STUDYING MICROBIAL INACTIVATION BY A NEW UV RADIATION SOURCE:

MICROPLASMA UV

by

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B.Sc., Sharif University of Technology, 2017

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

**Studying microbial inactivation by a new UV radiation source: microplasma UV**

submitted by Milad Raeiszadeh Oskouei in partial fulfillment of the requirements for the degree of Master of Applied Science in Chemical and Biological Engineering

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Abstract

Pathogenic microbes are quickly becoming one of the biggest human health threats of our time. Ultraviolet (UV) radiation enables the optogenetic control of microorganismal replication without requiring chemical addition. However, microbes with UV-resistant and repairable nucleic acids have challenged the germicidal efficiency (GE) of present UV sources. In this regard, targeting intercellular proteins responsible for nucleic acid excitation, repair, and infection can be the solution.

The newly-emerged microplasma UV technology is capable of irradiating far-UVC (200 – 240 nm) with unique spectral power distributions in a flat form, which opens new pathways for the development of novel UV disinfection systems. This study is the first to identify the mechanism-analyzed and kinetic-modelled GE of microplasma UV, radiating around proteins UV-absorption and decomposition peak. The microplasma UV lamp is initially characterized in terms of radiation profile and the impact of operating parameters on the power output. It is shown to be an instant-on and fast stabilized source. The radiant power output is a linear function of the electrical current and is not influenced by the lamp operating temperature and intermittent on/off cycles.

Afterwards, a protocol is also developed for obtaining reliable kinetic data for microplasma UV-induced reactions. An experimental setup is proposed for the kinetic studies, where the characteristics of the incident irradiance of the lamp, including uniformity, collimation, and divergence, are quantitatively evaluated. Two studied cases of microbial inactivation and the chemical photo-initiated oxidation in individual protocol-based setups confirm the reproducibility of the fluence-based kinetic data independent of the reactor size.
Eventually, the GE of microplasma UV is studied against two surrogates for challenging microorganisms, *Escherichia coli* (*E. coli*) and bacteriophage MS2, and compared with literature values for current UV sources: about 2-fold GE for MS2 inactivation and one-third repair for *E. coli* is achieved. Emitted microplasma UV photons induce significant nucleic acid repair-deficiency disorder and dramatic infection proteins excitation to enhance the genome inactivation. The reactive oxygen species are found to not play a role in this enhancement. Present results nominate promising inactivation sources for severely resistant microorganisms, thereby paving the way toward sustainable disinfection systems.
Lay Summary

Pathogenic microbes are threatening human health worldwide. Chemical disinfection materials, e.g. chlorine, have some hazardous side effects. Ultraviolet (UV) disinfection is another effective technology for the inactivation of microorganisms. UV is of great interest for industrial application because of no chemical usage. However, UV disinfection with current sources still has some problems: (1) some of the sources have mercury which is a hazardous material for environment, (2) some resistant microbes require high UV energy to be inactivated, (3) some other microbes can repair the UV-caused damage. A newly emerging UV source, microplasma UV, is mercury-free and has many special features that may facilitate UV disinfection. This study focused on accurately measuring the output of microplasma UV lamps and exploring its potential to bring more severe and long-lasting damage to microbial cells. The findings in this research will promote the practical applications of microplasma UV, such as in water treatment industries.
Preface

This research presented henceforth was conducted under the supervision of Professor Fariborz Taghipour in the Photo-reaction Engineering research group of Chemical and Biological Engineering (CHBE) department at the University of British Columbia (UBC). I, Milad Raeiszadeh, was responsible for conducting the literature review, identifying the knowledge gap, constructing and evaluating hypotheses, defining objectives, designing experimental protocols, performing experiments, compiling and interpreting results, and writing this thesis. The followings are the list of publications from this project in academic journals and international conferences, to the date of submitting this thesis:

A version of CHAPTER 4 has been published in Water Research:


A version of CHAPTER 5 has been submitted to a scientific journal:

- **Milad Raeiszadeh** and Fariborz Taghipour, Inactivation of resistant microorganisms by microplasma UV.

Moreover, the following conference papers have been lectured as podium presentations

- **Milad Raeiszadeh**, Fariborz Taghipour, Microplasma-UV lamp as a new technology for UV water purification and disinfection, 47th BCWWA Annual Conference, Victoria, Canada, May 2019

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**Roman**

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<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Area</td>
<td>cm²</td>
</tr>
<tr>
<td>D</td>
<td>Distance between solution surface and the microplasma UV lamp</td>
<td>cm</td>
</tr>
<tr>
<td>E</td>
<td>Irradiance</td>
<td>mW cm⁻²</td>
</tr>
<tr>
<td>G</td>
<td>Action spectra of the target microorganism</td>
<td>Unitless</td>
</tr>
<tr>
<td>H</td>
<td>Fluence</td>
<td>mJ cm⁻²</td>
</tr>
<tr>
<td>k or K</td>
<td>First order reaction rate constant</td>
<td>cm² mJ⁻¹</td>
</tr>
<tr>
<td>l</td>
<td>Solution depth</td>
<td>cm</td>
</tr>
<tr>
<td>n</td>
<td>Reflection index</td>
<td>Unitless</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>S</td>
<td>Survival ratio</td>
<td>Unitless</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
<td>sec</td>
</tr>
<tr>
<td>T</td>
<td>Transmittance</td>
<td>Unitless</td>
</tr>
<tr>
<td>v</td>
<td>Volume</td>
<td>cm³</td>
</tr>
</tbody>
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**Greek**

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<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
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<tbody>
<tr>
<td>α</td>
<td>Absorption coefficient</td>
<td>cm⁻¹</td>
</tr>
<tr>
<td>Φ</td>
<td>Quantum yield</td>
<td>Einstein mol⁻¹</td>
</tr>
<tr>
<td>τ</td>
<td>Irradiation time</td>
<td>Sec</td>
</tr>
<tr>
<td>ε</td>
<td>Molar absorption coefficient</td>
<td>Mol⁻¹ cm⁻¹</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
<td>nm</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>AOP</td>
<td>Advanced oxidation process</td>
<td></td>
</tr>
<tr>
<td>API</td>
<td>Absolute peak irradiance</td>
<td></td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>Collimation factor</td>
<td></td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming unit</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
<td></td>
</tr>
<tr>
<td>DF</td>
<td>Divergent factor</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
<td></td>
</tr>
<tr>
<td>ERM</td>
<td>Effectively repairable microorganism</td>
<td></td>
</tr>
<tr>
<td>Far-UVC</td>
<td>Far Ultraviolet C (in the wavelength range of 200 – 240 nm)</td>
<td></td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
<td></td>
</tr>
<tr>
<td>GE</td>
<td>Germicidal efficiency</td>
<td></td>
</tr>
<tr>
<td>GTIR</td>
<td>Germicidal to total irradiance ratio</td>
<td></td>
</tr>
<tr>
<td>IGI</td>
<td>Integrated germicidal irradiance</td>
<td></td>
</tr>
<tr>
<td>ITI</td>
<td>Integrated total irradiance</td>
<td></td>
</tr>
<tr>
<td>IUVA</td>
<td>International Ultraviolet Association</td>
<td></td>
</tr>
<tr>
<td>K-Microplasma</td>
<td>KrCl microplasma UV (irradiating monochromatic 221 nm)</td>
<td></td>
</tr>
<tr>
<td>LB broth</td>
<td>Lysogeny broth</td>
<td></td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
<td></td>
</tr>
<tr>
<td>LPMVL</td>
<td>Low pressure mercury vapour UV lamp</td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>Methylene blue</td>
<td></td>
</tr>
<tr>
<td>MPMVL</td>
<td>Medium pressure mercury vapour UV lamp</td>
<td></td>
</tr>
<tr>
<td>MVL</td>
<td>Mercury vapour UV lamp</td>
<td></td>
</tr>
<tr>
<td>Near-UVC</td>
<td>Near Ultraviolet C (in the wavelength range of 240 – 280 nm)</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>Petri factor</td>
<td></td>
</tr>
</tbody>
</table>
PFU  Plaque forming unit
P-Microplasma  Phosphor microplasma UV (irradiating polychromatic 220 – 280 nm)
QCE  Quantitative collimation evaluation
QDE  Quantitative divergence evaluation
QUE  Quantitative uniformity evaluation
RF  Reflection factor
RNA  Ribonucleic acid
ROS  Reactive Oxygen Species
SD  Sub-domain
SPD  Spectral power distribution
UBC  University of British Columbia
URM  UV-resistant microorganism
USEPA  The united states environmental protection agency
UV  Ultraviolet
UVA  Ultraviolet A (in the wavelength range of 315 – 400 nm)
UVB  Ultraviolet B (in the wavelength range of 280 – 315 nm)
UVC  Ultraviolet C (in the wavelength range of 200 – 280 nm)
UV-LED  Ultraviolet light-emitting diodes
VUV  Vacuum-UV
WF  Water factor
## Glossary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>•O$_2^-$</td>
<td>Superoxide radical</td>
</tr>
<tr>
<td>•OH</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>TEMPOL</td>
<td>4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl</td>
</tr>
</tbody>
</table>
Acknowledgements

I humbly express my enduring gratitude to my gentleman supervisor, Dr. Fariborz Taghipour, whose guidance and mentorship expertise is exceptional and inspiring. I thank him for giving me the privilege to work, learn, grow, and shape my professional career under his supervision.

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Last, but certainly not least, my family; mom, dad, and my lovely brother; to whom I owe all of my achievements throughout my life. Their emotional supports and prayers were always with me throughout all these years, pursuing my ambitions far from home. I am forever debtor for their sacrifices and thankful for that, although I cannot thank them enough.

The research presented in this thesis was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada.
Dedication

To my Lord, GOD ALMIGHTY, for endless feelable graces and blessings.

To my Beloveds; Parents, Brother, and Grandpa; for all sacrifices.

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And to all passed-away darlings on the Ukraine International Airlines Flight 752 crash on January 8, 2020, in Tehran, Iran. May all your Souls rest in Peace.
Chapter 1: Introduction

1.1 Background of ultraviolet disinfection

When calling his discovery in the electromagnetic radiation spectrum in 1801 “chemical ray,” Johann Wilhelm Ritter [1] would have never imagined that this finding would become a treasure in the field of biology, microbiology, and sustainability. Later, scientists determined that the exposure to this radiation, known as “ultraviolet” (UV) radiation, could prevent the growth of microbial cells within any medium.

Nowadays, pathogenic microbes are of the biggest human health challenges. The discovery of the hazardous byproducts associated with chemical antimicrobial agents opens a world-wide need for a sustainable disinfection technique against human health-threatening microorganisms [2]. As an example of this, bringing clean drinking water for the people in remote areas is one of the essential issues of the 21st century leading to a growing need for efficient and cost-effective water purifiers [3]. Water contaminants can only be removed to certain extents via conventional physicochemical methods, biodegradation, and chemical advanced oxidation; see Table 1-1 for a general comparison of different water purification methods [4-6].

UV disinfection has been a fast-growing sustainable disinfection technology over the past decades, thanks to its no requirement for chemical additives, low by-product formation tendency, and no overdosing risk [7]. Moreover, UV can degrade organic compounds by UV-based advanced oxidation processes assisted by the addition of oxidants [8] or photosensitizing agents such as photocatalysts [9]. The primary UV radiation sources employed in the municipal disinfection or advanced oxidation installations are mercury vapour UV lamps (MVLs). However, these lamps contain a considerable quantity of mercury, which leads to a significant amount of hazardous material after the lamp’s lifespan [10]. Other challenges associated with mercury lamps are long
warm-up time, filament distortion, and geometrical restrictions for the reactor design [11, 12]. More recently, UV light-emitting diode (UV-LED) technology has emerged as an alternative to conventional sources and attracted attention because it has no mercury usage, low energy consumption, compactness, and no warm-up time [13, 14]. The unique features of UV-LEDs make them a great candidate for point-of-use and point-of-care disinfection systems to be implemented in remote areas [15]. However, relatively low radiant power of the present UVC-LEDs has impeded their progress for large-scale applications.

**Table 1-1. A comparison on the different water purification techniques.**

<table>
<thead>
<tr>
<th>Disinfection Technique</th>
<th>Remove harmful microorganisms</th>
<th>Not changing the taste of water</th>
<th>Easiness of process</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV treatment</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Filters</td>
<td>×</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Chemical Treatment</td>
<td>✔</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Reverse Osmosis</td>
<td>✔</td>
<td>×</td>
<td>×</td>
</tr>
</tbody>
</table>

1.2 New microplasma UV technology

Microcavity plasma or “microplasma” UV is a new radiation technology that offers some advantages over current UV sources. The flat, thin microplasma UV lamps are driven by at least two interlaced arrays of tiny cavities (microcavities) with distinct dimensions fabricated into the interior surface of the lamp [16]. In this mercury-free technology, a rare gas occupies the volume inside the lamp and is in contact with the microcavity array. By turning on the lamp, the voltage interacts with the gas, such that a spatially uniform plasma is formed and confined within the microcavities; this is the microplasma. This microplasma produces UV-generating rare-gas excimer molecules which emit radiation in the deep-UV spectral region [17]. To date, available microplasma UV lamps use Xe₂, KrCl, and XeCl excimers to monochromatically radiate at 172,
222, and 308 nm peak wavelengths, respectively, and phosphor for a broad spectrum of 220–280 nm [18]. See Figure 1-1 for a schematic on the microplasma UV lamp and actual images of the available microplasma UV lamps with different sizes. The presence of the microcavities improves the power output and efficiency of the lamp by at least a factor of two compared with the previous sources for the mentioned peak wavelengths [16]. Additionally, the planar geometry of these lamps may help them to emit collimated and spatially-uniform beams.

The capability of microplasma UV lamps to irradiate unique UVC spectrums with different frequencies and high intensity in a flat form is outstanding. Therefore, microplasma UV technology possesses a great potential to create new opportunities for the development of novel planar UV-based water, air, and surface disinfection devices, thereby expanding the reach and applicability of UV technology.
Figure 1-1. (A) Cross-sectional schematic view of a microplasma lamp, showing the placement of different parts and position of the microcavities, adapted from manufacturer website [19]. (B) Current microplasma UV lamps with different radiation spectrum and sizes (large lamp is 4×4 in and small lamps are all 2×2 in)
1.3 Thesis layout

This research was conducted through standardized experimental protocols to achieve the objectives of this project. As a highlight, this dissertation includes the description of methodologies and experiments, illustration and discussion on the resultant data, and conclusion of thesis material along with recommendations for further studies on microplasma UV. The material is presented in 6 chapters, including an initial introductory CHAPTER 1 on the background of UV disinfection and introducing the newly-emerged microplasma technology for UV irradiation. The structure of the other chapters in this thesis is as follow:

CHAPTER 2 provides a literature review of the principles, applications, and challenges of microbial inactivation by current UV radiation sources. The significance of using microplasma UV is also articulated in this chapter. Furthermore, the knowledge gap is highlighted, and research objectives are stated based on the knowledge gap.

CHAPTER 3 demonstrates a description of general methodologies, fabricated setups, and analytical techniques used in this research to investigate the objectives. Detailed employed experimental and mathematical techniques are elaborated as Methodology sub-chapters within chapters 4 and 5.

CHAPTER 4 elaborates the newly-developed experimental protocols in this work to characterize microplasma UV lamps, study the effect of operating conditions to control microplasma UV’s consistent power output, and operate lamps in suitable kinetic study setups to report reproducible and setup-independent kinetic data.

CHAPTER 5 presents the investigation of microplasma UV irradiation with two different spectral power distributions for the inactivation of representative surrogates for UV-challenging microorganisms. This is in addition to studying the post-treatment microbial reactivation after
microplasma UV exposure. As the complimentary for a comprehensive study: (i) the results are applied to develop a simple kinetic model, (ii) compared with the ones available in the open literature for UV lamps and UV-LEDs, and (ii) the analyzed to obtain the general mechanism of microplasma far-UVC inactivation of microorganisms.

**CHAPTER 6** provides a summary and conclusion of the thesis material as well as some recommended research topics, which can potentially lead to the further advancement of the microplasma UV technology for microbial inactivation.

To our knowledge, this research is the first to investigate microplasma UV technology for the applications mentioned above.
Chapter 2: Literature Review

2.1 UV disinfection principles

UV is a form of electromagnetic radiation that covers the wavelengths in the range from 100 to 400 nm [20]. Within this range, as illustrated in Figure 2-1, the UV spectrum can be partitioned into UVA (400–315 nm), UVB (315–280 nm), UVC (280–200 nm), and vacuum-UV (VUV, 200-100 nm) subdivisions. The UVC section is itself bisected into near-UVC (280–240 nm) and far-UVC (240–200 nm) [21]. Among all UVs, the shorter the wavelength becomes, the more detrimental effect it poses to living cells. Intercellular components of living cells; e.g. nucleic acids, either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), and proteins; can absorb UVC with different sensitivities over the range. Hence, UVC photons are also called germicidal UV as they have the highest absorption by living organisms such as germs. The effect of UV irradiation toward microorganism is to “inactivate” it, and not killing, by preventing the microorganism from surviving or reproducing.

Figure 2-1. The region of UV radiation and its subdivisions in the electromagnetic region
During UVC exposure to microbial cells, the most sensitive targets of microorganisms are the DNA of bacteria, the DNA of DNA-viruses, and the RNA of RNA-viruses. UVC photons cause three critical damages to nucleic acids: cyclobutane pyrimidine dimers, (6–4) photoproducts, and protein-nucleic acid cross-links [22]. As the primary products of UVC-irradiated nucleic acid, pyrimidine dimers are formed between adjacent thymines in the polynucleotide chains of DNA (or uracil in RNA) [23]. Apart from cross-links with adjacent thymines, thymine may also form links with proteins, such as proteins in the capsid (in the case of some viruses). In addition to nucleic acid, other biological molecules with unsaturated bonds like coenzymes, hormones, and electron carriers may also be susceptible to UVC exposure [24]. Causing photochemical reactions in proteins and enzymes, UVC radiation damages other intercellular and viral components. However, proteins absorb UVC with a different wavelength-sensitivity pattern to nucleotides. The peak of UVC absorption is reached in the near-UVC range (around 265 nm), whereas the protein UVC absorption starts increasing below-240 nm wavelengths (far-UVC) and even surpasses DNA absorption at around 225 nm (see Chapter 5 and Figure 5-1 for more details).

**Figure 2-2.** Thymine dimerization (A) in the double-stranded DNA and (B) between DNA and adjacent protein molecules (reprinted with permission from the Ultraviolet Germicidal Irradiation Handbook, Kowalski 2009) showing how UV exposure disrupts the structure of DNA chain.
2.2 **Fundamental photochemistry terms**

The commonly-used terms in measuring and reporting radiation of UV sources are presented in this section. Terminologies used in this study were obtained from the “Ultraviolet Applications Handbook” [20]. Some of the frequently used terms in this study are presented hereunder due to increasing the clarity of the text:

Irradiance (mW cm$^{-2}$): Represents the total radiant power incident from all upward directions on an infinitesimal element of the surface of area (dA), containing the point under consideration, divided by the dA area. A radiometer probe can measure the irradiance.

Fluence rate (mW cm$^{-2}$): Defined as the total radiant power incident from all directions on an infinitesimally small sphere of cross-sectional area dA, divided by dA area. The fluence rate is the correct representation of the delivered UV energy to microbial cells inside the solution under irradiation.

Fluence (mJ cm$^{-2}$): Shows the energy from all the incident radiation from all directions to an infinitely small spherical volume divided by the cross-sectional area of the sphere. The other commonly-used name for fluence is UV dose. As it is clear from units, the product of fluence rate and exposure time yields the total delivered UV energy (UV fluence) that relates to the amount of microbial inactivation.

2.3 **Current UV disinfection challenges**

The great potential of UV or, more precisely UVC (200–280 nm), to disturb microbial replication has been attracting significant attention for use in various applications, such as water/air purification [7], surface decontamination [25], blood therapy [2], milk pasteurization [26], and poultry/agricultural product sanitization [27]. The low pressure mercury vapour UV lamp
(LPMVL) is the most commonly used commercialized UV source [23] (see Table 2-1 for a detailed operating characterization).

In spite of the great potentials of UV disinfection, two issues associated with current UV sources are still against the sustainability principles. First, the existence of a massive amount of hazardous mercury in conventional UV lamps has questioned the environmental-friendliness of these sources. Second, some microbes have challenged the inactivation efficiency of currently-used sources.

In principle, irradiated UV photons can simply inactivate microorganisms by disturbing their genetic nucleic acid structure [28] in either DNA or RNA. In practice, however, two types of microbes have slowed the further globalization of the currently available UV sources: microorganisms with (i) UV-resistant nucleic acid and (ii) effective post-irradiation repair mechanisms for nucleic acid lesions, which are designated hereafter by URM$s$ and ERM$s$, respectively. This leads to a higher required UV-dose regulated by global environmental protection agencies for adequate disinfection by UV sources [29], which, in turn, results in higher energy consumption and lower process efficiency. Further, the regulations usually require the addition of chemical disinfectants, such as chlorine and ozone, for providing a residual effect, which is against the purpose of sustainable UV treatment which is no adding of chemical agents.

Sensitivity of nucleic acid exciting and viral proteins in URM$s$ for UV with below 240 nm photons, known as far-UVC radiation [21], can be the key to increasing susceptibility by inducing damage to these components. In ERM$s$, the repair mechanism to maintain genome integrity consists of two main phenomena: intrinsic nucleotide excision repair [30] and light-initiated [31] repair, which are known as dark repair and photoreactivation, respectively. In photoreactivation, the repair is performed by an enzyme, called photolyase [32], which reverses UV-induced damage
in nucleic acids. In dark repair, the damage is reversed by the action of a number of different enzymes. The dark repair pathways do not directly reverse DNA damage but instead replace the damaged DNA with new fresh nucleotides. All of these enzymes are activated by an energy source which would be photons mainly in the wavelength range of 300–500 nm for photoreactivation, or existent nutrients within the cell for dark repair. Photoreactivation cannot completely reverse damage to DNA since UV may cause other types of photoproducts but it can effectively limit UV damage. Inactivating radiation at a broad range of UVCs has been claimed to be effective for reducing subsequent reactivation of microorganisms [31, 32].

2.4 Current solutions for UV disinfection challenges

Polychromatic medium pressure mercury vapour UV lamp (MPMVL) was the first to be studied to solve the two aforementioned roadblocks of UV disinfection as it irradiates far-UVC photons in its broad spectrum (see Figure 5-1A). MPMVL was shown to be more effective at inactivating URMs than LPMVL [31, 33]. When damage to nucleic acid was measured, the MPMVL and LPMVL were equally efficacious [34], which confirmed the enhanced inactivation of URMs by absorbing far-UVC with their proteins. MPMVL has also been shown to limit the degree of photoreactivation compared to LPMVL [35]. However, the biggest drawback of MPMVL compared with LPMVL is the overly broad spectrum (185–600 nm), which made them less efficient and more energy-consuming in providing the same germicidal dose in the UVC range. In addition, this broad emission gave rise to the possibility that the nucleic acid in a target pathogen may undergo simultaneous damage and repair when exposed to other wavelengths (UVA and visible light) during the disinfection process, decreasing the efficiency of MPMVL disinfection (see Table 2-1 for other characteristics). However, MPMVLs contain a considerable
amount of mercury, up to 2000–4000 mg per lamp (~100 folds more than LPMVL), which leads to a significant amount of hazardous material after the lamp lifespan.

Recently, the new mercury-free semiconductor-based UV-LED technology [36] has been developed with unique features (Table 2-1), making it a great candidate for point-of-use, point-of-entry, and point-of-care disinfection systems [37]. Low-power-consuming UV-LEDs have garnered increasing interest as a promising alternative to UV mercury lamps for disinfection [38]. However, UV-LEDs’ inability to radiate broad wavelengths, including far-UVC with a reasonable wall plug efficiency, has hindered their further application for URMs and ERMs. Some studies have successfully limited photoreactivation by adding UVA pretreatment to UVC-LED disinfection [28, 39]. However, energy and cost analysis demonstrated that this technique is still uneconomical due to the high dose requirement for UVA pretreatment [40].

Researchers have been encouraged to employ alternative far-UVC radiating sources, such as excilamps [41], UV lasers [42], and pulsed electric discharges [43]. However, in spite of exciting lab-scale achievements [44], critical drawbacks including low wall-plug efficiency, short lifetimes, and bulky configuration (see Table 2-1) along with a dearth of the reliable operating protocol have impeded their progress for practical applications.

2.5 Microplasma UV capability for solving UV disinfection challenges

Microplasma UV offers significant advantages over conventional sources (Table 2-1), allowing it to be utilized in commercial applications [16]. Within the microplasma UV lamp, UV-generating rare-gas excimer molecules, such as Xe₂ and KrCl, are produced, which monochromatically emit radiation in the far-UVC spectral region at 172 nm and 222 nm, respectively [18]. Ingeniously, a novel broad spectrum in the UVC region (220–280 nm) has also been generated by down-converting Xe₂ radiation with phosphor. These unique spectral power
distributions of mercury-free microplasma UV lamps, which are around proteins’ UV-absorption and decomposition peak, can help the current UV disinfection world to overcome the critical challenges mentioned in section 2.2.

As mentioned in section 2.3, some research papers reported the disinfection results of microorganisms by far-UVC irradiating excilamps. One of the well-known excilamps is KrCl containing ones nominally irradiating monochromatic 222nm photons [41]. However, the reported kinetic data for this source varies considerably. For example, the reported UV dose for a 4-log reduction of *Escherichia coli* bacteria varies in the range of 3 to 29 mJ cm\(^{-2}\) [45-48]. This is due to the lack of a standardized protocol to design appropriate setups for accurately calculating UV dose delivered by the excilamps. Typically the experiments were conducted in arbitrary distances, and fluence rates were not calculated inside the solutions.

As explained, there is not a standard work for the microbial inactivation by a 222 nm lamp to report general kinetic values. In the case of URM viruses, there is no standard work to report the kinetic values. There is only one recently published paper on the inactivation of bacteriophage MS2 virus by the 222nm KrCl excilamps, which claims 30 mJ cm\(^{-2}\) required for 4 log inactivation [49]. In the case of microbial reactivation, there is no systematic study showing the effect of far-UVC photons on the post-treatment reactivation. In addition, the effect of different far-UVC doses on reactivation is not studied, and the reactivation process is not kinetic-modelled for far-UVC sources so far, to the best of the author’s knowledge. Only several works qualitatively reported the suppression of photoreactivation by far-UVC sources comparing with UV lamps [47, 50].

In brief, to investigate the potentials of the microplasma UV, reliable and reproducible kinetic results should be gathered. The reliability of this investigation is not possible unless by first developing comprehensive protocols for accurately characterizing and controlling microplasma
UV lamps along with precisely measuring the microplasma UV lamp output in order to calculate the delivered UV fluence to microbial cells.

**Table 2-1. Operating characterizations of various germicidal UV sources [7, 11, 16, 18, 36, 41]**

<table>
<thead>
<tr>
<th>UV Source</th>
<th>Operating Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPMVL</strong></td>
<td>• Monochromatic (253.7 nm)</td>
</tr>
<tr>
<td></td>
<td>• ~30 mg of mercury for a 1.2 m lamp</td>
</tr>
<tr>
<td></td>
<td>• 2–7 min warm-up time</td>
</tr>
<tr>
<td></td>
<td>• 8,000–10,000 h lifetime</td>
</tr>
<tr>
<td></td>
<td>• Electrical to germicidal UV conversion efficiency of 35%–38%</td>
</tr>
<tr>
<td></td>
<td>• High power consumption (0.5 W/cm electrical input)</td>
</tr>
<tr>
<td></td>
<td>• Geometrical restrictions for reactor design (bulky cylindrical lamp)</td>
</tr>
<tr>
<td></td>
<td>• Operating surface temperature of 30–50 °C</td>
</tr>
<tr>
<td><strong>MPMVL</strong></td>
<td>• Polychromatic (185–600 nm)</td>
</tr>
<tr>
<td></td>
<td>• 2,000–4,000 mg of mercury for a 1.2 m lamp</td>
</tr>
<tr>
<td></td>
<td>• 4–10 min warm-up time</td>
</tr>
<tr>
<td></td>
<td>• 4,000–8,000 h lifetime</td>
</tr>
<tr>
<td></td>
<td>• Electrical to germicidal UV conversion efficiency of 10%–20%</td>
</tr>
<tr>
<td></td>
<td>• Very high power consumption (50–250 W/cm electrical input)</td>
</tr>
<tr>
<td></td>
<td>• Geometrical restrictions for reactor design (bulky cylindrical lamp)</td>
</tr>
<tr>
<td></td>
<td>• Operating surface temperature of 600–900 °C</td>
</tr>
<tr>
<td><strong>UV–LED</strong></td>
<td>• Quasi-monochromatic (Full width at half maximum (FWHM)~10), with selectable wavelength (250–360 nm) through semiconductor composition</td>
</tr>
<tr>
<td></td>
<td>• No mercury</td>
</tr>
<tr>
<td></td>
<td>• No warm-up time</td>
</tr>
<tr>
<td></td>
<td>• 10,000–20,000 h lifetime for UVC</td>
</tr>
<tr>
<td></td>
<td>• Electrical to germicidal UV conversion efficiency of 1%–5% for UVC</td>
</tr>
<tr>
<td></td>
<td>• Low power supply requirement (&lt;30V DC).</td>
</tr>
<tr>
<td></td>
<td>• Compactness for various reactor designs (point source)</td>
</tr>
<tr>
<td></td>
<td>• Low operating surface temperature</td>
</tr>
</tbody>
</table>
Excilamps, UV Lasers, and Pulsed Electric Discharges
- Monochromatic, selectable wavelength (entire UV) through excited dimer molecules
- No mercury
- No warm-up time
- Relatively short lifetime
- Electrical to germicidal UV conversion efficiency <15%
- High power supply requirement
- Geometrical restrictions for reactor design (bulky configuration)
- High operating surface temperature

Microplasma UV Lamps
- Both monochromatic (222 nm) and polychromatic (220–270 nm)
- No mercury
- No warm-up time
- >50,000 h lifetime
- Electrical to germicidal UV conversion efficiency >20%
- Low power supply requirement (<30V DC)
- Flat geometry in different sizes for designing planar reactors
- Low operating surface temperature (<40 °C)

2.6 Current operating protocols for UV sources

For any newly emerging radiation source, a standardized technique is required for the operation, characterization, and control of the radiation performance. For the older mercury UV lamps, several practical procedures for output characterizations have been previously suggested and adopted as the standard by the International Ultraviolet Association (IUVA) and the United States Environmental Protection Agency (USEPA) as standard protocols. [51, 52]. As for UV-LEDs, a distinct characterization protocol has been recently proposed for investigating the effects of operational conditions and measurement techniques [38].

In addition to the characterization protocol, a comprehensive protocol must be established for a UV radiation source to design appropriate apparatuses to facilitate obtaining accurate and consistent photo-chemical kinetic data which could be applied for the reactor design. For UV-induced reactions, the most accurate way to present the kinetic data is as a function of UV fluence.
received by the solution components [53]. For precise determination of the average fluence rate inside a solution, uniform radiation distribution should be obtained. This is possible whenever a uniform irradiation field on the solution surface is provided by collimating a set of vertically projected beams. In this regard, for UV lamps, the designing approach of standard setups, referred to as “collimated beams,” has been proposed and accepted by the IUVA and USEPA [54, 55]. In this method, the microbial sample needs to be located in at least 20 cm distance from the mercury UV lamp to obtain uniform radiation distribution inside the solution. At this distance, the incident radiation to the surface of the suspension was claimed to be collimated, which means that the in-solution fluence rate can be estimated by measuring irradiance on the solution surface.

Similarly, for UV-LEDs, a new method has been recently developed by systematically revising the UV lamp protocol considering the unique characteristics of UV-LEDs [56, 57]. A minimum distance of 13 cm from the UV-LED was recommended to guarantee the fluence rate calculation accuracy. Also, due to the low irradiance at this distance, utilizing optical lenses was suggested to obtain the collimation in much closer distances [56].

2.7 Knowledge gaps and research questions

Based on the literature review above, mercury-free far-UVC irradiating sources have a great potential to resolve the challenges of UV disinfection by enhancing the inactivation of URMs and suppress the repair mechanism of ERMs. However, the mentioned drawbacks of current far-UVC sources limit their progress for industrial applications. Microplasma UV lamps are new far-UVC irradiating sources which have the potential to be used in individual or in parallel with current UV sources, such as UV-LEDs to solve UV disinfection challenges. However, there has been no comprehensive study on the application of microplasma UV lamps for disinfection, and specifically for water, as they are newly-emerging radiation sources.
Therefore, the knowledge gap for using microplasma UVs for microbial inactivation and studying post-treatment reactivation is vast as, to our knowledge, there is no published work in the open literature. To fill this gap, the first and foremost strategy is to characterize the microplasma UV lamps and investigate the effect of operational conditions on the microplasma UV performance. Additionally, to reach valid and reportable photokinetic data for the inactivation of microorganisms by microplasma UV photons, a protocol is required to calculate the in-solution UV fluence. Without such protocol, the reported kinetic results from laboratory experiments and reactor designs based on those may not be reliable. The hypothesis is that due to the distinct geometry of microplasma UV lamps (thin flat lamp with spatially uniform microcavities within it), the collimation and uniformity of radiation on irradiated surfaces would take place in less distances comparing with UV lamps and UV-LEDs.

Based on the literature review and the knowledge gaps, the research questions can be stated as below:

**Question 1:** What are the key elements of a standard method to reliably characterize microplasma UV lamps? What is the effect of operating conditions on the specifications of microplasma UV lamps’ power output?

**Question 2:** What is the pattern of uniformity and collimation for microplasma UV generated rays on the surface of solutions under the irradiation?

**Question 3:** How a general protocol could be developed to determine the UV dose of the microplasma UV irradiation systems?

**Question 4:** What are the effects of the two typical types of microplasma UV sources (monochromatic 222 nm and polychromatic 220–270 nm) on the inactivation of representative
microorganisms for URMs and ERMs? And how is the germicidal efficiency of microplasma UV lamps compared with current UV lamps and UV-LEDs?

**Question 5:** What is the degree of microbial reactivation of a representative microorganism for ERMs after being irradiated with different UV doses by the two types of microplasma UV lamps (monochromatic 222 nm and polychromatic 220–280 nm)? And can the kinetic of this reactivation be modelled?

### 2.8 Thesis objectives

Based on the aforementioned challenges in the literature review associated with the UV disinfection by currently-used near-UVC sources and the knowledge gap on using microplasma UV, this research focused on studying the potentials of the newly-emerging UV radiation technology to overcome these challenges. UV dose determination is of great importance for the purpose of designing suitable microplasma UV irradiation apparatus to gain accurate, reproducible, and reliable photo-kinetic data. This microplasma UV dose determination highly depends on the proper operation of the lamp and the consideration of the microplasma UV characterization, as well as the setup configuration. Thus, the main objective of this research is to study the kinetics of the inactivation and reactivation of microorganisms caused by the microplasma UV irradiation. The overall objective will be achieved through the attaining the following sub-objectives:

1. Propose methodologies to operate and characterize microplasma UV lamps and accurately measure and control their power output.
2. Develop a protocol for accurately determining the delivered UV fluence rate to solutions irradiated by microplasma UV lamps.
3. Implement the developed UV dose determination protocol to study the kinetics and mechanism of the inactivation of representative microorganisms for URMs by two monochromatic and polychromatic microplasma UV sources.

4. Investigate, kinetic-model, and mechanism-study the post-treatment reactivation of representative microorganisms after irradiating by microplasma UV sources.
Chapter 3: Experimental Methodology

This chapter describes the overall experimental methodology, including utilized materials, designed experimental apparatus, general procedures, and analytical techniques to fulfill the objectives of this research. Specific experimental work and mathematical analysis for each sub-objective will be presented as part of the corresponding chapter, i.e. chapters 4 and 5.

3.1 Materials

Potassium iodate (KIO₃), potassium iodide (KI), sodium tetraborate decahydrate (Na₂B₄O₇.10H₂O), and methylene blue (MB, C₁₆H₁₈ClN₃S) were all supplied by Sigma–Aldrich with no further purifications. Hydrogen peroxide solution (H₂O₂, 30% w/w) was acquired from the Fisher Scientific Co. *E. coli* (ATCC 11229) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultivated following the supplier’s product data-sheet. All agents used in this study were analytical grade, and all solutions, apart from microbial suspensions, were prepared with deionized water.

3.2 Radiometry

A radiometry configuration was fabricated to measure the incident irradiance distribution on a plane using a detector. Spectral irradiance was measured with an Ocean Optics USB2000+ spectrometer equipped with a Sony ILX511B CCD detector. The detecting instrument has a measurement range of 200–850 nm with a resolution of 0.38 nm. To obtain more accurate radiometry data on the determined SDs, the area exposed to radiation was controlled using two methods: partially masking the detector-wide sensor and using a fibre-optic probe. In addition, since the radiation profile of UV sources is directional, the detector was equipped with a cosine corrector (with Spectralon diffusing material) to ensure that the measured number was the normal vector of the incident photon to the surface, according to the definition of irradiance.
The detecting instrument and its components were placed on a lifting stage to cover measurements in direction $y$. A multi-axis actuator was used to hold the microplasma UV lamp parallel with the detecting surface and move it in $x$ and $y$ directions to avoid spatial limitations in irradiance measurements across the surface area. The lamp and holding components were placed on a stage over a rail. By using the rail, the radiating instrument could reciprocate in $z$-direction toward the detector to simplify conducting the measurements in distances from the lamp with 0.1 cm intervals. Additionally, the average irradiance in different depths of the solution can be measured by providing the graduated rail. The entire radiometry apparatus was placed inside a frame built with UV-absorptive materials and covered with a piece of dark fabric to protect the detector from saturation by excessive light noises. The fabricated radiometry configuration is illustrated in Figure A-1.

3.3 Actinometry

The chemical actinometry is a widely used photochemical method to determine the photon flow entering a solution. Furthermore, as the actinometry solution can absorb radiations from all directions, it can demonstrate the fluence rate [58]. The absorption of the actinometer should be high enough at the germicidal range to absorb almost all of the photons that can be absorbed by the intercellular components of microorganisms. Among the currently accepted actinometers, the Potassium Iodide/Iodate actinometry is appropriate to be used for a source in the UVC region. It involves a set of photochemical reactions in which the UV direct degradation of triiodide ($I_3^-$) represents the fluence rate inside the well-mixed actinometry solution. Therefore, this chemical actinometry technique was applied to determine the fluence rate. Due to the high absorption coefficient of the actinometer in the UVC region, the photons are fully absorbed in a thin layer (less than 1 mm) on the top of the solution. Thus, the measured fluence rate with the chemical
actinometry is assumed as the fluence rate on the solution surface. The actinometry measurement was performed with 0.1 M KIO₃, 0.6 M KI, and 0.01 M Na₂B₄O₇·10H₂O solution following the standard procedure proposed in the literature by considering all the correction factors for fluence calculation on the solution surface [59]. The quantum yield of the iodide-iodate actinometry was corrected based on the SPD for microplasma UV lamps as it is a strong function of the wavelength [60]. To prevent any systematic errors, the container was filled entirely with actinometry solution to avoid wall reflectance and was stirred 30 s before each experiment to provide well-mixing conditions.

3.4 UV-degradation experiments

As an example of the UV disinfection process, inactivation of the *E. coli* bacterium and bacteriophage MS2 was studied as surrogates for enteric pathogens (see section 5.3.1). Details of stock preparation, cell culture, and colony enumerating methods are described in Appendix B. Additionally, a UV/hydrogen peroxide system was selected as a common UV-based photo-initiated oxidation. Degradation of MB was measured as a well-known model organic contaminant because its concentration can easily be calculated by measuring the absorbance of the solution at 664 nm with a UV-Vis(ible) spectrophotometer using the Beer-Lambert law.

The actual inactivation and degradation experiments were conducted in bench-scale, collimated, and uniformly irradiated, which was ensured by the developed protocol in chapter 4 and the UV apparatuses equipped with a 2 × 2 in microplasma UV lamp. The reaction solution was put into two quartz cylindrical containers with inner diameters of 5.5 cm and 1.8 cm, respectively (and depth of 1 cm), and placed on a stir plate, perpendicular to the incident radiation. A black sheet with an aperture was put on top of the container so that the beams entered the solution only from the container surface rather than the walls; this is particularly crucial when the container
size is smaller than the lamp. A schematic of the experimental setup is shown in Figure 3-1, and
the image of the actual apparatus is presented in Figure A-2 of Appendix A. The entire apparatus
was covered by a black box during the experiment to avoid any potential photodegradation or
photoreactivation by the ambient light.

Figure 3-1. Schematic of the setup for microplasma UV inactivation experiments using the black
sheet with an aperture to prevent UV beams from entering from the container walls.
Chapter 4: Protocols for Characterization and Kinetic Study

4.1 Introduction

As discussed in the literature review section, there are established protocols available for characterization and fluence-rate calculation of UV lamps and UV-LEDs. However, the aforementioned proposed protocols cannot be extended to microplasma UV lamps because the radiation source structure and the technology of UV emission are entirely different. In the present chapter, the aim is to develop a protocol for designing appropriate apparatuses to accurately investigate the kinetics and mechanisms of UV-induced reactions, which will be applicable to microplasma UV lamps of any intensity, size, and peak wavelength. As the primary stage, a comprehensive characterization methodology was proposed for the microplasma UV lamp in a non-UV-absorbing medium. This was achieved by introducing the lamp specifications and investigating the effect of operating parameters, such as lamp temperature and electrical current, on the lamp power output. Furthermore, a particular method was devised for studying the microplasma UV lamp incident irradiance pattern. Finally, after conducting systematic quantitative uniformity evaluation (QUE), quantitative collimation evaluation (QCE), and quantitative divergence evaluation (QDE) tests on the incident irradiation, a protocol was proposed for obtaining uniform radiation distribution inside solutions irradiated by the microplasma UV, for use in kinetic studies. Two UV-induced cases were also presented to evaluate the reproducibility of the kinetic data in two distinct protocol-based setup configurations for UV inactivation of E. coli (as a representative for microorganisms) and UV/H$_2$O$_2$ degradation of MB (as a model organic contaminant).
4.2 Methodology

In addition to what is presented hereunder, utilized materials and implemented methodologies for the radiometry, actinometry, and UV degradation experiments are elaborately discussed in chapter 3 (3.1 to 3.4, respectively).

4.2.1 Lamp characterization method

A 2×2 in microplasma UV lamp, with an active illuminating area of about 16 cm², was acquired from Eden Park Illumination Inc. The utilized microplasma UV lamp is shown in Figure 4-1A along with the electrical circuit for supplying power to the lamp. The lamp was connected to an analog DC power supply (Model: Aim TTI EX355R) so that the input voltage and current could be controlled.

Lamp temperature and flowing electrical current were considered as operational conditions and were studied over irradiation time. The effect of intermittent on/off cycles was also investigated as another essential characteristic of a radiation source. The output of a radiation source is usually presented by two groups of specifications, which are related to the irradiant power of the source and the spectral power distribution (SPD) of the beams. In the first group, the incident irradiance from the lamp on a given surface was studied. The important values included the following: absolute irradiance at the peak wavelength (absolute peak irradiance [API]), the integrated irradiance over the radiation spectrum (integrated total irradiance [ITI]), and irradiance over the germicidal spectrum of 200–300 nm (integrated germicidal irradiance [IGI]). In the second group of lamp specifications, peak wavelength, full width at half maximum (FWHM), and germicidal to total irradiance ratio (GTIR) of the emitted beams were evaluated.
Figure 4-1. (A) Schematic of the assembled operating microplasma UV lamp system and (B) spectral power distribution of the employed microplasma UV lamp with its related specifications.
4.2.1.1 Domain determination method

Typically, to study the irradiance received by surfaces in front of a radiation source, a method is required for dividing the total domain into certain constituent sub-domains (SDs). Radiometry measurements can be operated on the determined SDs to estimate the average irradiance on the domain.

Assuming the radiating surface of the commercial flat microplasma UV lamp as a square, it consists of many circular arcs with two diameters of 2.11 mm and 0.71 mm. These arcs are positioned side by side, so a small arc is adjacent to four larger arcs in four Cartesian directions and vice versa (Figure 4-2B). The main nonuniformity in the pattern of arcs is an arc-empty corner and a spacer at the center of the lamp where a set of the mentioned arcs are absent. Although the lamp face is square-shaped, a circular domain for radiant characterization is preferred, so that it can be applied to the standard solution containers used for photokinetic studies, which usually have circular surfaces (Petri dishes, beakers, etc.). The recommended method is elaborately described in Figure 4-2 in which each SD is in front of a circular area of the lamp face with a diameter of 5.26 mm and contains 4 small and 4 large (4-4) arcs (4-microcavity SDs) in most cases. To evaluate the effects of the abovementioned nonuniformities on the irradiance, there are some SDs in front of circles which contain 3-3 arcs (3-microcavity SDs), 2-2 arcs (2-microcavity SDs), and 0-0 arcs (0-microcavity SDs). Each domain (Circles 1 to 4) contains $8n + 1$ SDs, where $n$ is the circle number.

This domain determination method can be extended for lamps of any size, radiation pattern, and viewing angle because it is based on the lamp’s inherent structure, that is, the square shape and the two sizes of tiny microcavities. Furthermore, the method provides the capability of
conducting the irradiance uniformity evaluation tests for different solution container sizes by using the domains larger than the lamp (e.g., Circle 4 in Figure 4-2A).

**Figure 4-2.** The developed lamp structure-based method to determine (A) domains, Circles 1–4, and (B) their constituent SDs (an SD consists of 4 microcavities; the 4 larger circles are shown on the right-hand side) for irradiance measurements on the circular surfaces in front of the microplasma UV lamp.

### 4.2.2 Evaluation tests for the protocol development

In this protocol, three systematic evaluation tests were proposed to increase the accuracy of in-solution fluence rate calculation by providing uniform radiation distribution inside the solutions. In the QUE test, the distribution of the incident irradiance across the circular surface...
was evaluated to find a distance where the irradiance was distributed uniformly enough. Petri factor (PF) and coefficient of variation (CV) are represented as statistical parameters in the literature for quantitatively analyzing irradiance uniformity [54, 56]. The PF is described as the ratio of the average incident irradiance over the surface to the irradiance at the center. The CV is defined as the ratio of the standard deviation of the incident irradiance values to their mean value. A minimum PF of 0.9 and a maximum CV of 6.7% are reported to be appropriate for uniformity assurance. For the microplasma UV system, the PF and CV variables were calculated using the measured irradiance data on various SDs to evaluate irradiance uniformity (see Section 4.2.1.1).

Delivered beams to a planar surface may not all be parallel and perpendicularly incident. This could cause radiation gradients and reflections to be created nonuniformly inside the solution leading to the setup incompetency for reliable kinetic study. To quantitatively investigate the extent of collimation, a collimation factor (CF) is applied, which is equal to the ratio of the delivered irradiance to the fluence rate over the surface, where a CF = 1 means complete collimation. In the QCE test, CF would be calculated along the distance from the microplasma UV lamp (0–10 cm) to achieve satisfactory collimation where CF ≥ 0.99.

QDE is conducted due to the divergence of the beam as it passes through the depth of the solution container leading to the existence of a radiation gradient. In this regard, divergence factor (DF) was evaluated for solutions irradiated by the microplasma UV lamp, which is defined as the ratio of the average radiation inside the empty container to its value on the surface. DF was calculated based on an equation describing its definition (Equation 4.1) by measuring the delivered radiation at different depths of the container (with a 0.2 cm step size) and integrating it throughout the total depth (1 cm):
\[ DF = \frac{\int_{D}^{D+l} E(x)dx}{l \times E(D)} \] (4.1)

where \( D \) (cm) is the distance between the lamp and container surface and \( l \) (cm) is the container depth with \( dx \) as its step size. When the point source assumption can be made for the radiation source, the following simplified equation can be used:

\[ DF = \frac{D}{D + l} \] (4.2)

Hence, the QDF test can be used to find the minimum distance from the microplasma UV lamp, where the point source assumption becomes valid.

The abovementioned factors were measured for different solution-containing vessel sizes to propose a general protocol for designing quasi-collimated apparatuses.

4.3 Results and discussion

4.3.1 Characterization of microplasma UV

The measured SPD of the studied microplasma UV lamp is presented in Figure 4-1B. The FWHM of the studied 221 nm microplasma UV lamp emission spectrum was around 3 nm, which confirmed the monochromatic source assumption for this source. GTIR of the spectrum was about 0.92, based on the relative irradiation values, which was much higher than the one for the medium pressure mercury lamps commonly used in microbial inactivation applications. This showed that the source emitted much less photoreactivation-initiating radiation. Photoreactivation phenomenon is a molecular mechanism in which bacterial enzymes compensate for DNA damages induced by germicidal UVC by absorbing UVA (315–380 nm) and visible light [13].

4.3.1.1 Radiation pattern

Precise qualitative observation of the radiation distribution on planes in various distances from the microplasma UV lamp is illustrated in optical images in Figure 4-3. It can be understood
that, although the microplasma UV lamp had a square face, the radiation mostly had a circular radiation profile with higher intensities at the center and lower ones at the edges.

![Figure 4-3. Optical images of the radiation distribution on cross-sectional planes perpendicular to the source principal radiant emission direction, at 1 to 6 cm distances (A to F, respectively) from the microplasma UV lamp.](image)

Figure 4-3A shows the pattern of the IGI values over the distance from the microplasma UV lamp on nine SDs inside the Circle 1 domain. Equal values were observed for the measured irradiances on eight SDs with the same decreasing pattern along the distance. There were only tiny divergences at very close distances from the lamp, which would be due to the existent noises and errors for detector measurements at the near field readings [61]. However, on the centric SD, the irradiance values significantly increased (135%) from 0 to 0.4 cm and then decreased, similar to those of the other SDs. This is because of the absence of microcavities at the center of the lamp.
The pattern of delivered radiation to adjacent SDs in a row is analyzed in Figure 4-4B for a set of four SDs (see Figure 4-4D for SD locations). The resultant patterns demonstrated lower incident irradiance on near edge locations. In addition, SD4, which was outside of the lamp-size surface, showed a different pattern (maximum value at a distance other than 0) in comparison with SD1 to SD3, similar to the centric SD but with considerably lower values. This showed that, although the areas outside the lamp surface still received irradiance, the nonuniformities would be significant on a container with a larger surface area than the lamp up to a distance of about 6 cm from the lamp.

Four types of SDs were based on the number of constituent microcavities (0, 2, 3, and 4). Interestingly, the radiometry results on the lamp surface showed that irradiance on 3-microcavity and 2-microcavity SDs were nearly 3/4 and 1/2 of that on a 4-microcavity SD, respectively (see Figure 4-4C, distance = 0). By increasing the distance from the lamp, however, the abovementioned ratios were not observed among the delivered radiations on four types of SDs due to the cumulative effect of adjacent irradiating microcavities on each other. From a distance of about 6 cm, all SDs were almost equally-irradiated, which could lead to a uniform radiation distribution on larger-sized containers in front of the microplasma UV lamps. Similar observations can be seen on the cross-sectional optical images in Figure 4-3.
Figure 4-4. Pattern of integrated germicidal irradiance values measured on (A) Circle 1 SDs, a set of SDs (B) in a row, and (C) with different constitutive microcavity numbers over the distance from the microplasma UV lamp. (D) Schematic guide for the locations of the studied SDs in parts A to C.
4.3.1.2 Effect of lamp temperature

The irradiance received on a random 4-microcavity SD was studied in 1-second intervals over 8 min of illumination to evaluate the effect of lamp running time and temperature on its output specifications. Figure 4-5 shows a sample of resultant data at a 1 cm distance from the lamp; the same patterns were observed at other distances.

The lamp started illuminating instantly when it was plugged into an electrical source; that is, no warm-up time was required due to the lamp’s mercury-free design. The irradiance dramatically increased in the first 15 s of irradiating with no significant change in the lamp temperature. Afterward, although the temperature increased from the ambient temperature to 40 °C, the received irradiance increased relatively negligibly, 3% after 15 s and below 1% after 60 s, with regard to all absolute and integrated irradiances. This behaviour revealed that providing a short 15 s stabilization time was enough to reach a reasonably constant maximum output. It was significantly shorter than the reported physical warm-up time for UV lamps and stabilization time for UV-LED [38, 62].

While pursuing the reason for this stabilization time, it was observed that the flowing electrical current through the lamp circuit increased in the first 15 s and remained almost constant after that. According to Ohm’s law, in a constant input voltage, this change in current showed that the system resistance decreased in the first 15 s. Thus, providing a cooling system for the lamp powering circuit would be helpful for increasing power delivering efficiencies and decreasing the required stabilization time. The intrinsic instant-on and fast stabilized full-output characteristics of the microplasma UV lamp are crucial factors in using this UV radiation source for designing industrial-scale photoreactors with intermittent flow. Figure 4-5B demonstrates the effect of lamp
temperature on the lamp SPD specifications as there was almost no change in their values during the studied 8 min of irradiation, in spite of the significant temperature increase.

Although it was found that lamp temperature did not affect its output specifications in the studied range, raising the temperature to very high values may be detrimental to the lamp, either suddenly burning microcavities or gradually reducing the lamp’s practical lifetime. Hence, using a fan or any simple thermal management device is recommended to dissipate the generated heat and control the lamp temperature.

![Figure 4-5](image)

**Figure 4-5.** Effect of running time (and its associated increasing temperature) of the microplasma UV lamp on its (A) irradiance and (B) SPD specifications. (Irradiance measurements were conducted on a 4-microcavity SD at 1 cm distance from the lamp and constant current.)

### 4.3.1.3 Effect of electrical current

A range of operating currents from 0.1 A to the maximum allowable value was tested using a digital DC power supply. As with any other electrical device, there was a maximum allowable operating voltage for the microplasma UV lamp, which was set by the manufacturer (12V for the lamp studied here by flowing 0.65A current). A higher electrical current may alter the temperature...
increasing rate of the microplasma UV lamp. Therefore, in order to independently investigate the
effect of current, a constant temperature rising rate was maintained using a cooling fan. The
irradiance was measured after it attained a stabilized value based on what was described in Section
4.3.1.2.

As depicted in Figure 4-6B, SPD-related specifications of the emitted radiation showed no
significant variation from increasing the electrical current in the safe allowable range. Furthermore, in the studied current range, the over-distance incident irradiance pattern on different
locations (SDs) was not affected, and similar patterns, as illustrated in Figure 4-4, were observed.
However, all absolute and integrated irradiance values on an SD were considerably affected by
changing the electrical current (Figure 4-6A). This functionality can be extended to all SDs since
the relative radiation pattern was not altered by modifying the electrical current. Linear relations
were observed between the electrical current and all the studied peak-absolute and integrated
irradiances. Ultimately, these linear trendlines could be utilized as a calibration curve to provide
appropriate irradiances in a microplasma UV apparatus by adjusting the input current or,
conversely, measuring the unknown irradiance inside a photoreactor by simply controlling the
flowing current.

Note that delivering overvoltage values would have destructive effects on the lamp by
either causing significant damage to the lamp (possibly burning it out) or decreasing lamp lifespan
by degrading the window material. Hence, lower currents are recommended to extend the efficient
lifespan to 10,000 h. On the other hand, running a lamp on a very low voltage (therefore with less
light output) may extend its operating life, but is not an enhancement to efficiency.
Figure 4-6. Effect of electrical current on the (A) irradiance and (B) SPD specifications of the microplasma UV lamp. (Irradiance measurements were conducted on a 4-microcavity SD at 1 cm distance from the lamp and a constant lamp temperature raising rate.)

4.3.1.4 Effect of intermittent on/off cycles

Conventional UV lamps suffer from the drawback of not generating the same output after several cycles of switching on and off intermittently. To evaluate the potential of microplasma UV lamps in this regard, the effect of six on/off cycles was investigated for all incident absolute and integrated irradiance values. Each cycle was comprised of 30-second irradiation and 10-second off times. Figure 4-7 demonstrates the measured irradiance values versus time, revealing that the on/off cycle did not shift the lamp performance. In other words, incident irradiation and intensity did not change when turning on the lamp with a 15-second stabilization time. This characteristic of the microplasma UV lamp allows the treatment of intermittent flow because the lamp could only be in operation only when needed, which would lead to power usage efficiency, cost reduction, and prolonged lamp lifespan.
Figure 4-7. Effect of on/off cycles of the microplasma UV lamp on integrated and absolute irradiances. (Irradiance measurements were conducted on a 4-microcavity SD at 1 cm distance from the lamp.)

4.3.2 Development of a protocol for calculating fluence rate

4.3.2.1 Quantitative Uniformity Evaluation (QUE)

The calculated CV and PF values at different distances from the microplasma UV lamp are presented in Figure 4-8; the dashed horizontal lines compare the acceptable CV and PF values for uniformity assurance. The determined PF values using the proposed method showed a PF equal or greater than 1 for some domains and distances from the microplasma UV lamp. This could have been caused by a nonuniform radiation distribution at some circumstances since, based on the PF definition, PF > 1 is due to the higher irradiance at locations other than the center. PF is typically more applicable for the UV sources, which deliver the maximum irradiance at the center of the irradiated surface. Thus, evaluating the uniformity for the microplasma UV irradiation based on the PF values could be subject to errors and uncertainties. The new CV is a general statistical
variable known as the standard measure of any kind of distribution. It is applicable unbiasedly for any cases, including ones where there is no centric maximum irradiance. Therefore, the CV can substitute the PF for microplasma UV lamps to evaluate the radiation uniformity on an irradiated surface. Final demonstrated results of the QUE test were extracted from the curves in Figure 4-8 and listed in Table 4-1 as the conditions in which the irradiance uniformity was obtained for the studied microplasma UV lamp on different circular surface sizes. It is evident that at a distance of about 7 cm (or higher), the uniformity was met for all the domains.

Since the domain determination method was based on the lamp’s inherent structure, which is consistent among microplasma UV lamps of any size, these patterns can be scaled up or down for any lamp container size pairs by using a dimensionless parameter, $M = D/X$, in which $D$ (cm) is the diameter of the solution container, and $X$ (cm) is the size of the lamp side. By analyzing the required distance to obtain uniformity versus parameter $M$, it was revealed that, in the $0 < M < 0.4$ range, the uniformity was insured at a very short distance from the lamp ($< 0.2$). Afterward, the required distance for uniformity increased by an S-shape function of $M$, as demonstrated in Figure A-3.
Figure 4-8. Calculated PF and CV values for incident absolute peak irradiance (left column) and integrated germicidal irradiance (right column) at various distances from the microplasma UV lamp.
over different container surface sizes: (A–B) Circle 1, (C–D) Circle 2, (E–F) Circle 3, and (G–H) Circle 4 (See Figure 4-2 for circular domains). The values were calculated in triplicate, and the tolerance of results was less than 5% which is within the acceptable uncertainty.

Table 4-1. Circumstances of achieved irradiance uniformity on different container sizes based on the QUE test absolute peak irradiance and integrated germicidal irradiance values.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Diameter (mm)</th>
<th>Distance for uniformity (cm)</th>
<th>Incident irradiance at uniformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circle 1</td>
<td>17.9</td>
<td>&lt;0.2 &lt;0.2</td>
<td>API (μW.cm⁻².nm⁻¹) IGI (μW.cm⁻²)</td>
</tr>
<tr>
<td>Circle 2</td>
<td>30.5</td>
<td>2.2</td>
<td>126.91 558.07</td>
</tr>
<tr>
<td>Circle 3</td>
<td>43.1</td>
<td>5.4</td>
<td>37.84 143.61</td>
</tr>
<tr>
<td>Circle 4</td>
<td>54.7</td>
<td>7 7</td>
<td>24.04 106.97</td>
</tr>
</tbody>
</table>

4.3.2.2 Quantitative Collimation Evaluation (QCE)

To investigate the radiation collimation on containers of different sizes, the evaluation test was conducted by calculating CF for two available solution containers with diameters of 1.8 cm and 5.5 cm as representative of containers smaller and larger than the lamp, respectively. These systems are designated hereafter as D1.8 and D5.5, respectively.

To calculate the CF, the average irradiance and fluence rate on the solution surface should be determined. Thus, the delivered average irradiance to the solution surface was calculated using the radiometry results on the relevant domains, which were the Circle 1 and Circle 4 domains for the D1.8 and D5.5 systems, respectively. For instance, for the D5.5 system, the average irradiance was primarily calculated for the representative domain, Circle 4, by weighted averaging of the measured values on all its constituent SDs (33 SDs inside Circle 4 in Figure 4-2). After this, the average irradiance on the solution container area was computed by multiplying the value for Circle
4 into the $S_{D5.5}/S_{\text{circle4}}$ ratio, which was near 1. To calculate the average fluence rate on the solution surface, the actinometry experiments were performed for both systems.

The resultant CF values for the D5.5 system, as presented in Figure 4-9, showed poor collimation at very close distances, demonstrating that estimating the average fluence inside the solution by measuring the average irradiance on the solution surface can cause significant errors. In these cases, noncollimated beams on the solution surface create radiation gradients inside the solution, resulting in a complex mixture of radiation photon pathlengths and reflections from the container wall. The minimum distance to obtain a CF of more than 0.99 (a generally accepted value) for the D5.5 system was determined to be 6.2 cm where on-surface irradiance and fluence rate became identical, and the fluence inside the solution could be estimated by measuring the irradiance.

For the D1.8 system, the CF values demonstrated a different pattern over the distance. At near-field measurements, a high CF was observed claiming identical irradiance and fluence values, which may have been due to point-to-point irradiation at this very short distance between each radiating microcavity and a point on the parallel solution surface. This phenomenon took place for the D1.8 case as the size of the container was smaller than the lamp surface. Therefore, the entire solution surface was covered by these parallel point-to-point short beams, and the negative effect of lamp edges on collimation was avoided in this very short distance from the lamp. The flatness of both surfaces facilitated the incident beams to be straight enough and parallel enough between surfaces when the distance was very short. As seen in Figure 4-9, the CF for the D1.8 system started diminishing by increasing the distance and, consequently, creating nonparallel beams on the solution surface by the microcavities near the edges. After a certain distance, this decreasing trend stopped and changed to an increasing trend similar to the one for the D5.5 system.
Eventually, the standard collimation (CF > 0.99) was observed at 1.6 cm for the D1.8 system. A general schematic of the collimation evaluation test for the solution containers that were smaller and larger than the lamp is shown in Figure A-4, where the results can be extrapolated for any lamp container size pairs.

Note that, although the point-to-point irradiation may have caused parallel beams at very short distances for smaller size containers, it would be highly recommended to design the experimental setups based on the second collimation distance. This is due to the compassionate irradiance values to the distances in such a close range, which may cause severe errors in fluence measurements.

**Figure 4-9.** Calculated CF for D5.5 and D1.8 solution containers at various distances from the microplasma UV lamp.
4.3.2.3 Quantitative Divergence Evaluation (QDE)

Illustrated patterns in Figure 4-10 (A and B) compare the calculated DF values from the definition (Equation 4.1) and point source assumption (Equation 4.2) for different circular domains. The comparison demonstrates the minimum distance from the microplasma UV lamp, after which the point source assumption becomes valid. According to the resultant values, after 1 cm, the DF pattern became independent of the domain size because the divergence was created in the depth of the container and was not a function of its surface size. The pattern for both API and IGI values were nearly identical, indicating that the divergence was independent of the SPD of the source. To further study the point source assumption for lamps with possible smaller sizes, the QDE test was repeated for a reduced-size version of the lamp by masking its surface, as illustrated in Figure 4-10C. The inset graphs of Figure 4-10 (A and B) show the DF pattern for the smaller lamp on the Circle 1 domain compared with the point source assumption. It was revealed that the minimum distance to achieve a point source was much shorter for the smaller lamp (3 cm) than the one for the original size lamp (6.5 cm). A similar procedure can be applied to any lamp size to calculate the DF pattern, independent of the container size.
Figure 4-10. Calculated real DF for (A) API and (B) IGI values on different container sizes with 1 cm depth at various distances from the 16 cm² microplasma UV lamp compared with the resultant DF for the point source assumption. Inset graphs: DF values for (C) the reduced-size lamp (4 cm²).

4.3.2.4 Fluence rate calculation

After studying the irradiation uniformity and collimation over the solution surface, two more correcting phenomena must be considered to precisely calculate the average fluence rate inside the solution: (1) when a beam passes between two media, due to the change in refractive indices, a small part of the incident radiation is reflected back into the first medium obeying the Fresnel Law; (2) since aqueous solutions absorb UV with any wavelength of interest, a decrease in the irradiance arises as the beam passes through the solution according to the Beer–Lambert law. These two phenomena do not limit the setup designs; however, they must be considered to determine the average fluence inside the solution by correcting the irradiance on the solution surface by parameters termed reflection factor ($RF$) and the water factor ($WF$), respectively.
$RF$ can be calculated at each wavelength by using the reflection coefficient ($R$) of the aqueous solution as $RF_\lambda = 1 - R_\lambda$. $R$ can be computed for collimated incident radiation on the surface using Equation 4.3:

$$R_\lambda = \left( \frac{n_{1,\lambda} - n_{2,\lambda}}{n_{1,\lambda} + n_{2,\lambda}} \right)^2$$ (4.3)

where $n_{1,\lambda}$ and $n_{2,\lambda}$ are the refractive indexes for mediums 1 and 2 at the wavelength $\lambda$, respectively.

As the microplasma UV lamp is a well monochromatic source, refractive indexes in the peak wavelength, 221 nm in this case, can be readily used to calculate $RF$ without requiring any SPD-based weighting. According to the literature [63], the refractive index of water at 221 nm is 0.973.

Since the $RF$ is a function of the angle of incident beams, Equation 4.3 must be utilized after the collimation is ensured by the QCE test. Moreover, although the refractive index was reported to be a function of temperature, this effect was found to be negligible in our case as the refractive index was not sensitive in the temperature change range for solutions under microplasma UV lamp irradiation.

$WF$ is defined as the ratio of average fluence inside the solution to the average fluence at the surface and computed through Equation 4.4 for each wavelength, which is derived by integrating the Beer-Lambert law over the depth of solution:

$$WF_\lambda = \frac{1 - 10^{-\alpha_\lambda l}}{\alpha_\lambda \cdot l \cdot \ln(10)}$$ (4.4)

where $\alpha_\lambda$ (cm$^{-1}$) is the decadic absorption coefficient of the solution at each wavelength $\lambda$, and $l$ (cm) is the depth of the solution. For polychromatic sources, such as medium pressure UV lamps and some UV-LEDs, this correction must be SPD-weighted over the spectrum. However, as the
microplasma UV lamp has been shown to be a monochromatic source, the \( WF \) can be calculated at the peak wavelength (i.e., 221 nm in this work).

As an assumption, the fraction of photons that was reflected back into solution after passing through the solution depth was neglected in Equation 4.4. This is a fair assumption as beams that reach the bottom of the container are totally absorbed by the UV-opaque material of the container. \( WF \) should be calculated for the system after the collimation is ensured by the QCE test since its definition assumes that the incident radiation on the surface is collimated. Having a wholly mixed solution is a crucial factor in attaining a uniform \( WF \).

The decadic absorption coefficient in the \( WF \) formula is a function of concentration. Therefore, as the solution becomes either diluted or denser of the UV-absorbing species over time, the decadic absorption coefficient and, consequently, \( WF \) varies. This change in \( WF \) value during the UV-based degradation experiment was not considered in previous protocols for calculating UV fluence inside the solution. However, these changes are not negligible when studying chemicals with considerable UV absorptions. Therefore, assuming a constant \( WF \) over time could lead to the error-prone calculation of UV fluence, affecting the reported photokinetic rate constants. This error is more substantial in the degradation of organic contaminants and micropollutants through UV/oxidants, where the suspension of some chemicals could have considerable initial absorption. Furthermore, the UV-driven photolysis of the oxidant and secondary photo-initiated reactions among the generated active species and the chemical contaminant involved a total of 100 elementary reactions. Thus, the solution became a complex mixture of numerous species with various absorption coefficients toward peak wavelength, which could make the trend of \( WF \) over time extremely complicated and difficult to predict.
To address the time dependency effect of media absorbance during the experiments in calculating correct fluence inside the reactor, WF must be calculated in a time-weighted manner prior to computing the average fluence until the variations in WF become negligible ($\Delta WF < 5\%$). Toward this goal, the absorbance of the solution at peak wavelength was primarily measured at various time intervals with a spectrometer, $A_\lambda (t)$; then, the relative decadic absorption coefficient, $\alpha_\lambda (t)$, was calculated by using the definition of transmittance, $T_\lambda (t)$, and used for calculating the time-weighted $WF_\lambda (t)$, as described in Equations 4.5 to 4.7:

\[ T_\lambda (t) = 10^{-A_\lambda (t)} \]  
\[ \alpha_\lambda (t) = -\log \left( \frac{T_\lambda (t)}{l} \right) \]  
\[ WF_\lambda (t) = \frac{1 - 10^{-\alpha_\lambda (t) \cdot l}}{\alpha_\lambda (t) \cdot l \cdot \ln(10)} \]  

The actual WF is calculated by averaging the $WF (t)$ values over the time period ($\tau$):

\[ WF_\lambda = \frac{1}{\tau} \sum_{t=0}^{\tau} WF_\lambda (t) \]  

In collimated- and uniform-irradiance ensured circumstances, the overall average fluence inside the solution could be calculated using the following equation:

\[ H = E \times \left( \frac{DF \times RF}{CF} \right) \times (WF \times \tau) \]  

where $H$ (mJ·cm$^{-2}$) is the average fluence inside the solution, $E$ (mW·cm$^{-2}$) is the average incident irradiance on the solution surface, and $\tau$ (s) is the exposure time.

### 4.3.3 Case studies

To check the reproducibility of the photokinetic data in the quasi-collimated apparatus designed by the protocol, the UV inactivation/degradation experiments were conducted in two
distinct systems. The distance between the microplasma UV lamp and the solution was set to 2 cm and 6.5 cm for systems with D1.8 and D5.5 containers, respectively, to obtain all evaluation test factors, that is, $CV$, $CF$, and $DF$, in a satisfactory range. These values were then used together with complimentary UV fluence correction factors ($RF$ and $WF$) to determine the applied average fluence using Equation 4.9. To calculate the $WF$ of the solution in each case through Equation 4.4, the absorbance data of that actual solution sample toward 221 nm UV exposure was used. In addition, it was assumed that the $WF$ changes during the reaction time were not negligible, and $WF$ was calculated independently for each sample in time intervals.

### 4.3.3.1 Kinetic study for inactivating microbial cells

The average log reduction of viable cells in the D1.8 system is presented in Figure 4.11-A as a function of UV fluence. The overall mechanism of *E. coli* inactivation presented a lag phase for around 2.5 mJ cm$^{-2}$ fluence (shoulder region), followed by a linear loss of bacterial viability over UV exposure, which was consistent with the literature [47]. A tailing region was also observed at high log inactivations, which was reported in the literature as being the result of a significant shielding effect in aqueous media of high cell density ($10^6$ CFU mL$^{-1}$) [45]. Another reason for this tailing region was the decrease in cell concentration below the detection limit ($10^1$ CFU mL$^{-1}$).

Since the fluence-response curve of *E. coli* cells included a shoulder region, Equation 4.10 was employed as a multi-parameter mathematical model to report the incremental log inactivation as a function of the delivered fluence:

$$\frac{N}{N_0} = 1 - (1 - 10^{-k\cdot H})^m$$  \hspace{1cm} (4.10)
where $N_0$ is the initial *E. coli* cell concentration (CFU mL\(^{-1}\)) before exposure, $N$ is the final cell concentration (CFU mL\(^{-1}\)) in the UV-treated sample, and $H$ is the average fluence inside the suspension (mJ cm\(^{-2}\)). Also, variables $k$ and $m$ of the model represent the first-order inactivation rate constant in the linear region (cm mJ\(^{-1}\)) and the number of critical targets, respectively. The $k$ and $m$ variables would be determined by the nonlinear least-square curve fitting of the model with the experimental data. The best-fitted model curve is depicted in Figure 4.11-A (the red line), which resulted in $k$ and $m$ values of 0.494 cm\(^{-2}\) mJ\(^{-1}\) and 7.572, respectively, with a high correlation coefficient of 0.995 in the combined shoulder and linear regions. The shielding effect and below detection limit concentration prevented this model from predicting the tailing region. However, the standard for reporting UV dose requirements for any disinfection method is typically up to 4-log inactivation (99.99% removal), which was successfully predicted by the model.

For the alternative D5.5 system, the same pattern of the inactivation-fluence curve was observed (figure not shown because of overlapping with the one for the D1.8 system). The required fluence and time for incremental log inactivations were calculated based on the predicting model for both systems and are tabulated in Table 4.2. A less than 5% difference between fluence-based values showed the reproducibility of the log inactivation data regardless of the container size when collimated apparatuses were used. Furthermore, in a time-based version, it was clear that more UV exposure time would be required for the D5.5 system to achieve a particular log inactivation as the fluence rate was lower at that distance. Based on the results, an average value of 9.9 mJ cm\(^{-2}\) UV dose is required to achieve 4-log inactivation of *E. coli*, which is in close agreement with the one exhibited by 222 nm excilamps reported in the literature [47].
4.3.3.2 Kinetic study for eliminating chemical contaminants

The degradation of 5 mg L\(^{-1}\) MB in a UV/H\(_2\)O\(_2\) solution with different oxidant doses was investigated in the same apparatuses described in the disinfection section. The WF reached an almost constant value after 45 s of irradiation. The time-averaged WF at 45 s showed a 12% error compared with the WF at the initial condition, which confirmed the potential error of previous constant WF assumption, even in such a dilute sample of the organic contaminant.

When the MB solution was exposed to pure 222 nm, only a slight degradation (about 5%) was observed. However, notably high removals were obtained in the presence of H\(_2\)O\(_2\) oxidant since the organic contaminants were degraded much more effectively and extensively by UV-initiated oxidation than by direct UV photolysis. This was caused by the highly reactive radicals and oxidizing species generated under UV/H\(_2\)O\(_2\) conditions. The MB removal patterns in the D1.8 system by the UV/H\(_2\)O\(_2\) process with different initial H\(_2\)O\(_2\) concentrations are shown in Figure 4.11-B. The MB degradation at 1.2 mM and 0.6 mM doses of H\(_2\)O\(_2\) were comparable, 97.6% and 96.1% at about 195 mJ cm\(^{-2}\) UV fluence, respectively; but the removal rates at 0.3 mM and 0.1 mM were significantly lower at the same UV fluence, 86.6% and 65.8%, respectively. This showed that among the different H\(_2\)O\(_2\) concentrations tested, 0.6 mM was the suitable one as it achieved a comparable MB degradation to that of 1.2 mM, where lower H\(_2\)O\(_2\) concentration was balanced by the more 221 nm-UV transmittance of the 0.6 mM-H\(_2\)O\(_2\) solution.

To evaluate the kinetics of degradation, the first-order model was utilized for the analysis of experimental data. The linear fittings with reasonable correlation coefficients (\(R^2 > 0.98\))—dashed lines in Figure 4.11-B—indicate that all reactions followed first-order kinetics. The first-order rate constant values of the MB removal were calculated in the fluence-based manner to compare them between the two studied systems. As is shown in Table 4.2, identical fluence-based
rate constants (with error < 5%) were resulted for the D1.8 and D5.5 setups, which confirmed that the proposed protocol was able to calculate the correct average fluence inside the solution. The time-based rate constants also demonstrated the lower energy delivered to the D5.5 container, which was positioned farther from the lamp. The fluence-based k values can be reliably used as the general ones for MB degradation in any 221 nm microplasma UV reactor independent of its size as long as the QUE, QCU, and QDE evaluation tests ensure the uniformity of radiation distribution inside the solution. The same procedure can be utilized for any organic contaminant with any initial concentration and dose of oxidant. The most crucial factor in using the protocol for other solutions is to precisely determine the time-weighted WF based on the actual absorbance data of the solution.

Figure 4-11. Case studies of the 221 nm microplasma UV lamp in the D1.8 system: (A) direct UV-inactivation of *E. coli* and (B) UV/H$_2$O$_2$-degradation of MB with different H$_2$O$_2$ concentrations. (Initial concentrations: ~10$^6$ CFU mL$^{-1}$ *E. coli* and 5 mg L$^{-1}$ MB.)
Table 4-2. A comparison of the D1.8 and D5.5 case study systems based on required fluence and time for E. coli incremental log reductions and first-order rate constants of MB degradation in different H$_2$O$_2$ doses. Triplicate runs were performed for experiments reported in the table and the mean values are shown. The standard deviations were typically less than 10% for E.coli inactivation studies and typically less than 5% for MB degradation studies.

<table>
<thead>
<tr>
<th>System</th>
<th>E. coli inactivation</th>
<th>MB degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log reduction</td>
<td>H$_2$O$_2$ dose (mM)</td>
</tr>
<tr>
<td></td>
<td>Required UV Fluence (mJ cm$^{-2}$)</td>
<td>Required Time (s)</td>
</tr>
<tr>
<td>D1.8</td>
<td>3.76 5.82 7.88 9.95</td>
<td>7.99 12.38 16.77 21.15</td>
</tr>
<tr>
<td>D5.5</td>
<td>3.22 5.46 7.65 9.80</td>
<td>33.38 56.61 79.31 101.60</td>
</tr>
</tbody>
</table>

4.4 Conclusion

In this work, a protocol was proposed for operating the recently emerged microplasma UV lamp for studying the rate of microbial inactivation and chemical contaminant degradation in water purification applications. The proposed protocol can be applied to the kinetic study of the elimination of microbial and chemical contaminants using microplasma UV lamps of any size, power, and peak wavelength. For a more detailed conclusion on results, see Chapter 6.
Chapter 5: Microplasma UV Inactivation of Microorganisms

5.1 Introduction

It was mentioned in Chapter 1 that superbugs with highly UV-resistant and/or repairable nucleic acids; assigned as URMs and ERMs, respectively; have challenged the inactivation efficiency of current UV sources. In this regard, targeting intercellular proteins responsible for nucleic acid excitation, repair, and infection can be the solution. Here, I identified the mechanism-analyzed, and kinetic-modelled efficiency of the newly emerged far-UVC source, microplasma UV, which radiates around proteins’ UV-absorption and decomposition peak with unique spectral power distributions. In Chapter 4, a comprehensive protocol was developed for operating, output controlling, and UV-dose measuring of microplasma UV lamps to achieve reproducible photokinetic data [64]. In this Chapter, two novel microplasma far-UVC sources, monochromatic KrCl (K-Microplasma) and polychromatic Xe2-phosphor (P-Microplasma), are utilized in protocol-based apparatuses. The kinetics and mechanism of microbial inactivation and reactivation were investigated and compared with other commonly used UV sources.

5.2 Methodology

5.2.1 Microplasma UV irradiation setup

Two monochromatic and polychromatic microplasma UVC lamps, both 2 × 2 in in size, were obtained from Eden Park Illumination Inc. (Champaign, IL, USA). The emitted radiation from the lamps and corresponding SPD were measured using an Ocean Optics USB2000+ spectrometer equipped with a Sony ILX511B CCD detector. The measured normalized SPD and SPD-related specifications for both lamps are illustrated in Figure 5-1a.
A UV exposure apparatus was designed based on the previously developed characterization and kinetic study protocols [64] (see Chapter 4 for the protocol). In this setup, a microplasma UV lamp, either K-Microplasma or P-Microplasma, was located at a distance of 6.5 cm from a quartz solution container 5.5 cm in diameter, perpendicular to its surface normal vector. At this distance, according to the protocol, all the radiation uniformity, collimation, and divergence values are in their satisfactory standard ranges, and consequently, uniform distribution of fluence is delivered to microbial cells within the container. In addition, using a black sheet with an aperture on top of the container, the beams are controlled to enter the solution only from the container surface rather than the walls. To be sheltered from any extra ambient light-induced photoinactivation or photoreactivation, the entire experimental apparatus was covered with a black box. The inner wall of the box was also UV-absorbing to minimize the reflection. A schematic representation of the experimental setup is shown in Figure A-2.

As controlling the input voltage and electrical current is essential for constantly driving the microplasma UV lamps, each lamp, and its power supplying electrical circuit was connected to a DC power supply (Model: Aim TTI EX355R) separately. Although it was previously demonstrated that a lamp’s rising temperature does not change its output [64], an air cooling system was installed for heat dissipation and thermal management. The lamp surface temperature was consistently controlled to be at 27 °C.

5.2.2 UV inactivation experiments

For microbial inactivation experiments, the container was filled entirely with the microbial solution to prevent systematic error caused by wall reflectance of incoming radiation. During the UV exposure and 30 s before it, a magnetic stirrer was used for mixing the microbial samples to ensure a well-mixed consistency for uniform UV fluence distribution inside the solution. The
solutions were kept at room temperature (25 °C) without any significant change before and after UV treatment. All experiments were repeated with three biologically independent samples to obtain enough statistical data. The data in the figures are presented as averages with error bars representing the standard deviation. A two-tailed paired t-test statistical analysis was performed to determine the significance of the observed data at 95% confidence (p < 0.05).

5.2.3 Microbial reactivation experiments

The time required for 3-log and 4.5-log inactivation was back-calculated by using the resulted kinetic data in the inactivation phase of the work. When the assigned time of irradiation for each case was reached, the lamps were turned off, stirring was stopped, and the suspension container was immediately transferred to a separate dark repair or photoreactivation apparatus for studying the corresponding repair. For dark repair, the microbial sample was placed inside a light-isolated black box while continuously stirring for 4 h. In the photoreactivation apparatus, two parallel fluorescent lamps (Philips F15T8, 18W, cool white, 4100 K) were aligned at 10 cm above the microbial sample, which was stirring for 4 h. The Ocean Optics USB2000 spectrometer was used to measure the broad 300–700 nm emission spectrum of the fluorescent lamps. Fluorescent lamps were turned on 15 min before the actual photoreactivation experiment to warm up and to ensure the stabilized output for photoreactivation. Throughout this 15 min, the average irradiance was measured using a UVA-visible radiometer, the Newport Optical 1917-R, with a 918D-ST-UV detector. The irradiance at the beginning of the photoreactivation process was measured to be 4.2 mW/cm² at the suspension surface. After the reactivation experiments, the reactivation was evaluated through the colony-forming ability assay. In both dark and photo repair, to track the microorganism concentration over time, 30-min samples were taken at intervals, and each reactivation experiment was conducted in triplicate to obtain reproducibly insured statistical data.
5.2.4 Reactive oxygen species analysis

To elucidate the role of ROSs in far-UVC inactivation, radical scavengers were employed to create radical-free conditions. Hydroxyl radical (\(\bullet\)OH), superoxide radical (\(\bullet\)O\(^2\)\(\cdot\)), and hydrogen peroxide (H\(_2\)O\(_2\)) were scavenged by mannitol (C\(_6\)H\(_{14}\)O\(_6\)) 4-Hydroxy-TEMPO (TEMPO, or 4-hydroxy-2,2,6,6- tetramethyl piperidine-1-oxyl), and catalase, respectively. These scavengers were selected based on their reaction rate constants with radicals to ensure that the majority of primary radicals reacted with scavengers rather than with cells. All scavengers were obtained from Sigma Aldrich Co. The biological impact of scavengers was examined by adding them to microbial suspensions without UV irradiation. No change in concentrations was observed during these control experiments, thus indicating that scavengers are not intrinsically destructive for cells. A given concentration of each scavenger (1 mM TEMPOL, 0.5 M mannitol, and 1 g L\(^{-1}\) catalase) was added to the relevant reaction solution in advance. To make the scavenger dissolve in water and permeate into cells prior to the UV treatment, the scavenger containing the microbial sample was initially stirred in the dark for 30 min. Inactivation and reactivation experiments were conducted and compared in the presence and absence of scavengers to perceive the role of each ROS.

5.2.5 Microorganisms propagation and enumeration

As it is discussed in section 5.3.1, the disinfection potential of microplasma UV lamps was measured via examining two types of microorganisms, bacterium *E. coli* (ATCC 11229) and bacteriophage MS2 virus (ATCC 15597 with *E. coli* ATCC 15597 as the host). The dry powder form of each microbe was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultivated according to the supplier’s instructions. A spread agar plate and a double-layer agar plate technique, adapted from a standard protocol proposed by the United States
Environmental Protection Agency [22], were used to assess infectivity for \( E. \ coli \) and MS2, respectively, after UV inactivation or the post-treatment reactivation. The procedure is explained in Appendix B.

5.2.6 Calculation and modelling

The corresponding in-solution UV fluence for each case was calculated using the following equation, which was formulated throughout the developed protocol in Chapter 4 [64]:

\[
H = E \times \left(\frac{DF \times RF}{CF}\right) \times \left(WF \times \tau\right)
\]

(5.1)

where \( H \) (mJ·cm\(^{-2}\)) is the average in-solution fluence, \( E \) (mW·cm\(^{-2}\)) is the average incident irradiance on the solution surface, and \( \tau \) (s) is the exposure time. In brief, in the collimated and uniform irradiance assured distance from the lamp, first, the average irradiance was measured on the solution surface by the Ocean Optics USB2000 spectrometer. Then, the divergence factor \( (DF) \), reflection factor \( (RF) \), and collimation factor \( (CF) \) were calculated for the given container using their definitions. Finally, the water factor \( (WF) \) of the actual microbial sample was calculated to determine the UV transmittance and depth of the solution [56]. For monochromatic K-Microplasma, the aforementioned correction factors were calculated for the peak wavelength (221 nm), but for P-Microplasma, they were weight-averaged based on the broad SPD in the 220–280 nm range [58].

According to the second law of photochemistry, UV microbial inactivation is usually modelled based on the Chick–Watson first-order model [65]. Apart from the initial shoulder region for \( E. \ coli \), the relationship between microbial log inactivation and the delivered UV fluence is linearly modelled as:

\[
\text{Log inactivation} = \log\left(\frac{N_0}{N_t}\right) = K \cdot H
\]

(5.2)
where $N_0$ and $N_t$ represent the number of colonies (CFU mL$^{-1}$ for *E. coli* and PFU mL$^{-1}$ for MS2, respectively) before and after the UV exposure, $H$ (mJ·cm$^{-2}$) is the in-solution UV fluence, and $K$ (cm$^2$ mJ$^{-1}$) is the fluence-based inactivation rate constant.

A suitable calculation technique for the germicidal efficiency (GE) of a radiation source should be performed as a function of source radiating behaviour. On the other hand, every microorganism has a specific spectral sensitivity fingerprint based on its biological compositions. Therefore, the non-dimensional GE is defined by the over-wavelength integral of the convolution of source SPD ($E_\lambda$) and the normalized action spectra of the target microorganism for LPMVL ($G_\lambda$) divided by the integral of the source SPD [66]. Published action spectra weighted by LPMVL emission[67] were used in this work. In this work, this is called “theoretical GE” and calculated as follows:

$$\text{Theoretical GE} = \frac{\int E_\lambda * G_\lambda \, d\lambda}{\int E_\lambda \, d\lambda}$$

(5.3)

In parallel, GE for microplasma UV lamps was also calculated based on the experimental photokinetic data to check whether they corroborated theoretical GE values [67]. This so-called “action GE” is defined as:

$$\text{Action GE} = \frac{K_{F,\text{Microplasma}}}{K_{F,LPMVL}}$$

(5.4)

where $K_{F,\text{Microplasma}}$ (cm$^2$ mJ$^{-1}$) is the fluence-based first-order rate constant of log removal for each microplasma UV source and $K_{F,LPMVL}$ (cm$^2$ mJ$^{-1}$) is the corresponding value for LPMVL. Here, the $K_{F,LPMVL}$ values for studied microorganisms were collected and averaged from the data in the literature [68]. In a collimated and uniform irradiance assured experimental condition, theoretical and action, GE values should be equal.
The microbial reactivation, both dark and photo-initiated, was first quantitatively evaluated by defining the percentage of log repair as follows:

\[
\text{Log Repair} = \frac{\log_{10} N_{rt} - \log_{10} N_d}{\log_{10} N_0 - \log_{10} N_d} \times 100\% \tag{5.5}
\]

where \( N_0 \) and \( N_d \) are the cell numbers before and right after UV exposure, and \( N_{rt} \) is the cell number after reactivation for \( t \) period of time, all with unit colony forming units (CFU) mL\(^{-1}\) for \( E. \ coli \) and plaque-forming units (PFU) mL\(^{-1}\) for MS2. In addition, to model the reactivation process, the ratio of the colony-forming ability of cells is frequently defined as the survival ratio to express reactivation, which is calculated as follows:

\[
S = \frac{N_{rt}}{N_0} \times 100\% \tag{5.6}
\]

where \( S \) is the survival ratio at time \( t \), and \( N_0 \) and \( N_{rt} \) are the same as what was defined for Eq. (5).

Kashimada et al. assumed the photoreactivation phenomenon as a saturation-type pseudo-first-order reaction and suggested the first asymptotic kinetic model as follows [69]:

\[
\frac{dS_r}{dt} = k_1 \cdot (S_m - S_r) \tag{5.7}
\]

\[
S_r = (S_m - S_0)(1 - e^{-k_1 \cdot t}) + S_0 \tag{5.8}
\]

where \( S_0 \) and \( S_m \) are the survival ratios immediately after UV exposure and at ultimate saturation (maximum value in this model), respectively, and \( k_1 \) (time\(^{-1}\)) is the first-order reactivation rate constant. In this model, \( S_r \) is equal to total \( S \) as no decay phase is predicted. However, further investigations showed that this model could not predict the beginning of the photoreactivation curve when an induction period is observed. In this regard, Nebot Sanz et al. recommended a logistic regression model defined as [70]:

\[
\frac{dS_r}{dt} = k_2 \cdot (S_m - S) \cdot S_r \tag{5.9}
\]
\[ S_r = \frac{S_m}{1 + \left( \frac{S_m}{S_0} - 1 \right) e^{-k_2 S_m t}} \]  

(5.10)

where \( k_2 \) (time\(^{-1}\)) is the reactivation second-order rate constant. However, this model also supposed a maximum saturation for the photoreactivation process over a prolonged period (\( S_r = S \)) and may not predict the decay phase at the very end of the photoreactivation process.

A new general model was developed in our work and was applied to describe the photoreactivation. To achieve this goal, a new term was introduced to the Nebot Sanz et al. logistic model to account for the observed decay phase. As this phase is caused by the slight UVA in the fluorescent lamp (or sunlight) spectrum, the well-known first-order UV disinfection kinetics is applied for the total survival decay as follows:

\[ \frac{dS}{dt} = k_d \cdot S \]  

(5.11)

\[ S = S_{0,d} \cdot e^{-k_d t} \]  

(5.12)

where \( S_{0,d} \) is the survival ratio at the beginning of the decay phase (\( t_m \)), and \( k_d \) (time\(^{-1}\)) is the decay rate constant, which depends on the exposed radiation and resistance of the microorganism. During the photoreactivation, the survival decay begins at the time \( t_m \) where the term \( S_{0,d} \) coincides with the survival \( S_r \) of the Nebot Sanz et al. model. By combining corresponding models for the growth and decay phases, the following expression is proposed for the total survival:

\[ S = \frac{S_m e^{-k_d t}}{1 + \left( \frac{S_m}{S_0} - 1 \right) e^{-k_2 S_m t}} \]  

(5.13)

In this recommended model, \( S \) would be the total survival (\( S_r \neq S \) anymore), and \( S_m \) would no longer be the maximum survival. Instead, the new formula for predicting the maximum survival is presented in Equation 5.14.
\[ S_{max} = \frac{(k_2S_m - k_d) \left( k_d S_0 \right)^{k_d}}{k_2 S_m} \left( \frac{k_d S_0}{(S_0 - S_m)(k_d - k_2 S_m)} \right)^{k_2 S_m} \]  

(5.14)

5.3 Results

5.3.1 Microorganism selection

In the current work, indicator microorganisms for URM and ERM will be analyzed. For URM, bacteriophage-MS2, a single-stranded RNA (sRNA) virus, was used as a conservative UV-resistant representative. This was due to its lower susceptibility to far-UVC, according to its spectral sensitivity, compared with adenovirus, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Cryptosporidium parvum* as well-known regulated standards for pathogenic virus, gram-negative bacteria, gram-positive bacteria, and protozoa [44]. For ERM, a nonpathogenic strain of *Escherichia coli* (*E. coli*) is the most commonly used representative for investigating the protein-based dark and photo-initiated repair behaviour after UV irradiation [28, 31, 69-71]. *E. coli* can be inactivated with a fair UV-dose compared with other ERM, and it also contains nucleic acid protecting proteins for being studied under far-UVC irradiation.
Figure 5-1. (a) An extensive comparison of the spectral power distribution (SPD) of different germicidal UV sources along with relative action spectra of *E. coli* and MS2 (weighted by the LPMVL emission) in this region. Microplasma UV lamps were employed in this work, and others were common SPDs extracted from literature, but some alterations with respect to peak sharpness and full
width at half maximum (FWHM) may have varied slightly for extracted sources. Action spectrums were extracted from published values [67]. (b) A general absorption spectrum of microbial cell components (protein and nucleic acid) at the UV radiation range at the same concentrations [72]. Note that absorption of different proteins may slightly vary according to the constituent amino acids, and these figures are shown only for general insight into the difference between nucleic and protein UV-absorption behaviour. The same claim is also valid for the nucleic acid of different microorganisms as the general DNA absorption is depicted here.

5.3.2 Microbial inactivation

The non-dimensional GE of a UV source expresses the ratio of the effectiveness of that UV source compared with that of an LPMVL. Techniques for calculating the theoretical and action GE for microplasma UV lamps toward the studied microorganism are extensively discussed in section 5.2.6.

The dose responses for irradiation of E. coli and MS2 suspensions by microplasma UV sources are shown in Figure 5-2 (a and b). The fitted kinetic models with experimental data demonstrated that 4-log inactivation of E. coli was achieved at about 9.9 and 8.4 mJ.cm$^{-2}$ UV fluences for K-Microplasma and P-Microplasma, respectively, while, for the same UV source order, MS2 was 4-log reduced by 33.4 and 56.5 mJ.cm$^{-2}$ UV fluence.

Regardless of the microplasma source SPD, the fluence-response of E. coli showed an S-shaped pattern comprising a starting lag phase (shoulder region), followed by a linear loss of bacteria viability, and saturation (tailing region) at high fluences. The pattern is common among all UV sources in the literature.
In the linear region, P-Microplasma gained a higher first-order reaction rate as it targeted the genomic regions of the bacterium by irradiating UV with a broad spectrum containing the relative peak of DNA UV-absorption (Figure 5-1b), while the emitted photons of K-Microplasma were less destructive for DNA. Both microplasma lamps demonstrated a lower rate constant compared with LPMVL in this region. It is well documented that inactivation of *E. coli* by 254 nm UV occurs mainly by damaging DNA rather than proteins. However, ongoing from 254 to 205 nm, the absorption of intercellular proteins was found to increase significantly [73]. This resulted from the high absorbance of constituent aromatic (mainly tyrosine and tryptophan) and sulphur-containing (mainly cystine) amino acids, which usually show a peak absorption value at around 205 nm [74, 75]. For instance, the molar absorption coefficient of aromatic amino acids increased approximately one order of magnitude from 254 to 220 nm (Figure 5-1b). Absorption by proteins at wavelengths below 230 nm has been even shown to be identical to that by DNA at 260 nm [72]. Additionally, the decomposition quantum yield of aromatic amino acids highly increases (~5 times) from 254 to 205 nm [76]. Therefore, the exposed far-UVC photons to cells may be scavenged within the cell by being absorbed by proteins surrounding the DNA. This leads to lower DNA damage and higher protein damage in microplasma UV treatment cases compared with those using LPMVL. Higher inactivation by LPMVL may imply that UV absorption by proteins during inactivation has few consequences on the culturability of *E. coli* cells, and the inactivation is regulated mostly by the generation of DNA-scrambling pyrimidine dimers. Moreover, although P-Microplasma emits photons with broad SPD comprising both DNA and protein UV-absorption peaks, it could not enhance the total efficiency as its relative intensity near the DNA peak was lower than that of LPMVL.
However, in the shoulder region, microplasma lamps showed similar behaviour to LPMVL in spite of higher DNA absorbance by LPMVL photons (Figure 5-2c). In addition, the increase in the rate of reaction (slope) from the shoulder to the next linear region was lower for microplasma sources than for LPMVL. The existence of the initial shoulder was due to the intrinsic DNA protection or self-repair mechanisms of the microorganism by corresponding proteins, which reduced the amount of DNA damage [23, 28]. Therefore, these observations suggested the suppression of DNA protection and repair ability because of the higher susceptibility of proteins to far-UVC during the microplasma UV irradiation.

Furthermore, the theoretical GE values could assess the relatively lower action GE of microplasma UV lamps to LPMVL (Figure 5-2d), although the currently-known action spectra of E. coli were not extended to below 230 nm wavelengths, where the protein absorption takes place. This can be more evidence of the domination of DNA absorption in the E. coli inactivation process. According to Figure 5-2e, 265 nm UV-LED had the highest efficiency for E. coli among all studied sources as it illuminated near DNA peak absorption.

For the MS2 virus, the story was completely different. The loss of MS2 viability exhibited pseudo-first-order kinetics over the UV fluence without the initial shoulder region, which suggested the absence of the self-repair mechanism even in low UV fluence. In such cases, according to the second law of photochemistry, the interaction of radiated photons with the microorganism depends solely on photochemical reactions with intercellular species without any simultaneous repair mechanism. Both studied sources indicated outstanding log-reduction values (Figure 5-2b) with K-Microplasma as the superior microplasma UV source for MS2 inactivation with a more than two-fold action GE with respect to the LPMVL. This was not surprising when the spectral sensitivity of MS2 was considered, with a greatly enhanced sensitivity below 240 nm,
and it was also confirmed by the theoretical GE values, which are shown in Figure 5-2d. DNA and RNA had similar absorbance spectra with a slightly left-shifted one for RNA [24]. Therefore, the enhancement appeared to be correlated with the absorption spectra of aromatic amino acids (proteins), where it increased dramatically in the far-UVC region (Figure 5-1b), and not the one for the MS2 genome (RNA). The lower GE of P-Microplasma compared with that of K-Microplasma, in spite of its broad SPD, highlighted that the RNA direct damage was only partially responsible for the total loss of viral infectivity. Targeted proteins could be either those responsible for infection or protein capsid surrounding the sRNA genome of MS2. Based on the results of a quantitative PCR assay, Beck et al. claimed that the high sensitivity of MS2 to far-UVCs is genome-mediated and not related to the direct capsid protein damage [77]. This could indicate a mechanism whereby aromatic amino acids absorb far-UVC, transmit the excitation energy to nucleic acid through a UV-generated crosslink between the protein and the nucleic acid [50], and finally form an RNA lesion. Previous studies suggested an energy transfer from RNA to viral proteins in MS2 through the aforementioned linkages following the near-UVC radiation [78], within the range of RNA peak absorption. Hence, at far-UVC, where the UV-absorption of protein is immense, it is conceivable that the energy transfer will occur in the opposite direction. Regardless of pathways, this work illustrated that microplasma UV sources exhibited outstanding GE for the inactivation of a URM by feeding the energy to its intercellular proteins.

The theoretical GE corroborated the action GE of both microplasma UV lamps for both E. coli and MS2 inactivation when they were employed in well-collimated setups by using standard protocols for the fluence calculation. A similar agreement between the theoretical and action GE have been reported for previous UV sources, that is, UV lamps and various UVC-LEDs. Therefore, the theoretical GE was used in this study as a standard substitute to compare the action GE of
microplasma UV lamps with that of other UV sources, instead of comparing diverse experimental data in the literature (Figure 5-2e). It was shown that microplasma UV lamps presented higher GE for MS2 inactivation compared with that of all the studied UV sources.

Figure 5-2. (a) E. coli and (b) MS2 action log reduction fluence responses under irradiation of microplasma UV lamps (this work) and LPMVL (from literature). (c) The magnified shoulder region
of E. coli inactivation curve. (d) Comparison of calculated relative theoretical (Equation 5-3) and action (Equation 5-4) GE of the LPMVL for microplasma sources for both microorganisms. (e) Comparison of calculated LPMVL-relative theoretical germicidal efficiencies for different germicidal UV sources (see Figure 5-1a for the SPD of the studied sources). Dashed lines in (a) and (b): First-order model fittings (Equation 5-2) excluding shoulder region. Initial concentrations for experiments: \( \sim10^6 \) CFU mL\(^{-1}\) E. coli and \( \sim10^6 \) PFU mL\(^{-1}\) MS2 in sterile phosphate buffered saline (PBS). Error bars represent the standard deviation from triplicate runs. Results with statistically significant differences \((p < 0.05)\) are depicted in figures.

5.3.3 Microbial reactivation

For MS2, the previously noted absence of shoulder region in dose-response underlined the inability to achieve RNA repair. This is due to the lack of functional repair enzymes (proteins) within the MS2 cell [28]. Complementary dark and photoreactivation experiments were also conducted for MS2, and no repair was observed (data not shown).

The 4 h stirring of unirradiated E. coli samples were designed as control experiments in the dark and under fluorescent lamp irradiation, and no change in the E. coli concentrations was observed. The ability to achieve nucleic acid repair depends not only on the biological organization of the microorganism but also on the amount of UV damage inflicted on the cell. Therefore, the UV-doses required for 3- and 4.5-log E. coli inactivation were calculated from the dose-response curves (Figure 5-2a) for both microplasma UV lamps and applied to evaluate the effect of various UV-doses on the reactivation of E. coli, including photoreactivation and dark repair. Thus, four cases of E. coli reactivation were studied for each microplasma UV lamp.

As a quantitative summary, in all cases, results indicate lower E. coli reactivation values for the P-Microplasma irradiation compared with K-Microplasma irradiation (Figure 5-3 a and b).
However, noteworthy trends were observed over time for these sources, which were different from what had been common for the previous UV sources.

The 3-log-inactivated *E. coli* cells by K-Microplasma recovered up to 19% during 4 h of fluorescence lighting, while the repair was almost half for P-Microplasma. In addition, the photoreactivation reached its peak value during the first 150 min exposure to the visible light and decreased slightly thereafter. The recovery was much lower for 4.5-log cases, which was confirmed by the well-established effect of the increasing UV-dose on diminishing reactivation [70]. In 4.5-log cases, the increasing trend of repair values resisted for less than 120 min, and afterwards, the decline in *E. coli* concentration was significant. Surprisingly, for the P-Microplasma inactivated *E. coli* samples, after 210 min of visible light exposure, the concentration reduced to even lower than its value at the beginning of the photoreactivation process, exhibiting further inactivation under light exposure. This could have happened because of the slight radiation in the UVA range by the utilized fluorescent lamp (see emission spectrum in Figure A-5), which might not have been tolerated by the cells. This occurred in spite of UVA being ~10^5 times less efficient in inducing DNA damage than that of UVC because of the poor UVA-absorption by DNA to overcome the cell repair and protection mechanism. This observation can be interpreted as indicating that the high far-UVC dose for 4.5-log inactivation intensely damaged the DNA repair system in *E. coli* cells to the extent that they could not resist scant UVA irradiation.

Documented photo repair results for LPMVL and UVC-LEDs in the literature have shown a significant log repair of 60%–80% for *E. coli* [28, 32, 71], whereas dramatically lower values resulted for microplasma UV lamps in this work. This indicated the significant ability of radiation below 240 nm in microplasma UV lamps’ spectra to damage the photoreactivation capability of *E. coli* cells. Photoreactivation repair is initiated by a single enzyme called photolyase, which
reverses UV-induced lesions in the DNA. Photolyases are structurally monomeric proteins of \(\sim400-600\) amino acids in length with two noncovalently bound cofactors, which constitute the large flavoprotein family. Photolyase, owing to aromatic amino acids in its structure, has a far-UVC preferred radiation absorption behaviour, as discussed in the Germicidal Efficiency section. Although the destruction of proteins was shown to have a minor biological role in inactivating *E. coli*, inflicting damage to proteins responsible for DNA repair by photons near their UV absorption peak would be vital for the microorganism due to the presence of very few repair molecules in the cell [31]. It has also been documented that the structural dissociation of amino acids results in denaturation of the enzyme molecule and loss of its biological activity. For example, denaturation of the polymerase causes a loss in the multiplication ability of a microorganism, while, regarding the photolyase, the microorganism loses its capability to repair UV-induced damage. Thus, below-240 nm UVs can reduce the subsequent photo repair, possibly by causing a disorder in endogenous photolyase or other related enzymes. Previous research has also indicated that the broad far-UVC radiation in the MPMVL spectrum could potentially affect the regulation of the photolyase gene to have lower expression and consequently reduce the amount of photolyase in *E. coli* [32, 79].

Figure 5-3c displays the final UV inactivation (log reduction) when the post-reactivation is also considered. By comparing the total log removal of microplasma sources with those of the LPMVL and UV-LEDs, it was revealed that they were more efficient for the ERM indicator microorganism when the combined inactivation + photoreactivation process was considered.

The dark repair showed comparable trends after P- and K-Microplasma irradiations (Figure 5-3b). As for 3-log removal cases, dark repair reached a maximum value (9% and 6.5%) in the first 90 min of lighting with no further recovery afterwards. For 4.5-log removal cases, the data fluctuated over the lighting time with relatively lower values compared with the 3-log cases.
(Figure 5-3b). In this case, the enhancement compared with that of LPMVL and UVC-LEDs was less significant (15% to 9%), and it appears that microplasma UV lamps below 240 nm photons could not totally eliminate the *E. coli* dark repair. This is due to the mechanism of dark repair that involves complex pathways caused by the action of various mechanisms, some of which may not be blocked by the far-UVC irradiation.

As a part of the systematic approach for studying photoreactivation, new observed trends should be kinetically modelled. To date, most of the studies on microbial reactivation have only described the observed overtime process, and few studies have been conducted on kinetic modelling. Previously proposed kinetic models were obviously unable to predict photoreactivation trends after far-UVC inactivation as they did not include the damaged proteins consequences. In all previous cases reported in the literature, a final saturation phase was predicted for photoreactivation. However, the saturation model did not adequately predict the decay phase, which is shown in the curve-fittings of Figure 5-3 (d and e). Here, a new model that would be proposed takes into consideration all phases of microbial reactivation and decay, which is elaborately explained in section 5.2.6. Survival ratio values for *E. coli* photoreactivation versus time are well fitted with the new proposed model for all four studied cases (Figure 5-3). The experimentally observed maximum survival is also well predicted by the new model in this work, compared with the previous saturation models (Table 5-2). The corresponding decay rate constants (Table 5-2), extracted from modelling, emphasize the more severe damage to the self-repair mechanism by P-Microplasma.

This model can be used, in general, for any cases of photoreactivation following UV disinfection, including the ones with the LPMVL or UVC-LEDs (only by assuming $k_d = 0$), and cases with UVA pretreatment where the decay phase is also reported [28] ($k_d \neq 0$). This model can
be applied to photoreactivation under natural sunlight, which may also show the decay phase, as the sunlight contains significant UVA and UVB.

Figure 5-3. The trend over time of *E. coli* (a) photoreactivation and (b) dark log repair (Equation 5-5) after 3.0- and 4.5-log reduction under irradiation of two studied microplasma UV lamps. (c) The total *E. coli* log removal when the photoreactivation was in consideration for different UV sources after 3.0- and 4.5-log reduction. Corresponding values for the LPMVL and UV-LEDs are extracted.
from the literature [28, 32, 71, 79]. The kinetic modelling of survival values through the photoreactivation process after (d) 3-log and (e) 4.5-log reduction: SModel and DModel in the legends represent the previous saturation model in the literature (Equation 5-10) and the proposed model in this work based on the decay phase (Equation 5-13), respectively. Error bars display standard deviation from triplicate experiments. To avoid interweaving lines, error bars are omitted in parts (d) and (e). Results with statistically significant differences ($p < 0.05$) are depicted in figures.

Table 5-1. Maximum survival values and the decay phase reaction rate of photoreactivation after UV irradiation achieving 3.0- and 4.5-log removal from microplasma sources. SModel and DModel represent the previous saturation model in the literature (Equation 5-10) and the proposed model in this work based on the decay phase (Equation 5-13), respectively. The standard deviations were typically less than 10% for experimental data, and the coefficient of determination ($R^2$) values were more than 0.98 for model fittings (note that the SModel is fitted excluding the decay phase).

<table>
<thead>
<tr>
<th>Source</th>
<th>Reactivation Case</th>
<th>Experimental Observed $S_{max}$ (%)</th>
<th>SModel Predicted $S_{max}$ (%)</th>
<th>DModel Predicted $S_{max}$ (%)</th>
<th>$k_d$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-Microplasma</td>
<td>After 3-log</td>
<td>0.4027</td>
<td>0.4340</td>
<td>0.4061</td>
<td>0.0029</td>
</tr>
<tr>
<td></td>
<td>After 4.5-log</td>
<td>0.0069</td>
<td>0.0076</td>
<td>0.0067</td>
<td>0.0039</td>
</tr>
<tr>
<td>P-Microplasma</td>
<td>After 3-log</td>
<td>0.2570</td>
<td>0.3006</td>
<td>0.2522</td>
<td>0.0081</td>
</tr>
<tr>
<td></td>
<td>After 4.5-log</td>
<td>0.0055</td>
<td>0.0067</td>
<td>0.0054</td>
<td>0.0136</td>
</tr>
</tbody>
</table>

5.3.4 Further mechanism analysis

As mentioned in the introduction section, one of the methods claimed in the literature to increase the inactivation and limit the photoreactivation is using UVA pretreatment prior to the UVC (higher than 240 nm wavelength) inactivation. The mechanism is declared as UVA
pretreatment’s effect on the reactive oxygen species (ROS), mainly the hydroxyl radical, in the cell leading to the oxidative damage to cellular components, such as nucleic acid repair enzymes [28, 80]. Pursuing the actual mechanism for inactivation by microplasma lamps, the role of three principal ROSs, hydroxyl radical (●OH), superoxide radical (●O₂⁻), and hydrogen peroxide (H₂O₂), were studied using corresponding scavengers (see the section 5.2.4 for experimental details). Figure A-6 demonstrates the *E. coli* inactivation and photoreactivation for both microplasma UV lamps’ irradiation by adding various ROS scavengers. No significant difference was observed for either inactivation or photoreactivation in the presence or absence of scavengers, indicating that the ROS-initiated reactions are not involved in the mechanisms. This observation is noteworthy as it shows that, although far-UVC photons are more potent than UVA and highly absorbed by cell components, they do not affect the ROS balance in the cell. This can lead to lower probable disinfection by-products caused by advanced oxidation reactions among ROS and various intercellular chemicals. In short, the aforementioned enhancements that increase microbial inactivation and limit microbial reactivation are only attributed to the far-UVC’s impact on intercellular proteins.

### 5.4 Discussion

Biological applications of conventional germicidal UVC sources have been restricted by highly UV-resistant and reactivatable microorganisms. In this regard, below-240 nm UVCs can be an effective solution by providing alternative pathways for inactivation without requiring the addition of any chemical or radiation assistant agents. The success of microplasma UV lamps in suppressing the DNA repair and protection mechanism and attacking intercellular proteins could open new areas by virtue of their efficiency regarding the inactivation of highly UV-resistant microorganisms. *E. coli* is known as a highly photoreactive but UV-sensitive ERM with a
relatively weak DNA protection mechanism (during UV exposure) compared with other ERMs [50]. A short resistance shoulder region (<5 mJ.cm\(^{-2}\)) and easy 4-log-inactivation (<10 mJ.cm\(^{-2}\)) were reported for \textit{E. coli} even under 254 nm irradiation [68]. Therefore, DNA damage has been the dominant phenomenon, and the effect of reducing the shoulder region is not outstanding. However, for some of the germs, such as radiation-resistant fungi, moulds and bacteria, with dose requirements in the order of 1,000 mJ.cm\(^{-2}\) for 4-log reduction, the story could be different. The effective protection mechanisms in these microorganisms have been clearly shown by their long shoulder presented for the UVC inactivation curve. Under microplasma UV irradiation with intense far-UVC photons, this shoulder can be considerably lowered, and hence, the total UV fluence necessary for a respective inactivation could be reduced. This would be owing to the fact that, at higher UV fluences, various mechanisms of protein damage can presumably be significantly responsible for inactivation, in addition to the DNA damage. Therefore, if the UV fluence is high enough, many proteins would be damaged to such an extent that the damage leads to cell death, no matter how effective the DNA self-protection mechanisms are. Thus, higher GEs are predicted for severely-resistant ERMs, such as different \textit{Aspergillus} and \textit{Bacillus} species, which merits further research. The alternative indication for the assumption of protein and repair mechanism damage is the demonstrated low rate of photolyase-mediated photoreactivation in \textit{E. coli}, which is known as being strong among the ERMs, after irradiation with microplasma UV lamps. Based on the results and discussions in this work, the P-Microplasma is proposed as a new highly potent radiation source for bacteria, moulds, and fungi with strong in-treatment or post-treatment DNA-repair and protection mechanisms.

Additionally, the viral germs with higher UV resistance were shown to be inactivated more efficiently with microplasma UV lamps due to the indirect far-UVC-induced impact on the genome.
through absorption by viral proteins, which are responsible for the infection. The method in this work can be readily applied to other strongly resistant viruses that are mentioned in various disinfection standards, such as adenovirus [34] and T1UV [81]. Calculating the theoretical GE of K- and P-Microplasma for these two viruses, based on their published action spectra in the literature [67] (see Figure A-7), showed outstanding inactivation potential of microplasma sources in this regard (Figure 5-4). The results associated with K-Microplasma have been the most promising ones for viruses, as it is capable of enhancing adenovirus, MS2, and T1UV inactivation as much as 10, 2, and 1.5-fold, respectively, compared with LPMVL. Higher inactivation of adenovirus by far-UVC radiation comparing with one for near-UVC, predicted by the theoretical GE in this work, has been also reported experimentally in the literature [82]. This enormous potential of microplasma UV lamps could be also a relevant topic of research for further studies on germicidal efficiencies of microplasma UV lamps for various microorganisms.

The recent technological development that has been made possible by advances in the development of microplasma allows the chemical biology researchers and industries to benefit from a new disinfection source. P- and K-Microplasma can be utilized individually and in combination with each other or other available UV sources (such as UV-LEDs) to design optimized wavelength tailored disinfection systems for currently disinfection challenging microorganisms.
Figure 5-4. Comparison of calculated theoretical GEs (with respect to LPMVL) of different germicidal UV sources (see Figure 5-1a for the SPD of the studied sources) for standard regulating UV-resistant MS2, adenovirus, and T1UV viruses.
Chapter 6: Conclusion and Recommendations

6.1 Conclusions

In this study, the ultimate goal was to explore the potential for the application of the newly emerging far-UVC radiation technology — microplasma UV— to surpass the application limits of UV microbial inactivation and water disinfection. This would be possible by first developing a method to accurately determine the inactivation kinetics of microorganisms in microplasma UV setups. To this goal, the operation of microplasma UV lamps in a variety of operating conditions was studied. Then, a comprehensive protocol was proposed for the fluence rate determination and the reproducibility of resultant kinetic data was studied by comparing two different-sized reactors located in appropriate distances from the microplasma UV based on the developed protocol. Eventually, the impact of two available microplasma far-UVC exposures on both inactivation and post-treatment reactivation was analyzed by studying two representative microorganisms. In addition, the kinetics of reactions were modelled and compared with the ones available for UV lamps, and UV-LEDs and the mechanism of reactions were pursued. The main conclusions derived from the various tasks performed in this research are highlighted hereunder:

For CHAPTER 4:

- In the first stage, a method for measuring and controlling lamp radiant wavelength and power output was suggested. Lamp temperature, electrical current, and intermittent on/off cycles were investigated as potential output-altering operational conditions.
- The SPD-related specifications (peak wavelength, FWHM, and GTIR) and radiation pattern of the lamp were independent of operating conditions. The irradiance values were affected only and linearly by the electrical current. No warm-up time and typically
a 15 sec stabilization time were required for the lamp to reach the full and constant output.

- A quasi-collimated setup was obtained for the microplasma UV lamp, even without a collimating column. A protocol was proposed to determine the distance at which the collimation and uniformity would be insured for different lamp sizes and solution container scales. Furthermore, to calculate the average fluence inside the solutions, the WF concept was redefined for UV-initiated oxidation processes as a function of time.

- Reproducible photo-kinetic data was acquired for the inactivation of *E. coli* and the oxidation of MB in two containers with different diameters in protocol-determined distances from the microplasma UV lamp. *E. coli* was 4 log reduced by about 9.9 mJ cm\(^{-2}\) UV dose and the oxidation rate constants for MB were in the range of 85 to 186 cm\(^2\) mJ\(^{-1}\) corresponding to the initial concentration of utilized H\(_2\)O\(_2\).

For **CHAPTER 5:**

- The proposed protocol in chapter 4 was successfully implemented in several setups to investigate the effect of microplasma UV irradiation on the inactivation of *E. coli* and MS2.

- Both studied microplasma UV sources, K- and P-Microplasma, presented higher GE values for the URM representative microorganism (MS2) comparing with those reported for UV lamps and UVC-LEDs in the literature. Between two microplasma sources, K-Microplasma has the higher GE by having more than 2 fold GE than one for LPMVL. The mechanism was related to aromatic amino acids absorbing far-UVC,
transmitting the excitation energy to nucleic acid through a UV-generated crosslink between the protein and the nucleic acid, and finally forming a nucleotide lesion.

- For *E. coli* inactivation, microplasma UV lamps showed lower total GE than LPVML. This showed that UV absorption by proteins during inactivation has few consequences on the culturability of *E. coli* cells, and the inactivation is regulated mostly by the generation of DNA dimers. However, in the shoulder region of the fluence-response curve of *E. coli*, microplasmas had similar results with LPVML, which revealed that the self-repair mechanism was suppressed by the generated far-UVC by microplasma UVs.

- For both *E. coli* and MS2, the calculated theoretical GE, based on the microorganism action spectra and microplasma source SPD, corroborated the experimentally-resultant action GE which shows the correct calculation of the in-solution UV dose.

- For *E. coli* reactivation, both microplasma UV lamps demonstrated significant suppression with log repair values (less than 20%) much lower than ones reported for LPMVL and UV-LEDs (about 60%) by degrading the responsible proteins for reactivation. Between two microplasma sources, P-Microplasma shows a more dramatic reactivation reduction to the extent that the 4.5-log reduced samples completely lose their repair ability.

- Conventional models of microbial reactivation did not correctly fit our data, and thus a new general kinetic model was provided, which proved to be applicable for not only the results of this work but also ones reported in the literature for other sources.

- Application of microplasma UVs for the inactivation of other regulating URMs was also studied by calculating the theoretical GE based on their action spectra. Both
microplasma lamps indicated to offer very high GE values; for Adenovirus, for example, about 10-fold higher values comparing with LPMVL was calculated.

It is not every day anyone get to report on a structurally-new UV source, and thus I am excited to provide a new description of the disinfection efficiencies of UV microplasma source. The developed protocols in this study are expected to play a significant role in obtaining accurate kinetics data, which is essential in the design and fabrication of the next generation of UV disinfection reactors. The microbial inactivation/activation results from this work exhibit great potential for various applications in environmental engineering, such as in water treatment. This, in turn, is anticipated to generate a great deal of interest among researchers and entrepreneurs in the field of environmental science, health, and protection on the applications of a viable new UV source for novel UV treatment devices.

6.2 Recommended topics

- As declared in Chapter 5 of this thesis, microplasma UV lamps showed a great theoretical GE for waterborne URMIs. Studying other critical microorganisms can be an exciting topic to further ensure the potentials of these novel far-UVC sources and report reliable kinetic data measured with the proposed protocol presented in this thesis.

- Some airborne microorganisms, such as Aspergillus niger, are mentioned to be severely resistant to UV treatment by current sources (more than 300 mJ cm⁻² for 4 log reduction). Using microplasma UV sources for air purification applications could solve these challenges, but it needs a comprehensive study.
• The radiation modelling is a well-established research topic for UV sources, and there are many articles in the literature for MVLs and UV-LEDs. However, there is no validated model for the irradiance/fluence rate estimation of microplasma UV lamps. This could be interesting, but at the same time, a challenging topic as the UV generating structure of microplasma UV lamps possess distinctive structures.

• Designing continuous UV reactors based on MVLs and UV-LEDs systems has been tried widely not only in the literature but also in the industry. After modelling the microplasma UV radiation, it is valuable to optimize microplasma UV reactors using an integrated model of radiation, hydrodynamics, and kinetics that is presented in this thesis.

• It was shown in Chapter 4 that K-Microplasma could lead to the degradation of chemical contaminants in water by adding H₂O₂. As the absorption of these photosensitizers, such as H₂O₂, increases in the far-UVC region, it could be an exciting area of research to apply microplasma UV lamps for AOPs.
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Appendices
Appendix A  Supplementary figures and schematics

Figure A-1. The fabricated radiometry setup for irradiance measurements over the recommended domains and subdomains. Inset on the corner: lamp in the ON mode.
Figure A-2. The real image of the protocol-based designed experimental setup with using the black sheet with the aperture to avoid UV beams to enter from the container walls.
Figure A-3. The estimated minimum distance to ensure the irradiance uniformity on irradiated surfaces extendable for any lamp-container size pairs (X = lamps side size and D = container diameter).
(A)

Solution Container

Noncollimated Sections ✗

Well-collimated ✔
Figure A-4. A 2D schematic of the QCE test for (A) larger and (B) smaller solution containers than the microplasma UV lamp.
Figure A-5. Spectral power distribution of the employed fluorescent lamp for microbial photoreactivation studies. The UV part of the lamp radiated spectrum is shown as the cause for the decay phase in the survival ratio trend through the photoreactivation process (see Figure 5-3 in the main body of the thesis).
Figure A-6. Effect of adding different scavengers on the inactivation of E. coli under (a) K-Microplasma and (b) P-Microplasma irradiation and reactivation of E. coli after 4.5-log reduction by (c) K-Microplasma and (d) P-Microplasma.
Figure A-7. Action spectra comparison for MS2, adenovirus, and T1UV viruses extracted from the published values in the literature [67].
Appendix B  Microorganisms propagation and enumeration for UV disinfection

The *E. coli* ATCC 11229 solution was mixed with glycerol and stocked deep-frozen. For each experiment, an *E. coli* stock was thawed and inoculated into LB broth medium (L3522 from Sigma-Aldrich Co. LLC) and cultivated in a 37 °C and 200 rpm in a shaking-incubator. The optical density (OD600) was measured in 30 min intervals and used to determine the microbial growth log phase. Then, the optimally grown *E. coli* solution at the log phase was centrifuged and washed by phosphate buffered saline (PBS) three times and then resuspended and serial diluted in PBS to achieve absolute concentrations to study. After UV irradiation experiments, the *E. coli* samples were collected and serially diluted with PBS. Afterward, 20 µL of each dilution was spread on Luria-Bertani (LB) agar medium plates and incubated at 37 °C upside down for 18 h. Each dilution was plated in triplicate, and the plates with 20~200 colonies were enumerated for further analysis.

For MS2 cultivation, the MS2 powder was dissolved in the fresh log-phase *E. coli* host in LB broth with the addition of CaCl₂ to assist MS2 attachment to host *E. coli* cells. The suspension was incubated at 37 °C with 200 rpm shaking for 24 h. Then, the cultivated suspension was filtered by a 0.45-micron filter, and the supernatant was collected as MS2 stock, which was refrigerated and used within a month. For each experiment, an MS2 stock was collected and diluted with PBS. After the UV experiment, the irradiated samples were serially diluted and assayed using a double-layer technique. Briefly, *E. coli* host (ATCC 15597) was cultivated in LB broth to reach log phase by the same procedure mentioned for the *E. coli* ATCC 11229. A 1 mL MS2 dilution was transferred to an LB bottom agar plate. Then, the fresh *E. coli* host was mixed with LB top agar solution at 45 °C, poured on the bottom agar plate, and mixed with the MS2 dilution. After cooling down and hardening, the agar plates were incubated upside down at 37 °C for 18 h. Each dilution was plated in triplicate, and plates with 20~200 plaques were enumerated for data analysis. The
initial concentration of microbial samples for UV exposer experiments was approximately $10^6$ CFU/mL for *E. coli* and $10^6$ PFU/mL for MS2 in PBS.