Evaluation of Pulsed Xenon UV Lamp on Inactivation of *Listeria monocytogenes* on Stainless-Steel Surfaces

by

Nazanin Yasoubi

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Applied Science

in

Chemical Engineering

Waterloo, Ontario, Canada, 2023

Author's Declaration

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

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

Abstract

Foodborne diseases remain a major global public health concern, with *Listeria monocytogenes* posing a significant risk, particularly for immunocompromised individuals. Ensuring food safety and minimizing contamination has become a top priority for food production facilities.

UV-C disinfection has emerged as a safe and effective method for inactivating various microorganisms. With the phase-out of traditional mercury UV-C lamps due to the Minamata Convention on Mercury, pulsed xenon UV lamps (PX-UV) have gained popularity as a mercury-free alternative. PX-UV lamps offer high-intensity UV-C irradiation in a short time and a wider range of wavelengths, making them environmentally friendly and versatile.

To better understand the factors affecting the disinfection efficacy of PX-UV lamps, an investigation of operational parameters was conducted. The study focused on the impact of exposure time, frequency, UV fluence (dose), angle of irradiance, and shielding effects due to the presence of soiling agents on the reduction of *Listeria monocytogenes* on stainless steel surfaces.

Radiometry analysis revealed that pulse frequency, in the range of 1 Hz to 25 Hz, influenced the fluence delivered per pulse, with higher frequencies resulting in lower UV fluence per pulse. However, regardless of pulse frequency, angle, or exposure time, the total UV fluence received was the main determinant of the log reduction. Notably, a 5.59-log reduction was achieved with 30 seconds of exposure to UV light at 25 Hz, corresponding to a fluence of 151.2 mJ/cm^2.

Experimental studies conducted in the presence of soiling agents have demonstrated the significance of the type of soiling material. It not only could affect the growth of bacteria but also could play a role in shielding the bacteria from UV radiation. The composition of the soiling material has implications for both the susceptibility of bacteria to UV radiation and their ability to proliferate. The thesis focuses on three categories of soiling agents: proteins, carbohydrates, and lipids. Among these groups, it was observed that protein soils had the most significant impact on promoting bacterial growth, while the log reduction value remained unchanged. Carbohydrates also had an effect on bacterial growth, although to a lesser extent compared to proteins. On the other hand, lipids exhibited a shielding effect, leading to a reduction in the log reduction value of *L. monocytogenes*. These findings highlight the varying effects of different soiling agents on bacterial growth and the subsequent log reduction achieved during disinfection.

This research highlights the significance of UV-C fluence as the primary consideration when using PX-UV lamps for no-touch disinfection, emphasizing the importance of selecting appropriate operational parameters to achieve the desired fluence. However, further research in this field can enhance our understanding of this technology. Some potential areas of future study include investigating the efficacy of UV disinfection on different types of surfaces, exploring the disinfection of fresh food products, and examining a wider range of soiling agents to better comprehend the impact of material structure on UV efficacy. These studies would contribute to expanding our knowledge and improving the effectiveness of UV-based disinfection methods in various applications and particularly food industries.

Acknowledgements

Foremost, I would sincerely like to thank my supervisors, Dr. Valerie Ward, and Dr. William A. Anderson, for their constant support and guidance through this project. Thank you both for letting me to be a member of your wonderful research groups and training me to fulfill this project.

I would like to thank Dr. Marc Aucoin and Dr. Yilan Liu for reading my thesis and accepting to be my committee members.

I would like to specially thanks Dr. Reza Roushan and Dr. Carol Moralejo for their knowledge and support during this project. I would like to sincerely thanks their support both technically and mentally.

I would like to thank all those in the BioWorks Team, especially Dr. Marc Aucoin, for his support and taking interest in my project and providing insights and ideas in a way of promoting the project, Dr. Shazia Tanvir from Anderson's lab who helped me a lot in the experiments and all those in Ward's lab: Dr. Reza Roushan, Dr. Anirban Banerjee, Chuchi Chen, Jiaqing Li, Parisa Ahmadi, Nicholas Rasmussen and Kyle Vandermeulen for helping me with this projects with their comments and building an enjoyable environment in lab.

To my kind parents, Janet and Javad, and my lovely sister Yasaman; I thank you for all your unconditional love and support in my whole life which helped me to be where I am today.

Additionally, I gratefully acknowledge the financial support by Solaris Robots, NSERC Alliance program and University of Waterloo, which made this project possible.

Dedication

Dedicated to my wonderful parents, Janet, and Javad.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BSA	Bovine Serum Albumin
CDC	Centres for Disease Control and Prevention
CFU	Colony Forming Unit
FDA	Food and Drug Administration
IPL	Intense Pulsed Light
IR	Infrared
LED	Light Emitting Diode
LPM	Low Pressure Mercury
PBS	Phosphate Buffer Saline
PC	Positive Control
PES	Polyethersulfone
PL	Pulsed Lamp
PX-UV	Pulsed Xenon UV Lamps
QACs	Quaternary Ammonium Compounds
RTE	Ready To Eat
SS	Stainless Steel
TEM	Transmission Electron Micrographs

TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
UV	Ultraviolet
VIS	Visible Light

Chapter 1
Introduction

1.1 Research Background

Foodborne illnesses are a significant public health concern, leading to an estimated 600 million cases and over 420,000 deaths annually worldwide [1], causing significant health and economic burdens. Listeriosis is a foodborne disease caused by *Listeria monocytogenes*, a gram-positive bacterium. Although the disease only causes 1 to 9 cases per million individuals per year and 0.02% of all foodborne illnesses in United States, listeriosis accounts for 28% of deaths due to foodborne illness [2]. The U.S. Food and Drug Administration (FDA) has also reported the severity of this disease with a high fatality rate of 20 to 30 percent even with sufficient antibiotic treatments and more than 90 percent of those affected require hospitalization. In the United States, the Centres for Disease Control and Prevention (CDC) have estimated that approximately 1,600 people are infected annually, resulting in around 260 fatalities and 1500 people need hospitalization [3]. Pregnant women, infants, older adults, and immunocompromised individuals are at greater risk for severe disease [4]. In addition to the public health burden of foodborne diseases, there are significant economic costs associated with outbreaks and recalls. Canada experienced its worst outbreak of listeriosis in 2008 with 57 total cases and 24 deaths [5]. The medical, industrial, and government costs associated with this outbreak was $242 million CAD [5]. *L. monocytogenes* can be readily isolated from a broad range of ecological niches such as soil, water, and vegetation making it difficult to prevent contamination of foodstuffs [6]. Thus, food processing environments are rigorously monitored for *L. monocytogenes* as it can persist in the facility for a long period of time [6]. It

can resist many stressors, persist in cold environments, and grows in protective biofilms [6]. Regular ingestion of a small numbers of *L. monocytogenes* is expected [6], thus, foods containing more than 10^3 CFU/mL of *L. monocytogenes* are considered to be contaminated [2]. Therefore, some countries allow a small amount of contamination ($<10^2$ CFU/g) while others have a zero-tolerance policy [6]. Regardless, the seriousness of listeriosis in vulnerable populations makes it imperative to developed improved methods for the inactivation of *L. monocytogenes* in food processing facilities. Stainless steel (SS) is a preferred material in workplaces and kitchens due to its strong mechanical strength, corrosion resistance, longevity, and ease of fabrication. Its smooth surface allows for easy cleaning and sanitization, making it ideal for food-contact surfaces. With its non-porous nature, SS reduces the risk of cross-contamination and helps maintain high hygiene standards. It plays a vital role in ensuring food safety in the food manufacturing and service industries [7].

UV-C disinfection is a safe and effective method for the inactivation of bacteria, viruses, spores, and other microorganisms [8]. Pulsed xenon UV (PX-UV) lamps provide light at high intensity pulses over a wide range of wavelengths, unlike mercury or light emitting diode (LED) lamps which provide monochromatic light typically in a continuous wave, although UV-C LEDs can provide light in pulses as well [9]. The use of pulsed lamp (PL) technology for food or food contact surfaces decontamination was approved by FDA in 1996 for xenon flashlamps at fluences below 12 J/cm^2 [8]. There are a number of possible parameters that can affect the extent of disinfection using UV-C lamps including light intensity, wavelength, exposure time, characteristics of the microorganism, surface/sample properties,

light penetration, and shielding effects due to existence of particles which could shield the microorganisms from the UV light [10].

1.2 Research Objectives

The purpose of this thesis was to study the impact of PX-UV system on inactivating *L. monocytogenes* on SS surfaces under various disinfection conditions, including different radiation angles, levels of soiling agents, frequencies, and exposure times. The objective of the thesis could be divided into the following sections:

- UV lamp dosimetry.
- Quantify the UV disinfection kinetics for the PX-UV lamp and *L. monocytogenes*.
- Study the effect of various parameters on disinfection efficacy of PX-UV including frequency, exposure time, and radiation angles.
- Study the effect of the presence of soiling agents on disinfection efficacy of PX-UV.

Developing a mathematical expression to quantify disinfection can be helpful for sizing the device for various applications or to determine the minimum fluence needed to achieve a certain level of disinfection for a specific process and specific microorganism [11]. Therefore, the UV disinfection kinetics for the PX-UV lamp and *L. monocytogenes* was quantified.

1.3 Thesis Outline

This thesis consists of 5 chapters. Chapter 1 provides research background and objectives of this study, while Chapter 2 provides more information about the bacteria, disinfection methods applied in industry and UV types and mechanism through literature review. In Chapter 3 materials and methods followed in this project were described in detail. Chapter 4 presents and

discusses about the results of this research. Finally, conclusions were made, and recommendations for future studies were suggested in Chapter 5.

Chapter 2
Literature Review

2.1 *L. monocytogenes* Background

Listeria monocytogenes are gram-positive, facultative intracellular, facultative anaerobic and rod shape bacteria (Figure 1) which can survive in various environments and are found everywhere [12]. Using specialized media, *L. monocytogenes* can be easily separated and identified from soil, water, and plants, including unprocessed raw produce intended for human consumption [6]. During the processes of slaughtering and milking, hygiene vulnerabilities are the primary critical areas for *Listeria* contamination [13].

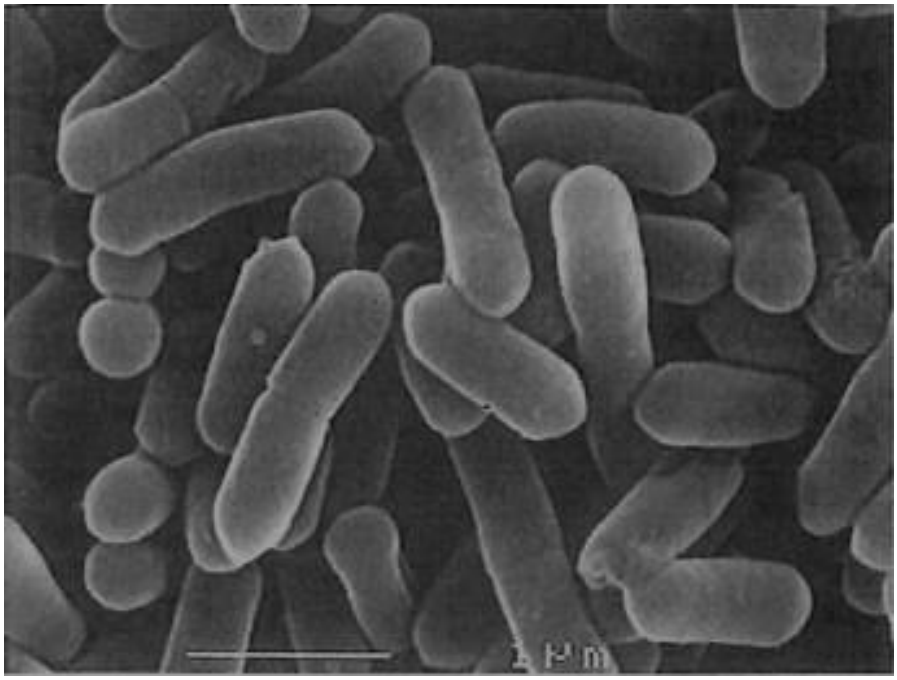


Figure 1 - Microscopic photo of *L. monocytogenes* [14].

L. monocytogenes has posed a significant challenge to the food industry as it can endure typical food processing conditions like high salt concentration, low water activity, extreme pH, and refrigeration temperatures. During food processing, it can adhere to surfaces that encounter food, such as SS [15], and can establish long-lasting subtypes within food production facilities. These subtypes have the potential to remain in the environment for a number of years [16]. This can increase the chances of food contamination during or after processing. The ability of the bacteria that can survive and thrive in food-processing environments, as well as multiply even in refrigeration temperatures, makes it pose a significant risk to public health. Moreover, it could cause an inevitable cost to the economy since contamination with *L. monocytogenes* is a major microbiological cause of food recalls, particularly in meat, poultry, seafood, and dairy products [13]. Furthermore, ingesting *L. monocytogenes* can result in different levels of gastroenteritis, and in immunocompromised individuals, the spread of the bacteria throughout the body can be fatal [17]. While all 13 serotypes of *L. monocytogenes* can result in human listeriosis, serotypes 1/2a, 1/2b, and 4b are responsible for the majority of cases (89 to 96% of cases) and most of the outbreaks were caused by serotype 4b [2], [18].

Hence, significant changes in plant layout, equipment design, cleaning and sanitization procedures, and personnel practices have been required to control the occurrence and proliferation of *L. monocytogenes* in these environments. Conducting microbiological tests of the processing environment and the equipment in use is essential to identify potential niches where *L. monocytogenes* might be present. For instance, hollow rollers on conveyors, support rods and on/off valves are some of the niches which mostly are made of SS. By managing the establishment and multiplication of this bacteria in such settings, it is possible to decrease, and

in some cases even prevent, the likelihood of product contamination through sanitation procedures [2].

In general, bacteria that adhere to surfaces tend to be less susceptible to cleaning and disinfection products compared to bacteria in suspension. Adhered *Listeria* cells, in particular, exhibit greater resistance to biocides compared to *Listeria* cells in suspension [19]. The presence of soil on the surface and prolonged adhesion can further reduce the susceptibility of *Listeria* cells to biocides. However, the influence of different types of food soiling on the sensitivity of surface-attached *L. monocytogenes* to different agents is not yet well understood [20].

2.2 Food Soils

Most food preparation equipment in the food industry is typically made of SS. It is essential to regularly clean this equipment to prevent the accumulation of organic material and microorganisms. Hygienic food contact surfaces can contain a mixture of organic material (food soil), inorganic material (cleaning agent residue), and microorganisms. The composition of this mixture, including both viable and inert components, will vary depending on the specific environment and conditions. [21]

The cleaning process aims to remove the accumulated material and microorganisms from the surfaces effectively. Proper cleaning procedures, including the use of suitable cleaning agents and techniques, are necessary to ensure the removal of both organic and inorganic residues, as well as the elimination of harmful microorganisms. Regular and thorough cleaning of food contact surfaces is crucial to maintain hygiene standards, prevent

cross-contamination, and ensure the safety and quality of the food products being prepared. [21]

When organic materials, with or without microorganisms, encounter surfaces, they can transfer onto the surfaces, a process referred to as soiling. Organic soiling of a surface can have various effects on cell-substrate interactions and introduce additional interactions between cells, soil, and the substrate. The presence of organic material on a surface can impact the properties of the substrate and affect the attachment and retention of cells. The interactions between cells, organic material, and the substrate can contribute to surface fouling, which is the accumulation of unwanted substances on the surface. Furthermore, the presence of organic material can influence the efficacy of cleaning regimes. [22]

Understanding the influence of organic soiling on substratum properties, cell attachment, and retention is crucial for managing surface fouling and developing effective cleaning protocols. By considering the impact of organic material on surface interactions, proper cleaning practices can be implemented to ensure the removal of organic soiling and maintain the cleanliness and functionality of the surfaces in various applications, including the food industry. [22]

Table 1 displays the results of a previous study investigating the effects of UV-C lamps operating at 254 nm on inactivating *L. monocytogenes* with various soiling agents on SS [7]. The data presented in the table illustrate the impact of different types of soiling agents on bacterial growth and log reduction values following treatment with three different UV fluences. Deionized water samples served as the control group with no soiling agents. The results

indicate that soiling agents, particularly those containing proteins or other nutrients, promoted bacterial growth, leading to higher initial loads. Furthermore, each type of soiling agent exhibited a distinct behavior regarding its effect on UV disinfection and the resulting log reduction values. The results presented in the table indicate that higher levels of protein and fat in pork and chicken juices can hinder the damaging effects of UV radiation on microorganisms present on stainless steel surfaces. This highlights the complex nature of food, which consists of various nutrients and forms a matrix that can influence the effectiveness of UV radiation in eliminating foodborne pathogens.

Table 1 - Results of previous study on the effect of soiling agents on inactivation of *L. monocytogenes* on SS using UV 254 nm method [7].

		Type of Soiling Agent					
	UV Fluence (mJ/cm^2)	Deionized Water	TSB	Pork Extract	Chicken Extract	Cabbage Juice	Milk
Log of Initial Load		4.23	5.52	5.87	6.14	4.31	6.66
Log reduction after 30 min treatment	424.8	1.83	1.57	1.07	1.18	2.83	4.85
Log reduction after 60 min treatment	849.6	2.32	3.71	1.81	1.48	2.83	5.18
Log reduction after 120 min treatment	1699.2	2.75	2.27	1.76	2.11	1.94	2.53

2.3 Surface Decontamination Methods

In the food manufacturing and service industries, significant efforts have been made to enhance food safety standards [23]. It has been traditionally recognized that surfaces that come into contact with food have the potential to harbor and support the survival and multiplication of pathogenic bacteria, leading to the risk of cross-contamination of food products [24].

Bacterial contamination on food-contacting surfaces can occur in various settings, including food processing facilities, catering establishments, and even domestic environments. This is particularly concerning as major foodborne pathogens have the opportunity to come into contact with and be transmitted through surfaces. Contamination can happen either through direct physical contact with contaminated objects or indirectly through the presence of airborne particles [25].

With the increase in foodborne disease outbreaks, particularly in the context of increasing antimicrobial resistance (AMR), there is an urgent need for innovative preventive control methods to restrict the distribution of pathogenic species. Microbial contamination can arise at any stage of food production, from pre-harvest to harvest and post-harvest preparation. To guarantee the production of safe food, it is critical to conduct sufficient investigation, detection, and monitoring of all production phases [26]. **Figure 2** shows an overview of various disinfection techniques currently being used in food industries either for sanitizing food contact surfaces or the food itself. In this section, the drawbacks or disadvantages of some methods mentioned in **Figure 2** are elaborated upon.

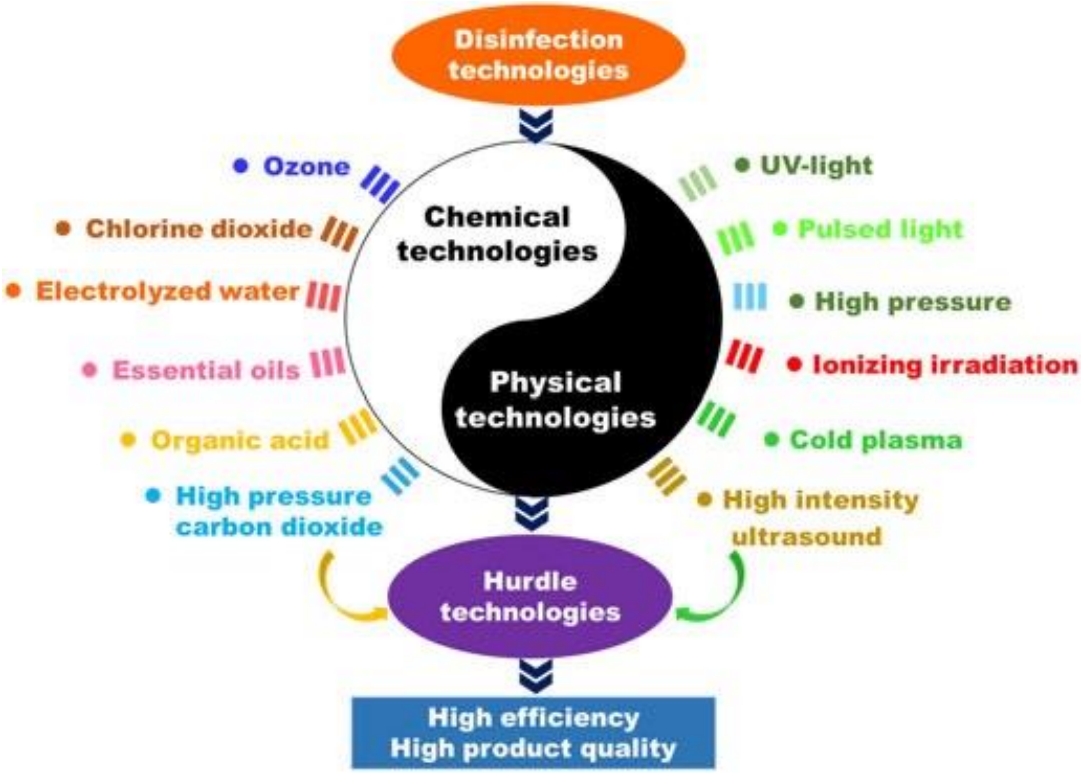


Figure 2 - Applicable food and food contact surfaces disinfection methods [27].

The currently approved sanitizers for food-contact surfaces, such as chlorine, iodine, and quaternary ammonium compounds (QACs), have shown limitations in effectively controlling outbreaks as they may allow pathogens, their spores, or toxins to persist after treatment. Furthermore, the residue of biocidal chemicals on surfaces can lead to chemical food pollution and unwanted alterations of the food products, including structural modifications, formation of free radicals, and textural changes in addition to causing corrosion in metals. Sub-lethal treatments may also enhance pathogenicity and antimicrobial resistance by triggering bacterial stress responses and altering gene expression within the species [28].

Plasma sterilization is a newer method of sanitization, but it involves subjecting food items to intense electric fields and reactive gas species, which can negatively impact the

physical and chemical structure of the food and materials. The effects of plasma sterilization on fresh fruits and vegetables include changes in color, while in meats, it can lead to protein denaturation and affect chemical quality. The severity of the treatment conditions plays a role in determining the extent of these effects [29].

Thermal methods are commonly employed for disinfection in the food industry, particularly for food products themselves. Traditional thermal-based food processing methods, such as appertization, pasteurization, and canning, rely on high temperatures to achieve extended shelf life and ensure food safety. While these thermal processes effectively eliminate microbes, they can also lead to undesirable modifications in the food matrix. These modifications include structural changes in proteins and polysaccharides, generation of free radicals, impact on food functionality and flavor, texture softening, and degradation of colors and vitamins [30].

Germicidal ultraviolet (UV) devices that emit UVC irradiation have found widespread use in surface disinfection. These devices come in various configurations, such as UV cabinets, portable area robot-like disinfection units, and overhead or lower room systems [31]. Compared to conventional approaches that involve using products containing chemicals, soap, or detergent, UVC surface disinfection offers several advantages. These include high effectiveness in inactivating microorganisms, no need for chemicals or detergents, and limited to no material corrosion. UVC irradiation has been proven to effectively inactivate a wide range of microorganisms, including bacteria, viruses, and fungi. Unlike chemical-based methods, UVC surface disinfection does not require the use of chemicals, eliminating the potential risks associated with chemical exposure and the release of harmful gases.

Additionally, UVC irradiation generally does not cause significant corrosion or damage to materials, making it suitable for use on various surfaces without compromising their integrity. Overall, UVC surface disinfection provides an efficient, chemical-free, and safe alternative for effectively eliminating pathogens on surfaces [32]. However, there are also some limitations in UVC application including but not limited to shadowing, pathogen coating and logistical challenges and costs. Pathogens that are shielded by shadows from objects are afforded protection as the UV radiation is unable to reach these pathogens. The disinfection effectiveness may be impeded by the shielding effect caused by the surrounding medium of the infectious microbe. As the size of the particles increases, the shielding effect becomes more prominent. The practical implementation of UV disinfection systems is hindered by logistical challenges, such as the operation, scheduling, and transportation of UV fixtures. These factors pose limitations to the widespread adoption of these disinfection systems. [33]

2.4 Ultraviolet Light

The use of UV light as a surface decontamination method has gained significant interest in recent years. Regulatory guidelines for the use of UV-C light, both continuous and pulsed, in the United States are provided by the FDA. In the European Union, UV-C light can be utilized; however, in Germany, its use is limited to specific applications such as water treatment, fruit and vegetable products, and stored hard cheeses [10].

2.4.1 Types of UV Lamps

There are various types of UV lamps including low pressure mercury lamps, UV LED, and PL technology.

PX-UV lamps are considered more environmentally friendly compared to continuous-wave UV lamps because they do not utilize mercury [34]. Conventional UV-C treatment methods have certain drawbacks, including limited penetration depth, low emission power, high mercury vapor content, and extended exposure times [35].

UV-LED lamps have emerged as another method for disinfection, also offering several advantages over mercury lamps. These advantages include the absence of toxic mercury, compact and flexible designs, instant operation without warm-up time, high durability, emission of monochromatic light at specific wavelengths, diverse wavelength options, potential for pulsed illumination, and the ability to maintain effectiveness at cold temperatures (such as in refrigeration). However, there are limitations to the use of LED lamps as a bactericidal technology. One limitation is the short penetration depth of UV light, which can reduce effectiveness in inactivating bacteria located deeper within solid or liquid media. Additionally, there is a concern regarding the potential reactivation of UV-injured bacterial cells following treatment with UV LED, which could compromise the overall effectiveness of the disinfection process and raise safety concerns [36].

PL technology offers several advantages compared to static UV treatment. One notable advantage is the rapid delivery of energy within a short time frame. Additionally, PL systems are cost-effective and generate minimal solid waste [37]. The benefits of PL include mitigating

the risk of foodborne pathogens on public health, extending the shelf life of products, and improving the economics of food distribution [38]. PL holds promise for applications in food processing that necessitate fast disinfection, particularly in scenarios where surface contamination poses a concern for microbial presence, such as with fresh produce, hard cheeses, meat slices, and other similar food commodities [39].

Table 2 presents a comprehensive overview of the inactivation of *Listeria* strains using various types of UV lamps, facilitating a more effective comparison with the findings of this study. Based on the table, it can be observed that the lamp utilized in this study achieved similar levels of bacteria inactivation compared to other PL lamps but with lower UV fluence values. Nevertheless, it is crucial to consider that the required UV fluence is highly influenced by both the irradiated surface and the characteristics of the UV lamp. Additionally, the log reductions attained with low-pressure mercury lamps are considerably lower when compared to PL and LED lamps.

Table 2 - Review of Listeria strains inactivation using various types of UV lamps.

Lamp Type[1]	UV Fluence (mJ/cm^2)	Exposure Time (sec)	Log reduction	Surface Type	Strain	Reference
PX-UV	60	45	4	Stainless Steel	*L. monocytogenes*	This study
PL	6000	NA	4.08	Stainless Steel	*L. innocua*	[8]
PL	12000	NA	<4	Stainless Steel	*L. innocua*	[40]
Intense pulsed light (IPL)	1600	180	6	Solid medium	*L. monocytogenes*	[41]
IPL	500	60	4	Solid medium	*L. monocytogenes*	[41]
PL	400	NA	7	Bacteria suspension	*L. innocua*	[42]
Pulsed UVC-LED	5	NA	3	Selective Agar	*L. monocytogenes*	[43]
Deep-UV LED	>20	300	6-7	Stainless Steel	*L. innocua*	[36]
UVC LED	7	70-80	>4	Bacteria suspension	*L. monocytogenes*	[44]
Low Pressure Mercury (LPM)	NA	1000	4	Solid medium	*L. monocytogenes*	[41]
LPM	5	NA	~2.5	Selective Agar	*L. monocytogenes*	[43]
LPM	7	22	4	Bacteria suspension	*L. monocytogenes*	[43]

1. Based on the explanation provided in the relative cited references, IPL, PL and PX-UV are all the same types of UV lamps containing xenon as their gas but with different lamp characterization which caused the writers named the lamps differently. Deep UV-LED radiates in the range of 200-300 nm while UVC- LED work in a monochromator way and pulsed UVC-LED operates at wider range of wavelengths (200-1100 nm).

2.4.2 UV Inactivation Mechanism

UV light is a potent agent for inactivating a wide range of microorganisms present in air, water, or on various surfaces. The mechanism of cell inactivation involves the damaging effects of UV light on nucleic acids, specifically DNA and RNA. UV light is absorbed by nucleic acids within the wavelength range of 200 to 310 nm. When nucleic acids absorb UV light, it leads to the disruption of chemical bonds, resulting in the formation of pyrimidine dimers. These dimers occur when adjacent pairs of thymine or cytosine pyrimidines on the same DNA or RNA strand become bonded together [45]. The formation of dimers from thymine (T) and cytosine (C) can occur in three possible combinations: T<>T, C<>T, and C<>C, listed in order of their occurrence. Thymine dimers are more likely to form due to two reasons: first, thymine has higher absorbance than cytosine in the germicidal range, and second, the quantum yield for the formation of T<>T is greater than that for the formation of C<>C and C<>T [46]. The formation of these dimers impairs the ability of cells to replicate their genetic material accurately. Consequently, microorganisms exposed to UV light and experiencing the formation of pyrimidine dimers become inactive and lose their ability to proliferate. This mechanism effectively hinders their growth and renders them unable to cause infections or contamination [45].

2.4.3 PX-UV Lamps Technology

PL technology is a non-thermal method that utilizes high-intensity light pulses for a short duration of time to achieve the decontamination of various surfaces and food products such as fruit juices, meat products, vegetables, and fruits. The PL system encompasses a broad range of wavelengths, spanning from 200 to 1100 nm. This range includes UV light with wavelengths

of 200-400 nm, visible light (VIS) with wavelengths of 400-700 nm, and the near-infrared region (IR) with wavelengths of 700-1100 nm [39]. PL offers significant advantages, such as rapid microbial reduction in a short treatment duration, minimal environmental impact, and great flexibility [35].

The process of microbial inactivation by PL lamps can be understood through the absorption of photons by DNA, which follows the principles of photochemistry and photophysics. These principles provide a foundation for characterizing PL processes. However, it is important to note that the effects of PL on microorganisms are complex, and further investigation is needed to fully understand these effects. The interplay between light and microorganisms involves various factors, and a comprehensive understanding of the mechanisms requires a multidisciplinary approach [47].

The inactivation mechanisms of PL can be categorized as follows [48]:

a) Photochemical effect: Microbial inactivation is primarily caused by chemical changes in the DNA and RNA. The formation of thymine dimers is a major photochemical change associated with microbial inactivation. Additionally, there may be other minor chemical bond formations and/or breakages in bacteria.

b) Photothermal effect: longer duration of PL treatments result in a significant increase in temperature. Due to the different heating rates of bacterial cells and the surrounding media, localized heating of bacteria occurs, leading to cell death. However, short-duration treatments lasting less than 10 seconds have a negligible photothermal effect, as there is only a minimal temperature increase.

c) Photophysical effect: The constant disturbance caused by high-energy pulses can cause structural damage to bacterial cells. Optimizing the pulse width and the number of pulses can enhance the effectiveness of PL treatment by maximizing the photophysical effect. However, this optimization was not done in the cited reference to mention here.

Figure 3 illustrates the examination of structural damage inflicted on *L. monocytogenes* cells to gain insights into the mechanism of PL treatments.

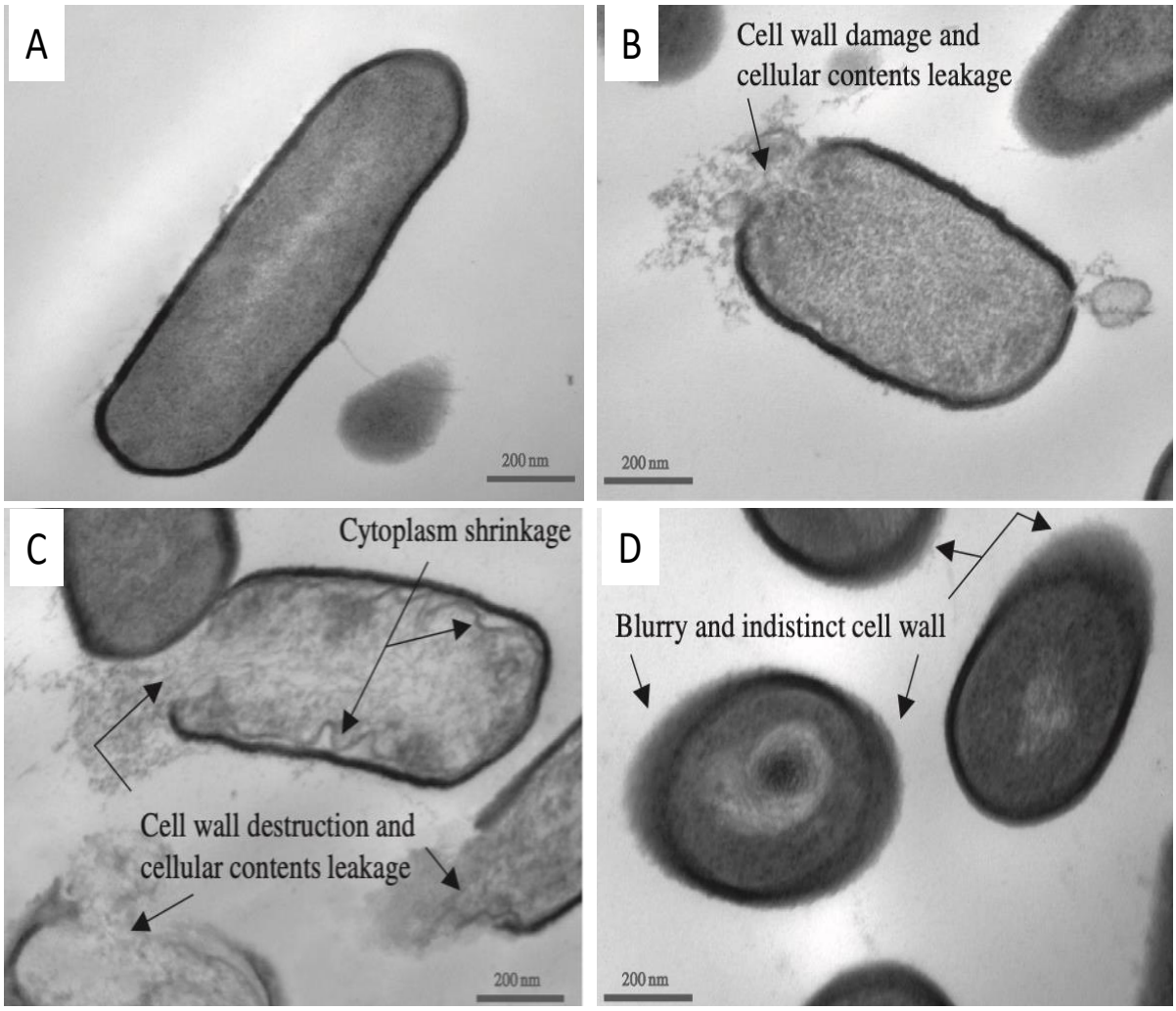


Figure 3 - Transmission electron micrographs of *L. monocytogenes* under treatment conditions of: (A) Untreated control cells, (B) Treated with PL for 150 pulses (30 s), (C) Treated for 900 pulses (180 s) under fluence of fluence of 1.75 mJ/cm^2 per pulse, (D) cells treated with UV-C for 1000 seconds at 254 nm. The bar indicates a length of 200 nanometers [41].

Transmission electron micrographs (TEM) analysis of *L. monocytogenes* cells treated with PL and UV-C revealed distinct structural changes. In the case of PL treatment, **Figure 3B** (150 pulses) and **Figure 3C** (900 pulses) demonstrated significant damage to the cells, including destruction of the cell wall, cytoplasm shrinkage, and leakage of cellular contents.

The extent of cell damage increased with the number of PL pulses. This damage induced by PL treatment could lead to cell death. On the other hand, *L. monocytogenes* cells treated with UV-C for 1000 seconds (**Figure 3D**) exhibited a similar cell shape to the untreated control cells (**Figure 3A**), with the exception of a blurry and indistinct cell wall [41].

This strongly demonstrates that the mechanism of inactivation for pulsed UV light differs from that of continuous UV light. Previous studies have suggested that pulsed UV light can be up to four to six times more effective in inactivating microorganisms compared to continuous UV light [49]. This increased effectiveness is believed to be due to the photophysical and photothermal effects associated with pulsed UV light. However, it should be noted that the current findings are based on a limited number of studies conducted in this particular field, and there remains a lack of research on the effectiveness of pulsed UV lights under various conditions. Therefore, further investigation and confirmation are necessary to establish a more comprehensive understanding in this regard.

The effectiveness of PX-UV disinfection is rooted in the technological principle of storing high-discharge voltage in a capacitor, which is then released in ultra-short bursts through a xenon-filled light source. This light source emits a broad-spectrum light flash, including approximately 25% in the UV range. PX-UV lamps has been shown to provide superior disinfection of food surfaces compared to LP-UV due to its high peak power and the ability to deliver stored energy in short pulses, typically at a rate of 1 to 10 pulses per second [50].

2.4.4 UV Limitations in the Food Industry

The limitations of UV technology as a surface food sanitizer are primarily associated with its inability to effectively treat uneven food surfaces that contain crevices where microorganisms can reside and remain inadequately decontaminated due to shading effects. Additionally, the presence of organic material and large microbial populations, such as in biofilms, can offer protection to spoilage and pathogenic microorganisms during UV treatments, again due to shading effect issues [52].

Additionally, there is a growing interest in the application of PL-technology to address complex challenges, such as foodborne parasites (e.g., the bumblebee trypanosome parasite *Crithidia bombi*). However, this poses additional challenges compared to conventional viable count methods. Evaluating the effectiveness of PL decontamination requires the use of animal infectivity or alternative quantitative PCR approaches, along with mammalian cell culture bioassays. This introduces difficulties in determining and interpreting the significance of inactivation kinetics, which were previously based solely on microbial culture-based methods. Nevertheless, accurate characterization of microbial inactivation kinetics is crucial for process optimization. As a result, an increasing number of researchers are utilizing resistant bacterial endospores to assess the performance of PL disinfection against foodborne parasites [26].

Safety considerations are also important when using UV technology. Direct human exposure to UV irradiation, especially from UVC devices, should be avoided during surface disinfection. Before applying UVC devices in occupied spaces, it is important to evaluate their safety. UV devices are known to pose risks to human skin and eyes. Exposure to these lamps

can cause erythema (skin redness) and photokeratitis (inflammation of the cornea) in individuals [51]. However, it is worth noting that reflected UV irradiation from surfaces can still pose health risks, particularly from highly reflective surfaces when using devices like UV wands and handheld devices [32]. Apart from concerns related to skin exposure, it is important to be aware that Far UVC devices (emitting at 200-230 nm) have the potential to generate ozone through photochemical reactions and electric discharges. This can lead to symptoms such as cough, throat irritation, and shortness of breath. Therefore, proper precautions should be taken to minimize these risks and ensure the safe use of UV devices. It is noted that commonly used xenon lamps are typically made from doped "ozone-free" quartz, which means they do not generate ozone during operation. The use of ozone-free xenon lamps helps to minimize potential health risks associated with ozone exposure [32], [53].

Indeed, the effectiveness of PL lamps for decontamination is influenced by various factors. The duration of exposure to pulsed UV light, the intensity of the light, and the specific wavelength used all play a role in determining the level of microbial inactivation. Additionally, different microorganisms have varying degrees of susceptibility to UV light, so their ability to withstand UV exposure can impact the effectiveness of the treatment [10].

The properties of the surface being treated also come into play. Surfaces with higher roughness or irregularities may provide hiding places or shielded areas where microorganisms can be protected from the UV light, reducing the overall effectiveness of the treatment [54].The penetration of UV light into the material or substance being treated is another important factor. UV light has limited penetration depth, so it may be less effective in inactivating

microorganisms that reside deeper within solid or liquid media [54].Furthermore, the presence of particles or debris can act as shields, blocking or absorbing the UV light and reducing its reach and effectiveness in reaching the microorganisms [54]. Considering all these factors is essential in optimizing the decontamination process and ensuring effective microbial inactivation with PL lamps.

One limitation of PL technology is the lack of standardization in PL system configurations and exposure conditions, particularly when using xenon light sources. This variation makes it exceptionally challenging, if not impossible, to compare research findings across different studies and applications of PL for food treatment [26]. To further elaborate on some of these variations, it can be mentioned that due to the wide range of parameters that can be adjusted in PL systems, such as pulse duration, pulse frequency, energy density, and wavelength, it becomes difficult to establish a consistent basis for comparison between studies. Additionally, the specific design and setup of PL systems can vary among research groups, further contributing to the lack of standardization.

Therefore, efforts towards standardizing PL system configurations, exposure conditions, and reporting methods are necessary to facilitate meaningful comparisons and enhance the overall reliability and applicability of PL technology in various food treatment scenarios [26].

2.4.5 Summary

The literature suggests that PX-UV lamps are widely recognized as an effective technology for microbial inactivation and surface disinfection. However, it is important to note that different types of UV lamps can have varying effects on different surfaces and microorganisms. Therefore, thorough studies are necessary to assess the efficacy of each lamp before implementing them on a large scale. Specifically, there is a lack of research on the application of PX-UV lamps for surface disinfection in the food industry, particularly in the presence of *L. monocytogenes* bacteria. This thesis aims to address these gaps by investigating the disinfection efficacy of PX-UV lamps and studying the impact of various factors mentioned earlier.

Chapter 3
Materials and Methods

3.1 Strain and Culture Conditions

The bacterial strain used in all experiments was *Listeria monocytogenes serotype 4b* ATCC 19115 (American Type Culture Collection, Manassas, VA). The strains were sub-cultured every 2 weeks on Listeria Selective Agar Base or Tryptic Soy Agar (TSA) and incubated at 37°C. The sub-cultured strains were then stored at 4°C until further use.

Listeria selective agar base was prepared by combining 17 g of Tryptone powder (Bio Basic, Markham, Ontario, Canada), 3 g of Peptone B Soy Protein (Bio Basic, Markham, Ontario, Canada), 6 g of yeast extract (Bio Basic, Markham, Ontario, Canada), 5 g of sodium chloride (VWR international, Radnor, Pennsylvania, United States), 2.5 g of dipotassium hydrogen phosphate (VWR international, Radnor, Pennsylvania, United States), 2.5 g of dextrose (Merck KGaA, Darmstadt, Germany), and 15 g of Agar A (Bio Basic, Markham, Ontario, Canada) in 1000 mL of ultra-pure water. The final pH of the medium was adjusted to 7.3 ± 0.2 at 25°C.

Tryptic Soy Agar (TSA) was prepared by combining 17 g of Tryptone powder, 3 g of Peptone B Soy Protein, 5 g of sodium chloride, 2.5 g of dipotassium hydrogen phosphate, 2.5 g of dextrose, and 15 g of Agar A in 1000 mL of ultra-pure water. The final pH of the medium was adjusted to 7.3 ± 0.2 at 25°C.

To prepare the overnight culture, a single colony of bacteria from the agar plate was transferred into a 125 mL flask containing 25 mL of Tryptic Soy Broth (TSB) media. The flask was then incubated at 37°C in an orbital shaker at a speed of 200 rpm for 16-18 hours.

TSB media was prepared by combining 17 g of Tryptone powder, 3 g of Peptone B Soy Protein, 5 g of sodium chloride, 2.5 g of dipotassium hydrogen phosphate, and 2.5 g of dextrose in 1000 mL of ultra-pure water. The final pH of the medium was adjusted to 7.3 ± 0.2 at 25°C.

After the incubation period, the culture was concentrated 10-fold into Phosphate Buffer Saline (PBS) by centrifuging 1 mL of the overnight culture for 2 minutes at 6500 rpm. The resulting pellet was washed with 100 µL of PBS. In experiments involving soiling agents, the unsoiled set was washed with 100 µL of PBS, while the soiled sets were washed with 100 µL of the corresponding soiling solution. The composition of the soiling solutions will be described in detail in Section 3.3.

PBS was prepared by adding 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of sodium phosphate dibasic, and 0.24 g of potassium phosphate monobasic in 1000 mL of ultra-pure water. The final pH of the solution was adjusted to 7.4 ± 0.2 at 25°C.

All the mentioned media were sterilized by autoclaving at 121°C for 20 minutes using the fluid cycle.

3.2 Stainless-Steel Coupons Preparation

Type 304 SS coupons with a diameter of 2.5 cm and a 2B dull finish on both sides were selected for all experiments to simulate the surfaces of SS commonly encountered in food processing environments.

To ensure the coupons were free from contaminants, they were immersed in 100% ethanol for a period of 1-2 days. Afterward, the coupons were placed in an aluminum-covered beaker and subjected to autoclaving at 121°C for 20 minutes using the dry cycle to sterilize.

Surface roughness of the coupons was measured using LEXT 3D measuring Laser Microscope (OLS5000) (Olympus Corporation, Tokyo, Japan). The parameter which represents surface roughness is root mean square height (Sq) which is a three-dimensional expansion of the line roughness of root mean square deviation (Rq) and the schematic is shown in **Figure 4**. The device calculates Sq using **Eq.1**:

$$Sq = \sqrt{\frac{1}{A}\iint_{A} Z^2(x,y)dxdy} \qquad \text{(Eq.1)}$$

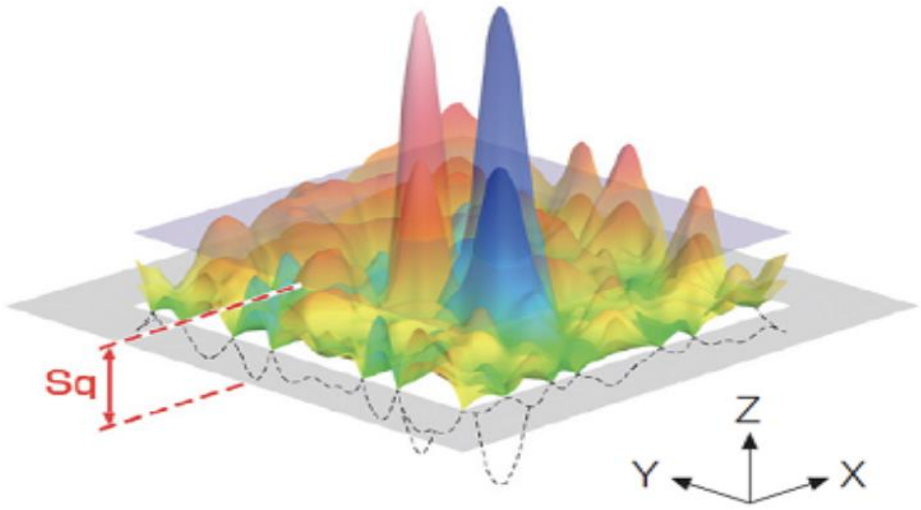


Figure 4 - Schematic of the root mean square height (Sq) [55]

3.3 Soiling Agents Preparation

For the study, three categories of soils were chosen: proteins, carbohydrates, and lipids. The specific substances used for each category were as follows:

1. Proteins:
    - Bovine Serum Albumin (BSA) (Sigma Aldrich, St. Louis, Missouri, USA)
    - Peptone-A (Bio Basic, Markham, Ontario, Canada)
    - Casamino Acid (Bio Basic, Markham, Ontario, Canada)
2. Carbohydrates:
    - D-Lactose monohydrate (Bio Basic, Markham, Ontario, Canada)
    - Glycogen (Fisher Scientific Company, Hampton, New Hampshire, USA)

- Potato Starch (Sigma Aldrich, St. Louis, Missouri, USA)
- Trehalose (Sigma Aldrich, St. Louis, Missouri, USA)

3. Lipids:
    - Triolein (Sigma Aldrich, St. Louis, Missouri, USA)
    - Fish Oil (menhaden from Sigma Aldrich, St. Louis, Missouri, USA)

To prepare the soiling solutions, the desired amount of each chemical was added to PBS to achieve the preferred concentration (%W/V). The solutions were then vortexed until complete dissolution was achieved. After complete dissolution, each solution was filtered using 0.2 μm polyethersulfone (PES) syringe filters (VWR international, Radnor, Pennsylvania, United States) to remove any remaining particles, ensuring a clear and homogeneous soiling solution.

3.4 UV Spectrophotometry of Soiling Agents

UV spectrophotometry was done using diode array spectrophotometer (Hewlett Packard 8452A, hp, Palo Alto, California, USA). This device is able to measure UV absorbance in the spectrum of 180 nm to 800 nm. Quartz cuvettes with 1 cm pathlength were used in all measurements. Measurements were done to study Beer-Lambert law **(Eq.2)** for each soiling agent and finding out the desired concentration of soils due to absorbance. Concentrations were selected based on the absorbance amount on 254 nm since UV fluences measured with a sensor calibrated at 254 nm and so this makes possible comparisons easier.

$$A = \varepsilon bc \quad \text{(Eq.2)}$$

Where, A = Absorbance, ε = molar absorbance coefficient, b = pathlength and c = concentration.

3.5 Inoculation and UV Treatment

Experiments were done following a modified ASTM E3135-18 method (American Society for Testing and Materials) [56]. The cleaned and sterilized SS coupons were placed in a sterile biosafety cabinet and ten 1 µL droplets of the concentrated culture were placed on each coupon and air dried at room temperature until they disappeared (15-20 minutes). In each case, three coupons were used as a positive control (no treatment), three were used as a negative control for sterility, and three were used for each treatment combination being tested. In experiments involving soiling agents, the negative controls consisted of ten 1 µL droplets of the respective soiling solution. After air-drying, each positive control and negative control coupons were placed in a 50 mL conical tube containing 10 mL of sterile PBS. Treatment coupons were exposed to a PX-UV lamp (Solaris Robots, Mississauga, ON, Canada) positioned at a distance of 19 cm from the target (unless otherwise specified). The exposure time and frequency were adjusted to achieve the desired UV fluence. Following UV treatment, the treatment coupons were transferred to separate 50 mL conical tubes containing 10 mL of sterile PBS. All tubes, including positive controls, negative controls, and treatment coupons, were agitated for 20 minutes in an orbital shaker set at 25°C and 150 rpm. This step facilitated the dislodging of any bacteria from the coupons into the PBS solution. To enumerate bacterial colonies, the spread plate method was employed. Ten-fold sterile dilutions of the samples were prepared using sterile PBS, and 100 µL of each dilution was spread onto Listeria selective agar base or

TSA plates. The plates were then incubated at 37°C for 24-48 hours for colony growth. The colonies were counted and expressed as colony-forming units per milliliter (CFU/mL) using **Eq. 3**.

$$\frac{Number\ of\ Colonies}{mL}\left(\frac{CFU}{mL}\right) = \frac{Number\ of\ Colonies}{0.1} \times \frac{1}{DF} \quad \text{(Eq.3)}$$

Where DF is the dilution factor defined by dividing the total volume by the volume of aliquot. Only plates with counts between 20-200 were used for calculating the log reduction. Log reduction was calculated using **Eq.4**.

$$Log\ reduction = \log_{10}(\frac{PC}{T}) \quad \text{(Eq. 4)}$$

Where, PC = number of colonies on positive control agar plate (CFU/mL) and T: number of colonies on treatment agar plate (CFU/mL).

3.6 Radiometry

UV fluence were measured using Flash application of ILT2500 radiometer (International Light Technology, Peabody, MA 01960, United States) which is designed to measure the light of pulsed light sources including PX-UV lamp. The radiometer is equipped with a SED240 detector which can measure pulsed light in the wavelength range of 220-300 nm (**Figure 5**) and 0-250 Hz of frequency. The detector was factory calibrated for 254 nm to make it possible to compare the results with low pressure mercury lamps that emit monochromatically at that wavelength.

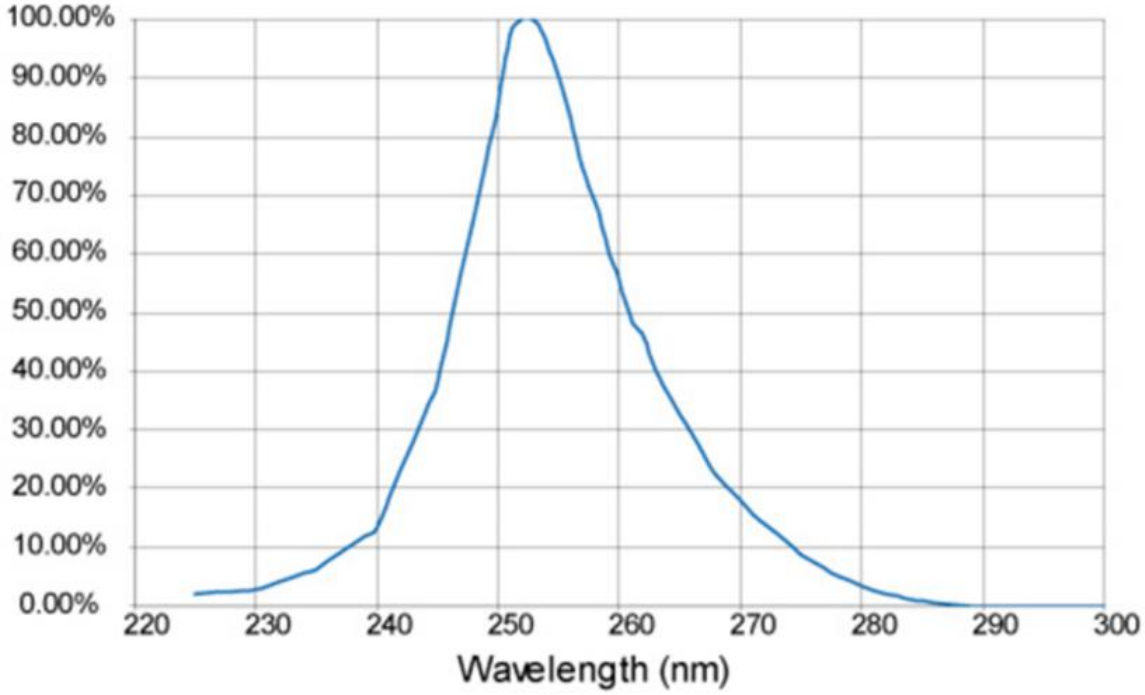


Figure 5 - Measurement range of the SED 240 radiometer sensor [57]

3.7 Mathematical Modeling of UV Disinfection

Bacteria inactivation was modelled in terms of survival (S) rather than log reduction which was calculated using **Eq. 5** [11]:

$$S = \frac{T}{PC} \qquad \text{(Eq. 5)}$$

In many cases, a bacterial population being inactivated with UV light will experience two stage decay where the first stage decay (fast decay) will be considered a vulnerable population (1-*f*) and the second stage (slow decay) will be the resistant population (*f*). The second stage decay model used in this work is as follows **Eq. 6** [11]:

$$S = (1 - f)e^{-k_1 D} + fe^{-k_2 D} \qquad \text{(Eq. 6)}$$

Where f is the UV resistant fraction (slow decay), k_1 is the first stage rate constant (cm^2/mJ), k_2 is the second stage rate constant (cm^2/mJ) and D is the UV fluence (mJ/cm^2). Bacteria survival was fit using linear regression via MATLAB's (MathWorks, USA) curve fitting toolbox.

Chapter 4
Results and Discussion

4.1 UV Lamp Dosimetry

In order to gain a deeper understanding of the UV lamp utilized in this thesis and investigate the impact of operational parameters on irradiance (fluence rate) and total UV fluence, radiometry measurements were conducted. These measurements were performed using different combinations of pulse frequency (ranging from 1 to 25 Hz) and total exposure times (1 to 5 minutes). The obtained results were then visualized in **Figure 6**.

As depicted in **Figure 6A**, it is evident that the UV fluence exhibits a linear relationship with increasing exposure time when the frequency remains constant which is expected. Furthermore, the slope of this relationship becomes steeper as the frequency of radiation rises. Similarly, in **Figure 6B**, it can be observed that increasing the frequency of the UV lamp also leads to a linear increase in fluence at a constant exposure time. However, examining the combined effect of frequency and exposure time in **Figure 6C**, some curvature is apparent within the tested ranges using the radiometer and at lower frequencies, a higher-than-expected UV fluence was received in the same amount of time than in high frequency trials.

The fluence per pulse is calculated and shown in **Figure 6D**. The results show that lower fluence per pulse is delivered at higher frequencies than the lower ones. This is presumably because at higher frequencies the system has less time to reset between pulses than at lower frequencies and the result is lower available energy emitted per pulse. However, in the lowest frequency which is 1 Hz has lower energy per pulse than 5 Hz which could be

related to the electronic properties of the device. To ensure the consistency of the results under various conditions, the fluence per pulse is measured at two different distances between the lamp and the radiometer sensor. The results are depicted in a single graph with two axes, enabling a clear observation of the trend.

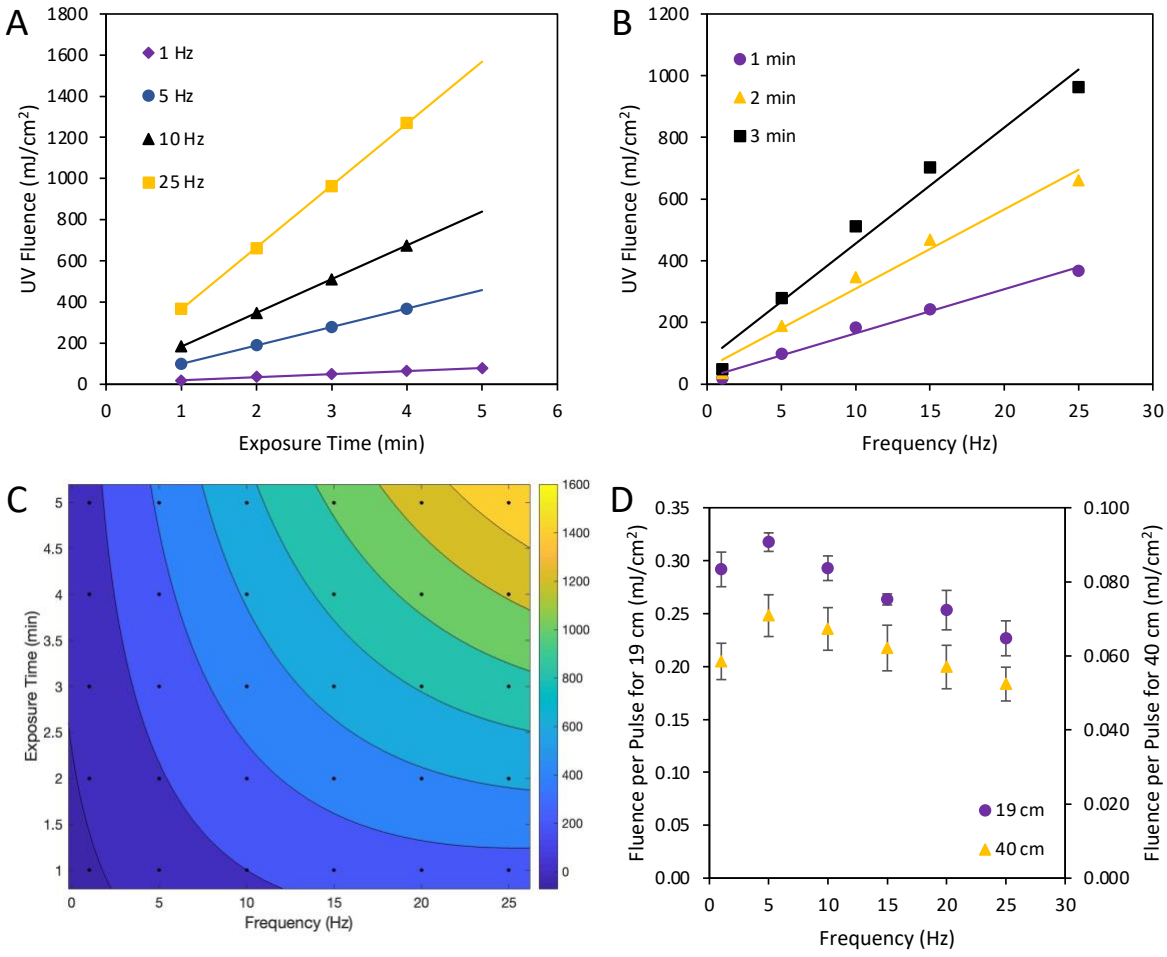


Figure 6 - Pulsed xenon UV lamp dosimetry at 19 cm from the lamp to the detector at (A) constant frequency and varied exposure time, (B) constant exposure time and different frequencies (C) contour plot of frequency and exposure time and (D) fluence per pulse at varied frequency in 2 different distances of lamp to the sensor (n=3).

4.1.1 *L. monocytogenes* Dose Response and UV Disinfection Kinetics

To enhance our comprehension of the specific reaction kinetics involved in the inactivation of *L. monocytogenes* using PX-UV, radiometry was employed to determine the UV fluence in all experiments. Subsequently, the collected data was graphically represented to analyze and explore the intricate dynamics of *L. monocytogenes* inactivation with PX-UV (**Figure 7**).

The overall pattern indicates the presence of two distinct rates influencing the disinfection process. A log-reduction of 3 was achieved with relatively low fluence (< 40 mJ/cm^2), beyond which the log-reduction increases gradually with higher fluences. The data exhibits a relatively high degree of variability, which is common in tests of this nature due to compounded errors from pipetting, variations in laboratory temperature or humidity, and the inherent variability in the density of the initial culture, which was only roughly adjusted.

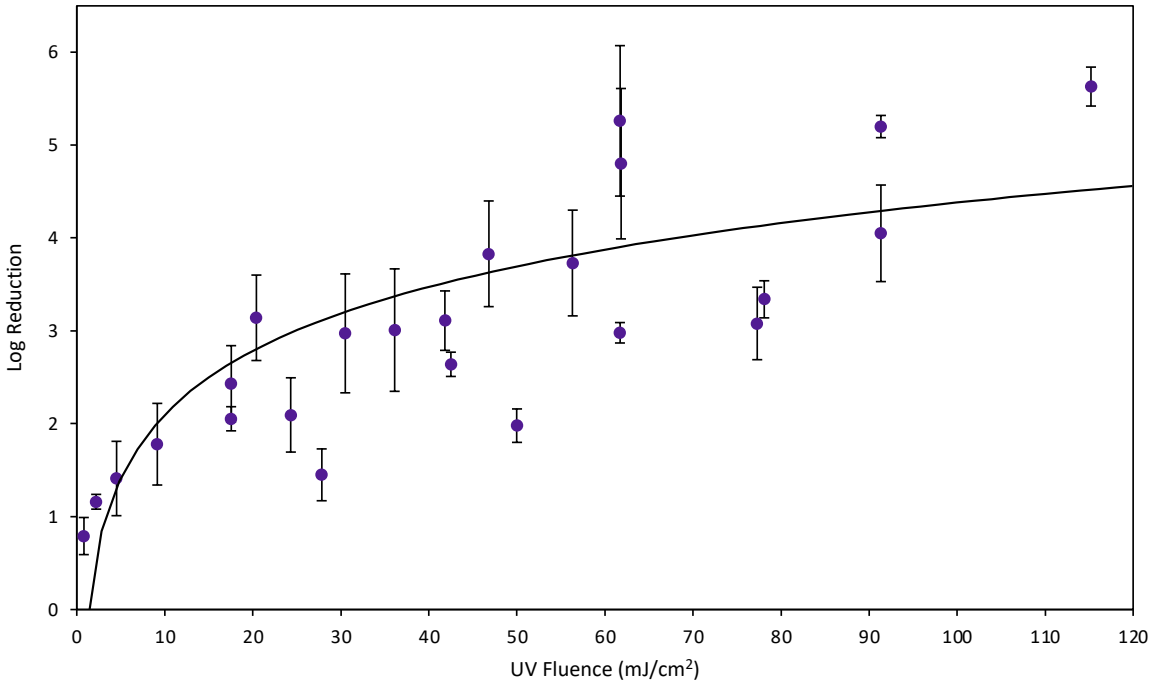


Figure 7 - Dose response of *L. monocytogenes* to Pulsed xenon UV lamp (n = 3).

To examine the potential impact of the initial inoculum concentration on inactivation outcomes, specifically in relation to shielding effects observed at higher densities, a comparison was made between two different inoculum densities across various fluences (**Figure 8**). The findings indicate that, in general, the results for lower fluences remain consistent regardless of the inoculum concentration (6 or 11 log of bacteria). However, at higher fluences, a higher log reduction is achieved when the initial concentration is higher. Though when the p-values calculated for each set of UV fluences, the differences between reductions in 62 mJ/cm^2 and 118 mJ/cm^2 were significant (P = 0.013 and 0.019, respectively).

The difference between reductions in 91 mJ/cm^2 was also marginally significant with P-value = 0.053. This suggests that the extent of inactivation could be influenced by the initial bacterial load on the surface being treated. For example, when examining the highest fluence of 118 mJ/cm^2, it becomes evident that achieving a log reduction greater than 6 is not feasible for a 6-log inoculum. Therefore, a higher level of log reduction can be attained if the initial bacterial load is higher (e.g., log of 11). If shielding effects were present, one would expect lower inactivation at higher initial loads due to the presence of dead bacteria that could still absorb UV light. However, this was not observed, as a consistent population of survivors was found even at high UV fluences (**Figure 9**).

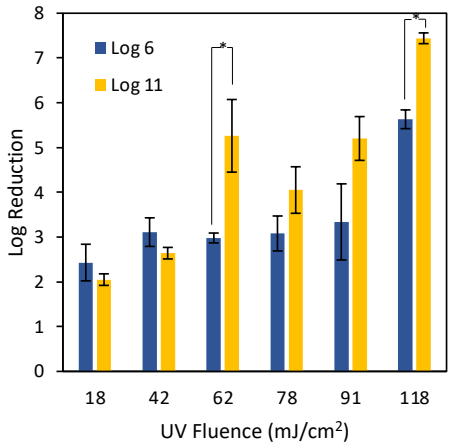


Figure 8 - Dose response of *L. monocytogenes* with two different initial loads (n=3). Asterisks indicate significant differences between log reductions in log 6 and log 11 of initial loads (*, P<0.05).

The disinfection rate can be influenced by various factors, including complexities such as shoulder effects, relative humidity, and photoreactivation, which can result in a two-stage decay. However, it can also be a simple single-stage exponential decay. Additionally,

variations in the exposure fluence can occur due to uneven irradiance fields, whether in air or on surfaces [11]. In this study, dose-response data was utilized to determine the UV disinfection kinetics of *L. monocytogenes* using the PX-UV lamp on SS. Based on the observed data (**Figure 7**), a two-stage decay model, represented by **Eq. 6**, was applied as the kinetic model. Survival fractions were calculated using **Eq. 5.** The data was fitted to **Eq. 7** using linear regression analysis, yielding a coefficient of determination (R^2) of 0.9983. This high R^2 value indicates a strong fit of the data to the equation, resulting in the following equation:

$$S = 0.918\, e^{-2.801D} + 0.082\, e^{-0.113D} \qquad \text{(Eq. 7)}$$

Figure 9 displays the predicted data obtained from **Eq. 7**, along with the actual data. The coefficients at 95% confidence intervals are summarized in **Table 3**.

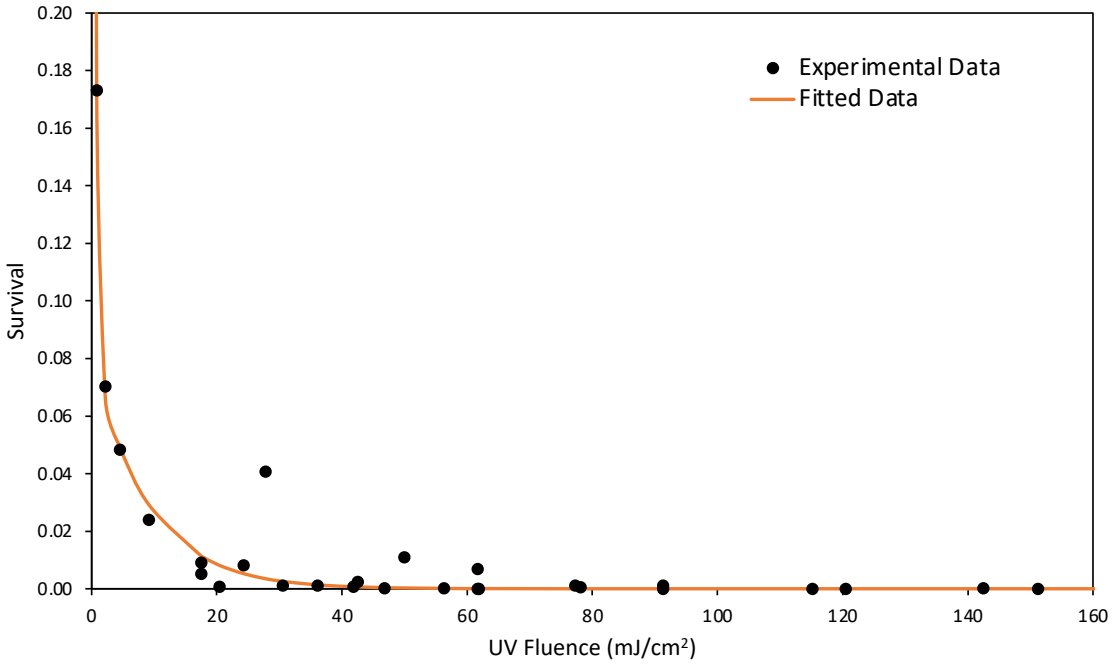


Figure 9 - Survival of *L. monocytogenes* to UV fluence and fitted kinetics.

Table 3 - Coefficients values of UV disinfection model with 95% confidence interval.

Coefficient	Values
f	0.082 ± 0.023
k_1	2.801 ± 0.305
k_2	0.113 ± 0.045

4.1.2 Effect of Exposure Time

Utilizing the insights gained regarding the influence of frequency and time on UV fluence, the PX-UV lamp was employed to treat SS coupons contaminated with *L. monocytogenes*. As anticipated, the log reduction demonstrated an increase as the exposure time increased for frequencies below 10 Hz. Notably, all treatments achieved a remarkable 5-log reduction (99.999%) with less than 5 minutes of UV exposure (**Figure 10**). The log reduction ranged from 2.5 to 7.44, depending on the frequency and exposure time. Particularly, the treatment with high frequency (25 Hz) PX-UV resulted in an impressive 5.59-log reduction in just 30 seconds and in contrast to the observed increasing trend at lower frequencies, there was a more gradual change in log reductions with increasing exposure time at this frequency (**Figure 10D**). This could potentially be attributed to the fact that 25 Hz represents a sufficiently high frequency capable of rapidly inactivating a significant portion of the bacterial cells within a short exposure time. Consequently, there may be a reduced number of bacteria remaining to be inactivated with longer exposure periods.

Furthermore, the observed results align with the expectations derived from the radiometry and dose response analyses. As anticipated, the UV fluence increased with longer

exposure times, leading to higher log reductions. This consistency reinforces and validates the previous findings.

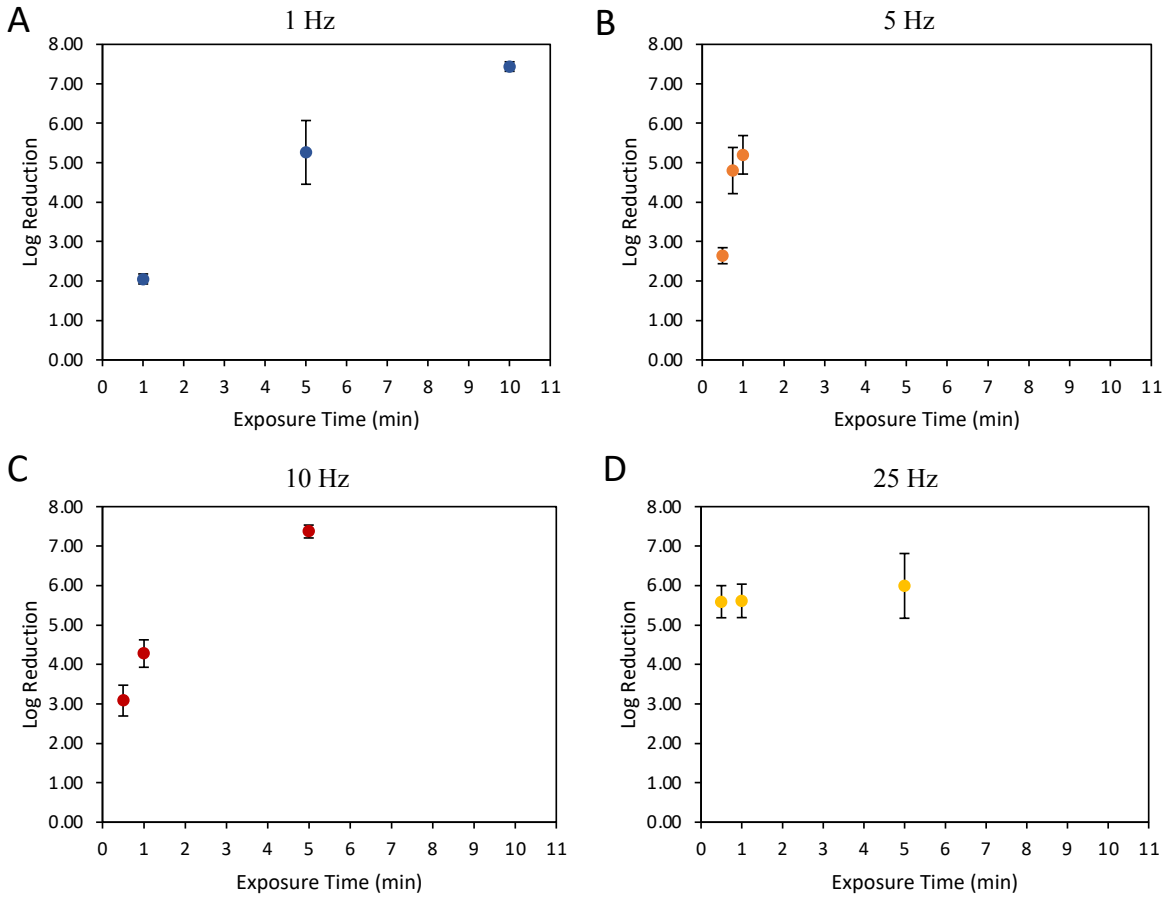


Figure 10 - Inactivation of *L. monocytogenes* on stainless-steel coupons at different PX-UV frequencies (n = 3).

4.1.3 Effect of Frequency at Constant UV Fluence

For further investigation on the impact of pulse frequency, additional experiments were conducted using a constant UV fluence (~20, 55, 100 mJ/cm^2) while varying the frequency and exposure time, as determined by radiometry. The results of these experiments are shown in

Figure 11. It was observed that when the UV fluence was kept constant, the level of bacterial inactivation remained consistent. The average log reduction for all tested frequencies was 2.57 ± 0.57 at 20 mJ/cm^2, 4.84 ± 0.39 at 55 mJ/cm^2, and 4.87 ± 0.16 at 100 mJ/cm^2. These findings indicate that a fluence of 55 mJ/cm^2 was sufficient to effectively eliminate the loaded bacteria from the surface, regardless of the frequency used. Conversely, 20 mJ/cm^2 was found to be too low, while 100 mJ/cm^2 was too high for optimal disinfection as it did not yield a higher reduction compared to 55 mJ/cm^2. Typically, optimal disinfection is achieved when a log reduction of higher than 4 is attained. However, this optimal disinfection depends heavily on the initial bacterial load, which in this thesis was typically around log-6 to log-8. Consequently, the fluence of 55 mJ/cm^2 was selected for further experimentation, encompassing a wider range of frequencies to explore their effects. It is important to highlight that the results obtained from experiments conducted with fluences of 55 and 100 mJ/cm^2 led to similar conclusions. This could be attributed to the fact that a significant portion of the bacterial population had already been inactivated at 55 mJ/cm^2, entering the gradual phase of inactivation as depicted in **Figure 7**.

The PX-UV lamp demonstrated efficacy within a frequency range of 1 to 55 Hz. However, maintaining the desired fluence became challenging at higher frequencies due to the corresponding decrease in fluence per pulse. Consequently, a frequency of 25 Hz was chosen as the highest frequency to study this effect. As frequency influences the fluence per pulse delivered by the PX-UV lamp, it is crucial to measure the actual fluence at the targeted

disinfection location to ensure that a sufficiently high fluence is received for the desired level of disinfection.

In general, higher frequency fluences achieved significant log reduction within a very short period of time, allowing users to minimize the duration required for this disinfection method. To a certain extent, it can be inferred that the photothermal effect does not have a significant impact on the efficacy of inactivation. This conclusion is supported by the consistent reduction observed at all frequencies when the fluence is kept constant. Additionally, it is possible that the photothermal effect becomes more relevant during longer exposure times. These findings align with those of previous studies and provide further support for the limited influence of the photothermal effect on the overall inactivation efficacy [58].

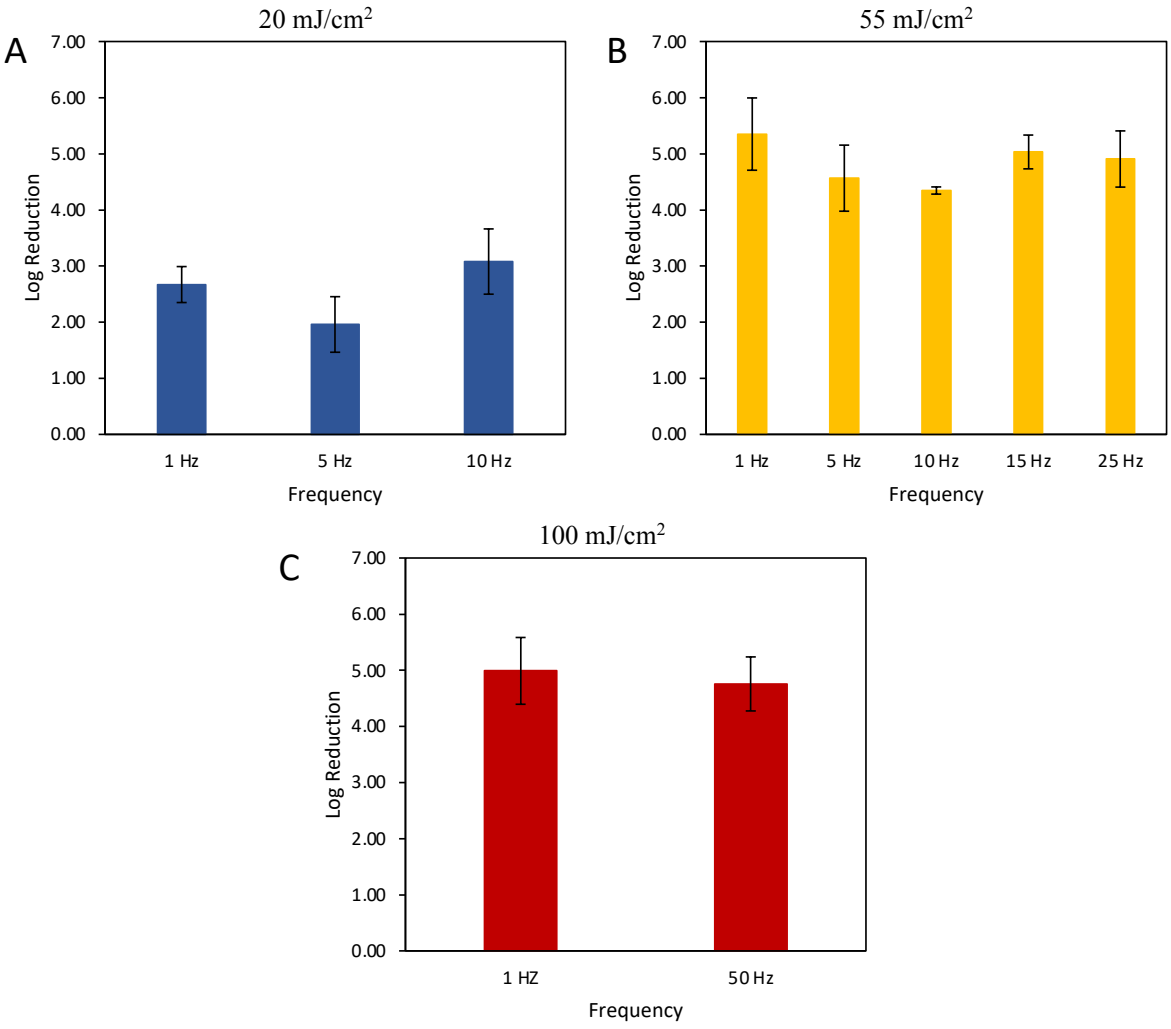


Figure 11 - Effect of frequency variation on inactivation of *L. monocytogenes* on stainless-steel with constant UV fluence: (A) 20 mJ/cm2, (B) 55 mJ/cm2 and (C) 100 mJ/cm2 (n = 3).

4.1.4 Effect of Radiation Angles at Constant UV Fluence

A limitation of UV disinfection is the requirement for light to effectively penetrate the target location for disinfection to take place. It has been hypothesized that the roughness of a surface could potentially impact the efficacy of UV inactivation by shielding certain bacteria from the UV light. To address this, the surface roughness of the SS used in this thesis was measured

according to the procedure outlined in Section 3.2. The results revealed a highly uniform surface with an Sq value of 0.242 ± 0.035 µm. The roughness map illustrating these findings is displayed in **Figure 12**. Given that the average deviation in height (roughness) is smaller than the average width of most bacteria, it is unlikely to have a substantial impact on the survival of the resistant fraction in this particular scenario.

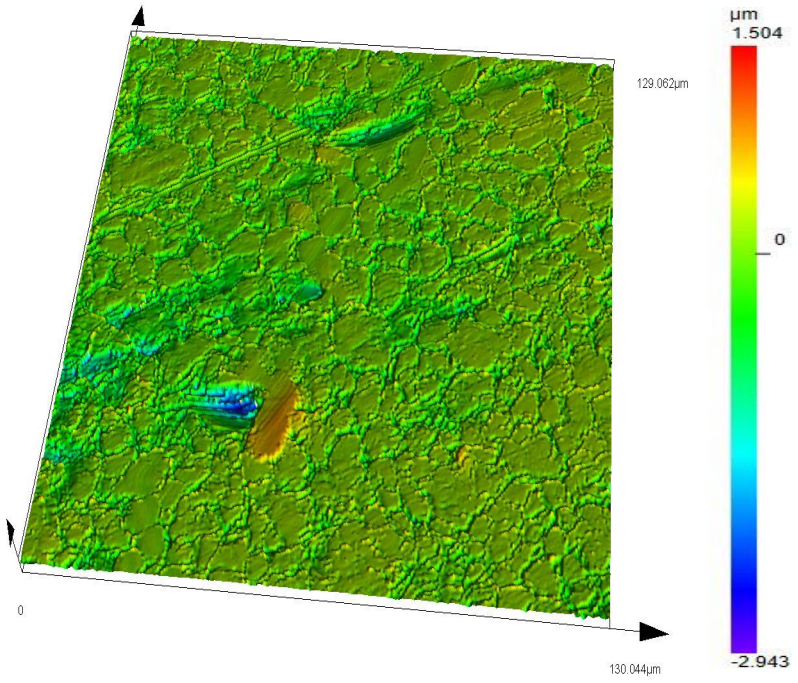


Figure 12 - Surface roughness 3D map.

To explore the impact of radiation angles and test the accuracy of the hypothesis, experiments were conducted where the radiation angles were varied from the standard 90° to the incident light (as shown in **Figure 13**). Radiometry measurements revealed a decrease in fluence from 92.89 mJ/cm^2 to 3.22 mJ/cm^2 when the radiation angle was reduced from 90° to 0°, as indicated in **Table 4**. Previous findings have indicated that as the fluence decreases,

bacterial inactivation also decreases. Consequently, experiments were carried out to investigate the effect of radiation angle on log reduction while maintaining a constant fluence of approximately 90 mJ/cm^2. Once again, consistent levels of bacterial inactivation were observed when the same UV fluence was applied, regardless of the angles tested except for 45° and 30° where the difference was significant (P value = 0.003). This further demonstrates that if the fluence is kept constant, the desired level of disinfection can be achieved (as depicted in **Figure 13**).

Hence, it is strongly advised to use radiometry measurements to accurately determine the UV fluence, particularly for surfaces that are not perpendicular to the light source. This is crucial because such surfaces can experience significant variations in the amount of UV fluence received, which can ultimately impact the effectiveness of disinfection and yield different results.

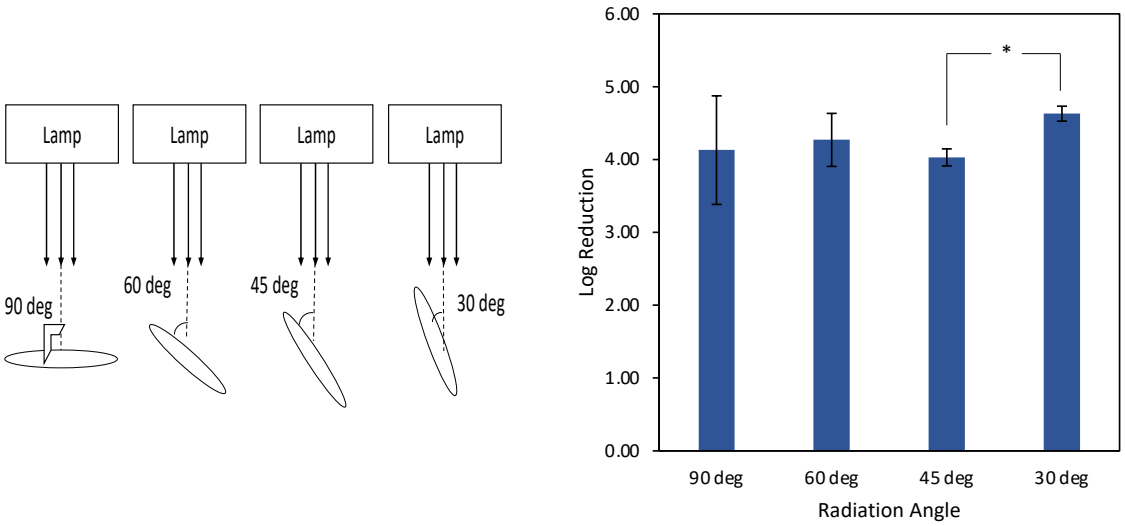


Figure 13 - (A) Schematic of the SS positions (B) Effect of radiation angle on *L. monocytogenes* inactivation on SS coupons (n = 3). Asterisks indicate significant differences between log reductions in mentioned angles of UV radiation (*, $P<0.05$).

Table 4 – Dosimetry of various radiation angles at 5 Hz frequency and 1 minute exposure time.

Radiation Angle	Fluence (mJ/cm^2)
90	92.89
60	51.53
45	44.86
30	33.67
0	3.22

4.2 UV Absorption of Soiling Agents

Prior to investigating the impact of soiling agents on UV inactivation efficacy, spectrophotometry measurements were conducted on each of the soiling solutions to analyze their absorbance spectra and verify the applicability of the Beer-Lambert law.

4.2.1 Protein Soils

Figure 14 illustrates the spectrophotometry results of the protein soils, namely Peptone A, BSA, and Casamino acid. A concentration of 0.2% w/v was chosen for plotting the absorbance spectra, as it provided an optimal balance between not being excessively high or too low in terms of absorbance. Interestingly, the absorbance spectra for all three protein soils appeared quite similar, with a peak absorbance observed around 280 nm on average, which is attributed to the presence of tryptophan, tyrosine, and phenylalanine residues. These amino acids are known to primarily absorb UV light, with specific absorbance maxima at 280 nm, 275 nm, and 258 nm, respectively [59]. Notably, BSA exhibited the highest peak absorbance compared to Casamino acid and Peptone A, respectively.

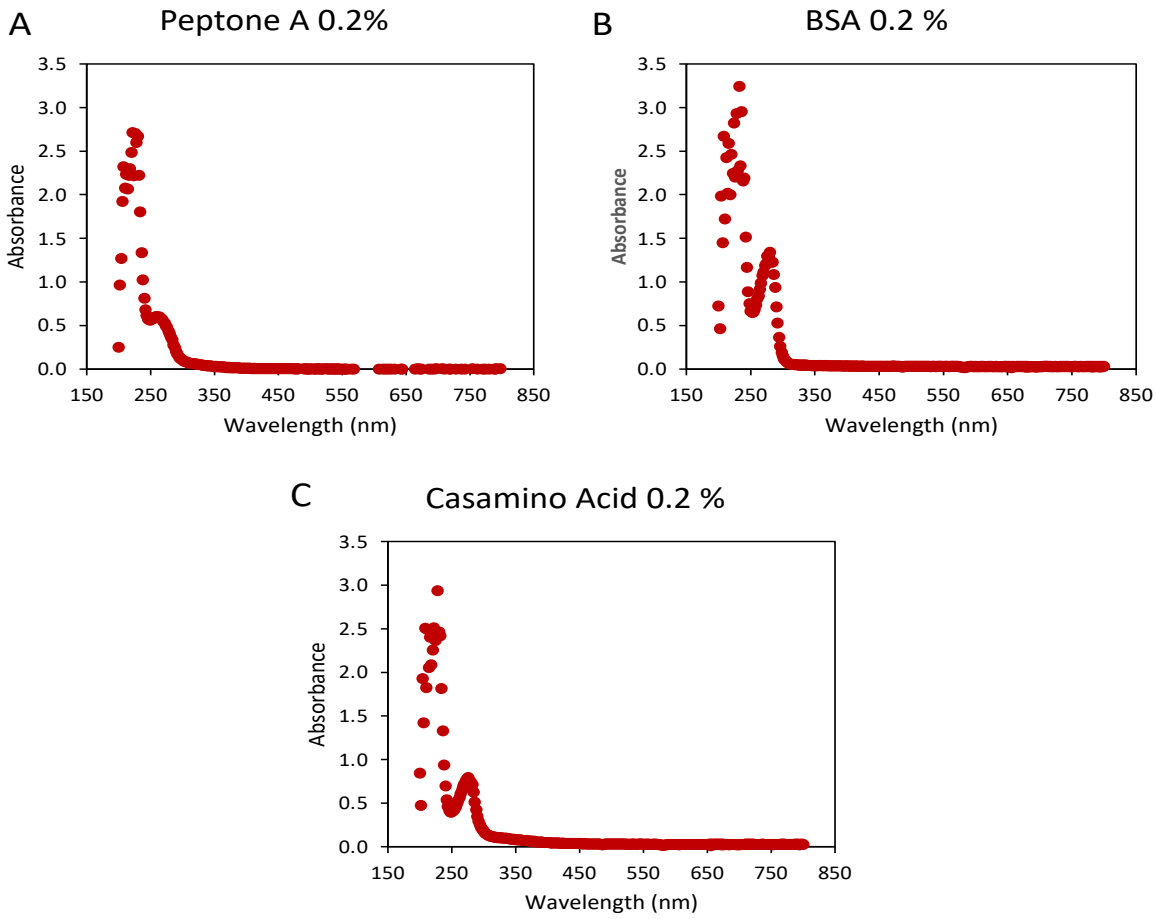


Figure 14 - Absorbance spectra of protein soils.

Subsequently, the Beer-Lambert law was examined for each solution, and the outcomes are presented in the graphs depicted in **Figure 15**. The relationship between absorbance and concentration was found to be consistent for both BSA and Peptone A, with similar slopes observed. These correlations were employed to determine the concentrations required to achieve the desired absorbance levels.

By establishing the Beer-Lambert law for each solution, it became possible to quantify the relationship between absorbance and concentration, enabling precise control over the

concentration needed to achieve specific absorbance values. This information is vital for subsequent experiments and ensures accurate preparation of the soiling solutions with desired optical properties.

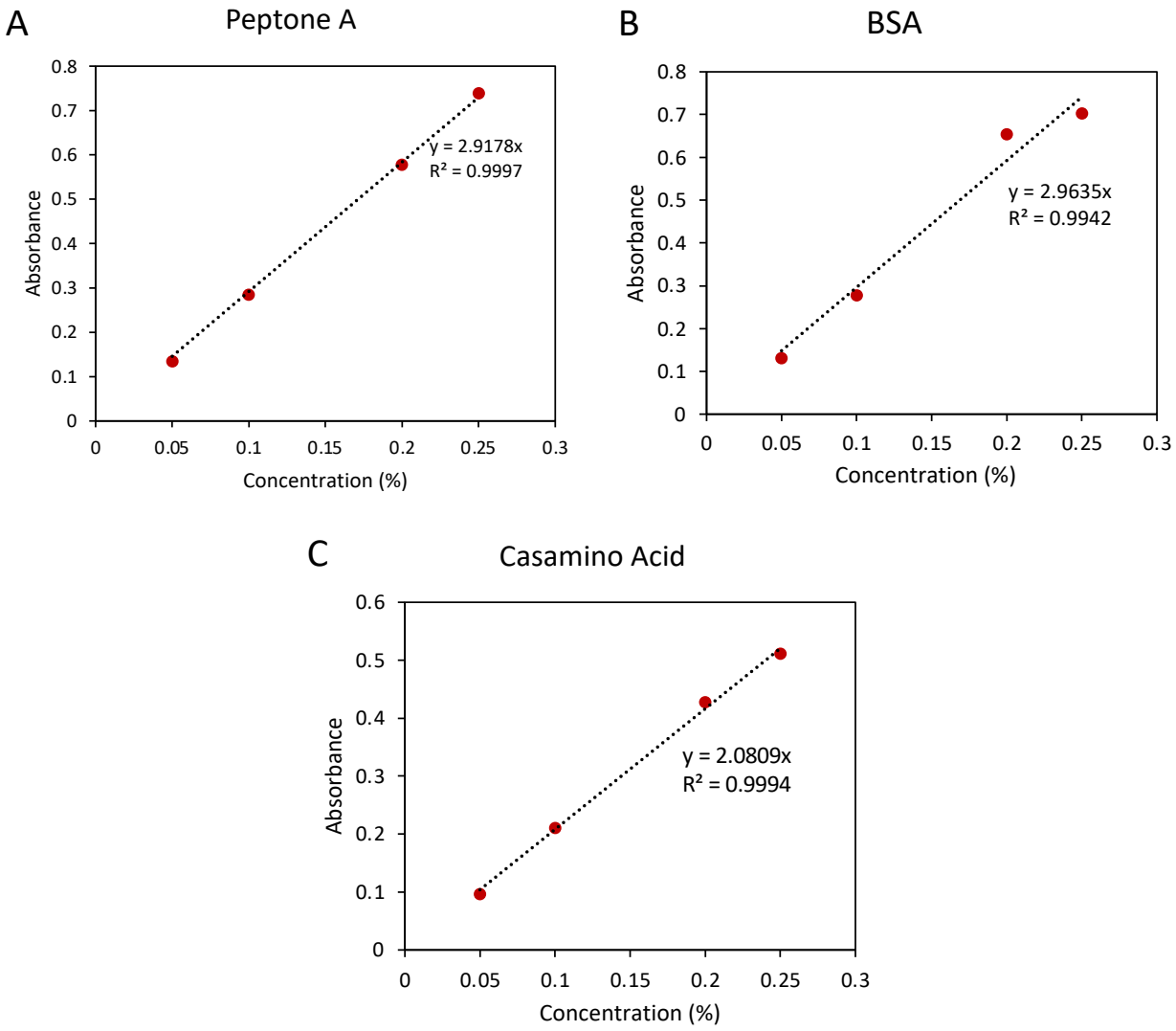


Figure 15 - Beer-Lambert law for protein soils at the wavelength of 254 nm.

4.2.2 Carbohydrate Soils

Similar spectrophotometry measurements were conducted for carbohydrate soils, including D-Lactose monohydrate, Potato starch, Glycogen, and Trehalose. The results of these measurements, as shown in **Figure 16**, reveal distinct absorbance spectra for each carbohydrate soil. In general, peaks were observed in the range of 250 to 280 nm. However, compared to protein soils there are more gradual peaks and unlike the protein soils, the absorbance behavior of the carbohydrate soils varied significantly among the different types. This variation can be attributed to the differences in the chemical structures of the carbohydrates. Notably, the absorbance spectrum of Potato starch exhibited a nearly linear trend across the measured wavelengths, distinguishing it from the other carbohydrate soils.

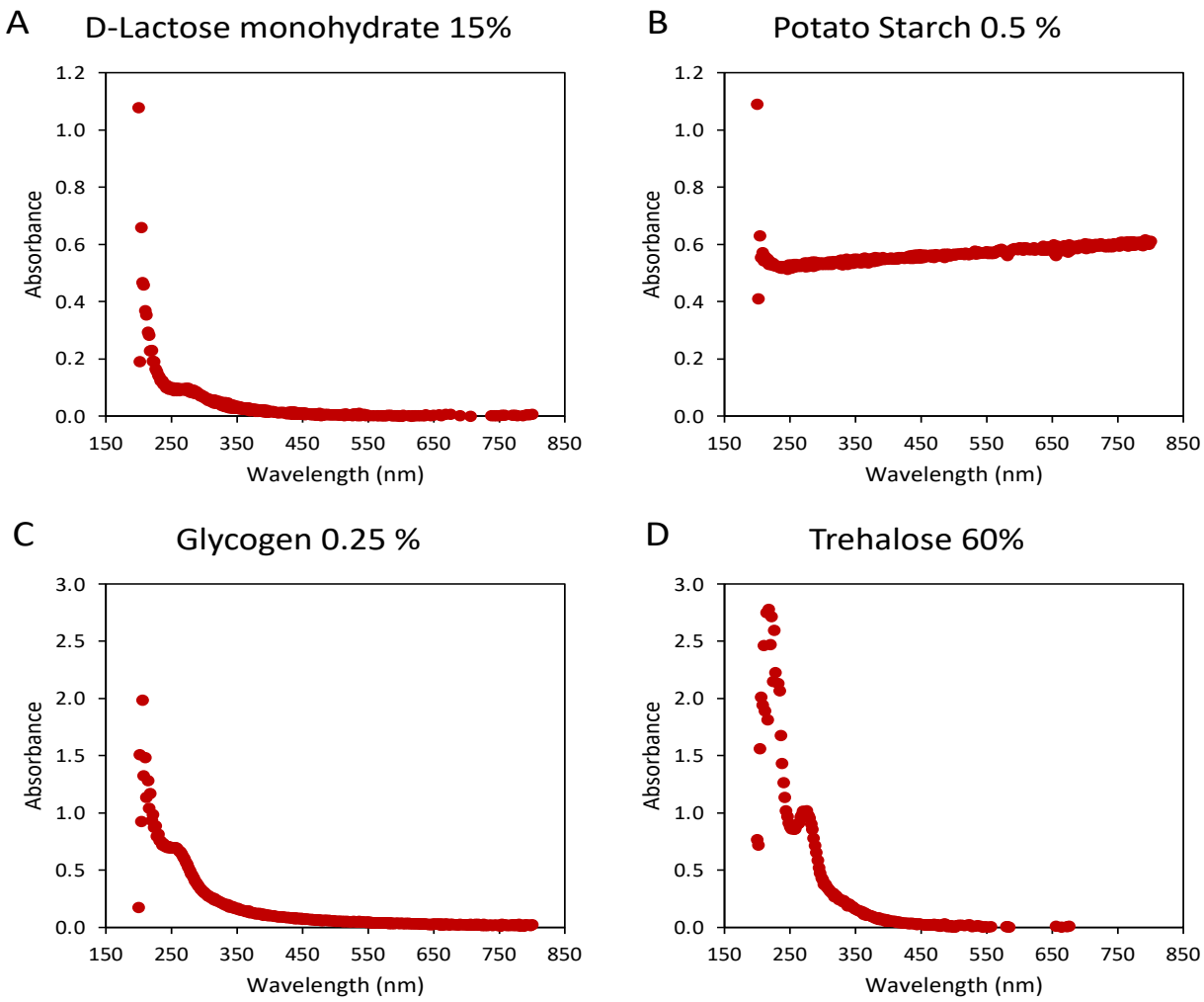


Figure 16 - Absorbance spectra of carbohydrate soils.

The analysis of the Beer-Lambert law correlations, presented in **Figure 17**, further emphasizes the differences among the carbohydrate soils. The correlations between absorbance and concentration were found to be unique for each carbohydrate type, highlighting the need for individualized concentration determination based on desired absorbance levels.

It is worth mentioning that the concentrations of the carbohydrate solutions differed notably, particularly for Trehalose and D-Lactose monohydrate, which have higher

solubilities. These variations in concentration reflect the different solubility characteristics of the carbohydrates and are essential for accurately preparing the soiling solutions used in subsequent experiments.

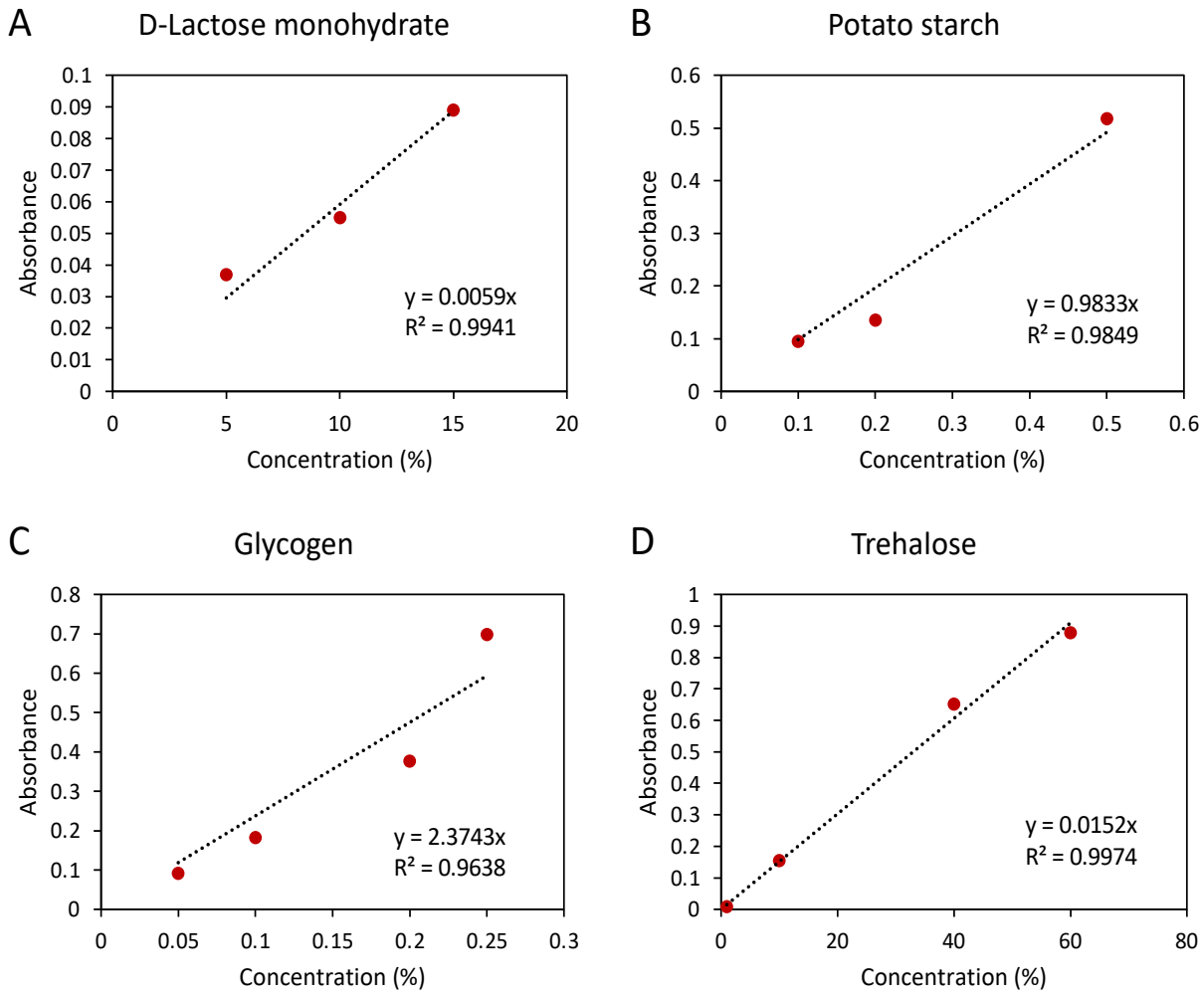


Figure 17 - Beer-Lambert law for carbohydrate soils at the wavelength of 254 nm.

4.2.3 Lipid Soils

The UV spectrophotometry measurements were also performed for the third category of soils, which consisted of lipids, specifically Fish oil and Triolein. In contrast to the previous two categories, no distinct peak was observed in the absorbance spectra of these lipid soils (**Figure 18**). However, both Fish oil and Triolein exhibited similar absorbance behavior across the measured wavelengths.

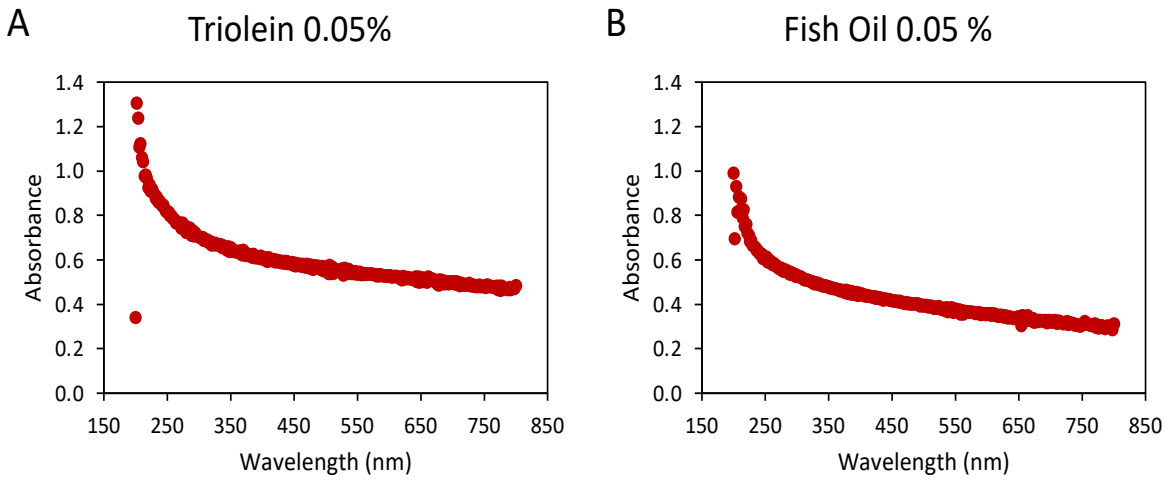


Figure 18 - Absorbance spectra of lipids soils.

The analysis of the Beer-Lambert law correlations, depicted in **Figure 19**, further explains the absorbance characteristics of the lipid soils. The correlations for lipids, represented by higher slopes, were found to be steeper compared to those observed for proteins and carbohydrates. This higher slope indicates a stronger relationship between absorbance and concentration, resulting in higher absorbance values for the same concentration of lipid solutions.

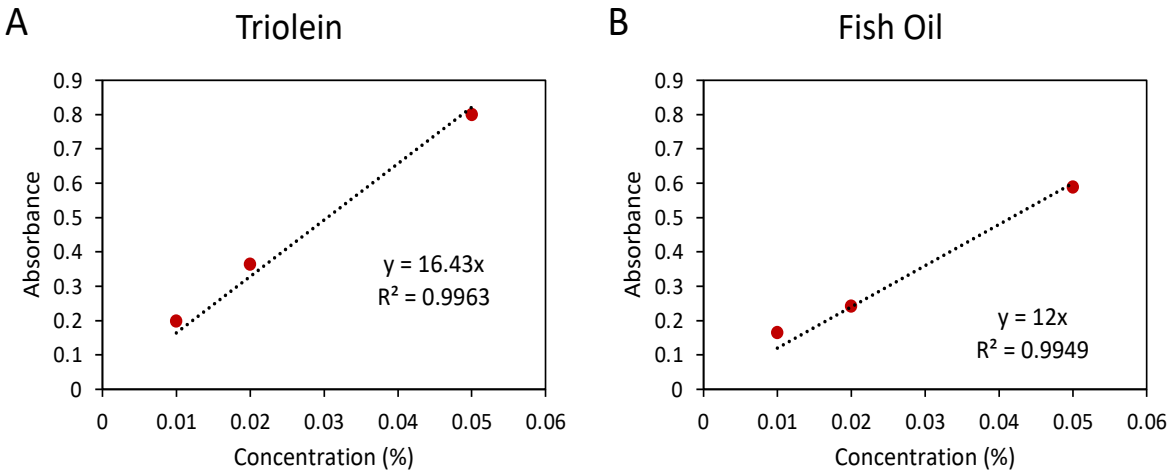


Figure 19 - Beer-Lambert law for Lipid soils at the wavelength of 254 nm.

It is noteworthy that the absence of specific peaks in the absorbance spectra of lipids suggests a different optical behavior compared to proteins and carbohydrates. The unique absorbance properties of lipids can be related to their chemical structure and molecular composition.

These findings emphasized the need for studying the UV inactivation efficacy under the presence of soiling agents.

4.3 Effect of Soiling Agents at Constant UV Fluence

After conducting UV spectrophotometry on different types of soiling agents, the study focused on investigating the impact of the presence of soiling agents in *L. monocytogenes* culture, on PX-UV germicidal efficacy. To enable meaningful comparisons, a UV fluence of 20 mJ/cm^2 was selected for the experiments, based on previous results. This fluence was chosen as it falls within a range that is neither too low nor too high, allowing for effective comparisons to be made. Consequently, all experiments in this section were conducted using this UV fluence.

4.3.1 Protein Soils

The concentrations of the soiling agents were determined based on the spectrophotometry results, aiming to achieve a UV absorbance of approximately 0.5 for protein soils. **Table 5** provides the concentrations that were prepared for each solution to attain the desired absorbance level. This allowed for the investigation of the PX-UV efficacy under a constant UV fluence and absorbance condition.

Table 5 - Concentrations of protein soils to achieve UV absorbance of ~ 0.5 and corresponding absorbance values.

Soiling Agent	Concentration (%)	UV absorbance at 254 nm
BSA	0.15	0.463 ± 0.003
Peptone A	0.2	0.567 ± 0.003
Casamino Acid	0.25	0.464 ± 0.006

Figure 20A depicts the impact of protein soils on bacterial growth. Although BSA appears to enhance bacterial growth, the difference is not statistically significant compared to the no soil condition. However, the positive control with BSA showed a significantly higher number of colonies than casamino acid (P value = 0.018) which is log 6.06 for BSA compared to log 5.63 for Casamino acid. In terms of log reduction (**Figure 20B**), there was no significant difference observed between no soil and different types of protein soils. This indicates that protein soils have no effect on the efficacy of UV treatment at this concentration level, and their only impact is on bacterial growth. Therefore, a higher number of bacteria colonies will

remain after UV treatment when the positive control has a higher bacterial count, while the log reduction remains constant.

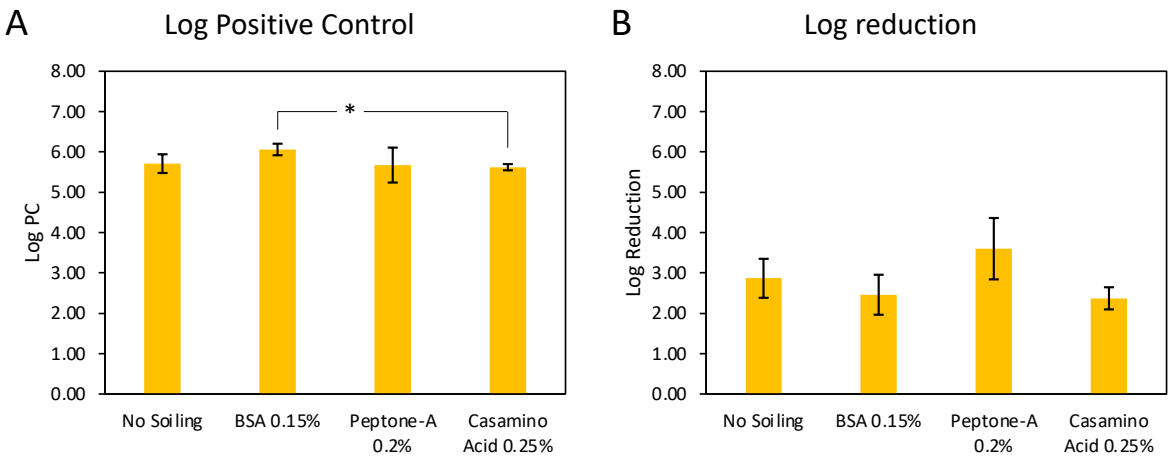


Figure 20 - Results of protein soil experiments with concentrations chosen to achieve a UV absorbance of approximately 0.5 (n = 3).

To explore the potential influence of soiling agent concentration on PX-UV efficacy, experiments were conducted using a higher concentration (1 W/V%) of soiling solutions. The absorbance of soiling solution is shown in **Table 6**.

Table 6 - UV absorbance of protein soils with 1 W/V% of concentration.

Soiling Agent	**Concentration (%)**	**UV absorbance at 254 nm (from Beer-Lambert law)**
BSA	**1**	2.96
Peptone - A	**1**	2.92
Casamino Acid	**1**	2.08

This led to a significant effect on bacteria growth, particularly when BSA was used as the soiling agent (log 6.61), compared to no soiling (log 5.41), Peptone A (log 5.96), and Casamino acid (log 5.12) (P values = 0.026, 0.011, 0.004, respectively). Among the soiling agents, BSA exhibited the highest impact on increasing *L. monocytogenes* growth, followed by Peptone A and Casamino acid. However, the effect of Peptone A and Casamino acid was not statistically significant. Notably, the bacteria growth was significantly higher when using Peptone A compared to Casamino acid (P value = 0.022). Refer to **Figure 21A** for a visual representation of the results.

Despite using a higher concentration of soiling agents, the log reduction achieved remained at approximately the same level (**Figure 21B**). The only statistically significant differences in log reduction were observed between the no soiling condition and Casamino acid (P value = 0.047), as well as between BSA and Casamino acid (P value = 0.022). Interestingly, Casamino acid exhibited a higher log reduction, which aligns with the minimal impact observed also on lower concentration on bacteria growth and inactivation using PX-UV. These findings suggest that Casamino acid may have a limited effect on both bacterial growth and the efficacy of PX-UV treatment.

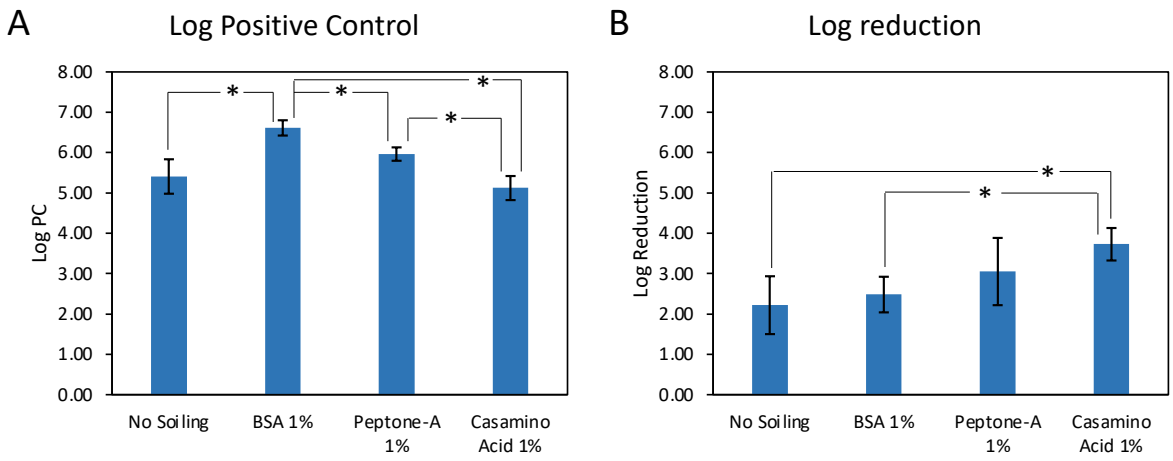


Figure 21 - Results of protein soil experiments with concentrations of 1 W/V% (n = 3).

Overall, the experiments using different types of protein soils demonstrated that the presence of these soils primarily influenced the growth of *L. monocytogenes*, while the log reduction remained consistent. This indicates that the efficacy of PX-UV in inactivating *L. monocytogenes* was not significantly affected by the presence of protein soils.

4.3.2 Carbohydrate Soils

The first set of experiments utilized D-Lactose monohydrate, Glycogen, and Potato starch as soiling agents. The concentrations of these agents were determined to achieve a UV absorbance of 0.1, which was measured using a UV spectrophotometer. The concentrations and corresponding absorbance values are presented in **Table 7**. An absorbance close to 0.1 was selected for the experiments because it was not feasible to prepare a solution of D-Lactose monohydrate with a concentration higher than 15% due to the solubility of this material.

Table 7 - Concentrations of carbohydrate soils to achieve UV absorbance of ~ 0.1 and corresponding absorbance values.

Soiling Agent	Concentration (%)	UV absorbance at 254 nm
D- Lactose monohydrate	15	0.089 ± 0.003
Potato Starch	0.1	0.07 ± 0.003
Glycogen	0.05	0.092 ± 0.002

In terms of promoting bacteria growth, Glycogen and Potato starch did not lead to significant changes in the growth of PC. However, D-Lactose monohydrate significantly increased the number of PC colonies to log 6.94 compared to the conditions without soiling (log 5.19) and with Glycogen as a soil (log 5.22), with p-values of 0.0005 and 0.0026, respectively (**Figure 22A**).

Similarly to protein soils, the log reductions of *L. monocytogenes* in the presence of carbohydrate soiling agents were not significantly different from the condition without soiling, even in the presence of D-Lactose monohydrate, which increased bacterial growth (**Figure 22B**).

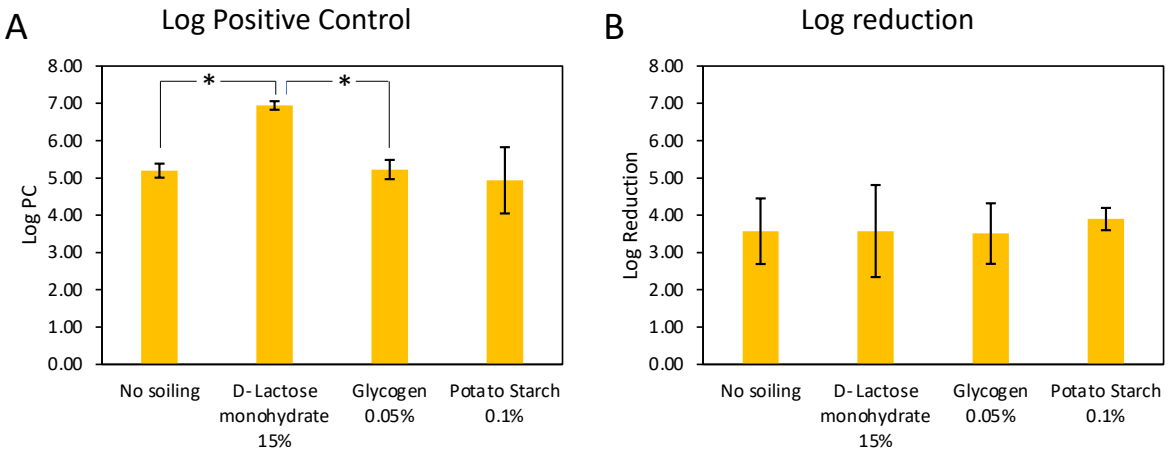


Figure 22 - Results of carbohydrate soil experiments with concentrations chosen to achieve a UV absorbance of approximately 0.1 (n = 3).

To ensure more consistent results, an alternative carbohydrate, Trehalose, was selected to replace D-Lactose monohydrate, as it was expected to have a lesser effect on bacterial growth. Additionally, higher concentrations (1% w/v) of Glycogen and Potato starch were used in the second set of experiments. Trehalose was employed at its highest concentration (60%) due to its low UV absorbance. UV absorbance values are mentioned in **Table 8**.

Table 8 - UV absorbance of carbohydrate soiling agents

Soiling Agent	Concentration (%)	Absorbance at 254 nm
Potato Starch	**1**	1.065 $\pm$0.037
Glycogen	**1**	2.870 $\pm$ 0.775
Trehalose	**60**	0.878 $\pm$ 0.011

However, results shown significant increase in the growth of *L. monocytogenes* with the presence of Trehalose to log 6.92 compared to no soiling (log 5.88) (P value = 0.0046), Glycogen (P value = 0.011) and Potato starch (P value = 0.0084) (**Figure 23A**). Also, it has been seen that increasing the concentration of Glycogen could affect the results since it significantly increased the growth to log 6.54 of PC with P value = 0.021.

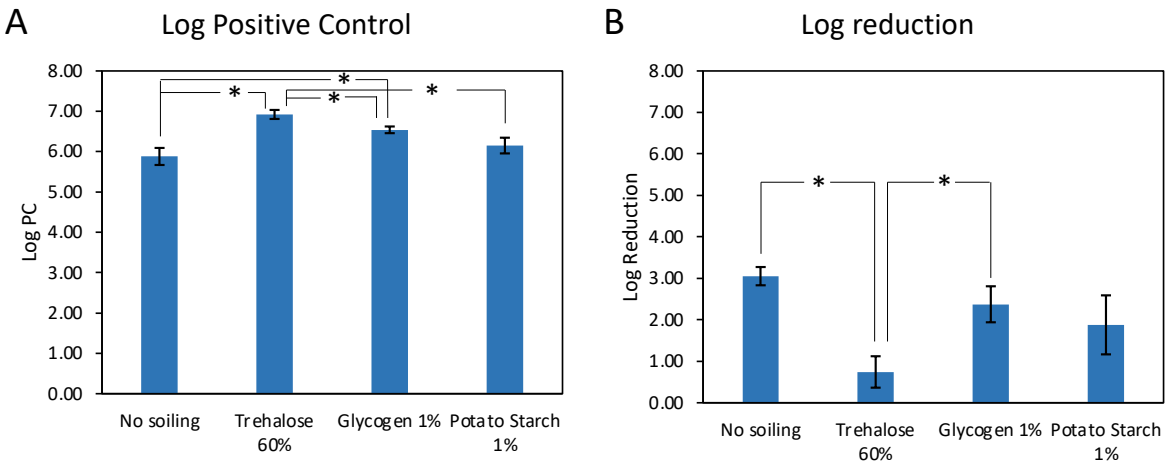


Figure 23 - Results of carbohydrate soil experiments (n = 3).

The results from the log reduction analysis (**Figure 23B**) indicate that the presence of Trehalose had a significant impact on PX-UV efficacy, reducing the log reduction from 3.05 (no soiling condition) to 0.74 (P value = 0.002). Similarly, the difference between reduction in glycogen and trehalose is significant reducing from log 2.37 to log 0.74 (P value = 0.008).

4.3.3 Lipid Soils

The last group of tested soiling agents was lipids including Triolein and Fish oil. Duo to previous results from protein soiling agents and carbohydrates, higher concentration of lipids was decided to use in the experiments regardless of the UV absorbance value. Therefore, concentration of 0.5 W/V% was chosen to conduct the experiments. UV absorbance values are shown in **Table 9**.

Table 9 - UV absorbance of lipid soiling agents.

Soiling Agent	Concentration (%)	Absorbance at 254 nm (From Beer-Lambert law)
Triolein	0.5	8.215
Fish Oil	0.5	6

Figure 24A demonstrates that Triolein had a significant effect on increasing the growth of *L. monocytogenes*, with the bacterial count rising from log 5.32 (under no soiling conditions) to log 6.12 (P value = 0.024). However, Triolein did not impact the efficacy of PX-UV inactivation, as the log reduction remained consistent at around 2 logs (**Figure 24B**). On the other hand, Fish oil reduced the log reduction significantly, from 2.54 (no soiling condition) to 1.22 log (P value = 0.001). Additionally, there was a significant difference in log reductions between the presence of Fish oil and Triolein (P value = 0.043).

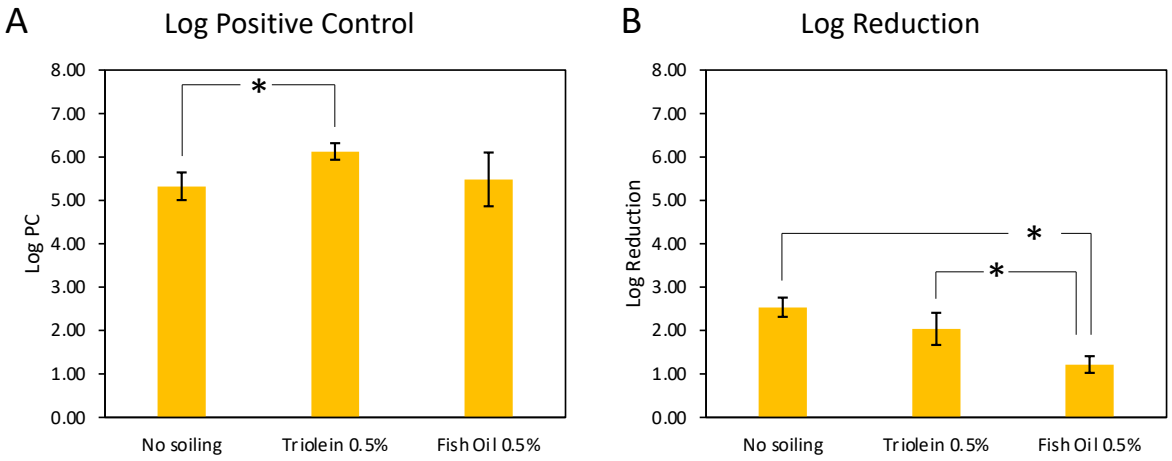


Figure 24 - Results of lipid soil experiments (n = 3).

Chapter 5
Conclusions and Recommendation

5.1 Conclusions

This study focuses on evaluating the effectiveness of PX-UV lamps, a modern UV technology, in inactivating *L. monocytogenes*, a major concern in foodborne illness, on SS surfaces commonly used in food production plants.

UV dosimetry was performed using a relevant radiometer to assess the UV fluence delivered by the PX-UV lamp. Dose-response experiments were conducted to determine the UV kinetics of *L. monocytogenes* inactivation on SS surfaces using this particular lamp, providing valuable insights into the optimal fluence required to eliminate a specific number of bacteria. Additionally, the study investigated the impact of exposure time, frequency, Radiation angles, and shielding effect with the presence of soiling agents on the efficacy of the PX-UV lamp.

The main conclusions of this thesis can be classified as follows:

1. Total UV fluence: The total UV fluence received from the PX-UV lamp at the surface is the most significant factor in achieving the desired inactivation of *L. monocytogenes*. This parameter should be carefully controlled to ensure effective disinfection.
2. Importance of Radiometry: Radiometry measurements are crucial in determining the optimal combination of frequency and exposure time required to achieve specific fluences. These measurements aid in optimizing the disinfection process. Accurate

radiometry measurements are essential for surfaces that are positioned at angles other than 90° relative to the lamp, as it significantly affects the fluence received by the surface. The angle of incidence plays a crucial role in determining the amount of UV fluence delivered to the surface. Therefore, conducting radiometry measurements for such surfaces is vital to ensure precise and reliable assessment of the UV fluence received, enabling accurate analysis of disinfection efficacy.

3. Efficiency of PX-UV Lamps: PX-UV lamps prove to be an efficient and environmentally friendly method for the inactivation of *L. monocytogenes* on stainless steel surfaces. The required fluence is significantly lower than the FDA-approved level for food and food contact surfaces decontamination, making it a promising alternative (~ 100 mJ/cm^2 in this study compared to 12 J/cm^2 approved by FDA).

4. Influence of Initial Bacteria Load: The log reduction achieved at higher UV fluences is influenced by the initial bacteria load. Higher log reductions were observed when the initial load of bacteria was higher, particularly for UV fluences exceeding 60 mJ/cm^2. This observation suggests that there is no limitation due to the shielding effect of other bacteria, as even greater reductions were achieved with higher inoculum loads. Therefore, considering the initial load of bacteria is crucial for ensuring effective disinfection outcomes.

5. Impact of Frequency and Radiation Angles: Changing the frequency and radiation angles does not significantly impact the inactivation of bacteria as long as the received fluence remains constant. Also, no inactivation was apparently achieved due to the

photothermal effect under the conditions used in this thesis since various frequencies resulted in consistent reduction in bacteria at constant fluence.

6. Effect of Soiling Agents: The presence of different types of soiling agents, such as protein, carbohydrate, and lipid, has varying effects on bacteria inactivation. Protein and carbohydrate soils can improve bacterial growth but do not affect the efficacy of the UV lamp itself. Lipid and certain carbohydrate soils have a shielding effect, inhibiting the bacteria from receiving the full UV light.
7. Extended Exposure Time for Soiled Surfaces: Soiled surfaces, particularly those with protein and carbohydrate soils, may require extended exposure time or improved treatment conditions to achieve better disinfection.

5.2 Recommendations

Based on the results obtained from this thesis and the literature review conducted, the following recommendations are made for future studies in this field:

1. Improved Fluence Measurement: Develop better methods for accurately measuring the actual fluence of UV received by the surface and considering possible reflections of UV radiation. This will help in optimizing UV disinfection processes and ensuring effective inactivation of microorganisms.
2. Soiling Agent Analysis: Conduct more studies on various types of soiling agents to understand the relationship between their structure, their effect on bacterial growth, and

their shielding effects. This will provide valuable insights into the mechanisms of UV inactivation in the presence of different types of contaminants.

3. Surface Material Investigation: Explore the efficacy of UV inactivation on different types of surface materials with varying roughness and hydrophobicity. This should include various plastics commonly used as packaging materials and fabrics used in kitchen environments. Comparing the effect of different surface materials on UV inactivation ability will contribute to the development of tailored disinfection strategies.
4. UV Inactivation of Fresh Products: Investigate the effectiveness of UV inactivation of bacteria on fresh products such as dairy products, fruits, vegetables, and ready-to-eat food (RTE). This research can have practical applications in the food industry, ensuring the safety and extended shelf life of perishable food items.

These recommended future studies will contribute to advancing our understanding of UV disinfection methods, optimizing their application, and expanding their potential use in various food processing and handling scenarios due to the lack of studies on UV application on surfaces and particularly on *L. monocytogenes*.

Letters of Copyright Permission

1. Requested permission for **Figure 1**:

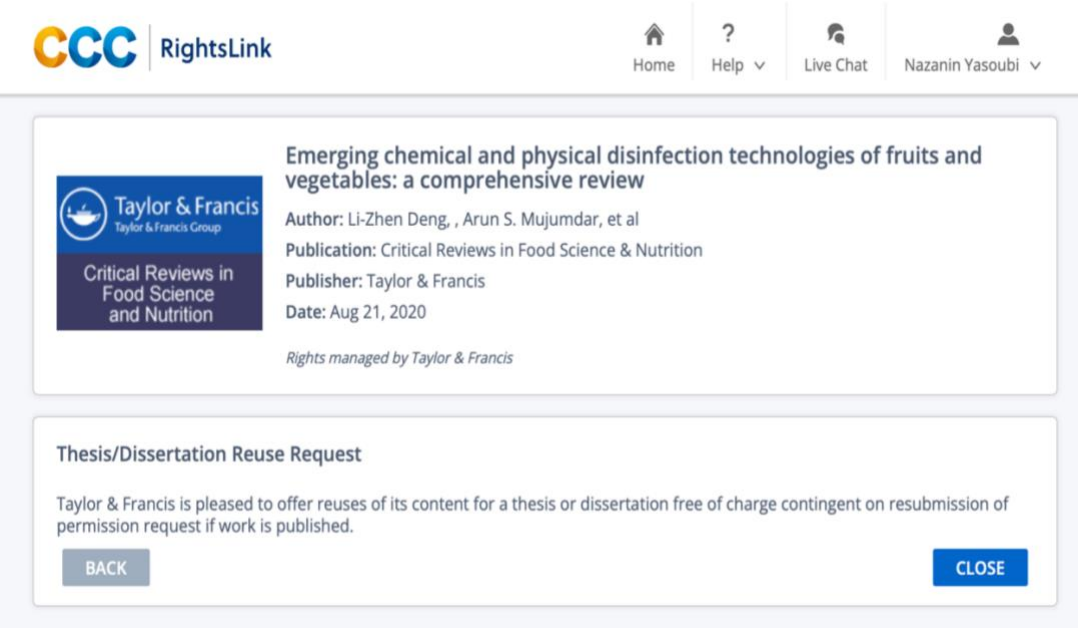
ELSEVIER LICENSE
TERMS AND CONDITIONS

Jun 08, 2023

This Agreement between Nazanin Yasoubi ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5562220702877
License date	Jun 04, 2023
Licensed Content Publisher	Elsevier
Licensed Content Publication	Elsevier Books
Licensed Content Title	Encyclopedia of Meat Sciences
Licensed Content Author	R.A. Holley,R.P. Cordeiro
Licensed Content Date	Jan 1, 2014
Licensed Content Pages	9
Start Page	348
End Page	356
Type of Use	reuse in a thesis/dissertation

2. Requested permission for **Figure 2**:



3. Requested permission **Figure 3**:

ELSEVIER LICENSE
TERMS AND CONDITIONS

Jun 08, 2023

This Agreement between Nazanin Yasoubi ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5559401251627
License date	May 31, 2023
Licensed Content Publisher	Elsevier
Licensed Content Publication	Food Control
Licensed Content Title	Comparison of intense pulsed light- and ultraviolet (UVC)-induced cell damage in Listeria monocytogenes and Escherichia coli O157:H7
Licensed Content Author	Chan-Ick Cheigh,Mi-Hyun Park,Myong-Soo Chung,Jung-Kue Shin,Young-Seo Park
Licensed Content Date	Jun 1, 2012
Licensed Content Volume	25
Licensed Content Issue	2
Licensed Content Pages	6

References

[1] WHO, "Food safety." https://www.who.int/news-room/fact-sheets/detail/food-safety (accessed Jan. 02, 2023).

[2] R. B. Tompkin, "Control of Listeria monocytogenes in the Food-Processing Environment," 2002. [Online]. Available: http://meridian.allenpress.com/jfp/article-pdf/65/4/709/1674591/0362-028x-65_4_709.pdf

[3] "Get the Facts about Listeria | FDA." https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-about-listeria (accessed Mar. 16, 2023).

[4] J. Szczawiński, M. E. Szczawińska, A. Łobacz, M. Tracz, and A. Jackowska-Tracz, "Modelling the growth rate of listeria monocytogenes in cooked ham stored at different temperatures," *Journal of Veterinary Research (Poland)*, vol. 61, no. 1, pp. 45–51, Mar. 2017, doi: 10.1515/jvetres-2017-0006.

[5] M. K. Thomas, R. Vriezen, J. M. Farber, A. Currie, W. Schlech, and A. Fazil, "Economic cost of a listeria monocytogenes outbreak in Canada, 2008," *Foodborne Pathog Dis*, vol. 12, no. 12, pp. 966–971, Dec. 2015, doi: 10.1089/fpd.2015.1965.

[6] W. F. Schlech, "Epidemiology and Clinical Manifestations of Listeria monocytogenes Infection," 2019, doi: 10.1128/microbiolspec.

[7] J. H. Yoon, J. E. Hyun, H. Song, J. Y. Kim, J. H. Kim, and S. Y. Lee, "Food residuals on the food-contacting surfaces of stainless steel and polypropylene influence the efficacy of ultraviolet light in killing foodborne pathogens," *J Food Saf*, vol. 38, no. 5, Oct. 2018, doi: 10.1111/jfs.12506.

[8] S. E. Woodling and C. I. Moraru, “Effect of Spectral Range in Surface Inactivation of Listeria innocua Using Broad-Spectrum Pulsed Light,” 2007. [Online]. Available: http://www.technicalglass.com/

[9] R. S. Bergman, “Germicidal UV Sources and Systems†,” *Photochemistry and Photobiology*, vol. 97, no. 3. Blackwell Publishing Inc., pp. 466–470, May 01, 2021. doi: 10.1111/php.13387.

[10] A. L. Holck, K. H. Liland, S. M. Drømtorp, G. M. Carlehö, and A. McLeod, “Comparison of UV-C and pulsed UV light treatments for reduction of salmonella, listeria monocytogenes, and enterohemorrhagic Escherichia coli on eggs,” *J Food Prot*, vol. 81, no. 1, pp. 6–16, Jan. 2018, doi: 10.4315/0362-028X.JFP-17-128.

[11] W. Kowalski, “Mathematical Modeling of UV Disinfection,” in *Ultraviolet Germicidal Irradiation Handbook*, Springer Berlin Heidelberg, 2009, pp. 51–72. doi: 10.1007/978-3-642-01999-9_3.

[12] G. S. Jones and S. E. F. D’Orazio, “Listeria monocytogenes: Cultivation and laboratory maintenance,” *Curr Protoc Microbiol*, no. SUPPL.31, 2013, doi: 10.1002/9780471729259.mc09b02s31.

[13] T. Jemmi and R. Stephan, “Listeria monocytogenes: food-borne pathogen and hygiene indicator,” 2006.

[14] R. A. Holley and R. P. Cordeiro, “MICROBIOLOGICAL SAFETY OF MEAT | Listeria monocytogenes,” *Encyclopedia of Meat Sciences*, pp. 348–356, Jan. 2014, doi: 10.1016/B978-0-12-384731-7.00039-8.

[15] J. W. F. Law, N. S. Ab Mutalib, K. G. Chan, and L. H. Lee, “An insight into the isolation, enumeration, and molecular detection of Listeria monocytogenes in food,” *Frontiers in Microbiology*, vol. 6, no. NOV. Frontiers Research Foundation, 2015. doi: 10.3389/fmicb.2015.01227.

[16] P. Di Ciccio, “LISTERIA MONOCYTOGENES: BIOFILM IN FOOD PROCESSING.” [Online]. Available: https://www.researchgate.net/publication/261885571

[17] F. Allerberger and M. Wagner, “Listeriosis: a resurgent foodborne infection,” *Clinical Microbiology and Infection*, vol. 16, no. 1, pp. 16–23, Jan. 2010, doi: 10.1111/J.1469-0691.2009.03109.X.

[18] J. M. Farber and P. I. Peterkin, “Listeria monocytogenes, a Food-Borne Pathogen,” 1991. [Online]. Available: https://journals.asm.org/journal/mr

[19] L. Gram, D. Bagge-Ravn, Y. Y. Ng, P. Gymoese, and B. F. Vogel, “Influence of food soiling matrix on cleaning and disinfection efficiency on surface attached Listeria monocytogenes,” *Food Control*, vol. 18, no. 10, pp. 1165–1171, Oct. 2007, doi: 10.1016/j.foodcont.2006.06.014.

[20] D. Bagge-Ravn, Y. Ng, M. Hjelm, J. N. Christiansen, C. Johansen, and L. Gram, “The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection,” *Int J Food Microbiol*, vol. 87, no. 3, pp. 239–250, Nov. 2003, doi: 10.1016/S0168-1605(03)00067-9.

[21] J. Verran and K. A. Whitehead, “Assessment of organic materials and microbial components on hygienic surfaces,” in *Food and Bioproducts Processing*, Institution of Chemical Engineers, 2006, pp. 260–264. doi: 10.1205/fbp06036.

[22] K. A. Whitehead, L. A. Smith, and J. Verran, “The detection of food soils and cells on stainless steel using industrial methods: UV illumination and ATP bioluminescence,” *Int J Food Microbiol*, vol. 127, no. 1–2, pp. 121–128, Sep. 2008, doi: 10.1016/j.ijfoodmicro.2008.06.019.

[23] “Existing and potential applications of ultraviolet light in the food industry – a critical review - Bintsis - 2000 - Journal of the Science of Food and Agriculture - Wiley Online Library.” https://onlinelibrary.wiley.com/doi/epdf/10.1002/%28SICI%291097-0010%2820000501%2980%3A6%3C637%3A%3AAID-JSFA603%3E3.0.CO%3B2-1 (accessed May 31, 2023).

[24] H. Yang, P. A. Kendall, L. Medeiros, and J. N. Sofos, “Inactivation of Listeria monocytogenes, Escherichia coli O157:H7, and Salmonella typhimurium with compounds available in households,” *J Food Prot*, vol. 72, no. 6, pp. 1201–1208, 2009, doi: 10.4315/0362-028X-72.6.1201.

[25] H. D. Kusumaningrum, G. Riboldi, W. C. Hazeleger, and R. R. Beumer, “Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods,” *Int J Food Microbiol*, vol. 85, no. 3, pp. 227–236, Aug. 2003, doi: 10.1016/S0168-1605(02)00540-8.

[26] M. Garvey and N. J. Rowan, “Pulsed UV as a potential surface sanitizer in food production processes to ensure consumer safety,” *Current Opinion in Food Science*, vol. 26. Elsevier Ltd, pp. 65–70, Apr. 01, 2019. doi: 10.1016/j.cofs.2019.03.003.

[27] L. Z. Deng *et al.*, “Emerging chemical and physical disinfection technologies of fruits and vegetables: a comprehensive review,” *Critical Reviews in Food Science and Nutrition*, vol. 60, no. 15. Taylor and Francis Inc., pp. 2481–2508, Aug. 21, 2020. doi: 10.1080/10408398.2019.1649633.

[28] K. Murray, F. Wu, J. Shi, S. Jun Xue, and K. Warriner, “Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions,” *Food Quality and Safety*, vol. 1, no. 4. Oxford University Press, pp. 289–301, Dec. 01, 2017. doi: 10.1093/fqsafe/fyx027.

[29] S. K. Pankaj, Z. Wan, and K. M. Keener, “Effects of cold plasma on food quality: A review,” *Foods*, vol. 7, no. 1. MDPI Multidisciplinary Digital Publishing Institute, Jan. 01, 2018. doi: 10.3390/foods7010004.

[30] F. Devlieghere, L. Vermeiren, and J. Debevere, “New preservation technologies: Possibilities and limitations,” in *International Dairy Journal*, Apr. 2004, pp. 273–285. doi: 10.1016/j.idairyj.2003.07.002.

[31] J. A. Byrne *et al.*, “A review of heterogeneous photocatalysis for water and surface disinfection,” *Molecules*, vol. 20, no. 4, pp. 5574–5615, Apr. 2015, doi: 10.3390/molecules20045574.

[32] B. Ma, S. Burke-Bevis, L. Tiefel, J. Rosen, B. Feeney, and K. G. Linden, “Reflection of UVC wavelengths from common materials during surface UV disinfection:

Assessment of human UV exposure and ozone generation," *Science of the Total Environment*, vol. 869, Apr. 2023, doi: 10.1016/j.scitotenv.2023.161848.

[33] I. Mehta, H. Y. Hsueh, S. Taghipour, W. Li, and S. Saeedi, "UV Disinfection Robots: A Review," *Rob Auton Syst*, vol. 161, Mar. 2023, doi: 10.1016/j.robot.2022.104332.

[34] V. M. Gómez-López, P. Ragaert, J. Debevere, and F. Devlieghere, "Pulsed light for food decontamination: a review," *Trends Food Sci Technol*, vol. 18, no. 9, pp. 464–473, Sep. 2007, doi: 10.1016/j.tifs.2007.03.010.

[35] G. Oms-Oliu, O. Martín-Belloso, and R. Soliva-Fortuny, "Pulsed light treatments for food preservation. A review," *Food and Bioprocess Technology*, vol. 3, no. 1. pp. 13–23, Jan. 2010. doi: 10.1007/s11947-008-0147-x.

[36] Y. Cheng *et al.*, "Inactivation of Listeria and E. coli by Deep-UV LED: effect of substrate conditions on inactivation kinetics," *Sci Rep*, vol. 10, no. 1, p. 3411, Feb. 2020, doi: 10.1038/s41598-020-60459-8.

[37] R. N. Pereira and A. A. Vicente, "Environmental impact of novel thermal and non-thermal technologies in food processing," *Food Research International*, vol. 43, no. 7, pp. 1936–1943, Aug. 2010, doi: 10.1016/J.FOODRES.2009.09.013.

[38] N. P. Ozer and A. Demirci, "Inactivation of Escherichia coli O157:H7 and Listeria monocytogenes inoculated on raw salmon fillets by pulsed UV-light treatment," *Int J Food Sci Technol*, vol. 41, no. 4, pp. 354–360, Apr. 2006, doi: 10.1111/J.1365-2621.2005.01071.X.

[39] M. L. Bhavya and H. Umesh Hebbar, “Pulsed light processing of foods for microbial safety,” *Food Quality and Safety*, vol. 1, no. 3. Oxford University Press, pp. 187–201, Sep. 01, 2017. doi: 10.1093/fqsafe/fyx017.

[40] A. R. Uesugi, S. E. Woodling, and C. I. Moraru, “Inactivation Kinetics and Factors of Variability in the Pulsed Light Treatment of Listeria innocua Cells,” 2007. [Online]. Available: http://meridian.allenpress.com/jfp/article-pdf/70/11/2518/1681202/0362-028x-70_11_2518.pdf

[41] C. I. Cheigh, H. J. Hwang, and M. S. Chung, “Intense pulsed light (IPL) and UV-C treatments for inactivating Listeria monocytogenes on solid medium and seafoods,” *Food Research International*, vol. 54, no. 1, pp. 745–752, Nov. 2013, doi: 10.1016/j.foodres.2013.08.025.

[42] A. Lasagabaster and I. Martínez de Marañón, “Impact of Process Parameters on Listeria innocua Inactivation Kinetics by Pulsed Light Technology,” *Food Bioproc Tech*, vol. 6, no. 7, pp. 1828–1836, Jul. 2013, doi: 10.1007/s11947-012-0855-0.

[43] D. K. Kim and D. H. Kang, “Elevated inactivation efficacy of a pulsed UVC lightemitting diode system for foodborne pathogens on selective media and food surfaces,” *Appl Environ Microbiol*, vol. 84, no. 20, Oct. 2018, doi: 10.1128/AEM.01340-18.

[44] A. Green, V. Popović, J. Pierscianowski, M. Biancaniello, K. Warriner, and T. Koutchma, “Inactivation of Escherichia coli, Listeria and Salmonella by single and multiple wavelength ultraviolet-light emitting diodes,” *Innovative Food Science and*

Emerging Technologies, vol. 47, pp. 353–361, Jun. 2018, doi: 10.1016/j.ifset.2018.03.019.

[45] M. Turtoi and D. Borda, "Decontamination of egg shells using ultraviolet light treatment," *Worlds Poult Sci J*, vol. 70, no. 2, pp. 265–278, 2014, doi: 10.1017/S0043933914000282.

[46] N. Giese and J. Darby, "SENSITIVITY OF MICROORGANISMS TO DIFFERENT WAVELENGTHS OF UV LIGHT: IMPLICATIONS ON MODELING OF MEDIUM PRESSURE UV SYSTEMS." [Online]. Available: www.elsevier.com/locate/watres

[47] V. M. Gómez-López and J. R. Bolton, "An Approach to Standardize Methods for Fluence Determination in Bench-Scale Pulsed Light Experiments," *Food Bioproc Tech*, vol. 9, no. 6, pp. 1040–1048, Jun. 2016, doi: 10.1007/s11947-016-1696-z.

[48] K. Krishnamurthy, J. C. Tewari, J. Irudayaraj, and A. Demirci, "Microscopic and spectroscopic evaluation of inactivation of staphylococcus aureus by pulsed UV light and infrared heating," *Food Bioproc Tech*, vol. 3, no. 1, pp. 93–104, Jan. 2010, doi: 10.1007/s11947-008-0084-8.

[49] F. Fine and P. Gervais, "Efficiency of pulsed UV light for microbial decontamination of food powders," *J Food Prot*, vol. 67, no. 4, pp. 787–792, 2004, doi: 10.4315/0362-028X-67.4.787.

[50] F. Schottroff, A. Fröhling, M. Zunabovic-Pichler, A. Krottenthaler, O. Schlüter, and H. Jäger, "Sublethal injury and Viable but Non-culturable (VBNC) state in microorganisms during preservation of food and biological materials by non-thermal processes,"

Frontiers in Microbiology, vol. 9, no. NOV. Frontiers Media S.A., Nov. 20, 2018. doi: 10.3389/fmicb.2018.02773.

[51] J. D. Sengillo *et al.*, "UV-Photokeratitis Associated with Germicidal Lamps Purchased during the COVID-19 Pandemic," *https://doi.org/10.1080/09273948.2020.1834587*, vol. 29, no. 1, pp. 76–80, 2020, doi: 10.1080/09273948.2020.1834587.

[52] N. J. Rowan, V. P. Valdramidis, and V. M. Gómez-López, "A review of quantitative methods to describe efficacy of pulsed light generated inactivation data that embraces the occurrence of viable but non culturable state microorganisms," *Trends in Food Science and Technology*, vol. 44, no. 1. Elsevier Ltd, pp. 79–92, 2015. doi: 10.1016/j.tifs.2015.03.006.

[53] H. Claus, "Ozone Generation by Ultraviolet Lamps†," *Photochemistry and Photobiology*, vol. 97, no. 3. Blackwell Publishing Inc., pp. 471–476, May 01, 2021. doi: 10.1111/php.13391.

[54] B. Y. Chen, H. M. Lung, B. B. Yang, and C. Y. Wang, "Pulsed light sterilization of packaging materials," *Food Packag Shelf Life*, vol. 5, pp. 1–9, Sep. 2015, doi: 10.1016/j.fpsl.2015.04.002.

[55] "Surface Roughness Measurement—Parameters | Olympus." https://www.olympus-ims.com/en/metrology/surface-roughness-measurement-portal/parameters/#!cms%5Bfocus%5D=007&cms[focus]=023 (accessed Apr. 10, 2023).

[56] ASTM E3135-18, “Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil”, doi: 10.1520/E3135-18.

[57] “ILT2400,SED240/NS254/TD | Germicidal & UV Disinfection Meters.” https://internationallight.com/products/ilt2400-hand-held-radiometer-germicidal-and-uv-hazard-measurements-254-nm (accessed May 10, 2023).

[58] N. J. Rowan, S. J. Macgregor, J. G. Anderson, R. A. Fouracre, L. Mcilvaney, and A. O. Farish, “Pulsed-Light Inactivation of Food-Related Microorganisms,” 1999. [Online]. Available: https://journals.asm.org/journal/aem

[59] A. B. Biter, J. Pollet, W. H. Chen, U. Strych, P. J. Hotez, and M. E. Bottazzi, “A method to probe protein structure from UV absorbance spectra,” *Anal Biochem*, vol. 587, p. 113450, Dec. 2019, doi: 10.1016/J.AB.2019.113450.