-1). The UV-LED and the heat sink were placed on a NEMA 17 stepper motor, which rotated the UV-LED with as high as 1600 steps per revolution, resulting in a very smooth radiation profile measurement. The UV-LED’s printed circuit board (PCB) was cooled down using a thermoelectric cooler (TEC) and a fan-cooled
heatsink. To enhance the heat dissipation, a silicon thermal paste was applied between the UV-LED, the TEC, and the heat sink contact surfaces. The UV-LED and heat management components were placed on an X/Y actuator. The X/Y actuator could move the UV-LED in x and y-direction to align the illuminating part of the UV-LED with the stepper motor shaft. Operating the stepper motor, the UV-LED was rotated from 0° to 360° on a horizontal plane.

Figure 3-1. Goniometer sketch – 1-UV-LED with PCB, 2-Heat management components, 3-Detector, 4-X/Y stage, 5-Stepper motor, 6-Rail and 7- lifting stage (the UV-LED’s driver and stepper motors’ microcontroller are not shown).

Spectral irradiance was measured with an Ocean Optics USB2000+ spectrometer equipped with a Sony ILX511B CCD detector with the resolution of 0.38 nm. The detector was equipped with 25 cm fiber optics (QP600-025-XSR) to measure closer distances of radiation distribution and to avoid spatial limitations. The fiber optics were equipped with a cosine corrector diffuser to assure that they measured irradiance. The detector and the attached components were put on a lifting stage, which was placed beside a rail. By using the rail, the UV-LED’s fixture could go back and forth toward the detector. All the components were placed inside a frame (100 cm × 50 cm).
that was covered with a blackout fabric to avoid detector saturation caused by excessive ambient light and to protect the detector from ambient light noises.

3.3 Radiometry

Radiometry is commonly used to measure the average irradiance on a plane with a detector [3], [80]–[87]. In this study, radiometry was used to calculate the output of UV-LEDs and to validate the mathematical model. A factory calibrated spectrometer (Ocean Optics USB2000+) was used as the detector to avoid using the sensor sensitivity since the photodiodes sensors are sensitive to the wavelength [13], [81], [88]. Since the detector’s response to photons incident angles is essential for non–collimated radiation measurements [89] and given that the UV-LEDs radiation is directional, the detector was equipped with a cosine corrector diffuser with 180° viewing angle to ensure irradiance measurement [38], [90].

The irradiance distribution on the petri dish surface was measured with an in-house designed and fabricated setup (Figure 3-2). This setup consists of two stepper motors which move the UV-LEDs on a planar. Since these stepper motors are controlled automatically, the detector can be moved with less than 1 mm resolution. Irradiance was measured in each position by integrating the measured spectral radiation distribution in a proper range of wavelengths.
Figure 3-2. Setup’s schematic view for radiometry and chemical actinometry experiments (in chemical actinometry experiments, the spectrometer was replaced with a petri dish containing the chemical actinometry solution) – 1) UV-LED and its thermal management components, 2) Spectrometer, 3) Lab jack to change the UV-LED elevation, 4, 5) stepper motors and rails to move the detector, and 6) setup frame which was covered with a UV absorptive cloth

### 3.4 Chemical actinometry

Chemical actinometry is a low cost, simple, and accurate method to measure the fluence rate inside a reactor [91]. Potassium Iodide-Iodate actinometry has been used widely for UVC region [92] since the actinometry solution absorbs all the radiation below 290 nm [93]. The actinometry measurement was performed with 0.1 M $\text{KIO}_3$, 0.6 M $\text{KI}$, and 0.01 M $\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$ solution prepared freshly before each experiment. A simplified reactor (petri dish) was used to measure the delivered radiation at different distances ($D$) from the UV-LED. Average photon fluence rate was calculated using the following equation [80], [94]:

\[
\text{Average photon fluence rate} = \frac{1}{D} \int I(D) \, dD
\]
\[
\bar{E}_{0p,\lambda}^0 = \frac{(\alpha_{352}^0 - \alpha_{352})v}{\varepsilon_{352} t \phi_{\lambda} A}
\]

Equation 3-1

where \(\bar{E}_{0p,\lambda}^0\) (Einstein cm\(^{-2}\)·s\(^{-1}\)) represents the spectral average photon fluence rate inside the solution at \(\lambda\) (nm); \(t\) (sec), \(v\) (cm\(^3\)), and \(A\) (cm\(^2\)) represent exposure time, solution volume, and petri dish plane area, respectively; while \(\phi_{\lambda}\) (Einstein·mol\(^{-1}\)) is the quantum yield of the reaction at the wavelength of \(\lambda\). The average photon fluence rate was calculated by integrating Equation 3-1 over germicidal wavelength. Since most of the photons were absorbed in the first 1–2 mm depth of the actinometry solution (\(\alpha > 4\) cm\(^{-1}\)), the measured average photon fluence rate inside the petri dish is equal to the average photon fluence rate at the surface of the petri dish.

\(\alpha_{352}^0\) and \(\alpha_{352}\) (cm\(^{-1}\)) are absorption coefficients before and after the UV exposure, respectively. 
\(\varepsilon_{352}\) (M\(^{-1}\)·cm\(^{-1}\)) is molar absorption coefficient of the actinometry solution. Since different molar absorption coefficients for this actinometry were reported, e.g., 27600, 27636, and 26400 M\(^{-1}\)·cm\(^{-1}\) [53], [82], [95], the concentration of potassium iodide was calculated by measuring absorption of the solution at 300 nm [93] and the molar absorption coefficient was corrected for the concentration based on Rahn et al. data [80]. The molar absorption coefficient was calculated between 27364 and 27600 M\(^{-1}\)·cm\(^{-1}\) for different experiments.

Quantum yield, \(\phi_{\lambda}\), of iodide-iodate actinometry is a function of wavelength [80], [96], [97] and in most of the UV-LEDs’ studies [53], [58], [98], it was used at the UV-LEDs’ peak wavelength. This is despite the fact that the spectral power distribution (SPD) of the UV-LEDs shows that these radiation sources are polychromatic [77]. In this study, the quantum yield was corrected over the spectral output of each UV-LED. The measured average photon fluence rate was
converted to average fluence rate using UV-LEDs’ spectral power distributions in order to compare the results with radiometry tests and validate the model.

The actinometry photon fluence rate measurement can be influenced by reflected radiation from the petri dish’s wall [99]. To prevent this, petri dish was completely filled with actinometry solution. The actinometry solution inside the petri dish was stirred 30 sec before each experiment while the UV-LED’s shutter is closed.

3.5 Spectrophotometry

Spectral absorption and the spectral transmittance of the microbial solution and the actinometry solution for deriving the growth curve and determining the fluence rate were measured with UV-Vis spectrophotometer (Cary 100, Agilent Technologies) with 1 cm quartz cuvette (Hellma Analytics).

3.6 Microbial tests

3.6.1 Microorganism preparation

Pure Escherichia coli (E. coli) (ATCC 11229) was used to prepare microorganism’s solutions for the first experiment following the suggested preparation procedure by the supplier. To store the stock solution for the other experiments, the pure E. coli solution was introduced to the culture medium which is Luria broth (LB broth) (Sigma-Aldrich). The E. coli and the medium solution were cultured for 24 hours in a shaker-incubator (Lab Companion™ SIF-5000) at 37 ºC and 200 rpm. Glycerol solution (50 %) was prepared and autoclaved at 121 ºC for 20 min (Mandel, BioClave 16). The Glycerol solution was added to the E. coli solution (1:1) in order to prevent E.
coli damage at low temperatures. Then, the mixture was distributed in centrifuge tubes (2 mL) and stored in a freezer (-40 °C) for a maximum of 6 months.

Before each disinfection experiment, one tube was taken from stock. After 5 min waiting for the solution to meltdown, 1 mL of the E. coli was added to 9 mL of the autoclaved LB broth solution. After 3–5 hours of cultivation in the shaker-incubator, the solution was centrifuged at 3000 rpm (Champion, F-33D) and washed with 10 mL of the phosphate buffered saline (PBS, 0.01M) (Sigma-Aldrich, tablet) solution three times to remove the nutrient medium. The 10 mL nutrient-free PBS solution was diluted in autoclaved PBS solution to reach the desired initial concentration of E. coli by measuring the absorption of the solution. The initial E. coli concentration for microbial study was maintained at 10^6 CFU by changing the PBS solution volume.

3.6.2 Microorganism enumeration

A variety of methods is available to enumerate microorganisms in laboratories avoiding the contamination of the culturing medium [100]. Among those, pour plating, spread-plating and spot plating [101] have more desirability due to the simplicity and reliability. However, for each method, the range of detection would be different. The range of detection limit of each method is presented in Table 3-1. These method limitations are important to be considered reporting reliable and reproducible data. For example, in one study, recently, more than 6.5 log inactivation was reported while based on the 10^7 CFU of the initial sample and 100 μL culturing sample, the maximum log inactivation would be 5 log [102]. Table 3-1 indicates that the pour plating method can enumerate microorganisms in a broader range and the human error is less
compared to the two other methods due to culturing higher sample volume. Pour plating was used for inactivation kinetics experiments in the current study.

Table 3-1. Common microorganism’s limitation for microorganism enumeration using plating methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Cultured sample volume (mL)</th>
<th>Minimum number of colonies in a single plate</th>
<th>Maximum log inactivation achieved for initial sample of $10^6$ CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spread Plating</td>
<td>0.1</td>
<td>20</td>
<td>3.7</td>
</tr>
<tr>
<td>Spot Plating</td>
<td>0.01</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Pour Plating</td>
<td>1</td>
<td>20</td>
<td>4.7</td>
</tr>
</tbody>
</table>

The concentration of E. coli was measured before and after UV exposure in the microbial tests. 1 mL of the E. coli sample was diluted in series by the dilution factor of 0.1 using a pre-autoclaved 9 mL of PBS solution. Based on the expected concentration of the E. coli, three respective diluted solutions were cultured (1 mL) in LB broth solution with Agar (Sigma-Aldrich, Miller) for 24 hours at 37 ºC in an incubator. The E. coli colonies appear as white dots on the plate (Figure 3-3). By counting the E. coli colonies, the concentration of the sample was back calculated and reported in colony forming unit (CFU) per milliliters. The log inactivation, then, was calculated by $\log \frac{N}{N_0}$ where $N$ and $N_0$ are the number of colonies before and after the UV exposure, respectively.
3.6.3 Photoreactivation control

Photoreactivation, in which the damaged DNA repair themselves using a single enzyme (Photolyse), has been reported for E. coli in room light after a couple of hours [103]. In our inactivation kinetics experiments, a series of samples were exposed to UV radiation in different time periods. To avoid the photoreactivation, all the samples were kept in a dark box after the UV exposure and while the other experiments were performed. Since the plating and culturing the samples are a time-consuming process, the extent of photoreactivation was measured. To do so, the first UV exposed sample was duplicated in two tubes, one was kept with the other samples in the box and the other one was kept in the room light to be exposed to the room light. The reason for choosing the first exposed sample was to maximize the effect of photoreactivation by keeping the sample in room light for a longer time period. These samples (duplicates) were
plated after all the other samples have been plated. The difference in the concentration of these two samples represents the extent of photoreactivation.
Chapter 4: Development of a Method for Characterization and Operation of UV-LED for Water Treatment

Despite the numerous advantages of UV-LED to conventional mercury lamps, unlike mercury lamps, no standard methods are available, from the manufacturers or in the literature, to measure and control UV-LED radiation sources outputs [104], [105]. A protocol is needed to measure and characterize UV-LED more accurately. Each UV-LED has three main specifications, which have to be included in the protocol: the radiant power, the radiation profile, and the spectral power distribution (SPD). Inaccurate measurement of the UV-LED’s radiant power or improper operation of the UV-LEDs results in recording and reporting inaccurately calculated UV doses for microbiological inactivation studies. The radiation profile of each UV-LED is unique [106], so the radiation profile of UV-LEDs has to be measured precisely in order to accurately determine the fluence distribution inside the UV reactors [107]. Moreover, the inactivation sensitivity of waterborne microorganisms are a function of wavelength [108], so the SPD of a UV-LED determines the disinfection efficiency of a UV-LED [26], [58]. In order to develop a comprehensive protocol, the effect of each parameter on these three specifications has to be investigated. To the author’s knowledge, studying the effect of operational conditions and measurement techniques for water treatment applications have not been reported in any detail in the open literature.

In this chapter, we investigated the impact of operational conditions and measurement techniques on determining the output of a variety of UV-LEDs with different radiant powers, radiation profiles, and SPDs; to develop a protocol to not only measure, but also control the output of UV-
LEDs. Operational conditions, such as case temperature and electrical current affect the performance of UV-LEDs, while the measurement techniques, such as detector size, measurement distance, and reflection from the environment, affect the measured spec of the UV-LED. The proposed protocol is an accurate method, which includes a set of guidelines to operate and characterize any UV-LED. Consequently, this protocol could be used to facilitate microbial inactivation or photochemical reaction studies by standardizing the characterization of UV-LED sources from different manufacturers, with a consistent method, and assist UV fluence determination. Further, it allows for an accurate kinetics study of UV-LED microorganism inactivation for applications such as reactor design. To the author’s knowledge, this is the first comprehensive study on developing a protocol for measuring and controlling UV-LED output for water disinfection applications.

4.1 Methodology

4.1.1 Experimental setup

The experimental setup for this chapter was presented in chapter 3, section 3.2.

4.1.2 UV-LED characterization

UV-LEDs from different manufacturers, which cover a wide range of viewing angles, radiant powers, and peak wavelength (Table 4-1) were selected and characterized in order to investigate the effect of operational conditions and measurement techniques on their outputs. Electrical current and case temperature were considered as operational conditions, while measurement distance, detector size, and reflection were considered as measurement techniques. Such
information is essential for the development of a standardized protocol, given that these parameters can potentially affect the performance of UV-LEDs; the efficiency of the experiments involving UV-LEDs are dependent upon UV-LEDs’ performance.
Table 4-1. List of characterized UV-LEDs in this study, with different radiation profiles and suggested operating conditions, which represent the typical available UV-LEDs in market

<table>
<thead>
<tr>
<th>UV-LED</th>
<th>Peak Wavelength (nm)</th>
<th>Viewing Angle (deg.)</th>
<th>FWHM (nm)</th>
<th>Voltage (V)</th>
<th>Current (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manufacturer</td>
<td>Measured*</td>
<td>Manufacturer</td>
<td>Measured†</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>LED1</td>
<td>265</td>
<td>266.3</td>
<td>130</td>
<td>130.2</td>
<td>11</td>
</tr>
<tr>
<td>LED2</td>
<td>275</td>
<td>278.1</td>
<td>135</td>
<td>120.6</td>
<td>10</td>
</tr>
<tr>
<td>LED3</td>
<td>275</td>
<td>274.4</td>
<td>124</td>
<td>128</td>
<td>12</td>
</tr>
<tr>
<td>LED4</td>
<td>285</td>
<td>282.7</td>
<td>130</td>
<td>128.2</td>
<td>13</td>
</tr>
<tr>
<td>LED5</td>
<td>365</td>
<td>366.6</td>
<td>116</td>
<td>116.1</td>
<td>9</td>
</tr>
<tr>
<td>LED6</td>
<td>365</td>
<td>365.5</td>
<td>110</td>
<td>111</td>
<td>9</td>
</tr>
<tr>
<td>LED7</td>
<td>365</td>
<td>365.2</td>
<td>3.5</td>
<td>3.6</td>
<td>9</td>
</tr>
<tr>
<td>LED8</td>
<td>365</td>
<td>364.1</td>
<td>120</td>
<td>115.2</td>
<td>9</td>
</tr>
</tbody>
</table>

* With maximum standard deviation of 0.29
† With maximum standard deviation of 0.1
‡ With maximum standard deviation of 0.22
4.1.2.1 Spectral power distribution measurement

The SPD of three different UV-LEDs with different peak wavelengths, 265 nm, 285 nm, and 365 nm, were measured. All of these measurements were performed at the distance of 25 cm from the UV-LEDs. The effects of case temperature and electrical current were investigated on the SPD of these UV-LEDs. It was assumed that measurement distance and detector surface size would not affect the relative spectral power distribution. This is a valid assumption; given the relative spectral power distribution is a function of the relative energy of photons and is not a function of radiation intensity.

In order to change the operational case temperature, TEC voltage was changed between −12 V and 12 V, for heating and cooling, with the caution of not exceeding the UV-LED’s maximum operational temperature. The relation between TEC voltage and UV-LED’s case temperature is specific to each UV-LED–TEC setup; Figure 4-1a shows a sample of this relation. A K-type thermocouple with an accuracy of ±0.45°C was placed on the cathode pin of the UV-LEDs to monitor the case temperature. The steady state temperature was reached almost 5 min after changing the TEC voltage, and it was double-checked by monitoring the deviation in online irradiance readings (Figure 4-1b). In addition, in order to evaluate the impact of TEC at the initial operation of UV-LED, two scenarios were tested; first, the TEC was turned on and after 10 s the UV-LED was turned on (TEC-LED); second, the UV-LED was turned on and after 10 s the TEC was turned on (LED-TEC). The comparison between two cases shows that although after adequate time period in both cases the UV-LED reaches the same radiant power, a drop in UV-LED radiant power can be observed before TEC is on (Figure 4-2). This drop in radiant
power can affect the lifetime of the UV-LED. Thus, in all the experiments in this study, the TEC was turned on before UV-LED.

Figure 4-1. a) UV-LED case temperature at steady state for different TEC voltages at room temperature (25°C) for LED4, b) Online measured irradiance during TEC voltage manipulation $T_1$ (52.8 °C) and $T_2$ (48.8 °C) are UV-LED’s case temperature at $t_1$ and $t_2$ time, respectively.

Figure 4-2. The impact of TEC on the radiant power of the UV-LED at the start of the UV-LED operation
UV-LEDs are electrical current driven devices. Although driver current and radiation output are related, excessive current will mitigate the UV-LED’s lifetime and its radiant output. The effect of the current on the SPD was investigated by changing it from zero to the maximum allowable current. The electrical current was controlled by a constant current driver (RCD-24-0.70), which was controlled by a potentiometer (analog mode) and a multi-meter, monitoring the current online. By controlling the TEC voltage, the case temperature was kept constant to ensure that the temperature would not interfere with the current on the SPD measurements.

The reflection of the walls, ceiling, ground, and setup components may affect the characterization of UV-LEDs. In fact, each surface reflectivity is a function of wavelength, and reflected radiation can affect not only the irradiance but also the radiation spectrum. The reflection effect was tested by repeating the experiment while the direct path of photons illuminated from UV-LEDs toward the detector was blocked by means of a non-transmittance UV material. The differences between the results with and without the blockage present the reflection effect.

4.1.2.2 Radiation profile measurement

The UV-LED radiation profile of eight UV-LEDs (Table 4-1), from different manufacturers, was measured by means of the designed goniometer (Figure B-1). In order to measure the radiation profile of the UV-LEDs with high accuracy and to derive a smoother radiation profile, irradiance was read every 5°. In the case of a steep change in the radiation profile, step sizes were decreased to 1°. Since the utilized detector was a spectrometer, the spectral irradiance output of the detector
was integrated to determine the irradiance at each angle ($\theta$, the angle between the UV-LED’s normal vector and the detector’s normal vector).

The effect of operational conditions and measurement techniques on the radiation profile of these UV-LEDs was investigated. In the same way as the SPD experiments, the effects of case temperature and electrical current were investigated in the allowable operational condition range for UV-LEDs with 124°, 120°, 116°, 110°, and 3.5° viewing angles.

The effect of the detector surface was investigated by measuring the radiation profile of two different UV-LEDs with wide and narrow viewing angles (120°, 3.5°). The two different detectors were a photodiode power sensor (NEWPORT 918D-ST-UV) with the active plane size of 0.81 cm$^2$, and the Ocean Optics detector, with the circular active site of 0.12 cm$^2$ in diameter. These measurements were performed at a distance of 5 cm away from the UV-LED. In addition, the radiation profile of these two UV-LEDs was measured at different distances (5–30 cm) to investigate the measurement distance effect. Along with these measurements, the reflection effect was tested by blocking the UV-LED-detector path for each angle (−90° to 90°) irradiance measurement.

**4.1.2.3 Radiant power measurement**

The output power of four UV-LEDs with different output powers (one high and one low output power of UVA LEDs, $LED_4$ and $LED_6$, and one high and one low output power of UVC LEDs, $LED_2$, $LED_3$) was calculated by measuring the absolute radiation profile on a dome surrounding the UV-LED. The effects of electrical current, case temperature, measurement distance, detector
size, and reflection were investigated on the calculated radiant power. Chemical actinometry and operating integrating sphere are two other methods to measure the absolute radiant power of the UV-LEDs. However, radiometry was chosen to keep the proposed characterization method simple and to avoid the uncertainty that causes by chemical actinometry solution quantum yield or reflection from the solution surface at different wavelengths.

The radiant power of a UV-LED \( P \) was calculated by integrating irradiance \( E \) on a sphere surface \( A \), enclosing UV-LED at distance of \( r \) and considering the radiation attenuation caused by medium absorption with the following equation:

\[
P = \oint \left[ \int E_\lambda 10^{-\alpha_\lambda r} d\lambda \right] dA
\]

Equation 4-1

where \( \alpha_\lambda \) represents the molar absorption coefficient of the medium at \( \lambda \). Since irradiance distribution is not uniform on the surface of this sphere, this integration was calculated by integrating on small elements. The area of these elements related to azimuthal angle \( \varphi \), polar angle \( \theta \), and the sphere radius \( r \) by:

\[
dA = r^2 \sin \theta \ d\varphi d\theta
\]

Equation 4-2

The irradiance distribution was measured at a constant distance from the UV-LED. Since UV-LEDs emit radiation in one side of their planar and absorption of the air in the UV range is negligible, Equation 4-1 was simplified as follows:
\[ P = r^2 \int_{0}^{\frac{\pi}{2}} \int_{0}^{2\pi} E(\theta, \varphi) \sin \theta \, d\varphi d\theta \quad \text{Equation 4-3} \]

Equation 4-3 was simplified for UV-LEDs with the symmetry assumption of the radiation profile in the azimuthal direction. This assumption has to be validated for each UV-LED since it is not valid for all UV-LEDs.

\[ P = 2\pi r^2 \int_{0}^{\frac{\pi}{2}} E(\theta) \sin \theta \, d\theta \quad \text{Equation 4-4} \]

The radiant powers of high and low power UV-LEDs in both UVA and UVC regions were calculated by solving Equation 4-3 numerically with MATLAB software. The results were compared with the reported value provided in the manufacturer’s datasheet. The reflection effect was also investigated.

4.2 Results and discussion

Investigating the effects of operational conditions and measurement techniques provided the information necessary for developing a protocol to systematically control and measure the output of UV-LEDs. The effect of operation and measurement parameters were taken into consideration for evaluating the SPD of the UV-LEDs listed in Table 4-1. Given that waterborne microorganisms’ inactivation is sensitive to spectral output of the radiation source [49], [50], [81], [109], [110] and based on the presented SPD of UV-LEDs in Figure 4-3, UV-LEDs have to be considered as a polychromatic radiation source. The SPD of UV-LEDs is usually presented by the peak wavelength and the full width at half maximum (FWHM.) For LED1, however, it was observed that although the peak wavelength and the FWHM were the same as those specified in the manufacturer datasheet, a local peak wavelength appeared around 400 nm. Almost 6% of this
UV-LED’s output was in the UVA and visible region, while the reported peak wavelength was in the UVC region. Utilizing such a UV-LED for water disinfection may result in inaccurate UV-LED output measurements and lower microorganism inactivation efficiency since UVA radiation and visible light can result in microbial reactivation [111]–[113]. Hence, an accurate and broadband measurement of the SPD was included in the context of the protocol.

Figure 4-3. SPD of the studied UV-LEDs. The magnified spectrum of the LED1 shows a local peak wavelength in the visible region.

Furthermore, measuring the irradiance output of the UV-LEDs over time showed that it can take around 5 min to reach a constant radiation output, as observed for the studied UV-LEDs (Figure B-2). This was different from the literature reports, which claimed that UV-LEDs do not need warm-up time [26], [30], [44], [82], [98], [114]. The path and the time period to reach the constant output are related to the thermal management of the UV-LEDs. For each UV-LED, this
warm-up time was measured (1% deviation from the steady state irradiance) and included in the proposed protocol.

4.2.1 Operational condition

The case temperature (as a representative of junction temperature) and electrical current effects on UV-LEDs performance were investigated in detail. Electrical current affects the electron and electron–holes recombination in UV-LEDs’ p–n junctions, while temperature influences the recombination’s efficiency.

4.2.1.1 Case temperature effect

The effect of the case temperature on the UV-LED’s SPD was evaluated by monitoring the peak wavelength and FWHM of the UV-LEDs. These increased with the increase in case temperature, as shown for LED4 in Figure 4-4. This phenomenon was observed for all the UV-LEDs in different UV regions (UVA, UVB, and UVC), but the severity was much greater for high power UV-LEDs. For instance, while increasing the temperature of LED4 (UVA LED) resulted in shifting the peak wavelength and the FWHM by 3 nm and 1 nm, as presented in Figure 4-4, increasing the temperature from 17°C to 47°C for LED1 (UVC LED) resulted in shifting the peak wavelength from 266 nm to 266.9 nm and shifting the FWHM from 10.5 nm to 10.9 nm. Since the action spectrum of a microorganism is related to the radiation spectrum [49], [81], [109], [110], the disinfection efficiency of UV-LEDs may be influenced by the case temperature, which indicated that temperature control of UV-LED systems is crucial for stable disinfection efficiency. However, some UV-LED manufacturers report the output of their UV-LEDs by peak
wavelength and FWHM without showing the effect of temperature. Since temperature can shift
the peak wavelength and change the radiation spectrum, and the extent of microbial inactivation
is related to the radiation wavelength, using the reported SPD from the manufacturers without
considering the effect of temperature might result in an inaccurate interpretation of the microbial
inactivation studies. The extent of the impact of UV-LED case temperature on the inactivation
studies depends on the microorganism sensitivity to wavelength on the studied range of
wavelength, and the relation between case temperature and the radiation spectrum.

![Graphs showing FWHM and peak wavelength of LED4's SPD at different case temperatures](image)

**Figure 4-4.** a) Full width at half maximum and b) Peak wavelength of the LED4’s SPD at different case
temperatures. All the data were collected at the steady output of the UV-LED.

The radiation profiles of 5 UV-LEDs, with a wide range of commercially available radiation
profiles (LED3, LED5, LED6, LED7, and LED8), were measured at different case temperatures.
These UV-LEDs were selected to cover a range of radiant power, viewing angle, and peak
wavelength. There was no significant difference between radiation profiles at different case
temperatures (Figure B-3). Based on the manufacturing of the UV-LEDs, a defect in illuminating
part of the UV-LED might happen, even in the operational temperature range. However, given the small size of the UV-LEDs’ die, this defect does not affect the radiation profile.

The radiant power of the studied UV-LEDs was significantly affected by the UV-LEDs’ case temperature (Figure 4-5a). Higher case temperatures resulted in lower radiant powers. For example, raising the case temperature from 11 °C to 47 °C for one of the studied UV-LEDs (LED1) reduced its output by 29%. Thus, there is a risk of reporting a considerably higher/lower UV dose if the case temperature effect is not considered in UV dose measurements for microbiological and photochemical studies. The temperature impact on the output of UV-LEDs is related to their material and internal quantum efficiency [104], [115]. The reverse-biased current leakage was found to be a function of temperature which affects the radiant power of LEDs [116]. The relation between the current leakage and the temperature is related to the threading dislocation density (TDD) of the p-n junctions ($10^9 – 10^{10}$ cm$^{-2}$ for GaN and Si) which means that for different semiconductors, the impact of temperature on UV-LEDs are different. That is, for different semiconductors, the impact of temperature on UV-LEDs is different. The relations among the current leakage, temperature, and TDD are available in the literature [116], [117] and more detailed investigation of this relationship is beyond the scope of this project.
4.2.1.2 Electrical current effect

The electrical current of the circuit is another parameter that can affect the output of UV-LEDs. An analog electrical driver was implemented to dim the UV-LEDs from 0 Amps to their maximum operational current. However, the higher electrical current increased the temperature of the UV-LEDs. Hence, in order to investigate the effect of the current independently, the constant temperature of the solder was maintained using the TEC. All the electrical dimming experiments were performed at the distance of 20 cm or 25 cm with the spectrometer aligned with the radiation source at $\theta = 0^\circ$.

The spectral power distribution of three UV-LEDs with a peak wavelength of 265 nm, 285 nm, and 365nm showed no significant effect of the electrical current on the emitted radiation spectrum. In addition, the measured radiation profile of UV-LEDs was not affected by the electrical current, as long as the UV-LED operated in a safe electrical current range.
As it was expected, the effect of the electrical current of the circuit on the radiant power of the UV-LEDs was significant, as illustrated for one of the UV-LEDs (*LED1*) tested in Figure 4-5b. Since the relative radiation profile of the UV-LEDs was not a function of the electrical current, the radiant power was calculated by measuring the irradiance at a specific distance at $\theta = 0^\circ$. A linear relation between the electrical current and the radiant power was observed for all the studied UV-LEDs. However, the slope of this line was different for each UV-LED. The irradiance–current relation (Figure 4-5b) has two main applications. First, by utilizing this property, a desirable radiation intensity inside a UV-LED reactor can be obtained by controlling the UV-LEDs’ current (at the same temperature). Second, the radiant power of the UV-LEDs can be monitored online by measuring the electrical current with a multi-meter, when the UV-LED is operating inside the reactor. A precise current driver is needed to operate the UV-LEDs as they are very sensitive to electrical current, and even a 1 mA change in the electrical current of low current UV-LEDs may cause a significant change in the UV-LED’s power output.

### 4.2.2 Measurement technique

Detector size, measurement distance, and reflection are considered as measurement techniques that affect the measured output of the UV-LEDs and not their performance. Not considering the effect of these parameters results in an inaccurate UV dose measurement in microbiological and photochemical studies.
4.2.2.1 Measurement distance

The SPD of two UV-LEDs, a UVC and a UVA LED, was measured in the range of 1–30 cm away from the UV-LED. As expected, there was no significant difference between the SPDs in the different distances. In addition, the relative radiation profile of four UV-LEDs with different viewing angles (135°, 125°, 120°, and 3.5°) was measured at different distances. Three of these UV-LEDs had different radiation profiles in different distances: LED3, LED7, and LED8. The radiation profile of the LED7 is shown in Figure 4-6a. The measured viewing angle of this UV-LED changed from 3.6° at 30 cm to 5.8° at 5 cm. The schematic view in Figure 4-6a explains this change. The detector read the average irradiance on its planar surface and reported this reading for its center point. In different distances from the UV-LED, the amount of radiation hitting the detector’s surface varied, which, in turn, affected the detector reading. This effect was higher at closer distances and for UV-LEDs with an instant change in their radiation profile; an example of such change is shown for LED7, which had a sharp peak in its radiation profile (Figure 4-6).
Figure 4-6. a) Measurement distance and b) detector planar size effect on radiation profile measurement of LED7. The pictures on top show the schematic view of the detector and UV-LED’s position and the surface of which the detector measures the irradiance.

The measured radiant power was calculated using the measured absolute radiation profile of the UV-LEDs. Since the measurement distance influences the radiation profile measurement, it subsequently affects the measured radiant power. The measured radiant power of LED3 in different distances (Figure 4-7) showed that there is an optimum distance beyond which the calculated radiant power becomes constant, which was expected for a non-UV-absorptive medium. Based on the UV-LEDs’ radiation profiles, a measurement closer than this distance may underestimate or overestimate the radiant power, given the UV-LED die cannot be considered as a point source at closer distances. This distance varies for each detector-UV-LED combination.
Figure 4-7. Radiant power of LED3 was measured at different distances from the UV-LED. Measurements at closer distances include error based on the detector size and UV-LED radiation profile.

4.2.2.2 Detector characteristics

The relative radiation profile and the radiant power of two UV-LEDs, LED7 and LED8, were measured with Newport and Ocean Optics detectors. These two detectors have different planar sizes. While consistent detector readings are expected when different detectors are used, Figure 6b shows a deviation between the measured relative radiation profiles from the two detectors at the same distance from the radiation source. In addition, the measured radiant power was influenced by the detector size. These differences were related to the amount of radiation hitting each detector’s surface (Figure 4-6b, schematic view). Using a chemical actinometry solution or an integrating sphere at closer distances will result in the same deviation in a measured radiation profile. Like the distance effect, there is an optimum distance after which the irradiance
measurement becomes independent of the detector size. Finding and reporting this distance for UV-LED characterization is vital.

Detector reading is a function of the incident angle of the photons to the planar surface of the detector. This function is not determined for many detectors and the detector manufacturers suggest using the detectors only for collimated radiations. Since UV-LEDs are directional radiation sources, at closer distances to the UV-LED the photons hitting the surface of the detector are not collimated. Trusting the detector reading, without considering the response of the detector to non-collimated radiation, at closer distances results in both an inaccurate radiation profile and an incorrect radiant power measurement. Utilization of a cosine corrector diffuser, which accounts for the photon incident angle and assures the irradiance measurement, is highly recommended.

4.2.3 Reflection

The reflection effect in each experiment was investigated by measuring the absolute radiation profile of each UV-LED. The absolute radiation profile was measured in two conditions: UV-LED OFF and UV-LED ON with a 1 cm² woody sheet between the UV-LED and the detector blocking the direct path of photons. If the results of these two conditions were significantly different, the setup configuration was modified by expanding the setup’s frame size, changing the frame cover material, or revising the setup component’s coating to reflect less UV radiation. To correctly characterize UV-LEDs, reflection effect has to be considered. This effect can be taken into account in a similar way suggested for UV mercury lamps, in the revised UV lamp measuring protocol [118].
4.3 A protocol to characterize UV-LEDs

The proper operation and accurate measurement of UV-LEDs are crucial, specifically for water treatment applications where a slight difference in SPD, radiation profile and radiant power measurements leads to the inaccurate UV dose determination, which results in incorrect microbial inactivation kinetics data. A standard protocol was developed to accurately control and monitor the UV-LEDs’ output. This protocol helps to perform more precise, reliable, and consistent UV-LED characterization in different laboratories and with different equipment. This is applicable to the characterization of UVA, UVB, and UVC LEDs in any shapes, radiant power, and radiation patterns.

4.3.1 Equipment

The main necessary equipment and devices are as follows:

*Thermal management* – A thermocouple and any combination of thermal management devices including a TEC, a heat sink, thermal paste, and a fan are needed to dissipate the generated heat of the UV-LED and to regulate and monitor the case temperature. Depending on the application, some of the thermal management equipment may be eliminated; for example, the fan for low output power UV-LEDs, or the TEC if the case temperature effect is not desired. For each UV-LED, a specific thermal management system has to be designed to control the temperature.

*Electrical driver* – A constant current driver (also known as DC/DC converter) followed by a constant voltage driver (to control the maximum voltage) is needed. The dimmable current driver
must be able to control the electrical current of the UV-LED. A multimeter is needed to monitor the current online.

**Detector** – A spectrometer equipped with a cosine corrector calibrated for the applicable wavelength range by a qualified third party, is needed. Alternatively, an integrating sphere can be used.

**Setup** – A setup has to be designed to measure the irradiance on the surface of a hemisphere. An example of this setup is presented in Figure 3-1.

### 4.3.2 Experimental procedure

Based on the experiments performed and the subsequent results, a proposed detailed experimental procedure for characterizing UV-LEDs is presented in Appendix A. In general, to characterize various UV-LEDs with different specifications, the following steps must be followed to obtain consistent, accurate, and reproducible results from the UV-LEDs’ experiments.

1- UV-LEDs are current-driven radiation sources, so a proper current regulator to maintain and control the UV-LEDs driving current is crucial to operate the UV-LED consistently and to evaluate the impact of the electrical current on the UV-LED’s performance.

2- Proper thermal management components are needed to maintain and control the temperature of the UV-LEDs. The temperature affects SPD and the radiant power of UV-LEDs. The temperature of the UV-LED has to be maintained during the experiment. To
minimize the impact of the temperature on the experiment results, measuring the LED
temperature before and after the experiment is suggested.

3- Radiant or irradiance of the UV-LED must be monitored during the first couple of
minutes of the UV-LED operation to determine the warm-up time, where the UV-LED
power reaches a steady-state value. Warm-up time is related to the structure of each UV-
LED and therefore varies.

4- The spectral power distribution of the UV-LEDs has to be measured along the UV region
with a spectrophotometer before performing any experiments, rather than relying on a
reported peak wavelength of the UV-LED by the manufacturer.

5- A proper setup has to be implemented to measure the radiation profile of the UV-LED in a
three-dimensional field. A two-dimensional relative radiation profile, which is usually
reported by the manufacturer, is only valid if the radiation profile is symmetric. Moreover,
the reflection of the radiations from the setup components has to be measured and
minimized.

6- The impact of the temperature and current on the SPD and the radiant power of the UV-
LED must be measured at constant current and temperature, respectively. The relationship
between these parameters is crucial for calculating the fluence rate of the UV-LEDs.

7- The impact of the detector size and the distance in which the characterization is
performed, have to be evaluated.
4.4 Conclusion

A protocol was developed to accurately control and measure the output of the UV-LEDs. In order to develop this protocol, the effect of the operational conditions and the measurement techniques were investigated. Operational conditions (temperature and electrical current) were found to significantly affect the radiant power of UV-LEDs. The SPD was affected only by temperature, and the relative radiation profile was found to be independent of temperature and electrical current. Measurement techniques (detector size and measurement distance) were found to considerably affect the UV-LED’s measured relative radiation profile and radiant power.

The proposed protocol helps to properly operate and control UV-LEDs. This protocol can be used for the accurate measurement of the UV-LED output power and radiation profile, and to quantify the performance of different UV-LEDs in water disinfection applications. The spectrum and radiation profile of UV-LEDs were accurately determined by the proposed protocol. These data are crucial for determining the microbiological inactivation kinetics of waterborne microorganisms, and for designing UV reactors. This protocol not only covers UV-LEDs’ water disinfection applications, but also can be applied to other applications of UV-LEDs, such as polymer curing, advanced oxidation processes, sensors, and more.
Chapter 5: UV-LED Radiation Modeling and its Applications in Dose Determination for Water Treatment

With the growing application of the ultraviolet light emitting diodes (UV-LED) for water treatment purposes, the significance of modeling their output for designing UV reactors becomes crucial. The aim of this chapter is to develop a model to predict the fluence rate and irradiance distribution of UV-LEDs, independent of their structure and radiation profile. To do so, the radiation distributions of two UV-LEDs with different radiation profiles were modeled. These UV-LEDs represent the two most common radiation profiles of UV-LEDs - balloon shape and heart shape. Irradiance and fluence rate distribution were predicted using a numerical method and experimentally validated by measuring irradiance and fluence rate.

Initially, these UV-LEDs were characterized based on the proposed characterization method in chapter 4 [77]. The actual radiation profiles of two UV-LEDs from two different manufacturers were measured. Then, the predicted fluence rate and irradiance were validated by experimental data from chemical actinometry and radiometry, respectively. Finally, the model was used to quantify some of the assumptions of the other models for UV-LEDs’ radiation profile. This model can be linked to a computational fluid dynamics (CFD) software to predict the fluence rate distribution inside the reactors for designing UV-LED reactors. The primary application of this model would be the accurate prediction of average fluence rate on the surface of the petri dish for microbial inactivation studies.
5.1 Methodology

5.1.1 Radiation sources

UV-LEDs are available in different radiation profiles (Figure 5-1) based on their chip orientation, material, and manufacturing technique [66], [119]–[123]. While most of the literature did not consider the effects of the UV-LEDs radiation profile, a few studies reported on their significant impact on the performance of the photoreactor [31], [78]. The radiation profile of most UV-LEDs can be categorized into two different groups: heart shape and balloon shape. The maximum irradiance of balloon shape radiation profiles is normal to the UV-LEDs surface, whereas that of the heart shape radiation profiles occur at any angle but the normal angle. In this study, two UV-LEDs, one representing each group, were chosen to investigate the accuracy of the model for different radiation profiles. LED1 had a heart shape radiation profile, and LED2 had a balloon shape radiation profile.

Figure 5-1. Relative radiation profiles of some of the available UV-LEDs.
5.1.2 UV radiation distribution model

Fluence modeling is needed to determine the inactivation kinetics of waterborne microorganisms. For UV mercury lamps, a method is proposed [13] to calculate the average fluence inside the water solution container (petri dish) with a collimated beam apparatus. In this method, the fluence is estimated by measuring the average incident fluence rate on the surface of the petri dish. This technique has been used for UV-LEDs with or without the columns in different distances from the UV-LEDs [55], [56], [58], [124], [125] without considering the radiation distribution on the petri dish surface. However, such consideration is essential given predicting the fluence rate on the petri dish surface accurately is crucial to determine the fluence–inactivation response of the microorganisms in a UV-LED setup.

UV-LED’s fluence rate and irradiance were modeled utilizing measured radiation profile data. Alternatively, the manufacturers’ reported relative radiation profile of UV-LEDs could be used as the model input. In this study, the three-dimensional radiation profiles of LED1 and LED2 were measured and the relative radiation profiles were used for modeling. In the model developed here, the relative radiation profile (RRP) of the UV-LEDs was calculated with the following equation:

\[
RRP(\theta, \varphi) = \frac{E_0(R_{ref}, \theta, \varphi)}{E_0(R_{ref}, 0, 0)}
\]

Equation 5-1

where \(\theta\) and \(\varphi\) are polar and azimuthal angles, respectively. \(E_0\) (mW·cm\(^{-1}\)) represents the fluence rate and \(R_{ref}\) (cm) is the distance at which the radiation profile was measured. The symbols and
terms used in this study were adopted from the “Glossary of terms used in photochemistry” and “Ultraviolet Applications Handbook” [38], [126].

The fluence rate distribution in the three-dimensional field in the air in the spherical coordinate system was calculated based on the availability of the radiant power, absolute radiation profile, and relative radiation profile. If the absolute radiation profile or relative radiation profile and one point fluence rate were available, the fluence rate distribution can be calculated with the following equation (scattering effect was negligible since there is no floating particle in air):

\[
E_0^0(r, \theta, \varphi) = RRP(\theta, \varphi).E_0^0(R_{\text{ref}}, 0, 0).\left(\frac{R_{\text{ref}}}{r}\right)^2.10^{-\alpha_\lambda(r-R_{\text{ref}})}
\]  

Equation 5-2

where \(\alpha_\lambda\) (cm\(^{-1}\)) is absorption coefficient of the air at \(\lambda\). Since the absorption of the air at UVC region is negligible, the last term on the right hand side of the above equation is equal to 1. If the absolute radiation profile of the UV-LEDs are not available, relative radiation profile (\(RRP\)) and total radiant power (\(P\)) of the UV-LED reported by the manufacturer can be used and the fluence rate distribution can be calculated with the following equation:

\[
E_0^0(r, \theta, \varphi) = RRP(\theta, \varphi).\frac{P}{r^2\int_0^{2\pi} \int_0^\pi RRP(\theta, \varphi) \sin(\theta) d\theta d\varphi}.10^{-\alpha_\lambda(r-R_{\text{ref}})}
\]  

Equation 5-3

In order to calculate the average fluence rate on the surface of the petri dish, Equation 5-2 was integrated on the planar surface (Figure 5-2):
\[
\overline{E_0} = \frac{\int_{A_p} E_0^0 \, dA}{A_p} = \frac{1}{\pi \hat{R}^2} \int_0^{2\pi} \int_0^\hat{R} \hat{r} \cdot E_0^0 \, d\hat{\phi} d\hat{r}
\]

Equation 5-4

where \( \overline{E_0} \) (mW·cm\(^{-2}\)) is the average incident fluence rate (in the air) on the surface of the petri dish and \( \hat{R} \) (cm) is the radius of the petri dish. In order to calculate the average fluence rate on the surface of the petri dish in the solution side, the reflected radiation, calculated from *Fresnel* equation, was deducted from Equation 5-4. The reflected radiation at the air–solution intersection is a function of incident angle of the photons and the medium’s refractive indices. Hence, the average fluence rate inside the solution was calculate by:

\[
\overline{E} = \frac{\int_{\lambda} E_{0\lambda} d\lambda}{\int d\lambda} = \frac{1}{\pi \hat{R}^2} \int_0^{\lambda_1} \int_0^{\lambda_2} \hat{r} \cdot E_{0\lambda}^0 \cdot (1 - R_\lambda) \cdot d\hat{\phi} \cdot d\hat{r} \cdot d\lambda
\]

Equation 5-5

where \( \overline{E_0} \) (mW·cm\(^{-2}\)) is the average fluence rate on the surface of the solution and \( R_\lambda \) is reflectance at \( \lambda \). All the integrations in this study were calculated numerically in MATLAB since the actual measured radiation profile was used.

**Figure 5-2. Model configuration and symbols**
Based on the petri dish and UV-LED configuration, the fluence rate calculated with Equation 5-3 can be converted to the Cartesian coordinate system by the following equation:

$$
\hat{E}_0^0(x, y, z) = RRP \left( \arccos \left( \frac{(z-Z)}{\sqrt{(x-X)^2 + (y-Y)^2 + (z-Z)^2}} \right), \arctan \left( \frac{y-Y}{x-X} \right) \right) \cdot \hat{E}_0^0(0,0, R_{ref}) \cdot \left( \frac{R_{ref}}{z-Z} \right)^2
$$

Equation 5-6

where $\hat{E}_0^0$ (mW·cm⁻²) is the fluence rate distribution in Cartesian coordinate system at x, y, and z point. X, Y, and Z are the UV-LEDs position coordinates.

Besides the fluence rate determination, the irradiance distribution was determined by considering the incident angle of a photon to the surface of the petri dish. Unlike fluence rate, irradiance is a function of the detector (target planar) orientation. To determine the incident irradiance distribution on the surface of the solution, the following equation was used:

$$
\bar{E}^0 = \frac{1}{4\pi R^2} \int_{0}^{R} \int_{0}^{2\pi} \hat{E}^0 \cdot \cos(\theta) \cdot d\phi \cdot d\hat{r}
$$

Equation 5-7

where $\bar{E}^0$ (mW·cm⁻²) represents the irradiance on the surface of petri dish. Using this equation, the irradiance distribution was predicted. These data were evaluated with radiometry, in which the detector measured the irradiance distribution in the Cartesian and Spherical coordinate system.
5.2 Results and discussion

5.2.1 UV-LED’s model

Figure 5-3 shows the predicted incident average fluence rates of LED1 and LED2, on three petri dishes with different diameters at different distances from the UV-LED (1 mm to 30 cm). From Figure 5-3a, it is evident that there is a lower sensitivity of the fluence rate to distance for LED1 at closer distances. UV-LEDs are directional radiation sources, and as the petri dish is placed farther from the UV-LED, less fluence rate is delivered to its surfaces. However, for LED1, moving the petri dish farther from the UV-LED, more average fluence rate was measured at near-field measurements. This can be justified by considering the heart shape radiation profile of LED1. The peak of its heart shape radiation profile reaches the edges of the petri dish surface, and it compensates a portion of the radiation loss due to the distance change.
Figure 5-3. Predicted average fluence rate of LED1 and LED2 using the model. a - Average fluence rate delivered to the surface of three different petri dishes in different distances (z). b - Average fluence rate delivered to the surface of different petri dishes (with a radius of R) in three different distances from the radiation source.

The average fluence rate on petri dishes with different radii (1 mm to 7 cm) was predicted at three different distances from the UV-LED (1, 5, and 20 cm) utilizing the model (Figure 5-3b). For a given distance from the UV-LED, lower average fluence rate is expected with a bigger petri dish, due to the UV-LEDs’ radiation propagation. The average fluence rate of LED1 for different petri dish sizes, however, has a peak in its radiation profile. This indicates that at some
distances from the UV-LED, with a bigger petri dish (larger volume of the target solution), a higher average fluence rate can be achieved, leading to more microbial inactivation. Note that for conventional mercury lamps and balloon shape UV-LEDs, the bigger the petri dishes, the lower the average fluence rates delivered.

Fluence rate distribution on the surface of a 2.5 cm petri dish at different distances (1, 5, 10, and 20 cm) from the LED1 and LED2 was predicted using the model (Figure 5-4). Although the average fluence rate at closer distances was high, the delivered fluence rate was not uniform. This suggests that more vigorous mixing would be needed inside the petri dish for these cases to increase uniformity inside the solution [127]. Since increasing the mixing has a limitation, increasing the distance between the UV-LED and the petri dish is an alternative method of obtaining more uniform delivered radiation distributions (a comprehensive discussion on determining the proper distance between UV-LED and the petri dish for obtaining a uniform delivered radiation distribution is presented in Chapter 6).
Figure 5-4. Fluence rate distribution on a petri dish with 2.5 cm radius at different distances.
5.2.2 Model validation

The model was validated with two methods: radiometry and chemical actinometry. Irradiance and fluence rate predictions on the surface of the petri dish were evaluated using a spectrometer and actinometry, respectively.

5.2.2.1 Radiometry

A simplified configuration of UV-LED and detector was chosen to compare the detector readings with the model predictions. The irradiance distributions on the surface of the petri dish at distances between 1.3 and 15 cm from the radiation source were measured with the spectrometer (measurements with 1 mm resolutions). Figure 5-5 shows the model prediction of average irradiance on a circular planar, with a diameter of 4.6 and 5.5 cm, for LED1 and LED2, respectively. Each experiment performed at least three times and the standard error was represented as error bar in Figure 5-5. The experimentally measured average irradiances show a good agreement with the model predictions. In addition, it was concluded that although the average irradiance at closer distances to the UV-LED is high, it is very sensitive to the distance in this tested range. Hence, closer distances are advised to be avoided for microbiological experiments. The absolute radiation distribution comparison between the model and the radiometry data is presented in App B.2.
5.2.2.2 Chemical actinometry

Average fluence rate of the two UV-LEDs at different distances (3–20 cm) from the radiation source was measured using iodide–iodate chemical actinometry and compared to the model predicted fluence rate. The petri dish diameters for LED1 and LED2 were 5.96 and 5.51 cm, respectively. Each actinometry experiment replicated at least three times and the standard error is presented as the error bar. Figure 5-6 shows a good agreement between the measured and predicted fluence rates. The closer the actinometry container was to the radiation source, the higher fluence rate was measured. At infinitely close distances to the UV-LED, the actinometry solution is able to measure the radiant power of UV-LED. In other words, the actinometry method, incorporated with reflection effect, acts like an integrated sphere for measuring the radiant power of UV-LEDs. However, since at closer distances to the UV-LED, the delivered irradiance to the actinometry solution is so sensitive to the distance, and the reflection of the
photons increases because of the wider incident angle of photons (the Fresnel law), it is not recommended to use chemical actinometry for measuring UV-LED’s radiant power. The determination of the spectral quantum yield of iodide–iodate actinometry at room temperature (20-24 °C) has been tried in literature, however, their relationship with temperature is not yet well determined for polychromatic radiation sources at UVC region. Thus, measuring the radiant power of UV-LEDs in the other temperature ranges using chemical actinometry is not possible due to unknown iodide–iodate quantum yield.

![Graph](image)

**Figure 5-6.** Model validation using iodide–iodate chemical actinometry to measure the average fluence rate at different distances for a) LED1 and b) LED2 UV-LED.

### 5.2.3 Application of the model

One of the applications of the developed model is to predict the radiation distribution on the surface of the microorganism’s container for microbiological experiments. This will be necessary for calculating the average UV dose delivered to the microorganisms. In this section,
the validity of point source assumption and symmetrical radiation profile assumption for UV-LEDs were investigated using the developed radiation model.

5.2.3.1 **Quantifying the symmetry assumptions for radiation profile of UV-LEDs**

Measuring the radiation profile of UV-LEDs in a three-dimensional field is challenging. It needs special equipment, and it is time-consuming. Researchers usually make two assumptions to simplify the radiation profile of UV-LEDs for implementing them in UV reactor designs, which are as follows:

1- Measuring/using the irradiance profile from $\theta = -90–90^\circ$ and assuming symmetrical radiation profile in azimuthal direction (Figure 7b).

2- Measuring/using the irradiance profile from $\theta = 0–90^\circ$ and assuming symmetrical radiation profile in azimuthal direction (Figure 5-7c).

The former assumption is valid for some UV-LEDs, e.g., LED2. However, since UV-LEDs are directional radiation sources, radiation intensity is dependent on both azimuthal and polar angles (Figure 5-7a). With respect to the latter assumption, Figure 5-7c depicts the mirrored radiation profiles of $-90^\circ–0^\circ$ and $0^\circ–90^\circ$ on the same graph, indicating that this assumption could cause an error in modeling and reactor designs.
Figure 5-7. Radiation Profile of LED1. Radiation intensity was measured on a hemisphere enclosing the UV-LED with 30cm radius. a) 3D radiation profile; b) 2D radiation Profile at two different azimuthal angles ($\phi$); c) 2D radiation profile (The mirrored data represent the radiation profile from -90° to 0°)

To quantify the errors related to these assumptions, the radiant power of LED1 and LED2 at different distances from the UV-LED was measured and compared with the calculated radiant power with these assumptions. The results show 5.3 ± 0.9% error for the former assumption and 15.1 ± 2.6% error for the latter assumption which considered symmetry in the radiation profile around the normal vector of the UV-LEDs. These error values are unique to each UV-LED and are related to the UV-LEDs’ radiation profile. The errors associated with these simplifying assumptions, which our study showed them to be significant, have to be evaluated and taken into account before using the LEDs for reactor designs.

5.2.3.2 Evaluating the point source assumption

The photonic detectors have different responses to the incident angle of photons, and they measure the average radiation hitting their planar. If the detector is equipped with a cosine corrector diffuser or if the incident photons are collimated and normal to the detector planar, the
detector reading would be average irradiance of its surface. This number is usually used to report the irradiance at the center point of the detector. This assumption results in an inaccuracy in measuring the irradiance distribution. To measure the detector surface size error, the irradiance was measured at the distance of 3 cm from the UV-LED. In these experiments, the detector was covered with different size apertures (Figure 5-8) and the irradiance was measured and reported. Figure 5-8 shows the difference between the measured irradiance with different covers. To evaluate the detector point source assumption, measurements at closer distances was needed, however, due to the spatial limitation and structure of the covers, the measurements with the covers were impossible with the available detectors. Utilizing the model, this error was quantified at closer distances, and the point source assumption for UV-LED was evaluated.

![Figure 5-8](image.png)

**Figure 5-8. The impact of detector cover on irradiance measurements at 3 cm away from the UV-LED (left) and detector with the covers (right)**

In order to quantify the detector reading error, radiation distributions on the surface of four different detectors were predicted and the average irradiances were calculated. These four
detectors include two available and two virtual detectors with 0.195, 0.455, 1, and 2 cm radii. The center point irradiance of these detectors was predicted using the model. The difference between a center point and detector reading was presented as the detector error in Figure 5-9. As it was expected, the error values are dependent on the radiation profile of the UV-LEDs. However, for both UV-LEDs, the near field detector reading is not reliable.

![Figure 5-9. Detector reading error calculated based on the difference between detector reading and predicted irradiance at the center point of the detector. R (cm) represents the detector radius. a) LED1, b) LED2.](image)

The calculated errors are helpful to evaluate the point source assumption for UV-LEDs as a UV radiation source. The center point irradiance of the detectors was predicted based on the inverse square relation between irradiance and distance of the radiation source. This relationship is valid only for point sources [90]. Ryer indicated that the radiation source could only be estimated as a point source when the distance between the radiation sources is at least five times the largest dimension of the radiation source; this being called the “five times rule”. In this section, the “five times rule”/point source assumption was evaluated for the UV-LEDs. The largest dimension of the illuminating part of the tested UV-LED was less than 3 mm and the minimum distance for
point source assumption of these UV-LEDs, based on Ryer statement, was 1.5 cm. Figure 5-9 shows 3% to more than 50% error at 1.5 cm away from the radiation source. This deviation from the “five times rule” is because of the detector planar size. To evaluate the point source assumption for the UV-LED–detector system, the minimum distance in which the detector reading had less than 0.5, 1, 2, and 5% error was determined (Table 1). For a maximum acceptable error of 1% for the point source assumption of UV-LED utilizing a detector with 1.95 mm radius, the five times rule has to change to a twelve and a seven times rule for LED1 and LED2, respectively. It is then concluded that near-field irradiance measurement with detectors does not result in accurate measurement for UV-LEDs. This inaccuracy is likely more significant for heart shape LEDs and irradiance measurement at near-field irradiance measurement has to be avoided.

Table 5-1. The minimum distance in which the detector reading error is less than acceptable error bound.

<table>
<thead>
<tr>
<th>UV-LED</th>
<th>DETECTOR RADIUS (MM)</th>
<th>ACCEPTABLE ERROR</th>
<th>0.5%</th>
<th>1%</th>
<th>2%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED1</td>
<td>1.95</td>
<td></td>
<td>6.9</td>
<td>3.6</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>4.55</td>
<td></td>
<td>16.1</td>
<td>8.2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>&gt;30</td>
<td>18.1</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>12.9</td>
<td>13</td>
</tr>
<tr>
<td>LED2</td>
<td>1.95</td>
<td></td>
<td>3</td>
<td>2.1</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>4.55</td>
<td></td>
<td>6.8</td>
<td>4.7</td>
<td>3.3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>15</td>
<td>10.4</td>
<td>7.2</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>29.9</td>
<td>20.7</td>
<td>14.3</td>
<td>8.8</td>
</tr>
</tbody>
</table>

5.3 Conclusion

A numerical model for predicting the fluence rate and irradiance distribution of UV-LEDs was developed in this chapter. To achieve a more realistic model for the radiation profile and address the previous models’ challenges, the actual radiation profile of UV-LED was used. Radiometry and chemical actinometry methods were implemented to validate the model. A setup was
designed to measure the irradiance distribution of UV-LEDs in a three-dimensional field and to validate the irradiance prediction of the model. The fluence rate prediction of the model was validated with iodide–iodate chemical actinometry. The model was used to evaluate the simplifying assumption of radiation profiles of the UV-LEDs and the results showed that the asymmetry assumption of the UV-LEDs is not valid in some cases. In addition, it was found that directional radiation illumination of UV-LED hugely affects the fluence rate distribution, especially for closer distances. Finally, the point source assumption for UV-LEDs was evaluated and “twelve times law” proposed to supersede the current “five times law” for UV-LEDs. This model can serve to predict the average fluence rate on top of a waterborne microorganism solution container for inactivation kinetics studies and help to develop a standard method for measuring average fluence rate inside a UV-LED reactor.
Chapter 6: Development of a Protocol to Determine UV-LED Fluence for Microbial Inactivation Studies

Fluence determination is necessary to evaluate ultraviolet (UV) reactors for water disinfection and to derive the inactivation kinetics of microorganisms. The ultraviolet light emitting diodes (UV-LEDs) are emerging UV sources with various advantages compared to conventional UV lamps. Unlike conventional mercury lamps, no standard method is available to determine the average fluence of the UV-LEDs. The conventional method to determine the fluence for UV mercury lamps is not applicable to UV-LEDs due to their relatively low output power, their polychromatic output, and their radiation profile. In this chapter, a method was developed to determine the average fluence inside a water solution in UV-LED bench-scale setup. In this method, the fluence rate on a petri dish surface was estimated by measuring the irradiance with a detector for a collimated and uniform radiation. The definition of parameters proposed in the fluence determination of the UV mercury lamps are revised and new parameters are proposed to measure and quantify the collimation and uniformity of the radiation. To study the effect of polychromatic output and radiation profile of the UV-LEDs, two UV-LEDs with the peak wavelengths of 262 nm and 274 nm and different radiation profiles were selected, as the representatives of typical UV-LEDs applied to microbial inactivation studies. The proper setup configuration for microbial inactivation studies, based on the defined correction factors, were determined.
6.1 Methodology

6.1.1 UV radiation source

UV-LEDs’ radiation profile is a function of their structure – chip orientation and lens. Typically UV-LEDs are categorized into two types, based on their radiation profile. UV-LEDs with flip-chip have a heart-shaped radiation profile. The maximum radiation intensity of these UV-LEDs is not normal to the UV-LEDs surface (LED1 in Figure 6-1). The lateral chip has a balloon shaped radiation profile. The maximum radiation intensity of these UV-LEDs is located normal to their surface (LED2 in Figure 6-1). A UV-LED from each type was selected to investigate the effect of radiation profile on the uniformity and collimation of radiation (Table 6-1).

![Figure 6-1. Heart shaped and balloon shaped relative radiation profiles of LED1 and LED2. Most of the available UV-LEDs have similar radiation profile to one of these LEDs.](image-url)
Table 6-1. Studied UV-LED specification extracted from their manufacturers’ provided data sheet

<table>
<thead>
<tr>
<th></th>
<th>Peak Wavelength (nm)</th>
<th>Nominal Radiant Power (mW)</th>
<th>FWHM* (nm)</th>
<th>Forward Voltage (V)</th>
<th>Forward Current (mA)</th>
<th>Viewing Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED1</td>
<td>275</td>
<td>10</td>
<td>12</td>
<td>8.5</td>
<td>100</td>
<td>124</td>
</tr>
<tr>
<td>LED2</td>
<td>262.3</td>
<td>12.5</td>
<td>11</td>
<td>11.6</td>
<td>300</td>
<td>127</td>
</tr>
</tbody>
</table>

* FWHM: Full width at half maximum

6.1.2 UV fluence calculation

To determine the UV response of a microorganism, uniform fluence rate has to be introduced to each microorganism in the suspension. Consequently, mixing the solution and obtaining uniform radiation distribution inside the petri dish is essential, however, there is limitation to reach complete mixing in the solution. Complete mixing in term of the hydrodynamics might be possible to achieve in long enough experiment time periods, but for a non-uniform radiation distribution inside the petri dish, even for long exposure times, the proportion of the non-uniform radiation distribution to the solution mixing causes non uniform radiation delivery to the target microorganisms. Obtaining collimated and uniform radiation beams on the surface of a petri dish and utilizing the low height of water in the petri dish are among the methods to increase the uniformity of radiation distribution inside the petri dish. However, achieving a completely uniform irradiance distribution is always a challenge. In order to evaluate the uniformity of the radiation distribution inside the petri dish, collimation and homogeneity of the radiation on the surface of the petri dish were measured at different distances from the UV-LED for LED1 and LED2 which have different radiation profiles. In order to quantify the uniformity and collimation of the incident radiation, to take into account the effect of radiation absorption of the solution,
and to take into consideration the radiation reflection of the solution surface, some correction factors were defined. Some of these correction factors presented here have been defined for UV mercury lamps [13]. In our study, new correction factors are introduced and some of the existing factors for UV lamp setup were redefined to fit the special characteristics of UV-LEDs radiation pattern and/or to increase the accuracy of measuring the fluence rate. These correction factors are as follow:

Petri factor (PF) – due to the non-uniform incident radiation distribution on the petri dish surface, non-uniform fluence distribution introduces to the microorganisms’ solution. Petri factor is defined as the ratio of average irradiance on the petri dish surface to the irradiance at the center point of the petri dish to quantify the uniformity of incident radiation on the surface of the solution. A simplified method to estimate PF is presented by Bolton et al. [128] which utilize irradiance measurements every 5 mm on two perpendiculars crossed lines intersecting at the center of the petri dish surface. To test the applicability of PF for UV-LED’s systems, in this study, the PF was calculated at different distances (0.1–30 cm - It was found later that the PF becomes independent of distance after 20 cm) from the LED1 and LED2 on a 5.1 cm diameter petri dish using the definition and the simplified method. The comparison between these two methods represents the accuracy of the simplified method to measure the PF for UV-LED systems.

Water factor (WF) – due to the UV absorption of the microorganism solution, irradiance decreases as photons pass through the solution based on beer–lambert law. WF is defined as the ratio of average fluence inside the petri dish to the average fluence at the surface of the petri
dish. Since UV-LEDs are polychromatic radiation sources, the WF was weighted based on the SPD of the UV-LED and spectral absorption of the solution. WF at the wavelength of \( \lambda \) was calculated using the following Equation:

\[
WF_{\lambda} = \frac{I_{\lambda} \times (1 - 10^{-a_{\lambda} \times l})}{I \times a_{\lambda} \times l \times \ln(10)}
\]  

Equation 6-1

Where \( I \) (mW.cm\(^{-2}\)) and \( I_{\lambda} \) (mW.cm\(^{-2}\).nm\(^{-1}\)) are UV-LED’s total radiant power and UV-LED’s radiant power at \( \lambda \), \( a_{\lambda} \) (cm\(^{-1}\)) is absorption coefficient of the solution at \( \lambda \), and \( l \) (cm) is the depth of the microorganism solution. To derive the WF equation, it was assumed that the incident radiation on the petri dish surface is collimated and all the radiation incident to the bottom of the petri dish is absorbed by the petri dish’s wall. The UV-LED’s monochromatic assumption’s impact on WF was also evaluated for LED1 and LED2.

Divergence factor (DF) – due to the directional radiation of UV-LEDs, the incident radiation on a circular planar at different distances from the UV-LED is a function of distance, which means a radiation gradient over the depth of the solution exists. DF was defined as the ratio of average fluence inside the petri dish in the absence of the solution to the average fluence on the surface of the petri dish. DF was calculated with two methods. First, the average fluence at different depths of the solution was measured and was integrated through the depth of the petri dish. In the second method, the point source assumption for UV-LED was made and the DF was calculated using the following Equation:
\[ DF = \frac{D}{D+l} \] 

where \( D \) (cm) is the distance between solution surface and the UV-LED and \( l \) (cm) is the solution depth. These correction factors were measured at different distances from the UV-LED, for different petri dish sizes, and for LED1 and LED2.

Reflection factor – due to the differences in refractive indices of the solution and air, a part of incident radiation to the surface of the petri dish reflects back into the air. The reflected radiation is a function of two media’s refractive indices and the incident angle of the photons. The refractive index is a function of temperature and wavelength, and the incident angle is a function of the distance between UV-LED and petri dish surface. Reflection factor at different distances was calculated by considering the incident angle distribution on the surface of the petri dish. In most water disinfection studies, UV-LEDs were considered as a monochromatic radiation source, while the full width at half maximum (FWHM) of the UV-LED’s spectrum is around 10 nm [77]. The impact of monochromatic assumption on RF for LED1 and LED2 with different peak wavelength was investigated in this study. In addition, the temperature impact on the refractive indices and consequently on the RF was investigated by using the refractive indices of water reported in the literature at different temperatures [129].

Collimation factor (CF) – due to the geometry of the radiation source, the incident radiation to the surface of the petri dish is not collimated, resulting in a gradient of fluence inside the petri dish. With a collimated incident radiation, fluence rate can be estimated by measuring irradiance. This concept has been used in conventional mercury lamp protocol to determine the average fluence rate on the petri dish surface by measuring the irradiance on the surface of the petri dish.
using a detector. In that protocol, collimation is achieved 20 cm away from the UV lamp [64]. Collimating the radiation for UV-LEDs has been tried recently utilizing a column with 3.3 cm diameter [58]. However, no method was utilized to measure and quantify the radiation collimation. Here, we defined CF as the ratio of irradiance to fluence rate at the surface of the petri dish in order to quantify the extent of collimation. CF of 1 means the measured irradiance and fluence rate are equal and a complete collimation occurs. Average irradiance and average fluence rate were measured by radiometry and chemical actinometry, respectively.

Consequently, the average fluence inside the microorganism solution in a petri dish was calculated by using these correction factors, the measured irradiance, and the measured fluence rate on the surface of the solution.

6.2 Results and discussion

The correction factors were determined at different distances from the UV-LEDs for different sizes of petri dishes, to investigate whether an acceptable collimated and uniform radiation can be obtained for the two UV-LEDs. The applicability of these factors was investigated for the UV-LED’s setup and finally, a method was presented to determine the average fluence inside the petri dish for microbiological studies.

6.2.1 Petri factor

Comparing the calculated PF from the definition (using the physically validated model [130]) and the simplified method, the results show a significant (up to 90%) difference between the calculated PFs from these two methods (Figure 6-2), while the acceptable uncertainty for PF is
One reason for this huge differences might be related to not considering the effect of irradiated area on average irradiance estimation in the simplified method. In the simplified method, the influence of one point irradiance measurement on the petri dish surface is considered independent of its position, while the area irradiated with that irradiance is proportional to the square distance from the center of the petri dish. Another reason might be related to the detector size and the measurements’ steps (5 mm) in the simplified method. As it was explained [130], the irradiance measurement is highly related to the detector surface size. Moreover, due to the non-uniform radiation distribution on the petri dish surface for UV-LEDs’ systems, by decreasing the measurement step sizes, different PF might be calculated. Another reason might be related to the radiation distribution on the petri dish surface which is not fully considered in the simplified method. In the simplified method, the irradiance is measured on a $x$–$y$ axis every 5 mm on the petri dish surface (Figure 6-3). For the other points on the petri dish surface, e.g., the point with $x_i$ and $y_j$ coordinates (Figure 6-3), irradiance is estimated with the geometric average between $(x_i,0)$ and $(0,y_j)$ points’ irradiances. However, the irradiance at $x_i$ and $y_j$ coordinates are more similar to those of $(\hat{x}_i,0)$ and $(0,\hat{y}_j)$ points due to the polar radiation symmetry. While in the PF measuring method using the definition, the average irradiance on the surface of the petri dish (using the model) was used instead of using the average irradiance of limited points.

To improve the simplified method, polar coordinates with weighted irradiance on the area are suggested. In this “Proposed method”, irradiance is measured for a polar discretized mesh and weighted for the mesh area with the following Equation:
\[ PF_{polar} = \frac{\sum_{r=0}^{R} \sum_{\theta=0}^{2\pi} A(r, \theta) \times E(r, \theta)}{E(0,0)} \]  

Equation 6-3

where \( A(r, \theta) \) (cm\(^2\)) and \( E(r, \theta) \) (mW.cm\(^{-2}\)) are the area and the irradiance of the polar mesh at \( r \) and \( \theta \) (Figure 4). As shown in Figure 6-2, there is a good consistency between the PFs calculated based on the definition and the proposed method in this study using polar coordinates.

Figure 6-2. Calculated petri factor and coefficient of variation (CV) of a) LED1 and b) LED2 at various distances from the UV-LED for a petri dish with 5.1 cm diameter. The petri factor was calculated based on the definition, simplified method, and newly proposed method in polar coordinate system.
Figure 6-3. The big circle shows petri dish surface. Filled/empty dots represents the detector position for irradiance measurement in order to calculate petri factor with the simplified method and the proposed method.

Although using the proposed method increased the accuracy of the PF determination, the PF of LED1 with a heart shape radiation profile shows a PF equal or higher than 1 in some distances from the UV-LED (Figure 6-2, as a result of irradiance being higher at locations other than the center of the petri dish), while the non-uniformity of radiation distribution at these distances was observed. It was concluded that the PF fails to quantify the radiation uniformity for UV-LED setup, and it needs to be revised. Based on the definition, PF is a unit-less average irradiance on the petri dish surface and not a true representative of the uniformity. However, PF is able to show the uniformity of radiation distribution in collimated beam apparatus to some extent, because of the specific configuration of the collimated beam apparatus. We proposed the coefficient of variation (CV) as a new parameter to represent the uniformity of radiation distribution for UV-LEDs. This suggestion is to take into consideration the variety radiation
profile of UV-LEDs which leads to not having the maximum irradiance at the center of the petri dish.

The weighted CV is proposed to substitute the PF for UV-LED systems to measure the uniformity of radiation on the petri dish surface. CV is the ratio of sample’s standard deviation to the sample’s mean value. Standard deviation statistically shows the dispersion/uniformity of a sample, but it is sensitive to the data magnitude. Dividing the standard deviation of samples to the sample’s mean value makes it possible to compare the uniformity of different systems. CV is widely utilized in polymerization, microsphere size distribution, etc. due to its advantages over other methods of dispersity evaluation [131], [132].

The CV for UV-LEDs was calculated using the weighted measured irradiance distribution on the surface of the petri dish in a polar coordinate system. The ratio of the standard deviation of these weighted measurements (samples) to their mean value was presented as CV [133]. Unlike the PF, the smaller CV, the better uniformity. A PF of 0.9 (90%) was presented as the minimum acceptable PF by IUVA, the value which is also adopted by the US-EPA [50], [128] for low-pressure mercury lamps. To find the maximum value for CV, radiation distribution of LED2 on the petri dish at different distances from the UV-LED was considered. This approach was taken, given the radiation distribution of LED2 on the petri dish surface is similar to that of low-pressure mercury lamps. The CV and PF of the setup at different distances between UV-LED and petri dish are presented in Figure 6-2. The maximum acceptable CV was determined when the PF is higher than 0.9. CV of 6.7% was determined as the maximum CV value to obtain
uniform irradiance distribution. The distance at which the radiation distribution was uniform was 4.4 cm and 7.5 cm for LED1 and LED2, respectively.

### 6.2.2 Water factor

In order to take account of radiation gradient through the depth of solution, WF was calculated for LED1 and LED2 with/without monochromatic radiation assumption (at the distance of 20 cm at which the radiation is collimated). With monochromatic assumption, the WF was calculated at the peak wavelength of the LED1 (275 nm) and LED2 (262.3 nm). Then, the calculated WF was compared to the weighted WF utilizing the SPD of each UV-LED using Equation 6-1. As can be seen in Equation 6-1, WF is a function of the sample UV absorption, SPD of the source, and the depth of the microorganism suspension. The impact of the sample depth is the same as that of the conventional UV dose determination method, and it can be easily shown that for smaller depth the WF is higher. For the UV-LED systems, the absorption of the sample at different wavelengths and polychromatic radiation of the UV source may affect the WF calculation.

The results showed (data not shown here) that at lower absorbance (e.g. for a sample of *E. coli* with absorption of 0.0211 at 285nm), the monochromatic assumption for both UV-LEDs is valid (differences were around 0.1%). But, for higher absorption of the solution, e.g., a high concentration of microorganisms [134], the monochromatic assumption caused more than 8% error. Hence, it is suggested to measure the UV absorption of the microorganism solution at different wavelength for each experiment prior to considering the monochromatic assumption for the UV-LEDs experiments.
6.2.3 Divergence factor

Figure 6-4 indicates that there is a minimum distance exist from the UV-LEDs after which the point source assumption becomes valid and the DF can be calculated from Equation 6-2. This distance was 5.5 cm and 8 cm for LED1 and LED2 (1% deviation), respectively. In addition, at closer distances, the irradiance gradient along the solution depth was so high leading to non-uniform irradiance distribution inside the petri dish. Thus, a proper distance which is dependent on UV-LED’s radiation profile, petri dish size, and the solution depth has to be determined for each UV-LED setup in order to get a uniform radiation distribution inside the petri dish.

![Figure 6-4](image)

Figure 6-4. Divergence factor calculated with point source assumption (PS) for a 1 cm depth petri dish in different distances from the UV-LED, and calculated divergence factor for LED1 and LED2 integrating the irradiance through the depth of the solution

6.2.4 Reflection factor

The effects of temperature, radiation profile, and SPD of the UV-LEDs were investigated on the RF determination. The effect of the temperature on RF determination was negligible (less than
1%) since the refractive index of the microorganism’s solution (PBS and microorganisms) was not sensitive to temperature in the range of 10–30°C.

To investigate the validity of monochromatic assumption for UV-LEDs, RF was calculated with monochromatic assumption and the results were compared to the weighted RFs based on UV-LED’s SPD. The negligible difference (0.7%) between these results were observed, indicating that monochromatic assumption is valid for reflection factor calculation of UV-LEDs.

The impact of radiation profile of UV-LEDs on the reflection factor was investigated by changing the distance between UV-LED and petri dish to obtain different radiation distributions and consequently different incident angles on the petri dish surface. RF was measured at different distances from the UV-LED for a 5.1 cm diameter petri dish. The reflection factor was considered 2.5% for low-pressure mercury lamps. However, as shown in Figure 6-5, for closer distances to UV-LEDs, the reflection factor is higher than 2.5%, meaning more radiation were reflected from the petri dish surface compared to UV-lamp setup. The closest distance to the UV-LED for having 2.5% reflection factor (with 5% deviation) was measured as 3.1 cm. Although reflection factor does not limit the setup designs, the reflection of the petri dish has to be considered when irradiance measured with a detector is being used to determine the average fluence inside the solution.
Figure 6-5. Reflectance (reflection factor = (100-reflectance)/100 calculated for LED1 and LED2 at different distances for a 5.1 cm petri dish

6.2.5 Collimation factor

CF was used to find the extent of collimation at different distances (0.1–30 cm) from the UV-LED. In addition, the impact of radiation profile was investigated on the radiation collimation on the petri dish surface by calculating CF for LED1 and LED2 (Figure 6-6).

In the literature, the fluence rate measurements for UV-LED systems have been made at 2–5 cm distances between UV-LED and microorganism’s solution (e.g. [56], [62], [75], [124], [135]). However, as presented in Figure 6-6, poor collimation occurs at these distances, indicating that the measured average irradiance or the measured average fluence rate has to be used with caution to estimate the average fluence inside the petri dish. Non-collimated radiation on the petri dish surface causes two main issues for average fluence rate determination. First, the average fluence rate and average irradiance on the surface of the petri dish is not equal, so, unlike the
conventional method for mercury lamps, the average fluence rate cannot be estimated by measuring the average irradiance with a detector. Second, non-collimated radiation causes radiation gradient inside the petri dish resulting in different radiation path lengths for photons and potentially reflected photons from the petri dish wall. The radiation distribution will not be uniform inside the petri dish (the non-uniformity cannot be quantified easily) and this setup cannot be used for measuring the inactivation kinetics of a microorganism. Average fluence determination, in this case, needs a complex modeling considering the petri dish wall reflection and radiation path lengths.

The minimum distance to obtain CF of more than 0.99 was determined for LED1 and LED2 to be 12.5 cm and 12.3 cm, respectively. It was concluded that even without a collimating column, quasi-collimated radiation can be obtained from UV-LEDs. This distance can be determined for different petri dish sizes and UV-LED systems utilizing the physically validated model. Given the studied UV-LEDs’ radiation profile covered most of the commercially available UV-LEDs, for a petri dish with 5.1 cm diameter, 13 cm distance is safe to obtain a uniform fluence distribution inside the petri dish for performing inactivation kinetics studies.
Figure 6-6. Collimation factor (irradiance–fluence rate ratio) for LED1 and LED2 at different distances from the UV-LED.

6.3 Fluence determination

Accurate determination of the fluence is essential for establishing the kinetics of microbial inactivation with UV radiation. This will, in turn, provide the necessary information for determining the log inactivation of target microorganisms as a function of fluence, as well as calculating the reduction equivalent dose (RED) that is needed for the design and validation of UV reactors.

In this study, the average fluence in a petri dish for a UV-LED setup was determined by using the average irradiance on the surface of the petri dish utilizing a spectrometer. Collimation and uniformity of the incident radiation were evaluated by defining and utilizing the CF and the CV. For CV lower than 6.7% and CF higher than 99%, the average fluence inside the petri dish was calculated using the following Equation:
\[ F_0 = \frac{\bar{E}_0 \times DF \times WF \times RF \times t}{CF} \]  

Equation 6-4

where, \( F_0 \) (mJ.cm\(^{-2}\)) is the average fluence inside the petri dish, \( \bar{E}_0 \) (mW.cm\(^{-2}\)) is the average incident irradiance on the surface of the petri dish, and \( t \) (s) is the exposure time. All of the parameters in equation 6-4 were estimated based on the measured irradiance and fluence rate on the surface of the petri dish. A diagram of the protocol is presented in Figure 6-7.
Figure 6-7. Proposed protocol for fluence (UV dose) determination for UV-LED’s system
For the studied UV-LEDs and a petri dish of 5.1 cm diameter, at distances greater than 13 cm from the UV-LED, collimation was achieved and all the DF, WF, and RF correction factors were in the acceptable ranges. Thus, even without a collimating column, the microbiological test can be performed at appropriate distances between UV-LED and solution surface. The average fluence rate at the distance of 13 cm from LED1 and LED2 were 0.01 mW.cm\(^{-2}\) and 0.02 mW.cm\(^{-2}\), respectively. These values correspond to utilizing about 3% of the radiant power of the UV-LED on the surface of the petri dish. Note that fluence rate in the collimated beam apparatus for low-pressure mercury lamps is usually an order of magnitude higher. Considering the low power output of current UV-LEDs, to achieve a comparable fluence from a UV-LED system, longer exposure times, utilizing optical devices, or using multiple UV-LEDs are recommended.

### 6.4 Conclusion

In this study, a new protocol was developed to measure the average fluence inside a petri dish solution for microbiological experiments. Correction factors were defined, or some existing ones for UV lamps were revised, in this protocol to measure the collimation and the uniformity of radiation on the petri dish surface containing microorganism solution. PF, which conventionally used for low-pressure mercury lamps, was revised for UV-LEDs and CV was proposed to measure the radiation uniformity. UV-LED monochromatic assumption and radiation profile of UV-LEDs’ impact on the correction factors and consequently fluence determination were investigated utilizing two UV-LEDs with different radiation profiles and SPD. This indicates that the monochromatic assumption for UV-LEDs has to be used with caution.
A minimum distance of about 13 cm was suggested for obtaining collimated radiation on the surface of the petri dish for UV-LEDs, with typical radiation profiles of either balloon or heart shape, and the viewing angles of about $125^\circ$.

Although radiation distribution in the petri dish is affected by the radiation profile of the source, the proposed protocol is independent of UV source radiation profile. Thus, this protocol can be implemented for any UV radiation sources. The only consideration would be accurately measuring the irradiance distribution and fluence rate on the surface of the petri. Consequently, all the correction factors can be calculated independently of the UV source radiation profile.
Chapter 7: Inactivation Kinetics Study of a Waterborne Microorganism under Different Radiation Distributions in Different Setups

This chapter presents the results of *E. coli* inactivation kinetics, considering the effect of radiation distribution on the petri dish surface. Inactivation kinetics of *E. coli* has been studied in the literature with UV-LED as the radiation source without considering the effect of radiation distribution inside the petri dish or on the surface of the petri dish. In chapter 6 a protocol was proposed as a guideline to determine the UV dose more accurately for UV-LED systems, which demonstrated the importance of radiation distribution inside the petri dish. In this chapter, after implementing this protocol to measure the inactivation kinetics of *E. coli* in a bench scale setup, radiation distribution on the surface of petri dish and its impact on the measured inactivation kinetics is investigated.

To investigate the effect of radiation distribution on the measured inactivation kinetics, different optical devices such as collimating columns with different UV reflectivity of their surfaces were implemented. Polyvinyl chloride (PVC), Teflon, aluminum, and quartz tubes in different diameters were used as the collimating columns. Moreover, the results were compared with the setup without any columns. PVC column was chosen as a non-UV-reflective material. To enhance the non-reflectivity of PVC column, the inner surface of the column was threaded. The aluminum column was used due to the high specular UV-reflectivity of its surface. Teflon column was used because of the high diffusive UV-reflectivity of its surface. Moreover, Teflon has recently been widely used as a collimating column, for the UV dose measurement in UV-
LED systems. Finally, quartz column was used as a mediocre UV-reflective material which can be potentially used in the UV reactors.

It was shown that at relatively far distances from the UV-LEDs, even without using a column, a collimated radiation can be occurred. However the intensity of the delivered radiation might not be sufficient. A UV collimating lens can be applied in order to enhance the radiation intensity on the petri dish surface and to improve the radiation distribution.

7.1 Experimental methodology

7.1.1 UV-LED setup

The setup presented in section 3.3 was modified to accommodate the collimating columns and the UV collimating lens (Figure 7-1). A beam was used to hold the columns; the columns were attached to this beam using some clamps. The position of the holder beam could be changed in order to align the column and keep the UV-LED at the center of the column by using some 3D-printed parts in all the experiments. The petri dish for the microbial tests was placed 1 cm away from the bottom of the columns and was centered with each column. To align the center of the petri dish with the column, some alignment parts (Figure 7-1) were designed and fabricated with a high precision 3D-printer. A manual shutter was added to the bottom of the column to control the UV exposure time for inactivation kinetics studies.
Figure 7.1. Setup sketch equipped with aluminum column for microbial inactivation studies-1) heat management components, 2) UV-LED/column alignment part, 3) Aluminum column, 4) Clamp, 5) Stirrer/petri dish alignment part, 6) Stirrer and 7) Leveler

7.1.2 Radiation source

A UV-LED with a peak wavelength (275 nm) close to the maximum absorption spectrum of E. coli was selected. The UV-LED module (PCB and the heat management components), columns, lens, and the petri dish were aligned and placed inside an enclosure. The operational conditions of the UV-LED such as electrical current and temperature were monitored and
controlled during each experiment based on the characterization protocol presented in chapter 4 to maintain a consistency UV-LED output. The data sheet of this UV-LED is included in B.6.

7.1.3 UV irradiation

E. coli is commonly used in drinking water researches as an indicator microorganism for water contamination. In this chapter, E. coli (ATCC 11229) was used for inactivation kinetics studies. Preparation and enumeration method for this microorganism have been explained in section 3.6. It was mentioned earlier that to prepare the microorganism solution, the microorganism has to not be at late stationary phase of the growth curve. To plot the growth curve for E. coli, usually, the absorbance of the microorganism’s solution at 600 nm is measured at different time intervals. The absorption of the solution represents the number of microorganism colonies. UV-Vis spectrophotometer was used to measure the absorbance. The 600 nm absorption was zeroed using an LB broth solution and then the absorption of the microorganism solution over one day was measured while the solution incubated at 37 °C. Figure 7-2 shows the growth curve of E. coli. For the further microorganism preparation, 4 hrs incubation was considered to achieve the proper concentration of the colonies and avoid the late stationary phase.
The microbial tests were performed in a 25 ml petri dish fully loaded with microorganism solution (Figure B-8). The exposure time for each experiment was estimated before each experiment by considering the fluence rate and the inactivation kinetics of *E. coli* with low-pressure mercury lamps, targeting at least 4 log inactivation. For example, for a setup with fluence rate of $E_0$ (mW·cm$^{-2}$), reduction equivalent dose (RED)–which represents the UV dose related to a specific microorganism log reduction– for 4 log reduction can be calculated from the *E. coli* inactivation kinetics of low-pressure mercury lamp [42], and, consequently, the maximum needed exposure time can be back calculated using the following equation:

$$Exposure\ time\ (sec) = \frac{RED_{4\ log}}{E_0}$$
\[ RED_{4\log} \approx 8 \text{ mJ} \cdot \text{cm}^{-1} \]

For better dispersion of the microorganisms inside the petri dish solution, 60 sec mixing was performed before each experiment with the closed shutter in a black box. The extent of photoreactivation during the plate preparation and the enumeration process in the laboratory operational condition was tested, in addition to the photoreactivation control experiments which were explained earlier (section 3.6.3). A set of the identical experiments were performed under different room lights and the results were compared. These experiments were performed under red light (at night), under regular lab’s florescent lamps off, under daylight (during the daytime with the curtains opened and the lights on). The absolute radiation spectrum on the working bench for these cases was measured as well. The room light spectrum in different conditions such as daylight with lab curtains closed/open, red light, and under florescent light is provided in Figure B-7.

**7.1.4 Experimental design**

**7.1.4.1 E. coli inactivation kinetics experiments**

Based on the protocol presented in chapter 6, a proper distance for the UV-LED was determined to meet the presented criteria of collimation and uniformity of the radiation distribution on the surface of a petri dish. For these set of experiments, no column was used (bare case) (Figure B-11). Microbial tests along with the radiometry (Figure B-12) and actinometry were performed at this distance in order to calculate the weighted coefficient of variation of the radiation on the surface of the petri dish, reflection factor, divergence factor, and the collimation
factor. The water factor was calculated based on the measured absorption spectrum of the microbial solution. These correction factors were used later to calculate the average UV dose inside the petri dish. Microbial tests for *E. coli* inactivation kinetics were performed at different time intervals with the highest exposure time to reach at least 4 log inactivation. The microbial tests were triplicated.

Most of the UV-LED inactivation kinetics studies, in the literature, are using a distance smaller or equal to 2 cm. In order to compare the measured inactivation kinetics reported in the literature with the inactivation kinetics measured with the proposed protocol, and to evaluate the applicability of the conventional UV dose determination protocol, identical inactivation kinetics studies were replicated by reducing the distance between UV-LED and the petri dish to 2 cm. the results were compared in terms of the actual kinetics.

### 7.1.4.2 Radiation distribution impact

To investigate the impact of radiation distribution, delivered to the petri dish, on the UV dose measurement, in addition to the bare case (without any column), some columns with a variety of materials, sizes, and roughnesses were added to the setup. A collimating column has been used for mercury lamps; further, recently the same approach has been used for UV-LEDs, in some studies, to measure the UV dose without considering the column impact on the delivered radiation distribution.

The delivered radiation intensity to the petri dish surface for UV-LED apparatus, where the proposed protocol is implemented, usually is considerably weaker than that of mercury lamps’
setup. The weak delivered radiation intensity requires very long exposure times for inactivation of more UV resistant microorganisms, such as some viruses. To increase the radiation intensity, using a column with high UV reflective material is of high interest. Aluminum, quartz, and Teflon columns were used for this purpose. Teflon columns have been used in UV-LED studies to measure the UV dose. Although a reflective column may increase the delivered radiation intensity, it might affect both the collimation and uniformity of radiation on the petri dish surface. Both qualitative and quantitative study was performed to evaluate the applicability of these columns for UV dose determination with the proposed protocol.

Moreover, PVC columns were used as a low UV-reflective material to block the divergent photons of the UV-LEDs. The same concept was considered to propose the conventional UV dose determination protocol. To increase the UV reflectivity, the inner surface of the PVC column was threaded and compared with the non-threaded column. Both qualitative and quantitative studies were performed to evaluate the applicability of these columns with the proposed UV dose determination protocol.

7.1.4.3 Radiation Intensity Enhancement

Another option to enhance the delivered radiation intensity from a single UV-LED was implementing an optical device like a UV transparent lens. The divergent radiation of UV-LEDs causes diminishing the delivered radiation intensity to the surface of the petri dish while the petri dish is positioned farther from the UV-LED. The idea here is collecting the radiation at the distances closer to the UV-LED (surface A) with a lens and to substantially collimate it toward the petri dish surface which is farther from the UV-LED (surface B) (Figure 7-3). For the
preliminary studies, the irradiance delivered to the center of the B surface was compared in two cases, with a collimating lens, and without a collimating lens. The results showed more than four times (~29 vs. ~121 μW. cm⁻²) higher irradiance when a collimating lens was implemented (Figure 7-3). A quantitative study was performed to evaluate the applicability of the collimating lens along with the proposed UV dose determination protocol by measuring the correction factors presented in the protocol and comparing the measured inactivation kinetics with the bare case.

Figure 7-3. Schematic view of the lens and UV-LED and the impact of using lens on the trajectory of the photons (rays direction with lens: solid lines, without lens: dotted lines)
7.2 Results and discussion

7.2.1 E. coli inactivation kinetics measurements

The microbial inactivation kinetics of the E. coli was measured based on the proposed protocol for fluence determination in a setup without a column. The distance of 16 cm was chosen based on preliminary results of the modeling of the UV-LED’s radiation profile. The correction factors proposed in the protocol were calculated by measuring the irradiance and fluence rate at the surface and different depths of the petri dish.

The absorption spectrum of the E. coli solution and spectral power distribution of the UV-LED were measured (200-320 nm) in order to calculate the weighted water factor (Figure 7-4). UV-LED was considered as a polychromatic radiation source for calculating the water factor using equation 6-1. In order to measure the uniformity of the radiation on the surface of the petri dish, the radiation distribution was measured with the radiometry method. The weighted coefficient of variation (CV) was calculated to quantify the uniformity. The calculated CV was 2.64 % which is within the acceptable range of uniformity (<6.7 %). The reflection factor was estimated using the radiation model by considering the incident angle of each photon on the surface of the petri dish (RF = 0.97). In order to calculate the divergence factor, instead of using the point source assumption, the average irradiance at different depths of the petri dish was measured with radiometry method. The ratio of these measurements to the measured average irradiance at the surface of the petri dish represents the water factor (WF = 0.96). The collimation factor was estimated by using the fluence rate measured by actinometry, and average irradiance measured with the radiometry (CF = 0.99).
After calculating the correction factors, the average fluence rate inside the petri dish was calculated based on the proposed protocol and by irradiating the E. coli solution at different time intervals. Figure 7-5 shows the inactivation kinetics of E. coli. At low UV doses, a shoulder can be seen. The shoulder can be explained with series-event inactivation kinetics in which an inactivation of a microorganism needs sufficient amount of DNA damage [136]. Following the shoulder region, there is a linear relation between UV dose and microbial log inactivation (Line slope = 0.75, line intercept = -2.34, and R² = 0.99). At higher doses, the slope of the inactivation decreases and the inactivation kinetics reach a state called tailing. Tailing for the E. coli inactivation might happen when inactivated E. coli cells clump to each other and shielding the viable cells. The early tailing region was observed in Figure 7-5. The further tailing region could not be measured due to the E. coli enumeration method limitations. The same inactivation kinetics trend was observed for the same E. coli strain at 254 nm (Figure B-13).
In addition, the inactivation kinetics experiments were replicated at a closer distance (2 cm) with two different protocols. First, the fluence rate was estimated using the proposed protocol in the current research. The correction factors were calculated and the inactivation kinetics was derived. Second, the fluence rate was determined using the protocol proposed by Bolton and Linden [13] for mercury lamps. This protocol has been used for UV-LEDs in most of the UV-LEDs’ studies published in the open literature. In order to determine the fluence rate for the latter
case, the correction factors were measured using mercury lamp’s method. The RF, DF, and PF were calculated using the measured radiation distribution on the surface of the petri dish using radiometry. The petri factor was measured by using the definition rather than the simplified method (proposed for UV mercury lamps) due to the error associated with the simplified method which was explained in chapter 6.

Although it is expected to calculate the same inactivation kinetics in different setup configurations, Figure 7-5 shows a huge difference between both studied cases inactivation kinetics. In the other words, the measured inactivation kinetics for a microorganism should be independent of the setup. The differences between the measured inactivation kinetics with the mercury lamp method and the proposed method are related to the way that the correction factors were calculated for 2 cm case. DF in the mercury lamps method was calculated based on the point source assumption, which appears not to be valid based on section 5.2.3.2. Moreover, the petri factor was way less than 0.9 (PF = 0.12) which indicates that the radiation distribution is not uniform. Besides, it was shown in chapter 6 that the petri factor is not suitable for measuring the uniformity of the radiation.

The deviation between the inactivation kinetics measurement at 2 cm and 16 cm by the same method (current research proposed method) is related to the correction factors’ value. At 2 cm the coefficient of variation was 154.89 % which is larger than the acceptable range. Similar to the CV, the collimation factor and the divergence factor were beyond the acceptable range. Being out of the acceptable ranges causes the error in inactivation kinetics measurements. It was
concluded that at the closer distances, the measured inactivation kinetics should be considered with caution.

Although at farther distances from the UV-LED, the fluence rate can be calculated with the proposed protocol, the intensity of the fluence rate delivered to the sample is not comparable to mercury lamp setup. For the rest of this chapter, alternative setups are studied to obtain more fluence rate intensity from a single UV-LED, while the correction factors are in the acceptable range.

Figure 7-5. The inactivation kinetics of E. coli measured in two different distances from the UV-LED using conventional method (ML - mercury lamp protocol) and this research proposed method to calculate the fluence rate
7.2.2 Qualitative comparison of the radiation distribution for various columns

One of the alternatives to achieve more radiation delivered to the petri dish, from one single UV-LED, is using columns. Besides, by using columns, divergent UV radiation from the UV-LEDs is blocked. In this case, safer setups can be designed, preventing UV exposure to the user. In this section, a qualitative comparison between PVC column, quartz column, Teflon column, aluminum column, and bare (no column) was performed by measuring the radiation distribution on the surface of the petri dish (bottom of the column) using radiometry method. The impact of the column roughness on the aluminum column and PVC column was investigated. Moreover, the impact of the column diameter for all the columns was investigated. The UV-LED used in the qualitative studies is different from the one used for inactivation kinetics studies, but its relative radiation pattern is the same.

7.2.2.1 Impact of the column material on the UV radiation distribution on the surface of the petri dish

The impact of the column material on the delivered radiation distribution on the surface of the petri dish was investigated by choosing PVC, quartz, aluminum, and Teflon as the columns. The columns are 15 cm long and the radiation distribution was measured 1 cm away from the bottom of the column, where the surface of the petri dish is expected to be positioned. For the case without any columns, it was shown that at the distance of 16 cm from the UV-LED, an
acceptable radiation distribution for measuring the fluence rate is occurred. Using these columns, the applicability of the protocol for determination of the UV dose was investigated.

Figure 7-6 shows a quite uniform radiation distribution on the surface of the petri dish for the bare case while for the cases like aluminum and quartz, the radiation distribution is not uniform. This nonuniformity is caused by the specular reflection of the UV radiation from the columns. However, the radiation intensity on the petri dish is much higher than the bare case. On the other hand, PVC and Teflon show a better radiation uniformity on the petri dish surface. PVC has a non-reflective surface for UV radiation and Teflon has a high diffusive reflectivity for UV radiation. The differences between the intensity of the radiation for these two columns are caused by the differences in their UV reflectivity.
Figure 7-6. Irradiance distribution on the surface of a petri dish 1 cm away from the bottom of a 15 cm columns

### 7.2.2.2 Impact of the column diameter on the delivered radiation distribution

To investigate the impact of the column diameter on the delivered radiation distribution to the petri dish surface, the radiation distribution was measured for columns with 47 mm and 76 mm diameters. Figure 7-7 shows a comparison between these two diameters for the columns with different materials. For the PVC column, the irradiance intensity is almost the same for both
diameters because PVC is not a UV-reflective material. For the Teflon column, the irradiance uniformity is lower for the smaller diameter column which is caused by the diffusive reflection of the column. For the smaller diameter, more photon incident happens inside the column and it leads to more nonuniformity of the radiation. For the quartz column, the radiation uniformity and irradiance intensity are almost the same. This could be a result of the transparency of the quartz column for UV radiation. Quartz has very high UV reflectivity for wider photon incidents, however, for the narrow incident photons, it is transparent. For the aluminum column, the smaller diameter gives lower irradiance intensity due to the specular reflectivity of the column. The number of photons’ incident increases for smaller diameter of the column and due to the partial reflectivity of the UV radiation from the column, less irradiance intensity delivered to the bottom of the column. Comparing the aluminum and Teflon which represents a specular reflective and diffusive reflective material, respectively, it can be found that more radiation is delivered to the bottom of the column for the aluminum column. This difference is a result of the reflection from the wall. For the diffusive reflection, the reflection angle can be in any directions while for specular reflection, all the reflection happens to face the petri dish (Figure B-6).
In general, for all of the studied columns, the column with wider diameter gives a better radiation distribution on the surface of the petri dish, either by having higher irradiance intensity or better uniformity. Besides, with the column with 76 mm diameter, larger petri dishes can be used with smaller sample depth. The smaller sample depth leads to better water factor for turbid water samples. Also the effect of the column’s bottom edge on the irradiance distribution is less for the
columns with a bigger diameter. Based on these advantages, for the next experiments, columns with 76 mm diameter were used.

7.2.2.3 Impact of the column inner wall roughnesses on the delivered radiation distribution to the surface of the petri dish

To qualitatively investigate the impact of the column’s roughness, four roughnesses were formed for the aluminum column include, as is, polished, rough, and smooth. “As is” (non-treated) column is the one without any changes to its inner surface. The polished column is the one that was polished with a cotton and microfiber cloth. The finishing is like a mirror surface. The rough and smooth columns were fabricated using turning process with a metalworking lathe with different settings (Figure B-9). Figure 7-8 shows that the column’s roughness can considerably affect the radiation distribution on the petri dish surface. There are two parameters affecting the radiation distribution under the columns for different roughnesses. One is the UV reflectivity of the surface and the other one is the direction of the reflected photon. Smoothing the surface of the aluminum increases the UV reflectivity, on other hand, treating the surface might change the roughness of the surface and leads to reflection of a photon in an undesirable direction (which is toward the UV-LED). Based on these two parameters, the four roughness variations studied can be explained. Due to no improvements in the radiation distribution, the characteristics of the aluminum surface roughness were not studied in details. Among the columns with different roughness variations, the polished column was used since it delivers more irradiance with relatively good radiation uniformity.
The impact of roughness was investigated on the PVC column, as well. Besides the regular (as is) PVC column, a PVC column with threads inside the column was used to block all the incident photons to the inner wall (Figure B-10). As it was expected, more uniform but lower intensity irradiance was delivered to the petri dish with this column. Since PVC column was chosen because of its non-UV-reflectivity characteristics, threaded PVC column was used in the further experiments.

![Figure 7-8. Cross-section radiation distribution on the surface of the petri dish for aluminum and PVC columns with different inner wall roughnesses](image)

### 7.2.3 Inactivation kinetics study using the selected columns

After a qualitative comparison of the radiation distribution using the columns, a quantitative comparison for delivering irradiance is made among the columns. The delivered average irradiance and CV were used as indices to select the potential columns for inactivation kinetics studies. Figure 7-9 includes the average delivered irradiance and CV calculated based on the measured irradiance by radiometry. Among these columns, aluminum has a high CV value,
however, its average irradiance is the highest among the column studied. Thus, this column was chosen to test the impact of the radiation nonuniformity on the measured average fluence rate for the inactivation kinetics tests. As for PVC column, the one with the threaded wall, was chosen due to its better radiation uniformity (lower and acceptable CV) and almost the same average irradiance as the PVC not threaded one. Quartz column was not an option because of the mediocre average irradiance and high CV. For the Teflon column, average irradiance is higher than both bare and PVC cases and the radiation is uniform. Moreover, Teflon columns have been implemented widely for UV-LED’s studies, recently. The inactivation kinetics studies using these columns have been compared with the bare case to investigate their applicability for fluence rate measurement and consequently measuring the inactivation kinetics of a target microorganism.
7.2.3.1 Impact of the radiation uniformity on the measured inactivation kinetics

The uniformity of the radiation on the petri dish surface influences the calculated average fluence rate inside the petri dish by causing a non-homogeneous radiation distribution inside the water sample. Based on the non-uniformity of the fluence rate inside the petri dish, the calculated average fluence rate with the proposed protocol might be under/overestimated. Here the inactivation kinetics of E. coli was measured for the setup with aluminum and PVC columns.
The results were compared with the bare case. In these three cases, the distance between the UV-LED and the petri dish surface was kept at 16 cm.

As expected, a similar kinetics trend was measured for the PVC setup and the bare case. PVC column causes a uniform radiation and hence, all the correction factors for this case were in the acceptable range. This column is an alternative for the bare case since it blocks the divergent photons and increases the safety of the setup without affecting the accuracy of the experiments.

On the other hand, for the aluminum column, a non-uniform radiation distribution was measured. All the correction factors for this case was within the acceptable range except CF and CV. CF was 0.93 and the CV was 93.60 %. Figure 7-10 shows that there is a significant difference between the inactivation kinetics measured with aluminum setup and the bare case, particularly in the high fluence regions of E. coli inactivation. This differences was caused by the inaccurate fluence rate determination for the aluminum setup. For example, at the fluence of 10.6 mJ.cm\(^{-2}\), there is 1.5 log difference between the aluminum and the bare case log inactivation. In addition to inaccurate fluence rate determination, the kinetics trend is also different from the bare case. For the aluminum kinetics experiments, the shoulder and linear phase combined which is a result of the non-homogeneity of the radiation inside the petri dish. To correct the fluence rate determination for this case, a validated mathematical model needed to trace each photon inside the petri dish considering the reflection of the walls and path length of each photon inside the water. Based on the result of the inactivation kinetics experiments, the aluminum column is not recommended for UV-LED setups. Moreover, for any setup which causes non-uniform radiation
distribution on the petri dish surface, the inactivation kinetics results should be considered with caution.

Figure 7-10. Inactivation kinetics of E. coli with aluminum, PVC, and bare case setups

7.2.3.2 The impact of the radiation collimation on the measured inactivation kinetics

One of the basic assumptions for proposing the fluence determination protocol was achieving collimated radiation on the surface of the petri dish. The collimation was quantified by using CF which was defined earlier. Not collimated radiation affects not only the CF, but also may cause lower DF. Lower DF means there is a gradient of average fluence rate/irradiance through the depth of the water sample. Moreover, it is expected that higher average irradiance for Teflon
column, compared to the bare case, is a result of reflection of the photons from the wall. Although in the setup with Teflon column the delivered irradiance is uniform, this uniformity was caused by the reflected photons from the Teflon, which means non-collimated radiation is happening.

The inactivation kinetics of E. coli was measured using the Teflon column and the bare case setups (Figure 7-11). Unlike aluminum column, with the Teflon setup, the shoulder and tailing were observed in the E. coli inactivation kinetics. However, the shoulder and the tailing were not match the bare case which means not only the fluence determination was not accurate, but also the delivered fluence rate was changing during different exposure times. It means that even by changing the fluence rate, in either cases, the measured inactivation kinetics cannot be mapped. This difference is a result of the fluence gradient through the water sample. Based on these results, it can be predicted that for a smaller length of the Teflon column, the deviation is even higher in terms of inactivation kinetics measurements. For the shorter Teflon columns, in order to achieve a uniform radiation distribution on the surface of the petri dish, photons with a wider incident angles have to reach to the petri dish surface, which leads to a severe fluence gradient through the depth of water sample. Thus, the Teflon column is not recommended for the microbial kinetics tests. The results from the short Teflon column which is widely used for UV-LED setups should be considered with caution.
7.2.4 Kinetics studies using an optical lens

A UV transparent lens was used to enhance the delivered radiation distribution and radiation intensity on the surface of the petri dish. To evaluate the feasibility of utilizing lenses to enhance the radiation intensity on the petri dish surface, the delivered radiation on the surface A (Figure 7-3) was measured using radiometry. Then, the delivered radiation on the surface B (Figure 7-3) was measured using a detector (with the lens). The total energy flux using the radiometry and a detector were 9.15 mW and 8.38 mW, respectively. Only 8 % difference in the energy flux indicates that this lens is effective to collimate the UV radiation from the UV-LED.
The irradiance distribution and the fluence rate were measured by radiometry and actinometry for the lens setup. Then, the correction factors for fluence determination were calculated and the inactivation kinetics was measured.

A Plano-convex UV Fused Silica lens (Thorlabs) was used to collimate the photons. The lens was placed 20 mm away from the UV-LED with aligned centers. The petri dish was placed 16 cm away from the UV-LED. Figure 7-12 shows a uniform radiation distribution on the surface of the petri dish with relatively higher irradiance compared to the bare case. The measured correction factors are presented in Table 7-1. It can be seen that all the correction factors are in the acceptable range. The higher intensity of the irradiance is caused by refraction of the collected photons at closer distances to the UV-LED.

**Table 7-1. Correction factors for lens setup**

<table>
<thead>
<tr>
<th>Correction factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>3.23%</td>
</tr>
<tr>
<td>PF</td>
<td>1.04</td>
</tr>
<tr>
<td>DF</td>
<td>0.93</td>
</tr>
<tr>
<td>CF</td>
<td>0.99</td>
</tr>
<tr>
<td>RF</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Figure 7-12. Irradiance distribution on the surface of a petri dish, 16 cm away from the UV-LED with a collimating lens in between.

Figure 7-13 shows the inactivation of E. coli with the lens setup and the bare case. It can be seen that there is a good consistency between these two cases due to the proper radiation distribution provided by the lens. Both tailing and shoulder regions were observed. The exposure times in lens experiments were about 4 times shorter than the bare case due to the higher average fluence rate provided by the lens setup. The higher fluence rate makes it possible to perform kinetics studies for more UV resistance microorganisms in shorter time periods.
7.3 Conclusion

From these studies, it was observed that the radiation distribution characteristics have a significant impact on the measured inactivation kinetics studies. The impact of radiation uniformity on the measured kinetics was investigated by using an aluminum column. The apparent kinetics with this column was not the same as the bare case in which all the correction factors are in the acceptable range. Moreover, the impact of collimation of the radiation was investigated by using Teflon column. It was shown that the collimation not only affects the fluence rate determination, but also affects the kinetics trends such a shoulder and tailing regions.
In general, it was concluded that none of the columns with UV-reflective surfaces can deliver desirable radiation distribution to the petri dish surface, which means inactivation kinetics studies cannot be performed with these columns. Using PVC column with non-UV-reflective surface resulted in a uniform and collimated radiation distribution. The measured kinetics with this column was conforming to the bare case.

Furthermore, the applicability of UV lens for microbial inactivation kinetics studies was tested. It was concluded that using a UV transparent lens to collimate UV-LED radiation can enhance the intensity of the delivered radiation to the petri dish surface with an acceptable uniform radiation distribution pattern.
Chapter 8: Conclusions and Recommendations

8.1 Conclusions

In this study, the ultimate goal was to develop a method to accurately determine the inactivation kinetics of waterborne microorganisms for UV-LED setups. To do so, the operation of UV-LEDs in a variety of conditions was studied. Then, the fluence rate determination of UV-LED systems was studied with the development of a mathematical model. Finally, a developed protocol was proposed for fluence rate determination and the effect of setup for fluence determination on the measured inactivation kinetics of E. coli, as a model microorganism was investigated. The main conclusions obtained from the various tasks associated with this research are highlighted below:

- A protocol was developed to properly operate UV-LEDs. In addition, this protocol includes a proposed method to characterize the output of different UV-LEDs. It was concluded that the reported output of UV-LEDs not only can be affected by the operational conditions, such as temperature and electrical current, but also it can be affected by the measurement techniques. The proposed protocol includes the impact of these parameters and present a guideline to operate and control UV-LEDs’ output accurately. Moreover, this protocol indicates/includes the importance of reporting operational parameters such as working temperature, measurement distance, detector characteristics, etc. Reporting these values are crucial to achieve accurate and reproducible results from similar UV-LED systems. Since the proposed protocol focuses on the operation of the UV-LED, it can be used for other UV-LED’s applications such as polymer curing, advanced oxidation processes, and sensors.
Next, a mathematical model was developed to estimate the irradiance and fluence rate distribution of UV-LEDs. The model used the output of the UV-LEDs in order to predict the average irradiance or average fluence rate on any surface in front of the UV-LED more realistically. The validated model, was used to evaluate some simplifying assumptions for radiation pattern of the UV-LEDs, such as assuming symmetry in azimuthal direction for UV-LED’s radiation profile. It was found that the symmetry assumption of the radiation profile of the UV-LEDs is not valid in some cases. In addition, it was found that directional radiation illumination of UV-LED hugely affects the fluence rate distribution, especially for closer distances. Further, it was concluded that the validity of the point source assumption for UV-LEDs is a function of the target surface (detector or petri dish). The “twelve times law” was proposed to supersede the current “five times law” for the studied UV-LEDs. This model can be helpful for designing UV-LED reactors, since it accurately predicts the irradiance/fluence rate in a three dimensional coordinates.

Subsequently, the developed model was used for average fluence rate determination inside a petri dish. In order to determine the average fluence rate for microbial inactivation kinetics studies, it was found that the conventional fluence determination protocol for mercury lamps is not applicable to UV-LEDs. Correction factors were revised and some new correction factors were defined to facilitate the fluence rate determination. Petri factor was found inaccurate to show the uniformity. A new coefficient was defined to substitute the petri factor for uniformity quantification. Water factor was revised and re-defined in order to account for the polychromatic spectrum of
UV-LEDs. The conventional equation to calculate the divergence factor was found to be driven based on the point source assumption. The method to measure the water factor was revised based on the data obtained from the model. Since in conventional fluence determination method, collimation of radiation was not considered, a new correction factor called collimation factor was defined to quantify the extent of collimation. Finally, a new protocol in the form of a flowchart was proposed to determine the fluence rate of UV-LEDs for microbial kinetics studies in a bench scale setup. Among the protocol steps for fluence determination, the source characterization is considered. In fact the petri dish delivered radiation characteristics determine the fluence calculation. Thus this protocol could be used for other UV sources with different shape, SPD, radiation pattern, and radiant powers.

- Finally, the proposed protocol was implemented in some setups with two intentions. First to evaluate the setups which have been used in the literature. Second, to enhance the radiation distribution and irradiance intensity on the petri dish surface. These included an apparatus with collimating columns and an apparatus utilizing an optical lens. It was concluded that the delivered radiation distribution to the petri dish surface is largely affected by the column material, surface finish, and column diameter. Moreover, it was observed that radiation uniformity and radiation collimation significantly affect the measured inactivation kinetics. Among the columns tested, the one with the non-reflective material can be used for fluence determination and kinetics studies. PVC as a non-UV-reflective column showed acceptable measured kinetics results. In order to enhance the irradiance intensity, a Plano-convex optical lens was used, where in addition
to the acceptable correction factors, it delivered more than 4 times higher irradiance to the petri dish compared to the bare case. It was concluded that for more UV-resistant microorganisms, lenses could be an option to be implemented for kinetics studies.

### 8.2 Recommendations

- One of the methods to measure the inactivation kinetics of a microorganism (or removal kinetics of a chemical) in a photochemical processes with a polychromatic radiation source, is utilizing the action spectra of that microorganism. In order to measure the action spectra no standard protocol was used, so far. It is recommended/valuable to utilize the proposed protocol to evaluate the available action spectra of different microorganisms.

- Considering the decreasing cost trend of UV-LEDs, ($100/mW at 2012, $10/mW at 2014, and ~3$/mW at 2017), UV-LED reactors for point-of-use applications are getting attentions. In this study, a validated model for irradiance/fluence rate estimation was presented. The UV reactor modeling for mercury lamp systems has been tried widely in the literatures. Since the only difference between mercury lamp reactors and UV-LED reactors (in terms of modeling) is the radiation pattern of the UV source, it is valuable to model and optimize UV-LED reactors using the an integrated model of hydrodynamics (from CFD), as well as kinetics and radiation, from the model presented in this thesis. The kinetics equation can be measured with the proposed protocol presented in this thesis. Moreover, this
model can be helpful to find the optimum setup configuration for UV fluence determination.

- In this study, the impacts of column material, roughness, and diameter were investigated on the inactivation kinetics of E. coli. It is suggested to replicate these experiments with a more UV-resistant microorganism to extend the inactivation period. Moreover, the inactivation of microorganism, which does not have the shoulder and tailing region, is suggested under different setups to evaluate the collimation and uniformity effect on fluence determination.

- It is recommended that the UV-LED inactivation kinetics of the indicator microorganisms are investigated with the proposed UV dose determination protocol and the results to be compared with those available in the literature.

- Fabricating and evaluating a setup, either equipped with a lens or a column including a variety of UV-LEDs are highly recommended. This setup can be sent out to different groups to perform a round robin test to evaluate its accuracy and reproducibility of the obtained data. This helps to increase the acceptability of the using the setup as a standardize method and it leads to more consistent and accurate microbial inactivation data.

- The inactivation of E. coli has been presented by action spectra, considering the DNA absorption. However, it is well established that both DNA absorption and protein absorption lead to inactivation of the cell. Utilizing the proposed protocol for accurate fluence determination in different wavelengths, it is recommended to
study the impact of both DNA and protein absorption at different wavelengths on the E. coli inactivation.
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1–18, May 2012.


[128] J. R. Bolton, S. E. Beck, and K. G. Linden, “Protocol for the Determination of Fluence (UV Dose) Using A Low-Pressure or Low-Pressure Protocol for the Determination of Fluence (UV Dose) Using A Low-Pressure or Low-Pressure High-Output UV Lamp in


Appendices

Appendix A  proposed protocol to characterize UV-LEDs

A.1  Experimental procedure

Setup precreation

1-  Prepare a setup with an adjustable distance between the UV-LED and the detector, measure the radiation profile in a 3D field, and regulate the UV-LED’s case temperature and electrical current. This can best be performed using a stepper motor and a rail (Figure 1). Align the UV-LED with the stepper motor’s shaft. Place the thermocouple in contact with UV-LED without blocking the radiation path. Align the detector with the UV-LED. Place the detector at the desirable distance from the UV-LED using the rail. Cover the setup with a non-reflective material.
2-  Zero the detector off-set before operating the UV-LED (based on the detector’s manual).

Warm-up time and aging measurement

3-  Turn on the UV-LED and set the current by means of the electrical driver’s potentiometer (or pulse width modulation for digital dimming drivers).
4-  Record the detector measurements every 10 seconds while the detector reading reaches a steady state. Note the time in which the UV-LED’s output reaches the steady state (warm-up time).
5-  Record the operational time of the UV-LED for all of the experiments (to be able to evaluate the effect of aging on the UV-LED’s output).
6-  Turn off the UV-LED.

Reflection impact measurement

7-  Rotate the UV-LED around the stepper motor’s shaft by using an automated controller and measure the irradiance every 5° from −90° to 90°.
8-  Place a circular non-reflective object (with an area of about 1 cm²) between the UV-LED and the detector to block the radiation path.
9-  Turn the UV-LED on and repeat step 7.
10- If there was any significant difference between the results of step 7 and step 9, the setup has to be revised to eliminate the reflection effect.
* Repeat steps 8–10 whenever the distance, the detector, or the UV-LED is changed.

UV-LED’s SPD and radiation profile measurement
11- Remove the non-reflective object, turn on the UV-LED, and measure the radiation profile of the UV-LED following step 7.
12- Repeat step 11 at different distances to find a distance after which the radiation profile does not change significantly ($D_{opt}$) (usually around 10–15 cm). This distance is a function of the viewing angle and the LED detector’s type and configuration. Use this relative radiation profile for further calculations.
   Step 13, step 14, and step 15 have to be followed if the effects of the case temperature and electrical current are of interest.
13- Measure irradiance and SPD at $D_{opt}$ and $\theta = 0^\circ$. Monitor the case temperature ($T_1$) and electrical current ($I_1$).

Temperature effect and electrical current measurement

14- Change the case temperature by changing the voltage of the TEC and let the system reach the steady-state temperature. Record the SPD and irradiance at all desirable case temperatures.
15- Change the electrical current by means of the electrical driver in the desirable range and record the SPD and irradiance. Keep the case temperature constant for all of the current values by changing the TEC voltage.
16- Rotate the UV-LED 10° around its planar normal vector and repeat step 11–15 until a half revolution has being tested.
17- Calculate peak wavelength, FWHM, and radiant power at each case temperature and electrical current. Radiant power is calculated with Equation 3 or alternatively, it can be directly measured with an integrating sphere.
18- Measure irradiance and SPD at $D_{opt}$ and $\theta = 0^\circ$ at the end of the experiments. Monitor the case temperature ($T_2$) at $I_1$.
19- If $T_1$ and $T_2$ are significantly different, revise the heat management components and redo the characterization.
A.2 Safety and handling

The safety concerns of UV radiation exposure have been explained in detail elsewhere [137]. If the setup consists of a stepper motor, a main shutdown power button is needed. UV-LEDs are sensitive to static electricity or surge voltage and must not be touched with a bare hand. Electrostatic discharge (ESD) gloves are needed for handling UV-LEDs. To store UV-LEDs for a long time, the researcher must follow the UV-LED manufacturer’s recommendations, since the storage instructions vary based on the structure and manufacturing techniques of the UV-LED.

A.3 Report content

A comprehensive report has to include the following data. These data are useful to compare two UV-LEDs and help to more accurately analyze microbial inactivation experiments.

- UV-LED’s detailed manufacturer’s datasheet.
- Detector specifications, such as radiation-sensitive planar size, manufacturer, grating, calibration data, etc.
- The optimum distance between UV-LED and the detector or the distance in which the measurements were performed.
- The position of the thermocouple.
- The warm-up time that was considered for each experiment.
- $T_1$, $T_2$, and operational time of the UV-LED for assessing the aging effects.
- The variation in peak wavelength and FWHM, as the result of the operational temperature and operational electrical current range.
- The relative radiation profile of the UV-LED in a 3-dimensional field (as a function of $\theta$, $\varphi$, and $r$).

Appendix B Supplementary experimental data
B.1 Characterization protocol supplementary data

In this appendix supplementary data related to the characterizing UV-LED protocol is presented.

Figure B-1. The measured relative radiation profile of the studied UV-LEDs
Figure B-2. A sample of warm up time for UV-LED’s system (temperature, current, and medium condition was constant)
Figure B-3. The impact of temperature on the absolute and relative radiation profile
B.2 Chemical actinometry sample data

Photo bleaching and exposure time controls were presented here by a sample of iodide-iodate actinometry results.

Table B-1 Chemical actinometry details for Klaran UV-LEDs over different exposure times

<table>
<thead>
<tr>
<th>Exposure Time</th>
<th>$A_{352 \text{nm}}$</th>
<th>Fluence</th>
<th>Fluence rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>sec</td>
<td>Dark cm$^{-1}$</td>
<td>UV exposed cm$^{-1}$</td>
<td>mJ.cm$^{-2}$</td>
</tr>
<tr>
<td>150</td>
<td>0.0225</td>
<td>0.1284</td>
<td>4.77</td>
</tr>
<tr>
<td></td>
<td>0.0156</td>
<td>0.1289</td>
<td>5.10</td>
</tr>
<tr>
<td></td>
<td>0.0120</td>
<td>0.1300</td>
<td>5.32</td>
</tr>
<tr>
<td>360</td>
<td>0.0224</td>
<td>0.2799</td>
<td>11.59</td>
</tr>
<tr>
<td></td>
<td>0.0161</td>
<td>0.2891</td>
<td>12.30</td>
</tr>
<tr>
<td></td>
<td>0.0133</td>
<td>0.2900</td>
<td>12.48</td>
</tr>
<tr>
<td>720</td>
<td>0.0240</td>
<td>0.5336</td>
<td>22.95</td>
</tr>
<tr>
<td></td>
<td>0.0182</td>
<td>0.5608</td>
<td>24.45</td>
</tr>
<tr>
<td></td>
<td>0.0142</td>
<td>0.5568</td>
<td>24.48</td>
</tr>
<tr>
<td>1200</td>
<td>0.0262</td>
<td>0.8547</td>
<td>37.30</td>
</tr>
<tr>
<td></td>
<td>0.0195</td>
<td>0.8954</td>
<td>39.46</td>
</tr>
<tr>
<td></td>
<td>0.0150</td>
<td>0.9063</td>
<td>40.21</td>
</tr>
</tbody>
</table>
Figure B-4 Fluence rate measurement for Klaran UV-LED at different exposure times
B.3 Radiometry vs. model (detailed data)

Radiation distribution of two UV-LEDs (LED1 and LED2) were measured using the radiometry method and were estimated using the model. The actual comparison of the radiation distribution is presented here:
Figure B-5 radiation distribution comparison between the model and the radiometry data
B.4 Model equation details

In this section, the derivative of equation 5-2, 5-3, and 5-6 is explained. Based on the definition of the relative radiation pattern (RRP, equation 5-1), the fluence rate at the position of \((r, \theta, \varphi)\) can be estimated by the following equation:

\[
E_0^0(r, \theta, \varphi) = E_0^0(r, 0, 0). RRP(\theta, \varphi)
\]

Equation B-1

On the other hand, based on equation 4-1:

\[
\frac{E_0^0(r, 0, 0)}{E_0^0(R_{ref}, 0, 0)} = \left(\frac{R_{ref}}{r}\right)^2 \frac{10^{-\alpha \lambda r}}{10^{-\alpha \lambda R_{ref}}}
\]

Equation B-2

Combining equation B-1 and B-2, equation 5-2 can be write as:

\[
E_0^0(r, \theta, \varphi) = RRP(\theta, \varphi). E_0^0(R_{ref}, 0, 0). \left(\frac{R_{ref}}{r}\right)^2 . 10^{-\alpha \lambda (r-R_{ref})}
\]

Equation B-3

Based on equation 4-3, the radiant power of a UV-LED can be calculated by the following equation:

\[
P = r^2 \int_0^{2\pi} \int_0^{\pi} E_0^0(r, \theta, \varphi) \sin \theta \, d\theta \, d\varphi
\]

Equation B-4

Combining equation B-1, B-2, and B-4, fluence rate distribution can be calculated by the following equation (equation 5-3):

\[
E_0^0(r, \theta, \varphi) = RRP(\theta, \varphi). \frac{P}{r^2 \int_0^{2\pi} \int_0^{\pi} RRP(\theta, \varphi) \sin(\theta) \, d\theta \, d\varphi} . 10^{-\alpha \lambda (r-R_{ref})}
\]

Equation B-5
In case the Cartesian coordinated is of interest, using the following parameters, the fluence rate distribution can be estimated by:

\[ r = \sqrt{(x - X)^2 + (y - Y)^2 + (z - Z)^2} \]  
Equation B-6

\[ \theta = \arccos \frac{z - Z}{\sqrt{(x - X)^2 + (y - Y)^2 + (z - Z)^2}} \]  
Equation B-7

\[ \varphi = \arctan \frac{y - Y}{x - X} \]  
Equation B-8

where \( x, y, \) and \( z \) are the coordinates of the position at which the fluence rate is being calculated and \( X, Y, \) and \( Z \) are the coordinates of the UV-LED position. Combining these equations with equation B-1, the fluence rate distribution in Cartesian coordinates can be estimated with the following equation (equation 5-6):

\[ \dot{E}_0^0(x, y, z) = RRP \left( \arccos \left( \frac{z - Z}{\sqrt{(x - X)^2 + (y - Y)^2 + (z - Z)^2}} \right), \arctan \left( \frac{y - Y}{x - X} \right) \right) \cdot \dot{E}_0^0(0, 0, R_{ref}) \cdot \left( \frac{R_{ref}}{z - Z} \right)^2 \]  
Equation B-9
B.5  UV-LED’s data sheet

The Klaran UV-LED data sheet is presented in this appendix as an example. Klaran is the most implemented UV-LED in this study which was used for microbial tests.

---

**Features**
- Delivers effective deep UV (UVC) germicidal radiation
- Viewing angle of 100 degrees
- Instant on/off
- RoHS-compliant and mercury-free

**Benefits**
- Compact size enables innovative product configurations
- Robust, rugged design for portable applications
- Flexible for on-demand operation using low energy DC power sources
- Safe disposal—no special handling
- Precise disinfection delivery

**Product Nomenclature**

Klaran LEDs are binned by germicidal power output \( P_o \).

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Peak Wavelength</th>
<th>Germicidal output at 400 mA</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLARAN-GER-P-501</td>
<td>250 nm – 280 nm</td>
<td>15 mW</td>
<td>15 mW</td>
<td>20 mW</td>
</tr>
<tr>
<td>KLARAN-GER-Q-501</td>
<td>250 nm – 280 nm</td>
<td>20 mW</td>
<td>20 mW</td>
<td>25 mW</td>
</tr>
<tr>
<td>KLARAN-GER-R-501</td>
<td>250 nm – 280 nm</td>
<td>25 mW</td>
<td>25 mW</td>
<td>30 mW</td>
</tr>
</tbody>
</table>

Notes:
1. Output power is defined as \( P_o \); see explanation in "Definition of \( P_o \)" for more information.
# LED Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unit</th>
<th>Typical</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viewing angle(^1)</td>
<td>degrees</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Forward voltage at 400 mA at T(_j) = 35 °C</td>
<td>V</td>
<td>8.45</td>
<td>10</td>
</tr>
<tr>
<td>Thermal resistance, junction-to-case at T(_j) = 35 °C</td>
<td>°C/W</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Power dissipation at 400 mA at T(_j) = 25 °C</td>
<td>W</td>
<td>3.4</td>
<td>4</td>
</tr>
</tbody>
</table>

Notes:
1. Viewing angle is twice of half-value angle. A half-value angle is the angle between axial direction and direction in which the light intensity value is half of the axial intensity.
2. T\(_j\) is defined as the temperature at the solder point. See Crystal IS AN010 for more information

## Absolute Maximum Ratings

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward current</td>
<td>mA</td>
<td>40</td>
<td>400</td>
</tr>
<tr>
<td>Reverse voltage</td>
<td>V</td>
<td></td>
<td>-5</td>
</tr>
<tr>
<td>Operating temperature</td>
<td>°C</td>
<td>-10</td>
<td>55</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>°C</td>
<td>-60</td>
<td>100</td>
</tr>
<tr>
<td>Junction temperature</td>
<td>°C</td>
<td></td>
<td>85</td>
</tr>
</tbody>
</table>

## Definition of \( P_o \)

\( P_o \) is defined by an integration of the measured LED emission multiplied by the ÖNORM standard spectra of \( B. subtilis \). Crystal IS application note AN005 provides more information on this specification.

## Safe Operating Area

The plot below represents the safe operating area for Klaran LEDs. Circuits should be designed for constant current. Please refer to the Crystal IS thermal management note AN010 for heat sink recommendations.
Typical Radiation Pattern

Klaran LEDs have a nominal viewing angle of 105°.

Typical Electrical Characteristics

The typical forward voltage is less than 10 V at an operating current of 400 mA.

Test Conditions: T_{J} = 25 °C
Pulse mode operation from 1 mA to 400 mA.

Test Conditions: I (CW) = 100 mA.
CW = Continuous Wave Mode

Typical Spectral Characteristics Over Current

The plot below shows the typical spectral emission curve for Klaran LEDs.

Typical Light Output Characteristics Over Current

The plot below shows the typical variation in light output with forward current.

Test Conditions: Solder temperature (T_J) = 25 °C
Pulse mode operation

Test Conditions: Solder temperature (T_J) = 25 °C
Pulse mode operation
Recommended Soldering Guidelines

The recommended solder reflow profile for Klarian UV C LEDs follows the JEDEC standard J-STD-020D. Hand soldering is not recommended for these devices.

<table>
<thead>
<tr>
<th>Profile Feature</th>
<th>Pb-Free Assembly</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREHEAT/SOAK</td>
<td></td>
</tr>
<tr>
<td>&gt; Temperature Min ($T_{\min}$)</td>
<td>150 °C</td>
</tr>
<tr>
<td>&gt; Temperature Max ($T_{\max}$)</td>
<td>230 °C</td>
</tr>
<tr>
<td>&gt; Maximum Time (t_R) from $T_{\min}$ to $T_{\max}$</td>
<td>60–120 seconds</td>
</tr>
<tr>
<td>Ramp-up rate ($T_1$ to $T_2$)</td>
<td>3 °C/second max.</td>
</tr>
<tr>
<td>Liquidus Temperature ($T_{L}$)</td>
<td>217 °C</td>
</tr>
<tr>
<td>Time ($t_1$) maintained above $T_{L}$</td>
<td>60–150 seconds</td>
</tr>
<tr>
<td>Maximum peak package body temperature ($T_{PK}$)</td>
<td>260 °C</td>
</tr>
<tr>
<td>Time ($t_2$) within 5 °C of the specified temperature ($T_{SP}$)</td>
<td>30 seconds</td>
</tr>
<tr>
<td>Ramp-down rate ($T_{PK}$ to $T_{L}$)</td>
<td>6 °C/second max.</td>
</tr>
<tr>
<td>Maximum Time 25 °C to peak temperature</td>
<td>8 minutes max.</td>
</tr>
</tbody>
</table>

All dimensions are in millimeters. Unless noted otherwise, all dimensions have a tolerance of ± 0.05 mm.
Reel Packaging Specification

Klaran UVC LEDs are packed in tape and reel for machine manufacturing.

### TAPE DIMENSIONS

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width</td>
<td>3.50 ± 0.05</td>
</tr>
<tr>
<td>Height</td>
<td>3.50 ± 0.05</td>
</tr>
<tr>
<td>Lead Width</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td>Lead Length</td>
<td>12.00 ± 1.00</td>
</tr>
</tbody>
</table>

**LED Position in Tape**

- **Cathode**
- **Anode**

Devices are placed with the cathode to the left so the polarity direction is cathode to anode.

### REEL INFORMATION

**END**

- **Trailer**
  - 140 mm (min) of unloaded tape

**Loaded with LEDs**

<table>
<thead>
<tr>
<th>Leader</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mm (min) of unloaded tape</td>
</tr>
</tbody>
</table>

**START**

<table>
<thead>
<tr>
<th>Leader</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 mm (min) of cover tape</td>
</tr>
</tbody>
</table>

Each reel includes a leader and trailer section that is not loaded with LEDs.
Handling Precautions

LEDs are sensitive to static electricity. When handling, proper ESD protection is required, including:

» Eliminating static charge
» Using grounded wriststrap, ESD footwear, clothes, and floors
» Grounded workstation and tools

Eye Safety Guidelines

During operation, the LED emits high intensity ultraviolet (UV) light, which is harmful to skin and eyes. UV light is hazardous to skin and may cause cancer. Avoid exposure to UV light when LED is operational. Precautions must be taken to avoid looking directly at the UV light without the use of UV light protective glasses. Do not look directly at the front of the LED or at the LED’s lens when LED is operational.

Attach the following warning labels on products/systems that use UV LEDs.

RoHS Compliance

The levels of environmentally sensitive, persistent biologically toxic (PBT), persistent organic pollutants (POP), or otherwise restricted materials in this product are below the maximum concentration values (also referred to as the threshold limits) permitted for such substances, or are used in an exempted application, in accordance with EU Directive 2011/65/EU on the restriction of the use of certain hazardous substances in electrical and electronic equipment (RoHS), as adopted by EU member states on January 2, 2013.

Disclaimer

The information in this document has been compiled from reference materials and other sources believed to be reliable and given in good faith. No warranty, either expressed or implied, is made, however, to the accuracy and completeness of the information, nor is any responsibility assumed or implied for any loss or damage resulting from the use or omission.

Each user bears full responsibility for making their own determination as to the suitability of Crystal IS products, recommendations or advice for their own particular use. Crystal IS makes no warranty or guarantee, express or implied, as to results obtained in end-use, nor of any design incorporating its Products, recommendations or advice.

Each user must identify and perform all tests and analyses necessary to ensure that it has used the application incorporating Crystal IS products will be safe and suitable for use under end-use conditions. Each user of devices assumes full responsibility to become educated in and to protect from harmful radiation. Crystal IS specifically disclaims any and all liability for harm arising from buyer’s use or misuse of UVC devices either in development or end-use.

We invite you to learn more about our UVC LEDs.
B.6 Microbial apparatus and related supplementary data

Figure B-6. Specular vs diffusive reflection of a surface
Figure B-7. Light spectrum on the working bench in different condition for microbial tests

Figure B-8. Schematic view of the petri dish with the dimensions for the microbial experiments
Figure B-9. Aluminum column in different roughnesses used for UV fluence protocol improvement

Figure B-10. Inner surface of the threaded PVC column used for microbial inactivation tests
Figure B-11. The bare setup for actinometry and microbial tests
Figure B-12. The radiometry setup for bare case experiments
Figure B-13. E. coli inactivation kinetics under low pressure mercury lamp radiation[42], [58], [138]–[143]