Biofilm Control Inside Secondary Water Storage Containers Using UV Light

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Abstract

This thesis investigates the feasibility of ultraviolet (UV) light treatment for biofilm control inside secondary storage containers used in humanitarian settings. A scoping literature review investigating real-world UV light treatment application revealed that, even though it is effective beyond controlled laboratory settings, it is underexplored as a method for biofilm control in humanitarian contexts. To address this gap, a ray tracing model was developed to simulate UV irradiance distribution within a representative jerry can, and experimental validation showed model predictions were within 17% of measured values. Lab-grown biofilms were then treated at locations inside the jerry can receiving moderate and low levels of UV irradiance. At a maximum UV dose of 16 mJ/cm², the location receiving moderate UV irradiance achieved 2.47 ± 0.75 (average ± standard deviation) log-reduction value (LRV) while the location receiving low UV irradiance achieved 2.99 ± 0.07 LRV. Comparisons with other UV/biofilm research showed that similar germicidal thresholds could be achieved even with the lower parameters incorporated into this research. These findings demonstrate that UV light treatment is a technically viable method for biofilm control inside secondary storage containers used in humanitarian settings. Future research investigating this treatment method under real-world conditions will prove to be the next key step in determining its feasibility for future implementation in humanitarian contexts.

Dedication

I would like to dedicate this study to my mom and dad. They have sacrificed to give me the best life anyone could ever ask for and I love them both with all my heart. I would also like to dedicate this to my sister, who has provided me with a lot of support and who I love very much. Finally, I would like to dedicate this to my grandma, grandpa, and great aunt, who have passed away during my studies but live on in my heart.

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List of Abbreviations

ANOVA – Analysis of variance

CBR – CDC Biofilm Reactor

CFU – Colony forming unit

DBP – Disinfection byproduct

EPS – Extracellular polymeric substances

HDPE – High-density polyethylene

LED – Light emitting diode

LIL – Low-irradiance location

MIL – Moderate-irradiance location

PSB – Phosphate buffered saline

TSA – Tryptic soy agar

TSB – Tryptic soy broth

UV - Ultraviolet

WASH – Water, sanitation, and hygiene

WHO – World Health Organization

Chapter 1: Introduction

1.1. Background

1.1.1. Biofilms

Biofilms are characterized by the presence of extracellular polymeric substances (EPS), which form a protective barrier around embedded communities of surface-attached microorganisms (Zhao et al., 2023). These microbial structures are commonly found in drinking water systems and are capable of harbouring pathogenic microorganisms, imposing health concerns to communities relying on this infrastructure. Biofilm formation typically follows a five-stage process: reversible attachment, irreversible attachment, EPS production, maturation, and dispersion (Sharma et al., 2023). Microorganisms may undergo reversible or irreversible attachment depending on the net balance of attractive and repulsive forces between the cell surface and the physical substrate (Muhammad et al., 2020). If repulsive forces are not overcome, microorganisms rejoin the surrounding environment (in the case of drinking water systems, the immediate flow regime), whereas overcoming them leads to irreversible attachment (Kostakioti et al., 2013; Muhammad et al., 2020). Irreversible attachment also includes cell-to-cell adhesion, where communication between microorganisms via chemical signals released within the population initiate biofilm formation (Muhammad et al., 2020). Microorganisms then begin to form microcolonies and reproduce, which leads to the secretion of EPS (Donlan, 2001; Flemming & Wingender, 2010). The continued development of microcolonies transitions the biofilm into the maturation phase, attracting planktonic microorganisms and regulating gene and protein expression to enhance its defensive properties (Muhammad et al., 2020; Sharma et al., 2023). Once the biofilm enters the dispersion stage, microorganisms separating from the biofilm may rejoin the surrounding environment (Rumbaugh & Sauer, 2020).

1.1.2. Ultraviolet (UV) light treatment for biofilm control

The literature has demonstrated that ultraviolet (UV) light is able to prevent, mitigate, and reduce biofilms (Gora et al., 2024; Luo et al., 2022). The primary mechanism responsible for this effect is the absorption of UV photons by microbial DNA (de Almeida et al., 2022). This prevents microorganisms from reproducing and interrupts the secretion of EPS. The UVC range (200-280

nm) has been found to be most effective for this process, as it induces pyrimidine dimers (blocking DNA replication) while simultaneously degrading integral constituents of the EPS matrix (Chevremont et al., 2012; Luo et al., 2022).

1.1.3. Drinking water in humanitarian settings

In humanitarian contexts, where centralized treatment plants and piped distribution networks are often unavailable, drinking water must be obtained through alternative means. This could include traveling to communal distribution points or collecting water from protected wells or surface water (Agensi et al. 2019; Jagals et al., 1997; Jagals et al., 2003; Roberts et al., 2001). Secondary storage containers are typically used for collection of drinking water and storage at the household level. These containers may become microbially compromised through contaminated source water, transport back to the household, and poor hygienic practices (Agensi et al. 2019; Jagals et al., 1997; Jagals et al., 2003; Roberts et al., 2001). Free-floating microorganisms suspended in bulk water or residual microbial contaminants on the inner walls of secondary storage containers may transition into a biofilm state, increasing the risk of re-contaminating previously treated drinking water or further degrading drinking water over prolonged storage. Increased surface roughness on the interior container surfaces may also enhance biofilm formation, as rough features similar in size to microorganisms provide additional contact points that promote initial attachment (String et al., 2021; Uzoma et al., 2023; van Loosdrecht et al., 1995).

1.2. Research Objectives

The main goal of this research is to examine UV light treatment for biofilm control inside secondary storage containers commonly used in humanitarian settings. To achieve this goal, the following objectives were established in this research:

1. *Literature review*: A scoping literature review was conducted to identify real-world applications and simulations of UV light treatment for bulk water disinfection and biofilm control to demonstrate this method works beyond the laboratory. This review was meant to demonstrate that UV light treatment has not yet been applied for these purposes in humanitarian settings. By synthesizing findings from the identified studies, a design concept for future research in humanitarian contexts was established.

2. *Ray tracing model*: Building on the design concepts established through the literature review, a digital three-dimensional model was developed to analyze the distribution of UV light irradiance within a representative secondary storage container commonly used in humanitarian settings. This approach provided an initial understanding of how UV irradiance, a fundamental parameter in UV-based treatment for biofilm control, varied spatially along the inner walls of the container.
3. *Experimental validation*: A physical twin of the ray tracing model was assembled in the laboratory to collect UV irradiance readings for the purpose of determining the 3D model's accuracy. Additionally, locations of interest were established for subsequent biofilm experiments.
4. *UV light treatment of lab-grown biofilms*: Non-pathogenic *E. coli* biofilms were cultivated under controlled laboratory conditions to represent microbial contamination on the inner walls of secondary storage containers commonly used in humanitarian settings. Coupons of these biofilms were then installed at the previously identified locations of interest and exposed to UV light at varying UV doses to determine if treatment would have germicidal effect.

1.3. Thesis Structure

This thesis is divided into four chapters including this first introductory chapter. Chapter 2 presents a theoretical evaluation of UV light treatment for biofilm control in humanitarian settings through a scoping literature review. This includes a systematic search of the literature to identify other real-world applications of UV light treatment. The identified research is used to better inform potential future research design that could address the established knowledge gap. Chapter 3 builds on the outcomes from Chapter 2 by examining the technical viability of UV light treatment for biofilm control inside a representative secondary storage container commonly used in humanitarian settings. A ray tracing model is developed to analyze the distribution of UV light inside a jerry can (commonly used in humanitarian settings). This provides an initial understanding of how UV irradiance varies with space since the intensity of UV light plays an important role in biofilm growth control. The ray tracing model is replicated in the laboratory for data collection which can be used to determine the accuracy of the model. The information

gathered from these steps is used to design the UV light treatment experiment, where biofilms cultivated under lab conditions are exposed to UV light inside the jerry can to determine the level of biofilm-bound cell inactivation. Chapter 4 concludes the thesis by providing a general summary of Chapters 2 and 3, tying significant outcomes together. Additionally, the final chapter will also discuss limitations of the research and future research directions.

Chapter 2: Literature Review

2.1. Introduction

Humanitarian response provides aid and relief to people affected by conflict, natural disasters, or other emergencies (Ejiohuo et al., 2024). One of the most significant issues in these settings is safe water supply. In many cases, water quality in these contexts can deteriorate to the point where consumption may cause illness, disease, or death (Roberts et al., 2001; Steele et al., 2008; Swerdlow et al., 1997; Walden et al., 2005). Humanitarian settings often lack adequate water safety barriers to protect against transmission of waterborne pathogens, and large populations in small areas with insufficient water, sanitation, and hygiene (WASH) services are especially prone to outbreaks of communicable diseases (Connolly et al., 2004). Water can be contaminated at the source, during transport, and in households (Agensi et al., 2019; Bain et al., 2014; Günther & Schipper, 2013; Jagals et al., 1997; Jagals et al., 2003; Mellor et al., 2013; Odeleye & Idowu, 2015; Roberts et al., 2001). Since water is often kept in secondary storage containers for many hours or days, microbial contaminants can transition from a planktonic (free-floating) phase to a biofilm phase (and vice-versa), further contributing to the deterioration of stored water quality over time in households (Usman et al., 2018).

The World Health Organization (WHO) recommends a comprehensive strategy to ensure safe drinking water that encompasses supply, treatment, and distribution (WHO, 2022). The Sphere Handbook (Sphere Project, 2018) helps adapt this approach to humanitarian contexts, offering recommendations for protecting water safety in such settings. These actions are summarized in Figure 1.

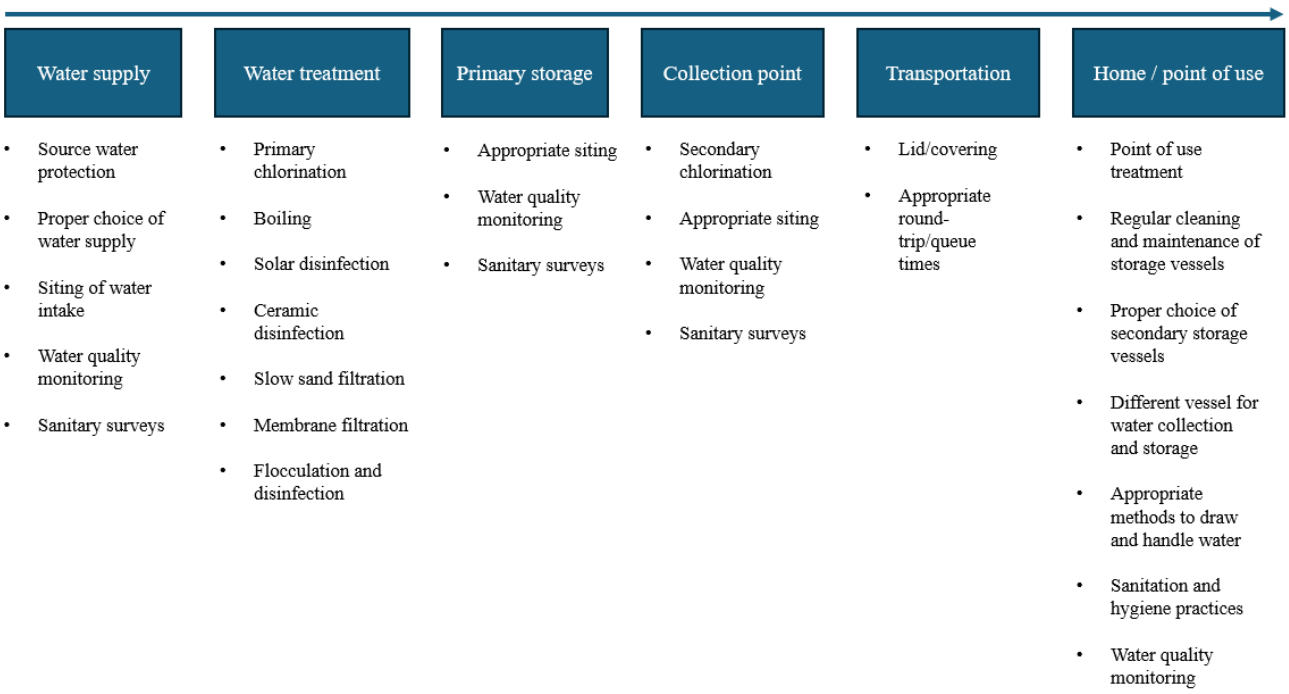

Figure 1: Water safety actions commonly implemented in humanitarian settings. This is not a comprehensive list, but highlights key interventions promoted by authoritative sources on water safety such as Sphere Project (2018) and WHO (2022).

A common water treatment method used in humanitarian settings is chlorination (Ali et al., 2021; Meierhofer et al., 2019). This method provides disinfection and residual protection, as free residual chlorine continues protecting drinking water from microbial contaminants after initial treatment. Drawbacks of this technique include alteration of taste and odour of drinking water and the potential for the formation of disinfection byproducts (DBPs), which present health risks if consumed (Ali et al., 2019).

The transportation and storage of water after collection exposes the water to the risk of recontamination. Various treatment approaches are commonly applied to secondary storage containers to protect both freshly collected water and previously treated water from microbial threats, which commonly includes biofilms. String et al. (2020, 2021) conducted a series of laboratory experiments to evaluate the efficacy of a subset of these methods for both bulk water protection and storage container treatment (Table 1).

Table 1: Summary of Locally-Available Bulk Water Disinfection and Storage Container Cleaning Methods from String et al. (2020, 2021)

Method	**Description**	**Treatment mechanisms**	**Results of method**
Chlorination of bulk water (String et al., 2021)	Aquatabs (effervescent chlorine tablets) added to water inside jerry can	Household water treatment	Biofilm growth significantly inhibited
Chlorine cleaning solution (String et al., 2021)	5.25% liquid chlorine (hypochlorite) diluted to ~ 0.5% by volume; added inside jerry can and shook	Disruption of biofilm on the inner jerry can walls	Biofilm growth inhibited
Rocks (String et al., 2021)	Pea pebbles; added inside jerry can and shook	Disruption of biofilm on the inner jerry can walls	Use of rocks for cleaning increased the roughness of interior wall, leading to more biofilm growth
Sand (String et al., 2021)	Sand; added inside jerry can and shook	Disruption of biofilm on the inner jerry can walls	Not effective
Rocks and chlorine (String et al., 2021)	Combination of pea pebbles and chlorine cleaning solution; added inside jerry can and shook	Disruption of biofilm on the inner jerry can walls	Reduced biofilm thickness and increased surface roughness

Sand and chlorine (String et al., 2021)	Combination of sand and chlorine cleaning solution; added inside jerry can and shook	Disruption of biofilm on the inner jerry can walls	Minimal efficacy, not as effective as chlorine or rocks/chlorine
Bleach (String et al., 2020)	0.52% sodium hypochlorite, pH = 12.4	Tap cleaning (TC) and storage container coupons cleaning (SCCC; Coupons provided uniform test surfaces for evaluating biofilm growth and cleaning efficacy)	TC: Removed biofilm structures after most cleaning methods SCC: Inconclusive results
Previously boiled water (String et al., 2020)	Boiled at ~80 °C, pH = 5.47		TC: Removed biofilm structures via soaking SCC: Inconclusive results
Soapy water (String et al., 2020)	20 g/L soap concentration, pH = 11.5		TC: Did not remove biofilm structure after all cleaning methods SCCC: Not tested
Vinegar (String et al., 2020)	5% acetic acid, pH = 2.4		TC: Removed biofilm structures after scrubbing methods SCC: Inconclusive results

Germicidal ultraviolet (UV) light is a disinfection method that has potential applications at multiple stages of drinking water systems in humanitarian settings. Absorption of UV photons by microorganisms in water and biofilm damages DNA and suppresses further growth (de Almeida et

al., 2022). This method has been widely adopted for urban drinking water and wastewater infrastructure contexts but is underexplored in humanitarian settings. In addition to its germicidal effects, UV light treatment offers additional benefits as a water safety solution since it does not alter the taste or odour of the water and does not produce DBPs.

To optimize the potential of this method, the UV dose delivered to intended targets must be maximized. However, achieving optimal dose delivery can be difficult. For example, different UV light sources have different limitations relating to irradiance reduction, limited wavelength range, or output inconsistencies. External factors, such as limited power from wall outlets and particulate contamination in the ambient environment, could also affect treatment. While these are important considerations in any application of UV light treatment, the question of whether targets receive sufficient irradiation is paramount. A better understanding of what goes into determining an effective UV dose can be found by analyzing its two main variables: intensity and treatment time. The formula for UV irradiance is given by,

$$I = \frac{P}{4\pi x^2} \tag{1}$$

Where I denotes UV irradiance (mW/cm^2), P denotes the power emitted from the UV light source (mW), and x denotes the distance between the source and target (cm). Since the power emitted from the UV light source is usually constant, the distance between the source and disinfection target greatly influences UV irradiance. Shorter distances ensure a greater light intensity is received by the target. Braga et al. (2020) demonstrated this, as their study found greater biofouling reduction at shorter distances during experimentation. This, in tandem with the duration of treatment, directly impacts the amount of UV light delivered to a target, known as UV dose. The relationship between UV dose, UV irradiance, and treatment time can be seen in the following equation,

$$D = I * t \tag{2}$$

Where D denotes UV dose (mJ/cm^2), I denotes UV irradiance, and t denotes treatment time (seconds). Optimizing UV intensity could lead to a reduction of treatment time and power requirements, ultimately improving overall treatment efficiency.

UV light disinfection has demonstrated efficacy for inactivating planktonic microorganisms (Bowker et al., 2011; Li et al., 2019; Song et al., 2016) and surface-bound biofilms (Gora et al., 2024). It therefore has the potential to address microbiological contamination of bulk water and biofilm growth in storage containers simultaneously. For example, *Escherichia coli* (*E. coli*), a common contaminant and indicator of fecal pollution present in drinking water from humanitarian contexts (Nowicki et al., 2021), is susceptible to UV disinfection in its planktonic state (Lanzarini-Lopes et al., 2019a) and biofilm state (Torkzadeh & Cates, 2021). The use of UV light to control mixed bacterial cultures, which better represent the microbial contamination present in humanitarian settings, has also been explored at the lab scale (See Table 3).

The goal of this paper is to theoretically explore how UV light treatment can be applied for the prevention, mitigation, and reduction of biofilm growth in secondary storage containers commonly used in humanitarian settings. Four objectives were carried out to achieve this goal. First, a scoping literature review identifies real-world applications and simulations of UV light treatment that demonstrate effectiveness beyond controlled laboratory environments, accompanied by definitions and summaries of key parameters relevant to these studies. Second, an evaluation of existing research determines the extent to which current literature addresses biofilm control inside secondary storage containers used in humanitarian settings and confirm whether this is a knowledge gap. Third, an outline of practical and contextual factors highlights essential considerations for implementing UV light treatment in humanitarian contexts. Finally, a synthesis of insights from the reviewed studies provides guidance for designing future UV light treatment research that is feasible, operationally realistic, and contextually appropriate for humanitarian settings.

2.2. Methodology

A scoping review was conducted to systematically identify relevant literature which examined UV light treatment for bulk water disinfection and/or biofilm control in real-world settings and simulations. Since contamination by planktonic microorganisms in bulk water is closely connected to biofilm development, both processes were examined together to provide a more integrated understanding of UV light treatment performance. Given the extensive research that has been done to establish the technical foundations and effectiveness of this treatment method through controlled lab experiments (Gora et al., 2024; Luo et al., 2022), the next step involves collecting literature

that translates this knowledge into practical application. In doing so, it becomes possible to bridge the gap between theoretical validation and real-world impact, and to continue building on how this treatment approach could be adapted to the challenges associated with humanitarian settings.

Scopus was systematically searched to identify relevant literature using the following search string: "TITLE-ABS-KEY (ultraviolet) OR TITLE-ABS-KEY (UV) AND TITLE-ABS-KEY (light) OR TITLE-ABS-KEY (exposure) OR TITLE-ABS-KEY (UV-disinfection) OR TITLE-ABS-KEY (UV-C) AND TITLE-ABS-KEY (disinfection) OR TITLE-ABS-KEY (biofouling) AND TITLE-ABS-KEY (biofilm) OR TITLE-ABS-KEY (biofilms)." Initially, "AND TITLE-ABS-KEY (humanitarian)" was included in the search string from Section 2 but was removed after the search yielded no results. Article titles and abstracts were screened through Scopus. Full-text reviews were subsequently conducted by retrieving articles from Scopus or other online repositories such as Omni and Engineering Village. The search was limited to peer-reviewed articles published in English from 2004 to 2025.

All literature identified from the search string underwent title and abstract screening to determine which articles were relevant for this study. Articles passed title and abstract screening if it was evident that the purpose of the study was to investigate UV light treatment for water and biofilms. Studies were included when they demonstrated a clear focus on UV light treatment of water and biofilms under practical, real-world conditions. Practicality was defined in by the setting of the experiment and the origin of the microbial community. Eligible studies were conducted either in full-scale natural environments or in laboratory systems explicitly designed to replicate real-world operational features. Studies were also included when they used microbial communities or samples sourced directly from environmental or operational settings, as these reflect naturally occurring, unaltered microbial populations. In addition to meeting these contextual criteria, studies were required to apply UV treatment in a manner consistent with real-world use, meaning the UV exposure method, configuration, and operational parameters were implemented in a reliable, repeatable, and practically relevant way.

Articles deemed irrelevant to the present study after screening were removed and the rest were retrieved and subjected to full-text review. Articles were excluded during this step if they focused primarily on mechanistic or highly controlled laboratory research rather than practical, real-world applicability. Specific conditions could include (but not limited to) ideal temperature for growth,

presence of nutrients/supplements, and stressors to develop durable biofilms. Research from these articles did not evaluate UV light treatment for direct practical application, nor did they evaluate treatment in conditions that reflect real-world operational environments.

2.3. Summary of Findings

Data items (parameters) for the present review are highlighted in Table 2. These data items were chosen due to their relevance in UV light disinfection studies and because they were the most commonly available data items reported in the relevant literature. After full-text reviews, the parameters that were most frequently revealed in each article were recorded and brought together in Table 3.

Table 2: Parameters Associated with UV Light Disinfection Research

Data Item	**Significance**
UV Dose (mJ/cm^2)	The total amount of UV light delivered to the target
Microorganisms	Microorganisms existing in a planktonic state or embedded in biofilms have different health risks and resistances to UV light. Embedded microorganisms face the possibility of gene mutation as well. Vulnerability of microorganisms to UV light is vital to the efficiency of the treatment process
Treatment Time (min, s)	The amount of time the UV source irradiates the medium/surface
UV Source	Impacts intensity/output of UV light
UV Delivery Configuration	The way UV light is delivered to the target medium/microorganisms/surface will determine more efficient approaches to disinfection
Wavelength (nm)	UVC wavelengths (200-280nm) are germicidal since microorganism DNA/RNA will become damaged by UV photons in this range. Energy associated with wavelengths <230nm are mostly absorbed by proteins and, therefore, not typically considered for germicidal applications (Poster et al., 2021)

In total, 16 articles were systematically identified. Figure 2 demonstrates the PRISMA flow diagram from the article selection process.

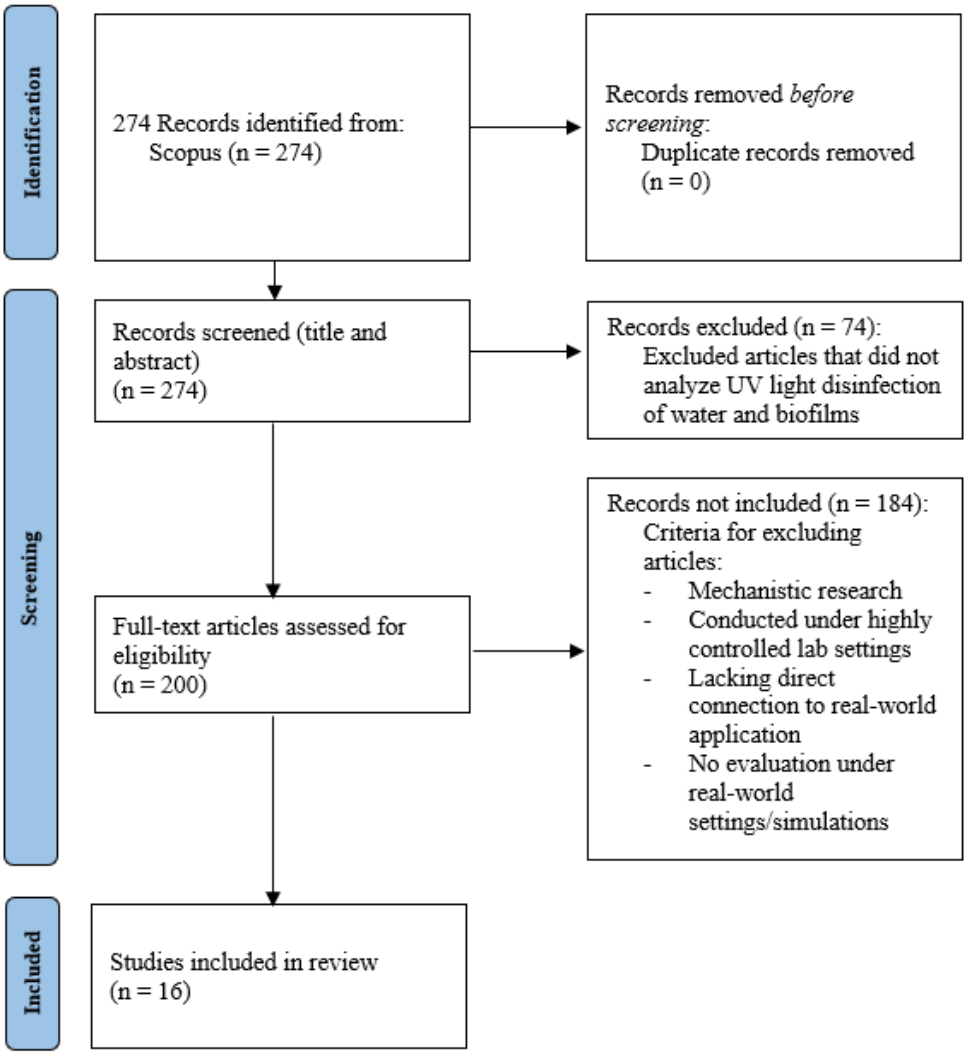

Figure 2: PRISMA flow diagram demonstrating the methodology behind identifying relevant literature.

After evaluating the identified literature, it was evident that UV light treatment for biofilm control inside secondary storage containers commonly used in humanitarian settings has not been explored, establishing this topic as a clear knowledge gap.

The following subsections will highlight key findings from the literature identified as per the methodology stated in Section 2. Table 3 provides a summary of data items extracted from this literature.

Table 3: Summary of Extracted Data Items from Systematically Identified Research Articles

Application	Reference	Dose (mJ/cm^2)	Microorganisms	Treatment Time (min)	UV Source/Configuration	Wavelength (nm)
Catheter Disinfection	Bak et al. 2009	1500 (0-2160)	Mixed culture	0.5, 1, 2, 6, 15, 30, 45, 60, 90	UVC germicidal lamp placed atop viewing cabinet	254
	Bak et al. 2010	7.86	*P. aeruginosa*	15-300	UV LED placed at one end of biofilm-contaminated Teflon/catheter tubes	265
	Bak et al. 2011	0.41×10^{-4}	*P. aeruginosa*	300	UV LED placed at one end of biofilm-contaminated catheter	265
Underwater Antifouling	Braga et al. 2020	-	Mixed culture from seawater	Continuous for 2 months or 1 min/day for 1 month	UVC lamp placed at the center of Nautilus shell	254
	Patil et al. 2007	43.8-2646	Mixed culture from tap water	1, 5, 15, 30	Glass coupons mounted around UV lamp in a seawater pond	254
Pilot-Scale Distribution Network Disinfection	Lehtola et al. 2005	-	Mixed culture from drinking water	-	Biofilm collected from pilot distribution network with polyethylene, copper, and composite plastic pipes and subjected to UV disinfection	254
Membrane Antifouling	Sperle et al. 2020	2	Mixed culture from tap water	-	Flow-through reactor equipped with UVC LEDs prior to interaction with low-pressure reverse osmosis membrane	278
Household Surface Water Treatment	Garcia et al. 2022	Up to 120	Mixed culture from river water	-	Mesita Azul flow through device utilizing a low-pressure UVC lamp attached to a household slow sand filter	254
Groundwater Treatment	Murray et al. 2015	$3x10^6$ and $6x10^6$	Mixed culture from well water	30 or 60	Stratilinker 2400 crosslinking chamber	254
Greywater Treatment	Ongena et al. 2023	$7.26x10^6$ (mJ/L)	Mixed culture from greywater	11	Membrane bioreactor (MBR) coupled with UV-lamp	254
Facial Protheses Disinfection	Malateaux et al., 2021	-	Mixed culture	10	UVC LED light (Clean Bag)	-
	Malateaux et al., 2024	-	Mixed culture	5, 10, 20	Clean Bag II (O2 Led)	254
HVAC Cooling Coil Disinfection	Luongo et al., 2017	-	Mixed culture	-	UVC lamp installed ten inches away from cooling coil in HVAC test apparatus	-

Side-Emitting Optical Fibers (SEOF)	Rho et al. 2022	-	*P. aeruginosa*	-	Reverse osmosis membrane equipped with SEOFs (with UV LED connected)	265
	Zhao et al. 2023a	-	*P. aeruginosa*	-	Flow-through reactor equipped with SEOFs (UV LED equipped) and UVC light at one or both ends	275
	Zhao et al. 2023b	-	*P. aeruginosa*	-	Pipe loop apparatus with multiple reactors; not all reactors equipped with SEOFs (UV LED equipped)	275

2.3.1. Catheter disinfection

Studies by Bak et al. (2009, 2010, 2011) demonstrated the effectiveness of UVC irradiation for biofilm destruction on catheters. Bak et al. (2009) collected patient urinary catheters that had been colonized by various microorganisms. After cutting the catheters into smaller pieces and exposing them to treatment from an UVC germicidal lamp for different intervals between 0 to 90 minutes, Bak et al. (2009) found that mean destruction rates were 89.6% for 0.5 minutes, 98% for 2 minutes, 98.79% for 60 minutes, and 98.53% for 90 minutes. Bak et al. (2010) conducted experiments on catheters left intact by using a LED to guide UVC light into the tube from one of its ends. They found that *Pseudomonas aeruginosa* (*P. aeruginosa*) biofilms grown inside Teflon catheters of the same length did not experience regrowth after treatment for 30, 60, 80, 90, and 300 minutes (treatment for 15 and 120 minutes left very minimal regrowth). Bak et al. (2011) utilized a similar UV configuration to that of Bak et al. (2010) and found that nearly 100% destruction was achieved in the entire lumen of 20 cm tubes contaminated with planktonic bacteria and biofilm.

2.3.2. Underwater antifouling

Both Braga et al. (2020) and Patil et al. (2007) found important trends while utilizing UVC light for in-situ underwater antifouling. Braga et al. (2020) found that the mode of UVC exposure and distance to the light source played a significant role in inhibiting biofilm colonization and destruction of established biofilms. Continuous UVC exposure on submerged polyvinyl chloride panels prevented microorganisms in seawater from surface colonization at all distances while continuous UVC exposure after allowing for biofilm formation reduced biofouling, especially at

shorter distances (Braga et al. 2010). Patil et al. (2007) found that, while UV intensity was important, the manner in which UV light is delivered was more critical since microorganism colonization and proliferation could occur between periodic treatment cycles.

2.3.3. Pilot scale distribution network disinfection

Lehtola et al. (2005) investigated UV disinfection in distribution networks connected to pilot-scale waterworks and found that this treatment significantly reduced heterotrophic plate counts in both the pilot waterworks and the outlet waters of polyethylene and copper pipes.

2.3.4. Membrane antifouling

UV pretreatment for biofouling control in membrane systems was explored by Sperle et al. (2020). By treating feed water with UV LEDs before the liquid was allowed to pass through the membrane, this study found that biofilm formation was significantly delayed and hydraulic resistance was reduced. The researchers explained that inactivation of viable planktonic microorganisms upstream from the membrane inhibited biofilm formation potential on the membrane layers, which establishes the role of UV disinfection as a proactive fouling control method, rather than relying solely on post-fouling cleaning strategies.

2.3.5. Household water treatment

Garcia et al. (2022) explored the effectiveness of an intermittent household slow sand filter which integrated post-treatment UVC irradiation via low-pressure lamp. This research demonstrated that the system was highly efficient, with most of the remaining bacteria from the filtration process inactivated by UV treatment without regrowth for 15 days. One of the factors Garcia et al. (2022) attributed to the remaining bacteria after UV treatment was residual water turbidity and colour, as this could hinder UV transmittance.

2.3.6. Greywater treatment

UVC irradiation has also been explored as a post-treatment method in a study comparing different greywater treatments conducted by Ongena et al. (2023). UV-lamps were coupled to a membrane bioreactor (MBR) and moving bed biofilm reactor (MBBR) for effluent treatment. UV treatment reduced *E. coli* in MBR effluent to non-detectable levels in highly turbid (54.4 NTU) water while

E. coli in the MBBR effluent was disinfected despite the presence of suspended biomass that could have hindered UV transmittance (Ongena et al., 2023).

2.3.7. Groundwater treatment

Murray et al. (2015) investigated the response of biofilms grown inside two groundwater wells to UVC treatment. In-situ growth enabled biofilms to develop under real-world conditions, thus allowing researchers to investigate how the natural biofilm structures responded to UV treatment. After submerging biofilm-coated coupons in well water and exposing them to UVC light, Murray et al. (2015) found that, while treatment was effective, the turbidity of well water was most likely impacting biofilm inactivation rates.

2.3.8. Facial prothesis disinfection

Malateaux et al. (2021, 2024) analyzed how UVC treatment could be used for biofilm reduction on facial protheses. They found that 10 minutes of UVC irradiation was a suitable alternative to other treatment methods, as an adequate level of biofilm reduction was achieved while being straightforward and quick to apply. Malateaux et al. (2021) noted that UVC treatment was only fully effective when the entire surface was exposed to irradiation. Malateaux et al. (2024) carried on this research by exploring multiple UVC treatment times (5, 10, and 20 minutes) for facial protheses and found that a 20-minute UVC exposure time yielded similar effectiveness when compared with other standard disinfection treatments such as 0.12% chlorhexidine and dimethyl sulfoxide. An alteration Malateaux et al. (2024) made based on their previous study was remodelling the configuration of UVC LEDs used in treatment, as this influenced results by delivering a more uniform distribution of treatment across the facial protheses.

2.3.9. HVAC cooling coil disinfection

Luongo et al. (2017) investigated how ultraviolet germicidal coil cleaning (UVG-CC) of HVAC cooling coils could increase heat transfer effectiveness and decrease static pressure drop across the coil. These researchers found that treatment led to a reduction in biofouling, with surface microbial loading reduced by 55% on average. UVG-CC increased heat transfer effectiveness in wetted conditions and had no effect on static pressure drop under mild climatic conditions (Luongo et al., 2017).

2.3.10. Side-emitting optical fibers (SEOFs)

Rho et al. (2022) and Zhao et al. (2023a, 2023b) explored applications of UV light treatment using side-emitting optical fibers (SEOFs). SEOFs combine UV LEDs with optical fibres that have been modified to increase UV light transmission along and next to the fibre. They can deliver UV light to hard-to-access locations like inside of pipes. Rho et al. (2022) found that SEOFs delivering UVC light to a reverse osmosis membrane mitigated biofilm formation by down-regulating quorum sensing (cell-to-cell communication promoting biofilm formation) and surface attachment genes, creating thinner, but denser, biofilms. Zhao et al. (2023a) also found UVC SEOFs inhibited biofilm formation in a pressurized water system with flowing water containing *P. aeruginosa*. In areas of high UVC irradiance, Zhao et al. (2023a) found that the expression of functional genes such as energy DNA repair and quorum sensing were inhibited while areas of low UVC irradiance upregulated genes associated with microbial survival. Finally, by utilizing a newly developed SEOF fabrication method, Zhao et al. (2023b) increased emission of UVC light by 10 times and reduced the amount of biofilm formation inside the tubing walls of their experimental apparatus.

2.4. Considerations for UV-Based Biofilm Control in Humanitarian Settings

2.4.1. Implementation of UV treatment for biofilm control in humanitarian settings

UV light treatment in humanitarian settings can be applied to secondary storage containers at four key stages: within the central water treatment system, at the water dispensing area, at the household, or potentially at an intermediate point where secondary storage containers are decontaminated between uses. A combination of these methods may prove most effective so that multiple biofilm control mechanisms (bulk water disinfection, prevention of biofilm attachment, or control of pre-existing biofilms) can be leveraged and because it is also necessary to consider how the process will be implemented. One approach may involve establishing designated treatment stations within communities, where containers can be exchanged for disinfected containers and water. Alternatively, a decentralized approach could involve response teams visiting individual households to disinfect secondary storage containers on-site, ensuring that containers remain with their owners. For point-of-use based disinfection, an exchange system could be implemented, where used secondary storage containers are replaced with new or sanitized ones,

followed by UV treatment after water collection. This approach would enhance water safety by providing cleaner containers that offer improved protection against contamination.

Table 4 highlights the advantage and disadvantages of each potential location for treatment.

Table 4: UV Disinfection for Biofilm Control at Different Locations in Humanitarian Settings

	Household	**At the Collection Point**	**At the Intermediate Point**
Advantages	Inhibits biofilm colonization potential by inactivating planktonic microbes in bulk water	Inhibits biofilm colonization potential by inactivating planktonic microbes in bulk water	Inhibits biofilm colonization potential by inactivating planktonic microbes in bulk water
	Eliminates established biofilms on inner walls of storage containers	Eliminates established biofilms on inner walls of storage containers	Eliminates established biofilms on inner walls of storage containers
	Point-of-use treatment lowers the risk of consuming contaminated water	Increases the number of containers treated	
	Can investigate/mitigate sources of contamination at the household level		
Disadvantages	Limited household access may hinder efforts	Transporting treated water/containers to the home may introduce recontamination	Transporting treated water/containers to the home may introduce recontamination
	Household visits are time-consuming, reducing treatment capacity		Distant treatment sites may discourage user engagement

2.4.2. Design considerations for the implementation of UV light for biofilm management in secondary storage containers

As Malateaux at el. (2021) noted, UV light treatment can only be fully effective when an entire target is exposed to germicidal irradiation. Achieving this in secondary storage containers in humanitarian setting is challenging due to the geometry of these containers. Geometrical obstructions such as handles can cause sections of the bulk water and parts of the interior surfaces to receive little to no UV irradiation. These areas are known as "dead zones" and may facilitate biofilm growth and recontamination of the bulk water. Figure 3 demonstrates potential dead zones in common secondary storage containers that are receiving UV light treatment from a traditional point source.

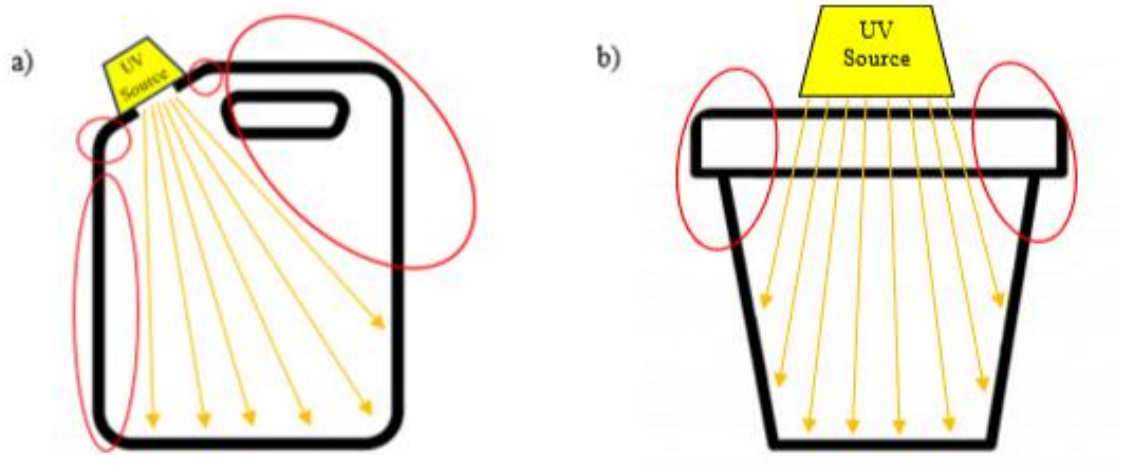

Figure 3: "Dead zones" that may result from conventional point source UV light emitting devices that are positioned over storage containers. Diagram depicts a) a jerry can, and b) a bucket.

As Malateaux et al. (2024) found, the positioning of a UV light source can influence the effectiveness of treatment. Figure 3 demonstrates that positioning UV devices above the opening of the containers will only allow a certain amount of area to receive light. This can create dead zones in the red highlighted areas (Figure 3) and reduce the effectiveness of the intended UV treatment. Therefore, to deliver UV light into dead zones, UV light sources must be designed and positioned in a way to overcome geometrical obstructions and ensure uniform exposure throughout the target area(s). To address this, there are two potential alternatives for the UV light source configuration. The first alternative involves inserting the UV light source into the container. Figure

4 demonstrates how the UV light source could be set up so that it is able to irradiate more area inside secondary storage containers.

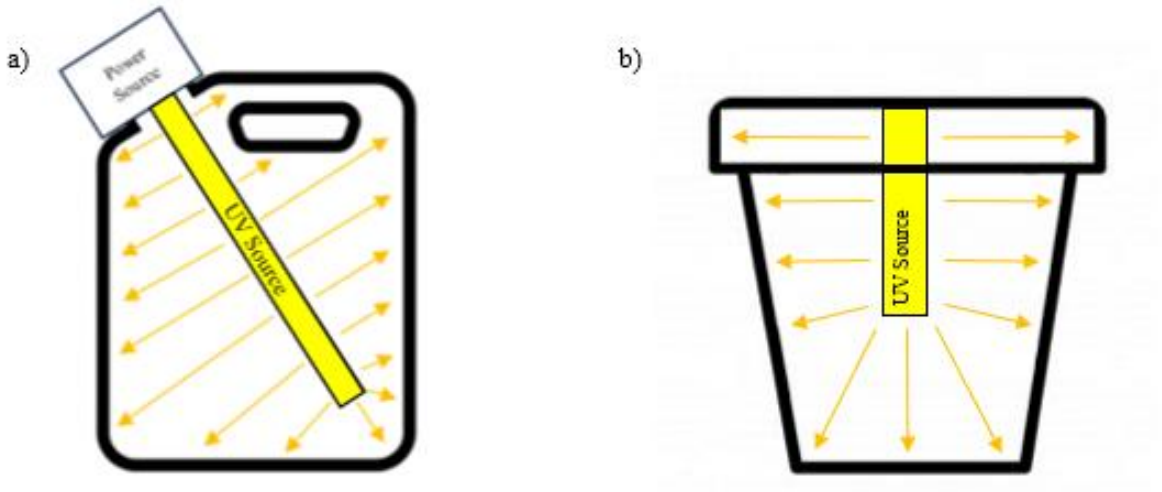

Figure 4: Alternative configurations demonstrating UV light sources inserted into storage containers for increased irradiation. Diagram depicts a) a jerry can, and b) a bucket.

As Figures 3a and 4a demonstrate, the neck/mouth of secondary storage containers may be small and/or narrow. Inserting the UV source into the container bypasses the main design constriction of these vessels. Additionally, the distance between the UV light source and target decreases and allows for greater UV intensity. This could help decrease the amount of time it takes for a germicidal UV dose to be delivered, in line with the findings of Braga et al. (2020), where shorter distances between UV light sources and treatment targets were associated with improved results.

In addition to the UV light source presented in Chapter 3.2.2., UV-SEOFs utilized in Rho et al. (2022) and Zhao et al. (2023a, 2023b) represent promising technology for effectively implementing the configurations highlighted in Figure 4. The slender shape of these fibers can bypass the restrictions imposed at the mouth/neck of these containers and deliver UV light to an increased amount of stored water and inner container walls. However, further advancements must be made to this technology before wide-scale application. While detailed SEOF fabrication steps can be found in O'Neal Tugaoen et al. (2018) and Lanzarini-Lopes et al. (2019b), this technology is developed by replacing the original outer coating and cladding of optical fibers with a new coating containing nanoparticles. This allows light travelling through SEOFs to be partially

refracted into the new coating and absorbed or scattered to the surrounding area (O'Neal Tugaoen et al., 2018). Since a single UV-LED is positioned at one end of the SEOF, the gradual decrease of UV light intensity through the fiber is a significant design constraint. While Zhao et al. (2023a) demonstrated that positioning UV-LEDs at both ends of the fibers can prevent intensity drop off at the distal end of the device, this may not always be applicable in different contexts (including secondary storage containers). However, recent advances in the method of SEOF fabrication made by Zhao et al. (2023b), which explored how increasing the surface roughness of the outer UVC-transparent polymer layer, have demonstrated that it is possible to significantly increase the device's side emissions without sacrificing physical flexibility.

UV sources inserted into secondary storage containers can also be effective when the containers have a large neck/mouth, as seen in Figures 3b and 4b. While the issue of geometrical obstruction is not as prevalent in containers of this design, the size of the UV light source may not be adequate to cover the increased surface area of the inner walls (See Figure 4b). As such, this introduces the second potential alternative for UV light source/configuration which involves using a larger sized UV device for treatment. Figure 5 demonstrates how a larger UV light source could also address the issue of dead zones in larger secondary storage containers.

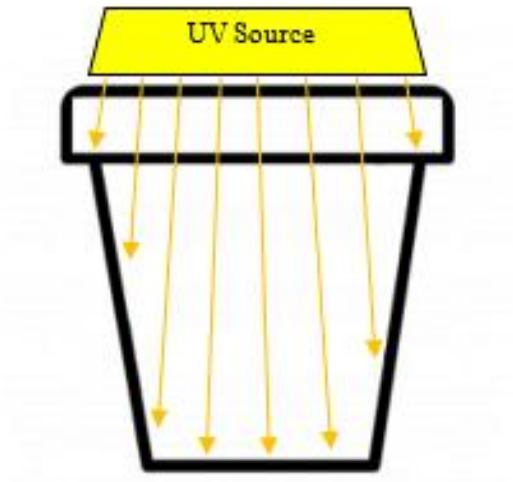

Figure 5: Alternative configuration demonstrating larger UV light sources increased irradiation of larger storage containers (e.g., bucket).

While the distance between the UV light source and the target of disinfection may increase through this alternative, there is a less risk of dead zones. An increase in power requirement could reduce

the time it takes for the intended UV dose to be delivered for treatment. It is important to note that treatment time can vary based on the context in which UV light treatment is applied, as shown by Bak et al. (2009, 2010). For example, highly turbid water could obstruct UV irradiation from reaching certain targets since the particles causing turbidity block/absorb UV rays. Under such conditions, original treatment specifications become obsolete. While it could still be possible for UV treatment to be effective in highly turbid water (Ongena et al., 2023), different environments will yield unique water characteristics that must be integrated into research considerations.

2.5. Conclusions

Contamination of drinking water in secondary storage containers remains a significant threat to the health and well-being of people living through humanitarian crises. Microorganisms introduced to drinking water during collection, transport, and/or storage at home can remain in a planktonic state inside containers or attach themselves to the inner walls of the vessel, leading to the formation of biofilms. This review demonstrates that UV light is a viable treatment strategy to address this issue, supported by its successful use in various practical applications. These applications include medical device disinfection, underwater antifouling, pilot scale distribution network disinfection, membrane antifouling, various water treatment applications, facial prothesis disinfection, and HVAC cooling coil disinfection. A scoping review of existing literature on this topic revealed that the use of UV-based treatment for drinking water safety in humanitarian settings remains largely unexplored, highlighting a critical gap in current knowledge that should be addressed. Developing an effective strategy is essential for implementing UV light treatment at scale and this includes pinpointing where treatment should be carried out (e.g., household, collection point, intermediate location between household and collection point). Key findings from the identified literature were synthesized to better inform research design considerations for adapting UV light treatment to humanitarian settings. Most notably, the restrictions imposed by the geometry of storage containers remains an important design constraint as structural obstructions can lead to dead zones which receive little to no UV light. Technology that is long and slender in shape can overcome these restrictions and maximize the efficiency of the treatment process by ensuring potential dead zones receive a sufficient amount of UV treatment to disinfect bulk water and to prevent, mitigate, and/or reduce biofilm growth on the inner container walls. Ultimately, incorporating the various considerations highlighted in this paper can ensure that UV

is used to promote drinking water safety while also making this treatment more applicable across a wider range of humanitarian settings.

Chapter 3: Modelling and Validation of Biofilm Control Using UV Light in Secondary Drinking Water Storage Containers

3.1. Introduction

Biofilms are surface-attached microbial communities embedded within a matrix of extracellular polymeric substances (EPS), which serves as a protective barrier against antimicrobial agents and environmental stressors (Donlan 2001; Fleeming & Wingender, 2010). These microbial structures are commonly found in aqueous environments, where hydrodynamic shear stress, surface physicochemical properties (e.g., hydrophobicity, surface roughness), and environmental conditions (e.g., nutrient availability, temperature, pH) promote microbial surface adhesion and initiate the biofilm formation process (Kilic, 2025). In addition to surface adhesion, cell-to-cell adhesion occurs through quorum-sensing, where microorganisms release chemical signals within the population to produce EPS and form the biofilm structure (Muhammad et al., 2020). The EPS matrix is essential to biofilm integrity and serves multiple functions including maintaining microbial structure and surface adhesion, shielding embedded microorganisms from external stressors, facilitating the transport of water and nutrients, and promoting bacterial aggregation to support reproduction (Flemming & Wingender, 2010).

Different microorganisms present in water may attach or become entrapped in biofilms (USEPA, 2002). This includes primary bacterial pathogens capable of causing waterborne disease (e.g., *Shigella, Vibrio cholerae,* Enterovirulent *Escherichia coli, Legionella pneumophila*, *Salmonella* spp.), opportunistic bacterial pathogens naturally occurring in water (e.g., *Pseudomonas aeruginosa, Enterobacter* spp*., Klebsiella pneumoniae, Acinetobacter* spp*., Serratia marcescens*), and indicator organisms which can signify the presence of faecal contamination and pathogens in water (e.g., *Escherichia coli*, total coliforms, heterotrophic plate counts) (Health Canada, 2021; Motlagh & Yang, 2019; USEPA, 2002). The biofilm formation potential, attachment, growth, and/or time of survival of these microorganisms has been extensively documented in the literature (Abberton et al., 2016; Bjergbæk et al., 2021; Boyle et al., 1991; Fass et al., 1996; LeChevallier et al., 1987; Lehtola et al., 2005; Pandove et al., 2016; Schaefer et al., 2013; van der Merwe et al., 2013; Williams et al., 2003). Once the biofilm structure begins to deteriorate, viable microorganisms may be released back into the immediate flow regime and

lead to water quality deterioration, downstream biofilm formation, or increased risk of exposure to pathogenic species.

Humanitarian settings are especially vulnerable to microbial contamination in drinking water due to poor water, sanitation, and hygiene (WASH) conditions, contributing to high levels of endemic and epidemic waterborne disease in these settings (Brainard et al., 2018; Monje et al., 2020; Moren et al., 1991; Nguyen et al., 2017; Okello et al., 2019; Qaserah et al., 2021; Roberts et al., 2001; Steele et al., 2008; Walden et al., 2005). Microbial contaminants in planktonic phase (Akhter et al., 2020; Alazzeh et al., 2019; Chalchisa et al., 2017; Gerla et al., 2015; Jagals et al., 1997; Mekonnen et al., 2019; Moropeng et al., 2021; Roberts et al., 2001; Yohannes et al., 2025) and biofilm phase (Bae et al., 2019; Budeli et al., 2018; Jagals et al., 2003; Judah et al., 2024; Mellor et al., 2013; Momba & Kaleni, 2002) have been found in stored water and on the inner walls of storage containers used in these settings, respectively. Research has shown that these containers have been linked to the degradation of microbiological drinking water quality, with water being recontaminated after previously being treated or already being contaminated and degrading further (Agensi et al., 2019; Jagals et al., 2003; Judah et al., 2025; Meierhofer et al., 2019; Mellor et al., 2013; Mintz et al., 1995; Momba & Kaleni, 2002; Roberts et al., 2001; Steele et al., 2008; String et al., 2021; Usman et al., 2018; Walden et al., 2005).

Bulk water treatment and container disinfection are well-documented in the literature as effective measures against microbial contamination in humanitarian settings. The use of chlorine-based disinfectants, through bulk treatment of water (Ali et al., 2015; Ali et al., 2021; Branz et al., 2017; Crider et al., 2022; Gärtner et al., 2021) or manual cleaning of storage containers (Gärtner et al., 2021; Meierhofer et al., 2019; Steele et al., 2008; Walden et al., 2005), is a common method for drinking water protection in these settings. In addition to bulk chlorination, other household water treatment methods such as ceramic filters (Clasen & Boisson, 2006), flocculant-disinfectant (Doocy & Burnham, 2006), solar disinfection (Conroy et al., 2001), boiling (Ram et al., 2007), membrane filtration (Ensink et al., 2015), and slow sand filtration (Graham & Hartung, 1988) are also identified in the Sphere Handbook (2018), the normative minimum standards for humanitarian response. String et al. (2020, 2021) investigated the efficacy of container disinfection methods commonly used in these settings, highlighting that abrasive

materials, liquid disinfectants, or a combination of both can be added to vessels and agitated to dislodge biofilm from interior surfaces.

Although widely adopted in conventional water treatment systems, ultraviolet (UV) light treatment is not commonly deployed in humanitarian settings and was not included among the recommended household water treatment interventions in the Sphere Handbook (2018). While UV light treatment has demonstrated efficacy in reducing microbial contamination in both planktonic (Song et al., 2016) and biofilm (Gora et al., 2024; Luo et al., 2022) phases, it remains underexplored as a strategy for bulk water and container disinfection in humanitarian settings. Moreover, in recent years, UV light emitting diodes (LEDs) have gained traction as a sustainable alternative to conventional mercury-based UV lamps (Nicolau et al., 2022). With this technological shift, UV LED-based devices intended for drinking water protection are becoming more accessible for household point-of-use applications. This treatment strategy does not require chemicals, nor does it alter the taste or odour of water. The mechanism behind this treatment centres on absorption of UV photons by free-floating microorganisms in water or embedded microorganisms in biofilms, leading to suppression of DNA replication and microbial growth (de Almeida et al., 2022).

To investigate the technical viability of UV light treatment for biofilm control in secondary storage containers in humanitarian settings, the present study had the following three objectives:

1. Ray tracing model: To understand UV irradiance at the inner walls of a representative secondary storage container commonly used in humanitarian settings (a jerry can), a digital 3D model was developed to carry out ray tracing simulations. These simulations would predict the intensity of UV light at the surfaces where biofilms are most likely to form and provide insight as to whether germicidal thresholds could be achieved.
2. Experimental validation: To confirm the accuracy of the model, laboratory experiments were conducted under conditions replicating the digital setup.
3. UV light treatment of lab-grown biofilms: Non-pathogenic *E. coli* biofilms were cultivated under laboratory conditions and installed at the previously identified locations to determine if the measured UV irradiance levels produced germicidal effects.

3.2. Methodology

3.2.1. Study design

Analyzing UV irradiation on the inner surfaces of the jerry can established an understanding of spatial variability during UV exposure, allowing for identification of low-irradiance locations. The ray tracing model was developed to simulate a practical arrangement of a UV light source and jerry can, incorporating input parameters that closely reflected their real-world properties. The digital model was replicated with the physical objects in the laboratory to conduct the validation experiment. The findings from these steps guided the design of subsequent UV light treatment, which first required biofilm cultivation to approximate real-world contamination from inner jerry can surfaces. These biofilms were then installed at the previously identified low-irradiance locations and exposed to UV light to determine biofilm-bound cell inactivation in zones facing greater vulnerability to microbial colonization. These results were statistically analyzed and fitted to a multiple disinfection models to characterize the relationship between UV dose and biofilm-bound cell concentration after treatment.

3.2.2. UVC LED light source

The UVC LED Clean Light (Type: CS-UV011; JackSupermall) was used as the UV light source. UV light was emitted from this source at 254 nm through a 25 cm cylindrical quartz glass tube. The total length of the device was 33 cm with a 0.64 cm diameter. Power consumption of the light was listed at 11 W. The device can be seen in Figure 6.

Figure 6: The UVC LED light source. UV light only irradiated from the transparent cylindrical tube.

3.2.3. Ray tracing model

The jerry can geometry was digitally reconstructed using SolidWorks 2025 (Student Edition; Dassault Systèmes: Waltham, Massachusetts, USA) and transferred into TracePro (version 25.3.0; Lambda Research Corporation: Westford, Massachusetts, USA) to assign material and

surface properties for a full solid-body jerry can characterization. Material properties included the index of refraction and absorption coefficient, which determine how light refracts, transmits, and attenuates within a medium. Surface properties incorporated specular reflection and absorptance, describing the proportion of incident light reflected or absorbed at each interface. The UVC LED light source was digitally recreated using the geometry feature in TracePro. The surface source feature was used to convert this geometry to a UV light emitting source by assigning the flux emission type, representing a uniformly emitting surface with total radiant power evenly distributed and emitted randomly across the surface area. A total of 1,000,000 rays were traced during the simulation to smooth out noise and variance in the results. The number of rays traced can be adjusted based on user preference or model demands, but becomes redundant past a certain threshold due to saturation of smoothing effects. Dry conditions were applied to the model for reasons stated in Section 3.2.4.

3.2.4. Experimental validation

UV irradiance readings were taken using a Flame Series Miniature Spectrometer (Model: FLAME-T-UV-VIS-ES; OceanOptics), a fiber-optic coupled instrument equipped with a 0.635 cm probe to acquire optical readings. A rectangular method was used to determine the irradiance of the UVC LED light source spectrum from 250-400 nm. Readings were recorded in triplicates.

All UV irradiance readings were collected inside a locally-purchased 20 L high-density polyethylene (HDPE) jerry can (intended for fuel). Holes slightly larger than 0.635 cm were drilled at 47 locations along the jerry can walls and the spectrometer probe was inserted for data collection. Hole locations (Figure 7) were determined by identifying potential hotspots for microbial accumulation (e.g., corners and edges), areas that may be prone to geometrical obstructions due to the shape of the jerry can, and other points that would typically be submerged after water collection. The UVC LED light source was inserted through the neck of the container and aligned with the neck angle, ensuring no wire was inserted through the mouth. The experiment was conducted in dry conditions, as it was not possible to hold water in the jerry can given the position of the holes. The experimental setup is depicted in Figure 8.

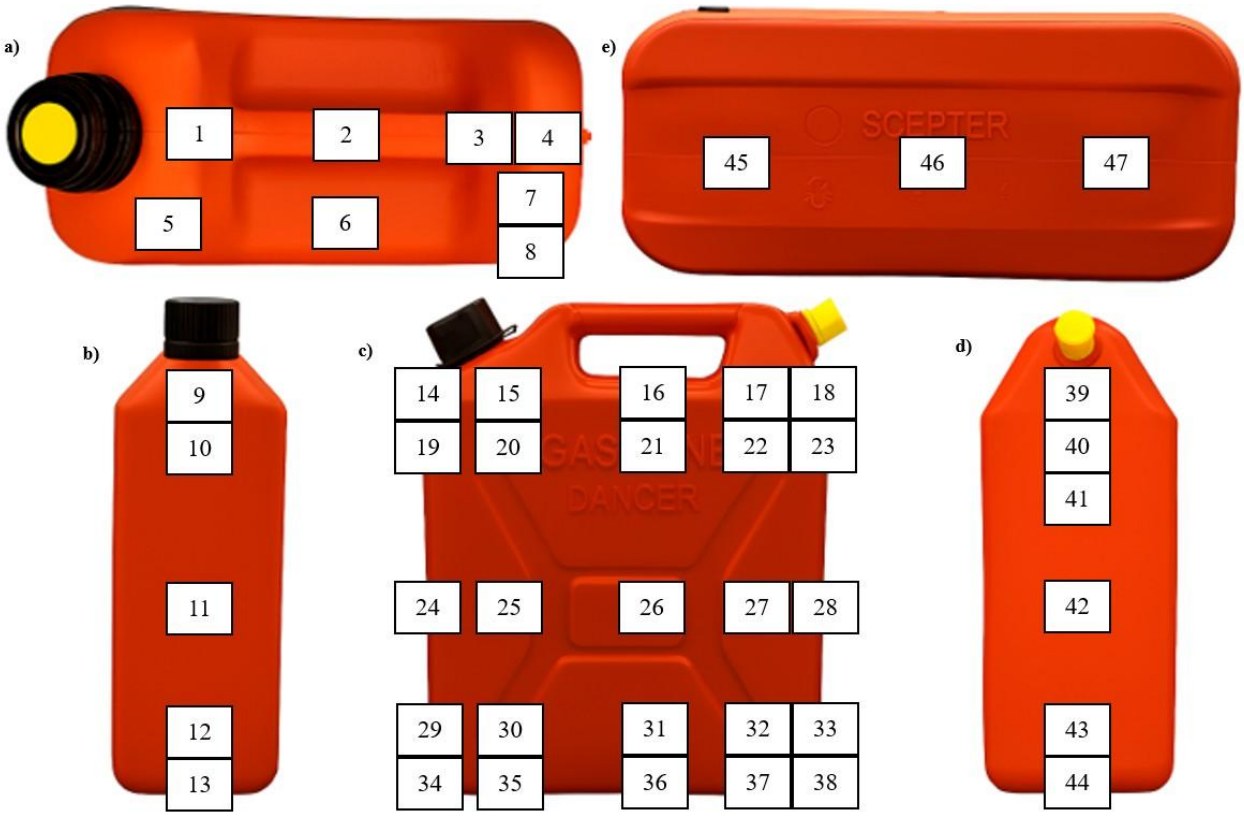

Figure 7: Hole locations in the jerry can experimental setup. Readings were taken from the a) top; b) front; c) side; d) back; e) bottom (location 34 is at the front of the jerry can while location 38 is at the back).

Figure 8: The yellow outline highlights the position of the UVC LED light source inside the jerry can. It was positioned at the same angle as the neck and extended to the center of the container.

Percentage root mean square error (%RMSE) was used to compare modelled UV irradiance readings (from TracePro) to experimental readings (from the spectrometer). %RMSE is defined by the following:

$$\%RMSE = \frac{\left(\frac{\sum_{i=1}^{N} y_i}{N}\right)}{\sqrt{\frac{\sum_{i=1}^{N} \|y_i - \hat{y}_i\|^2}{N}}} \quad (3)$$

Where y_i is the observed value, $\hat{y}_i$ is the predicted value, and N is the number of observations.

3.2.5. UV light treatment of biofilms

3.2.5.1. *Escherichia coli* reactivation and bacterial stock preparation

Escherichia coli (*E. coli*) ATCC 25922 (Cedarlane Labs: Burlington, Ontario, Canada) was used in all microbial experiments due to its non-pathogenic nature and rapid biofilm growing ability (Torkzadeh et al. 2021). The stock culture vial containing the lyophilized bacterium was kept in frozen conditions until experiments began. Sterile disposable inoculation loops were used to

transfer small pieces of bacteria from the vial into falcon tubes containing 5 mL of autoclave-sterilized 30 g/L tryptic soy broth (TSB) media (Becton, Dickinson and Company: Franklin Lakes, New Jersey, USA). The tubes were cultured in a shaking incubator at 115 rpm and 37°C for 18 hours.

3.2.5.2. CDC Biofilm Reactor (CBR)

Biofilm growth occurred inside the CDC Biofilm Reactor (Model: CBR 90; BioSurface Technologies Corporation: Bozeman, Montana, USA). The CBR holds a maximum of 24 coupons (1/2" diameter) which can be installed across eight polypropylene rods held in place by an ultra-high molecular weight lid. A central glass rod attached to the underside of the lid holds a baffled stir bar in place to create constant mixing and shear force to propel planktonic microorganisms onto the coupon surfaces and initiate biofilm formation. The CBR was autoclave-sterilized prior to every use.

3.2.5.3. Viable *Escherichia coli* density determination

Microbial experiments conducted in the CBR were completed by following the CDC Biofilm Reactor operator's manual (Biosurface Technologies Corporation, 2022). Since the manual was tailored for biofilm growth using *Pseudomonas aeruginosa* (*P. aeruginosa*), a series of growth kinetics experiments were completed to identify ideal conditions for biofilm growth using *E. coli* ATCC 25922. The previously prepared bacterial stock was diluted on 40 g/L tryptic soy agar (Becton, Dickinson and Company: Franklin Lakes, New Jersey, USA) using the streak plate method to isolate bacterial colonies. Plates were stored in an incubator at 37°C for 24 hours. The CBR operator's manual specified that the viable bacterial density of the seed culture to initiate biofilm growth should be greater than 10^8 colony forming units (CFU) per mL. Various TSB concentrations ranging from 100 mg/L to 3,000 mg/L were tested to determine the ideal nutrient concentration to meet this threshold. Each seed culture was inoculated with three isolated bacterial colonies, homogenized using a vortex, and placed in a shaking incubator at 115 rpm and 37°C for 24 hours. Optical density at 600 nm readings verified that *E. coli* growth in all concentrations reached the stationary phase after 24 hours. Plate counts revealed that a TSB concentration of 600 mg/L yielded the desired 10^8 CFU/mL, thus establishing the ideal nutrient concentration for the *E. coli* seed culture.

3.2.5.4. Biofilm growth apparatus and procedure

The biofilm growth apparatus (Figure 9) consisted of an influent carboy connected via tubing to the CBR, with the reactor subsequently connected to an effluent carboy for the collection of excess liquid. A peristaltic pump was used to carry influent liquid to the reactor while a stir plate was used to keep the baffled stir bar in constant rotation.

Figure 9: Fully assembled biofilm growth apparatus. Nutrient media is pumped out of the influent carboy (bottom left) and into the reactor. The CBR is attached via tubing to the effluent carboy, where excess liquid is stored once it is carried out of the reactor

E. coli biofilms were grown in the CBR according to the methods described in the CBR's operator manual (Biosurface Technologies Corporation, 2022). Specific modifications were made to this procedure to accommodate the growth characteristics of *E. coli*. All biofilms were

grown on polycarbonate coupons that underwent sonication twice (once in 70% ethanol and once in DI water) for 5 minutes prior to installation in the CBR and autoclave-sterilization. Polycarbonate was used as a substitute for HDPE because it demonstrated comparable UV inactivation performance, similar reflectance properties, and increased surface roughness that better supports biofilm attachment (Mullin et al., 2025). A bottle containing 510 mL of 600 mg/L TSB was autoclaved-sterilized to prepare the seed culture and batch phase liquid. A volume of 10 mL was transferred to a sterile falcon tube and inoculated with three isolated *E. coli* colonies before incubating at 115 rpm and 37 °C for 24 hours. An influent media of 7.2 L of 200 mg/L TSB was autoclaved-sterilized with the CBR and tubing. The remaining 500 mL of previously prepared liquid was poured into the CBR and inoculated with 1 mL of *E. coli* seed culture. The reactor was stirred at 120 rpm and maintained at 23°C during the 24 hours of batch operation. Following this initial growth phase, the CBR was switched into continuous phase operation. The previously prepared 7.2 L of influent media was then fed into the reactor at a flow rate of 5 mL/min over 24 hours.

3.2.5.5. UV light treatment apparatus and procedure

After 24 hours of continuous phase reactor operation, coupons were subjected to UV light treatment via the UVC LED light source. Rods containing biofilm-coated coupons were removed from the CBR and rinsed with 1 mL of phosphate buffer saline (PBS) to remove excess planktonic microorganisms. To simulate biofilm presence on the inner walls of secondary storage containers and enable subsequent UV light treatment, larger holes were drilled at two locations in the jerry can to facilitate coupon installation and exposure. Location 38 (low-irradiance location, LIL), was selected since the lowest UV irradiance reading was recorded at this location. Location 26 (moderate-irradiance location, MIL), was selected to verify results as the UV irradiance recorded at this location was order of magnitudes greater than LIL. LIL was measured to be 20 cm from the UV light source while MIL was 5 cm from the light source. The coupon face without biofilm growth was attached to an adhesive and installed for treatment. Chen et al. (2025) investigated the effects of UV_{254nm} light treatment on surface attached *E. coli* ATCC 25922 at UV doses of 2, 4, 8, and 16 mJ/cm^2. Given the use of the same wavelength and microorganisms in the present study, these UV doses were applied to the coupons installed in the jerry can. Since UV irradiance was known from previous measurements, the treatment time required to achieve each dose could be calculated by rearranging Eq. 4 into Eq. 5:

$$D = I * t \quad (4)$$

$$t = \frac{D}{I} \quad (5)$$

Where t represents treatment time (s), D represents UV dose (mJ/cm^2), and I represents UV irradiance (mW/cm^2). Table 5 summarizes the parameters described above.

Table 5: Experimental parameters at each location

		Treatment Time			
Location	**UV irradiance**	**2 mJ/cm^2**	**4 mJ/cm^2**	**8 mJ/cm^2**	**16 mJ/cm^2**
MIL	0.392 mW/cm^2	5 s	10 s	20 s	40 s
LIL	0.0163 mW/cm^2	120 s	240 s	480 s	960 s

3.2.5.6. Biofilm recovery, resuspension, and plating

The methods described by Gora et al. (2019) were utilized for biofilm recovery and resuspension. Untreated (for initial quantification) and treated biofilms were swabbed with sterile cotton swabs and transferred into falcon tubes containing 5 mL of PBS. Tubes were vortexed for 30 seconds to homogenize the microbial resuspension. Serial dilutions were carried out to prepare samples for quantification via CFU counts. TSA plates were divided into equal sections depending on the number of dilutions for each microbial resuspension. Each section was inoculated with 5 µL droplets of the corresponding dilution three times. All plates were wrapped with parafilm and stored in an incubator at 37°C for 18-24 hours.

3.2.5.7. Plate counting

Biofilm response to UV light treatment was quantified using plate counts, which measures the inactivation of biofilm-bound cells rather than changes in biofilm biomass, structure, or EPS composition. This method is reliable and widely used in UV/biofilm research to assess the viability of culturable cells (Gora et al., 2024). Therefore, to quantify UV light treatment performance, log reduction value (LRV) was calculated at each UV dose for each experimental trial. LRV is defined by the following formula:

$$LRV = \log_{10}\left(\frac{N_0}{N}\right) \quad (6)$$

Where N_0 is the initial biofilm-bound cell concentration (CFU/mL) and N is the biofilm-bound cell concentration after treatment (CFU/mL).

3.2.5.8. Biofilm-bound cell inactivation analysis

LRVs were analyzed through hypothesis testing at a 5% significance level. The Shapiro-Wilk test was used to determine if datasets followed a normal distribution. This would dictate whether to continue analysis with parametric or non-parametric tests. Upon confirmation of normality, a one-way analysis of variance (ANOVA) was used to test central tendencies of datasets, Levene's test was used to test variances of datasets, and a two-way ANOVA was used to test the main and interaction effects of two independent variables (location and UV dose) on a single dependent variable (LRV). Tukey's Honestly Significant Difference (HSD) was used for post hoc analysis.

3.2.5.9. Disinfection models

Previous UV/biofilm research from Gora et al. (2019) and Ma et al. (2022) fit their UV dose response data to the Chick-Watson model and the Geeraerd model, while the research of the former also fit their data to the multi-target model. As such, the UV dose response from the current study was fitted to these three models.

The Chick–Watson model was used to quantify the linear relationship between UV dose and biofilm-bound cell inactivation. The model is defined by the following:

$$\frac{N_t}{N_0} = 10^{-kD} \tag{7}$$

Where N_t represents biofilm-bound cell concentration at a given UV dose (CFU/mL), k represents the inactivation rate constant (cm^2/mJ), D represents UV dose (mJ/cm^2), and N_0 represents the initial biofilm-bound cell concentration before treatment (CFU/mL). The inactivation rate constant was estimated using Microsoft Excel's Solver function by minimizing the residual sum of squares between the observed and predicted surviving biofilm-bound cell concentrations.

The Geeraerd model (Geeraerd et al., 2000) was used to describe biofilm-bound cell inactivation given its ability to characterize non-linear behaviour. This model accounts for tailing and shouldering effects caused by a resistant proportion of the microbial population and an initial lag time between the start of treatment and the start of microbial inactivation, respectively. In the

present study, no apparent shoulder phase was observed. Therefore, the model is described by the following (Geeraerd et al., 2005):

$$N_t = (N_0 - N_{res})e^{-kD} + N_{res} \quad (8)$$

Where N_t represents biofilm-bound cell concentration at a given UV dose (CFU/mL), N_0 represents the initial biofilm-bound cell concentration before treatment (CFU/mL), N_{res} represents the resistant biofilm-bound cell population (CFU/mL), k represents the inactivation rate constant (cm^2/mJ), and D represents UV dose (mJ/cm^2). Model parameters (N_{res} and k) were estimated using Microsoft Excel's Solver function by minimizing the residual sum of squares between the observed and predicted surviving biofilm-bound cell concentrations.

A multi-target model was used to represent biofilm-bound cell inactivation as a process requiring multiple effective "hits," while also capturing shouldering behaviour observed in non-linear survival curves. The model is defined by the following:

$$\frac{N_t}{N_0} = 1 - (1 - 10^{-kD})^n \quad (9)$$

Where N_t represents biofilm-bound cell concentration at a given UV dose (CFU/mL), N_0 represents the initial biofilm-bound cell concentration before treatment (CFU/mL), k represents the inactivation rate constant (cm^2/mJ), D represents UV dose (mJ/cm^2), and n represents that number of effective hits. The inactivation rate constant and number of hits were estimated using Microsoft Excel's Solver function by minimizing the residual sum of squares between the observed and predicted surviving biofilm-bound cell concentrations.

The goodness-of-fit of each model was evaluated using R^2, root mean squared error (RMSE), and mean squared error (MSE). These metrics are defined by the following:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{N}(y_i - \bar{y}_i)^2} \quad (10)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\|y_i - \hat{y}_i\|^2}{N}} \quad (11)$$

$$MSE = \frac{\sum_{i=1}^{N}\|y_i - \hat{y}_i\|^2}{N} \quad (12)$$

Where y_i is the observed value, $\hat{y_i}$ is the predicted value, $\overline{y_i}$ is the mean of the observed values, and N is the number of observations.

3.3. Results

3.3.1. Ray tracing model and experimental validation

The ray tracing model can be seen in Figure 10. This geometry contained 212 surfaces, as rounding of edges (a "fillet") added new surfaces to the established model. HDPE material and surface properties were adjusted through an iterative process to best replicate the physical jerry can used during experimental validation. The parameters were refined through trial-and-error calibration until the model produced consistent and realistic behaviour. Table 6 summarizes the final material and surface property values used in the model.

Figure 10: 3D TracePro model of the jerry can with the UVC LED light source (cylindrical object positioned on an angle).

Table 6: Material and surface properties used in the ray tracing model

Property	Parameter	Value
Material	Index of refraction	1.54
	Absorption coefficient (1/mm)	10
Surface	Specular reflection	0.55
	Absorptance	0.45

Results of the ray tracing analysis were provided as irradiance maps, where incident UV irradiance was visualized using a spectral colour gradient (rainbow scale on black). The %RMSE between the modelled UV irradiance readings (from TracePro) and the experimental readings (from the spectrometer) was 17%. Sample results taken from the front and side of the jerry cans are presented in Figure 11 and Figure 12, respectively. Measurements recorded from the front of the jerry can in both instances showed greater difference between modelled and experimental outcomes than the readings from the side. This was especially apparent at locations 9 and 10, as the UV irradiances predicted by the TracePro model were less than that measured in the lab. The UV irradiance profiles taken from the side of the jerry can in both instances demonstrate similar distributions, showcasing how well TracePro is able to reproduce experimental measurements. The UVC LED light source did not produce uniform UV light irradiation along the full length of the device, leading to variation in irradiance readings. For example, locations 20 and 26 were directly beside the device, yet, the UV irradiance was different between these points since the device produced different intensities at these respective positions. Readings from the back (locations 17, 18, 22, 23, 27, and 28) and bottom (locations 29-38) of the jerry can were found to be lowest since these areas were furthest from the UV light source.

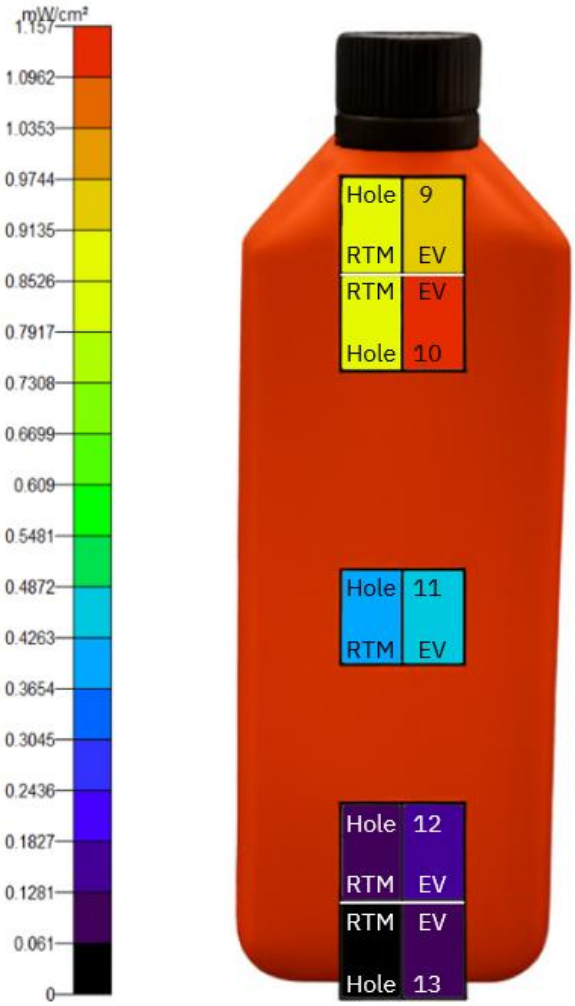

Figure 11: UV irradiance from the ray tracing model (left panel in each box, “RTM”) vs UV irradiance from experimental validation (right panel in each box, “EV”) both taken from the front of the jerry can.

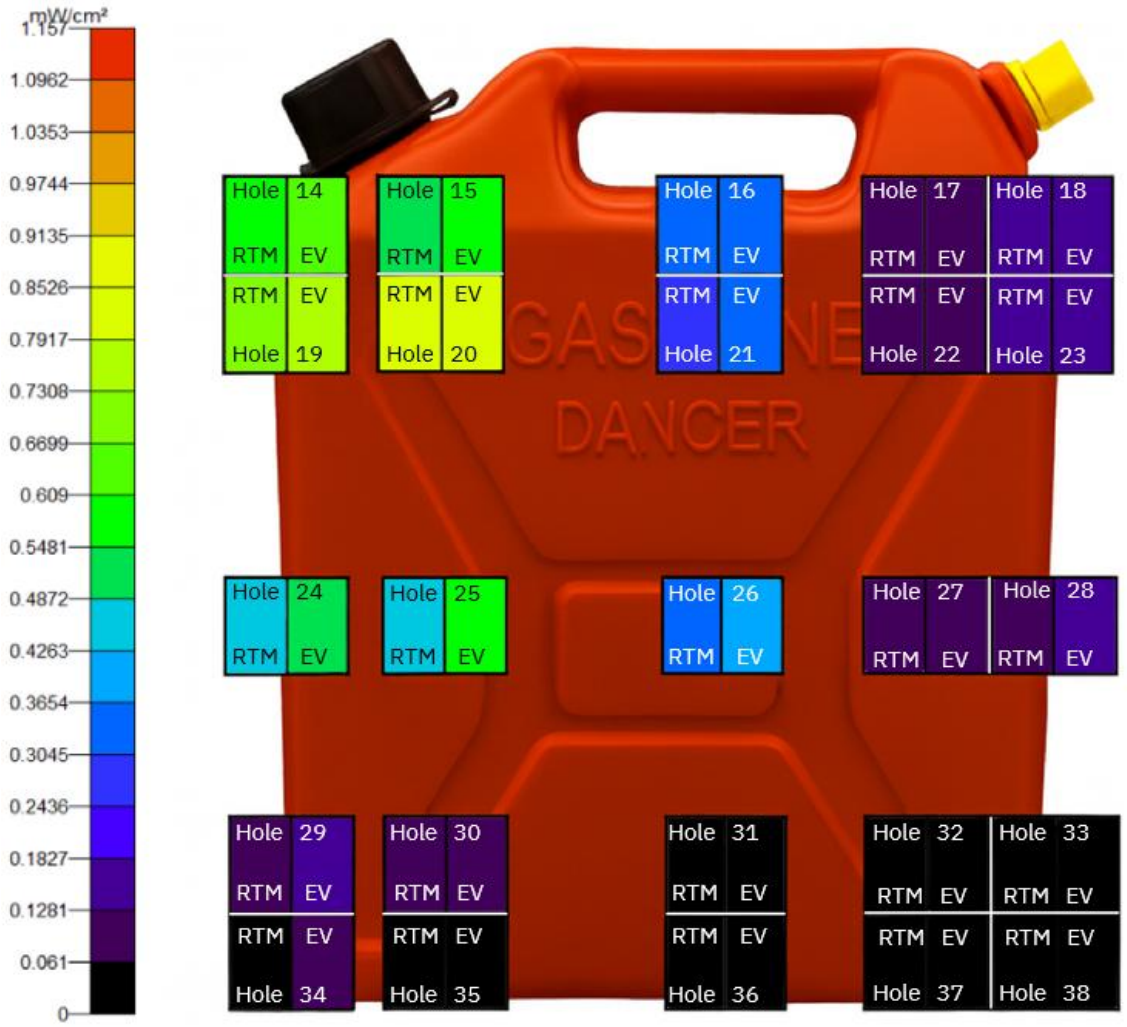

Figure 12: UV irradiance from the ray tracing model (left panel in each box, "RTM") vs UV irradiance from experimental validation (right panel in each box, "EV") both taken from the side of the jerry can.

3.3.2. UV light treatment of lab-grown biofilms

3.3.2.1. Biofilm-bound cell inactivation analysis

Location-specific LRVs were grouped by trial (2 locations, 4 trials per location, 4 LRVs per trial), and hypothesis testing was conducted to determine if it was reasonable to consider trials as replicates of each other. The Shapiro-Wilk test results indicated that the trial data met the assumption of normality. At MIL, no statistically significant differences were found when comparing the means ($p = 0.60$; one-way ANOVA) and variances ($p = 0.70$; Levene's test) of each set of replicates. Likewise, at LIL, no statistically significant differences were found when comparing means ($p = 0.41$; one-way ANOVA) and variances ($p = 0.79$; Levene's test) of each

set of replicates. This indicated that it was reasonable to treat trials as replicates of each other at each location.

Table 7 and Figure 13 summarize the results of the experiments conducted in different locations and at different UV doses.

Table 7: LRVs at each location (average ± standard deviation)

Location	2 mJ/cm²	4 mJ/cm²	8 mJ/cm²	16 mJ/cm²
MIL	0.34 ± 0.22	0.78 ± 0.42	1.29 ± 0.43	2.47 ± 0.75
LIL	1.08 ± 0.47	1.68 ± 0.31	2.51 ± 0.27	2.99 ± 0.07

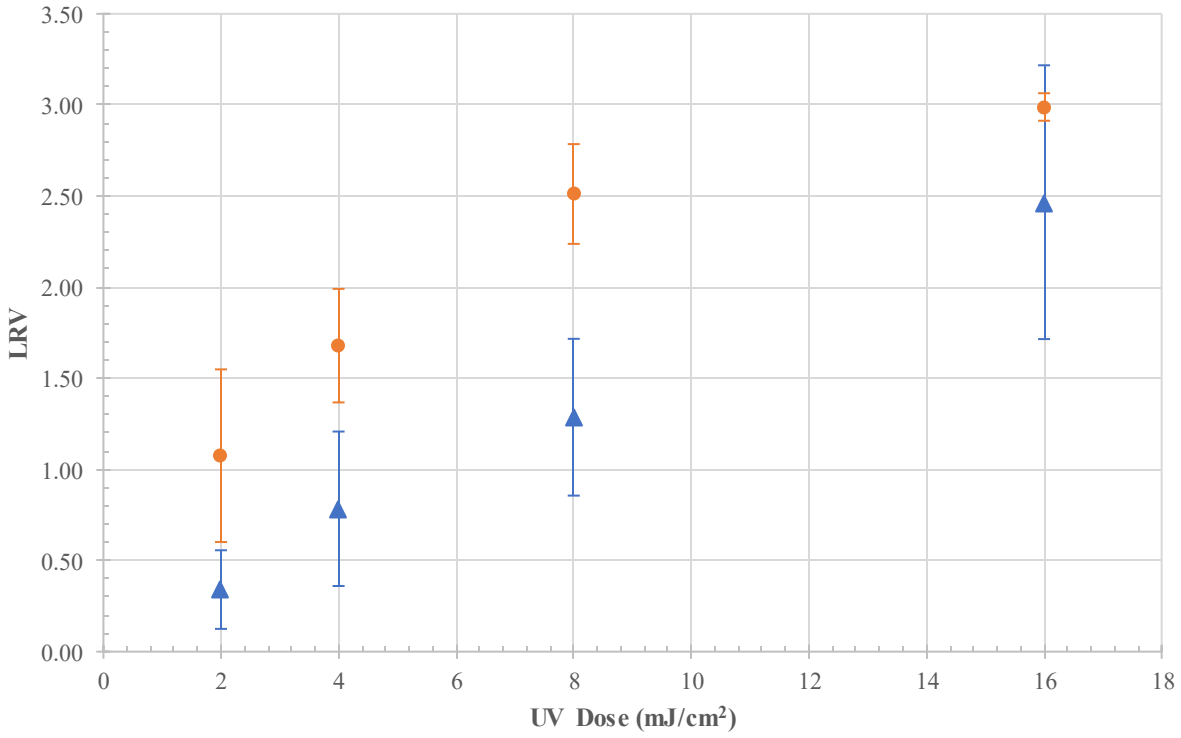

Figure 13: Average LRV with standard deviation bars from experiments conducted at MIL (triangles) and LIL (circles).

After observing the differences in LRVs at each location and the differences in LRVs between UV doses at each location in Figure 13, hypothesis testing was carried out to confirm if these discrepancies were statistically significantly different. The Shapiro-Wilk test results confirmed that the data met the assumption of normality. It was found that there were significant differences

in LRVs between MIL and LIL ($p < 0.05$; two-way ANOVA and Tukey's HSD). It was also found that there were significant differences in LRV between UV doses at each location. Post hoc pairwise testing revealed that, at MIL, significant differences were present between 2 and 16 mJ/cm^2, 4 and 16 mJ/cm^2, and 8 and 16 mJ/cm^2 ($p < 0.05$; two-way ANOVA and Tukey's HSD). At LIL, significant differences were present between 2 and 8 mJ/cm^2, 2 and 16 mJ/cm^2, and 4 and 16 mJ/cm^2 ($p < 0.05$; two-way ANOVA and Tukey's HSD). The interaction between location and UV dose was not significant ($p = 0.59$, two-way ANOVA), indicating that the effect of UV dose on LRV was consistent across both locations.

3.3.2.2. Disinfection models

The relationship between UV dose and surviving biofilm-bound cells is often non-linear (Gora et al., 2024), aligning with a modelling approach presented in Geeraerd et al. (2000). Experimental data from the current study were fitted to the Geeraerd model to characterize this relationship (Figure 14).

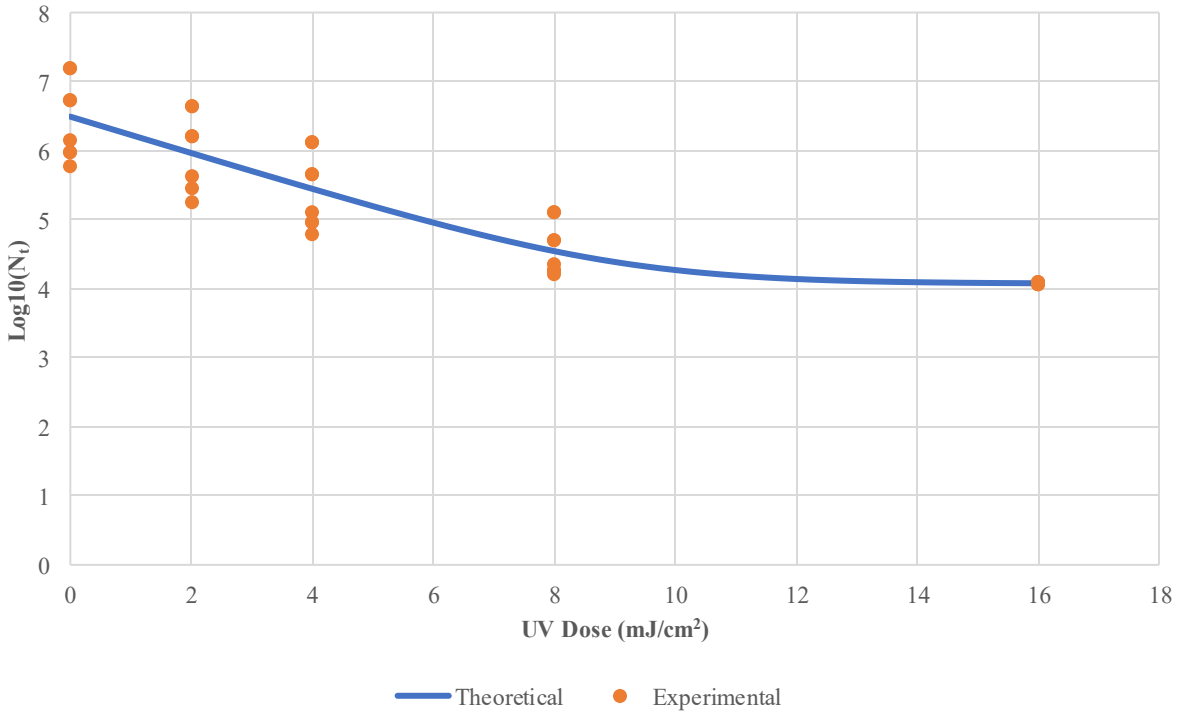

Figure 14: Data from the present research fitted to the Geeraerd model ($R^2 = 0.76$, RMSE = 0.30, and MSE = 0.090).

The Geeraerd model represented a reasonable fit for the data from the current study (R^2 = 0.76, RMSE = 0.30, and MSE = 0.090) with estimated microbial kinetic parameters N_{res} = 1.18 x 10^4 CFU/mL and k = 0.61 cm^2/mJ. The data was also fitted to the Chick-Watson model (Figure 15) and the multi-target model (Figure 16) to evaluate how well other disinfection models could capture the relationship between UV dose and surviving biofilm-bound cells. The evaluation metrics for the Chick-Watson model (R^2 = 0.73, RMSE = 0.42, and MSE = 0.18) and the multi-target model (R^2 = 0.73, RMSE = 0.42, and MSE = 0.18) confirmed that the Geeraerd model better captured the dose-response relationship.

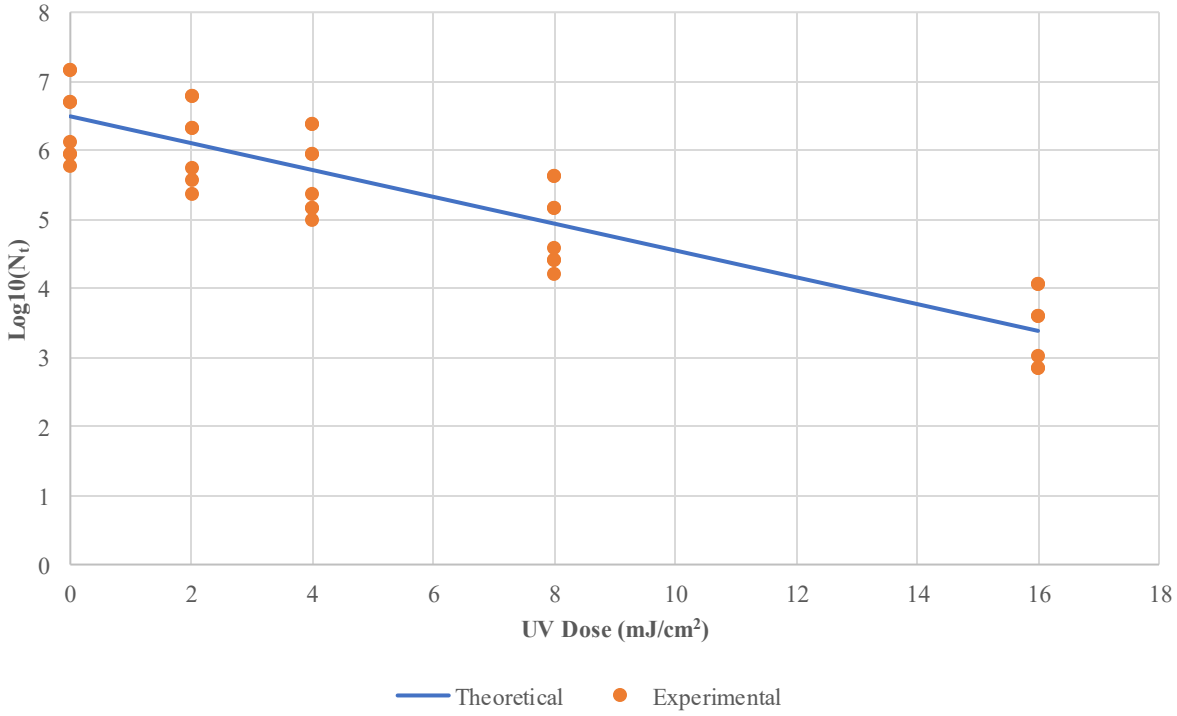

Figure 15: Data from the present research fitted to the Chick-Watson model (R^2 = 0.73, RMSE = 0.42, and MSE = 0.18).

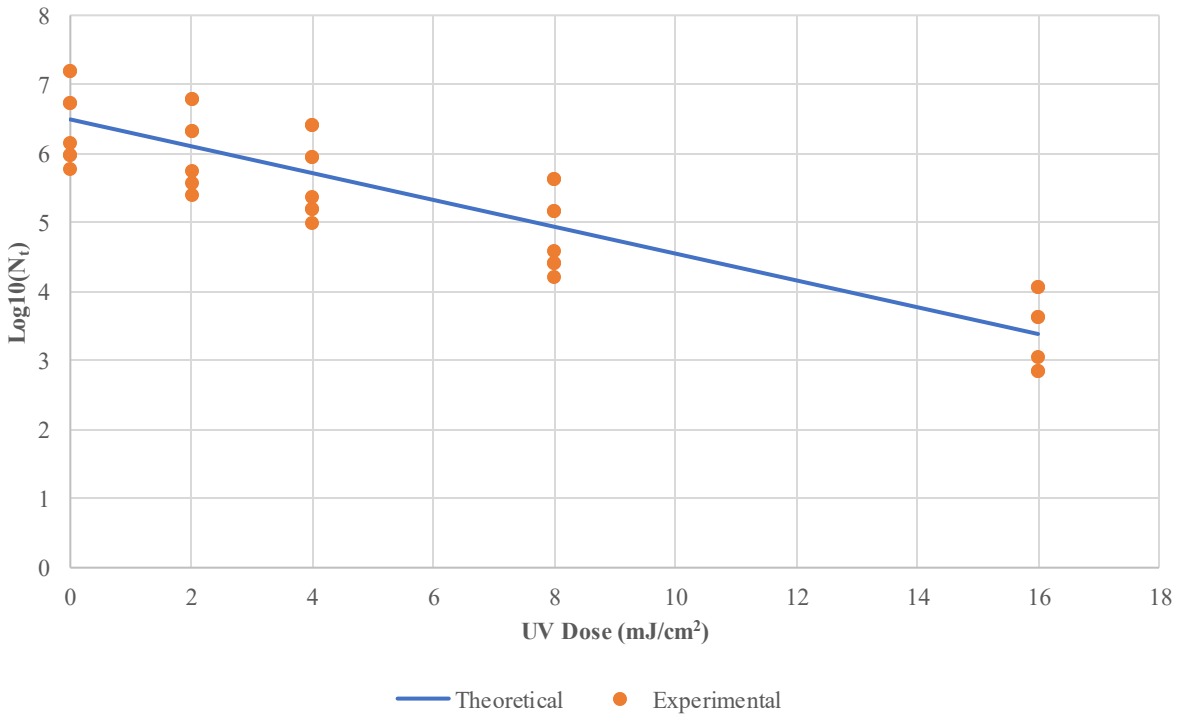

Figure 16: Data from the present research fitted to the multi-target model (R^2 = 0.73, RMSE = 0.42, and MSE = 0.18).

3.4. Discussion

3.4.1. Ray tracing model and experimental validation

The application of 3D modelling to obtain UV irradiance profiles is novel within the field of UV/biofilm research. This method could help researchers anticipate how target surfaces will be irradiated during treatment, supporting informed adjustments or redesigns if the predicted exposure is inadequate. To further improve the accuracy of these simulations, incorporating detailed material and surface properties (such as specific material absorptance, surface absorptance, spectral reflection, and spectral transmittance from manufacturers) would allow for more precise representation of the target's optical behaviour. Additionally, accounting for spatial gradients in the UV light source output could better represent a non-uniform profile.

No obstructions were present between the UV light source and the target surfaces, as the experiment was conducted under dry conditions (eliminating water-related losses) while the jerry can geometry did not impede light transmission. These kinds of obstructions are more commonly

encountered in narrow neck containers due to the limited line-of-sight between conventional UV light sources (too large to fit through the neck and must be positioned at the mouth of the container to irradiate inwards) and microbial targets. The neck acts as a collimator for light dispersion which can cause shadowing effects and uneven UV irradiance profiles. However, the literature also suggests that these containers may be preferable for drinking water protection due to the limited accessibility they provide for potential sources of contamination to the bulk water (Levy et al., 2008; Mellor et al., 2013; Mintz et al., 1995; Quick et al., 1996). As a result, UV light sources must be slender and long in design to overcome this restriction and optimize delivery. This could include the UV light source used in the present research or the UV side-emitting optical fibers (SEOFs) described in O'Neal Tugaoen et al. (2018), Lanzarini-Lopes et al. (2019), and Zhao et al. (2023b). UV-SEOFs distribute UV light along the entire length of a repurposed optical fiber and can be inserted into tight spaces and confined areas that would otherwise be restricted by the geometry of their surroundings (Lanzarini-Lopes et al., 2020).

Overall, the ray tracing model and experimental validation provided an important understanding of the distribution of UV irradiance inside the jerry can. While some of readings were low compared to other positions around the jerry can, biofilm inactivation is still possible at the measured irradiances as Torkzadeh et al. (2021) found that the biovolume of *E. coli* ATCC 25922 biofilms could be reduced by 95% at a UV_{254nm} irradiance of 0.0505 mW/cm^2 over 48 hours. The lowest of these readings was established as the "worst-case scenario," and could act as a reference point for comparisons during subsequent UV light treatment.

3.4.2. UV light treatment of lab-grown biofilms

3.4.2.1. Biofilm-bound cell inactivation analysis

Although equal UV doses at the same UVC wavelength were delivered to biofilms at MIL (higher irradiance, shorter exposure time) and LIL (lower irradiance, higher exposure time), biofilm-bound cell inactivation at the former was less than that at the latter (Figure 13). These results are in apparent contradiction to Bunsen-Roscoe law (Bunsen & Roscoe, 1863), which states that the effect of light on a system is proportional to the total energy dose, which is the product of light intensity and exposure time. According to this principle, equivalent doses should produce equivalent photochemical or biological effects, regardless of whether they are obtained from increased light intensity applied for a short amount of time or decreased light intensity

applied for a longer amount of time (Bunsen & Roscoe, 1863). In the context of a UV-biofilm system, equivalent UV doses applied to biofilms should produce the same inactivation (LRV), regardless of whether the UV doses were obtained from high UV irradiance and low treatment time or low UV irradiance and high treatment time. A contradiction of this principle when treating *E. coli* biofilms at UVC wavelengths was also seen in Shen et al. (2025). Shen et al. (2025) proposed a revised expression of the Bunsen-Roscoe law for UV light treatment where UV irradiance and treatment time are not just treated as two multiplicative factors for UV dose (Eq. 4), but rather conjugate variables that collectively and non-linearly influence biofilm inactivation (Eq. 13).

$$Log\ inactivation = f(I) * f(t) \tag{13}$$

Where f(I) describes how UV irradiance alone influences log inactivation and f(t) describes how treatment time alone influences log inactivation.

UVC wavelengths (200-280 nm) have strong bactericidal effect since they are close to the maximum DNA absorption rate and results in the formation of pyrimidine dimers (Chevremont et al., 2012). However, within a biofilm, microorganisms are protected by both the structural architecture and the surrounding EPS matrix (Shen et al., 2025). This results in shielding effects, where UV photons are not as easily absorbed by embedded microorganisms to suppress further growth (Argyraki et al., 2016; Elasri & Miller, 1999; Gora et al., 2019; Ma et al., 2022; Shen et al., 2025; Torkzadeh et al., 2021). More specifically, the shielding effects could be UV light absorption and scattering in the EPS matrix, as proposed by Luo et al. (2022). This can become problematic for UV light treatment in the UVC range since lower wavelengths have weaker penetration power (Chevremont et al., 2012; Shen et al., 2025). Ma et al. (2022) found that UV transmittance in five-day old *P. aeruginosa* biofilms decreased when the UVC wavelength of the treatment device decreased, while Shen et al. (2025) found that UV transmittance in *E. coli* biofilms decreased when lowering the wavelength from 275 nm to 268 nm. In such cases, UVC wavelengths may require more intense irradiation or longer treatment times to sufficiently penetrate biofilms and achieve greater microbial inactivation. Therefore, even though UV irradiance was much greater at MIL than LIL, the low treatment times at the former may not have allowed UV rays sufficient penetration of the biofilm structure to achieve greater microbial inactivation, causing significant differences in LRV between locations. Alternatively, it is

possible that inconsistent initial biofilm-bound cell concentrations on the polycarbonate coupons contributed to the counterintuitive LRV results. Consistent CBR operation across multiple experiments does not always ensure uniform attachment of planktonic microorganisms to coupons. This variation in initial biofilm-bound cell concentration could ultimately influence the relative inactivation observed following UV light treatment, even if absolute inactivation is similar.

Even though there were significant differences in biofilm-bound cell inactivation at each location in the jerry can, the results for both locations (at multiple doses) were still comparable to other UV-biofilm treatment studies conducted with UV_{254nm}. Some of these studies (Bae & Lee, 2012; Bak et al., 2009; Ma et al., 2022; Murray et al., 2015; Prado et al., 2019; Rand et al., 2007), summarized in Table 8, achieved LRV values ranging from 0.82 to 4.16. As per the results of the current study, UV doses of 8 and 16 mJ/cm^2 at MIL and all UV doses at LIL achieved LRVs within this range.

Table 8: Comparison of published UV/biofilm research

Study	**Biofilm growth method**	**Wavelength (nm)**	**Treatment time (mins)**	**UV irradiance (mW/cm^2)**	**UV dose (mJ/cm^2)**	**LRV**
Bae & Lee (2012)	*E. coli* O157:H7 on polypropylene coupons	254	30	0.236	425	0.82
			60	0.236	850	1.21
			120	0.236	1.70 x 10^3	2.93
			180	0.236	2.55 x 10^3	4.16
Bak et al. (2009)	Mixed culture on silicone urinary catheters	254	60	0.400	1.50 x 10^3	2
Elasri & Miller (1999)	Alginate matrix to simulate *P. aeruginosa* biofilms	256	Not specified	Not specified	2.5	~0.1
					5	~0.22
					10	~1
					12.5	~1.3
					15	~1.7
					17.5	~2

					20	~2
Gora et al. (2019)	*P. aeruginosa* on polycarbonate coupons in CBR	265	1.8	0.0742	8.1	1.3
			1.8	0.0742	4	0.9
Ma et al. (2022)	*P. aeruginosa* on polycarbonate coupons in CBR	254	Not specified	Not specified	11.3	1.47
		270			8.2	2.3
		282			14	2.38
Murray et al. (2015)	Mixed culture from groundwater well (#1 and #3) on stainless steel coupons	254	30 (Well 1)	1.67×10^3	3.00×10^6	2.4-2.7
			60 (Well 1)	1.67×10^3	6.00×10^6	1.9-2.8
			30 (Well 3)	1.67×10^3	3.00×10^6	1.4-2.8
			60 (Well 3)	1.67×10^3	6.00×10^6	1.2-1.6
Prado et al. (2019)	*Alicyclobacillus acidocaldarius* on stainless steel (SS) and rubber (R) coupons	254	30	1.46	2.62×10^3	3.03 (SS) and 3.26 (R)
Rand et al. (2007)	Natural culture growth on polycarbonate coupons	254	120	0.0125-0.0139	90-100	2.05
Roy et al. (2021)	*Vibrio parahaemolyticus* on shrimp (S) surfaces and crab (C) shells	260	Not specified	Not specified	5 (S)	1.37
					10 (S)	1.56
					30 (S)	1.84
					60 (S)	2.53
					5 (C)	0.75
					10 (C)	0.94
					30 (C)	1.37
					60 (C)	1.94

Other factors that impact biofilm-bound cell inactivation include but are not limited to: surface material and properties, microorganisms, biofilm age, growth media and UV exposure media, and measurement/characterization methods (Gora et al., 2024). However, UV dose remains one of the most common and standard explanation parameters in the literature. Gora et al. (2024) highlight this and compared LRV achieved during the direct irradiation of pre-existing biofilms across 23 different studies based on UV dose, ultimately concluding that the relationship between UV dose and LRV for biofilm remains poorly understood because of major differences in experimental conditions used in past studies. Table 8 highlights four studies (Elasri & Miller, 1999; Gora et al., 2019; Ma et al., 2022; Roy et al., 2021) that delivered UV doses between 2 to

16 mJ/cm^2 to bacterial biofilms relevant to drinking water. The associated LRVs ranged between 0.10 and 2.38, which is comparable with the three lowest UV doses at MIL and the two lowest UV doses at LIL. Gora et al. (2019), Ma et al. (2022) and the current study grew biofilms in a CBR with a subsequent UV dose of ~8 mJ/cm^2, resulting in somewhat similar LRVs of 1.30 (Gora et al., 2019), 2.30 (Ma et al., 2022), 1.29 (MIL), and 2.51 (LIL).

As a result of the holes at MIL and LIL, the jerry can was not able to hold water. It is possible that these dry conditions during the UV light treatment experiments may have enhanced treatment effectiveness. As Bolton & Linden (2003) explain, if water absorbs UV light, then it is necessary to account for a decrease in UV irradiance. This attenuation can be accounted for through a water factor, presented in Bolton & Linden (2003). This consideration was not necessary in the present research; however, from Table 8, Elasri & Miller (1999), Murray et al. (2015), and Rand et al. (2007) treated biofilms with UV light under submerged conditions. It is possible that these three studies incurred water-related losses due to UV light absorption and highlights that the differences in exposure conditions should be considered when comparing treatment efficiencies across studies.

3.4.2.2. Disinfection models

The results of the present study were a good fit to the Geereard model ($R^2 = 0.76$, RMSE = 0.30, and MSE = 0.090, $N_{res} = 1.18 \times 10^4$ CFU/mL, k = 0.61 cm^2/mJ.), which is in line with past UV/biofilm research (Gora et al, 2019; Ma et al., 2021; Mohsin et al., 2023). Mohsin et al. (2023) found an inactivation rate constant for *E. coli* ATCC 25922 of 1.06 cm^2/mJ after UVC light treatment, noticeably greater than that of the current study. This difference is expected as Mohsin et al. (2023) focused on surface-bound cells in nutrient-rich agar environments prior to biofilm formation. While experimental parameters were similar in the two studies, this difference further underscores UV shielding in biofilms amid the presence of EPS. Gora et al. (2019) fitted their data to the Geeraerd model, finding it was able to capture non-linear behaviour which included tailing due to resistant microbial subpopulations. Seeing as Gora et al. (2019) used *P. aeruginosa*, the ability of this model to capture the UV dose response of different microorganisms could promote application in future UV/biofilm research.

The Chick-Watson model and the multi-target model produced identical results ($R^2 = 0.73$, RMSE = 0.42, and MSE = 0.18), which may be attributed to the absence of shouldering effects during UV light treatment experiments. Excel estimated n = 0.44 in the multi-target model, indicating that the curve does not exhibit the classic multi-hit shoulder and instead behaves closer to a log-linear inactivation pattern. Peleg (2021) explains that when the shape factor (given by "n" in the multi-hit model) falls below 1, the survival curve displays downward concavity ("tailing") rather than a shoulder. Since the fitted n value lies below this threshold, the multi-target model was unable to express the non-linear shoulder phase it is designed to capture, resulting in a curve that overlapped the Chick–Watson model.

3.4.2.3. Operational implications in humanitarian settings

The distribution of UV light during treatment depends on the geometry of the container, device being used, and the position of the device. Curves, shadows, or tight spaces can lead to uneven coverage and biofilm being left untreated. If these biofilms were to grow into previously treated areas of the containers, microbial contamination could become a threat again. Power supply may also be a challenge in remote areas, requiring UV systems to deliver sufficient treatment with limited energy or alternative sources like batteries or solar panels. The type of container material could also impose a challenge, as some materials absorb or reflect UV light more than others. Beyond these technical and operational issues, capital investment and ongoing operation and maintenance costs are major considerations for humanitarian deployment. Periodic contamination checks and additional UV light treatment may be required to ensure continued protection but may be difficult to maintain in the field. Finally, response teams deploying this treatment may need additional training to position the devices correctly, follow safety guidelines, and make sure treatment is done in the most effective way every time. Successful integration would also require behavioural change among responses and communities, as water handling practices must adapt to include UV light treatment as needed. Supply chain reliability is another critical factor, as replacement parts, UV LEDs, and power sources must be available in remote settings. Ultimately, before these systemic challenges can be addressed, the fundamental viability of this approach must be demonstrated. For example, a prototype UV light device positioned inside a jerry can must be able to achieve biofilm inactivation under real-world storage conditions, and especially in submerged conditions, rather than the controlled lab conditions in the present study.

3.5. Conclusions

This research investigated the technical viability of UV light treatment for biofilm control inside a representative secondary storage container commonly used in humanitarian settings. A ray tracing model of a practical treatment setup, which included a UVC device positioned inside a jerry can, was developed to evaluate spatial variability in UV irradiance at potential biofilm hotspots along the inner walls of the container. This provided an initial understanding of whether germicidal thresholds could be met during treatment. Subsequently, experimental validation was conducted to verify the accuracy of the ray tracing model. By recreating the digital model in the laboratory, and collecting UV irradiance readings using a spectrometer, the modelled UV irradiances could be compared to experimental UV irradiances. It was found that the ray tracing model had reasonable predictive capacity. Through this process, a position ("LIL") inside the jerry can was identified where UV irradiance beneath the water line was lowest. This point served as a "worst case scenario" baseline for evaluating whether germicidal effects could be achieved at a low irradiance position. A comparison position was established ("MIL") to determine if biofilm-bound cell inactivation at the same UV doses would be consistent despite differences in irradiance at different locations. Biofilms approximating real-world contamination were grown in a CDC reactor, affixed to holes at these two locations, and treated with UV light. A general trend in results showed that biofilm-bound cell inactivation increased with an increase in UV dose. Statistically significant differences in LRVs were found between a UV dose of 2 and 16 mJ/cm^2 and 4 and 16 mJ/cm^2 at both locations, with 8 and 16 mJ/cm^2 and 2 and 8 mJ/cm^2 also revealing significant differences at MIL and LIL, respectively. The most noteworthy outcome was that the biofilm-bound cell inactivation between the two locations were different, as MIL consistently had lower and significantly different LRVs than LIL. This may be explained by the differences in UV irradiance and treatment times at each location, combined with the lessened biofilm penetrating ability at 254 nm. It may also be possible that inconsistent initial biofilm-bound cell concentrations led to these differences in results. Comparisons to previous UV/biofilm experiments in the literature highlighted substantial differences in experimental parameters leading to similar results in microbial inactivation, demonstrating that additional factors are impacting treatment. The Geeraerd model was found to reasonably describe the non-linear relationship between UV dose and surviving biofilm-bound cells, further demonstrating the disinfection model's applicability in this field of research. While this research has proven the

technical viability of UV light treatment by meeting germicidal thresholds inside a secondary storage container, performance under real-world conditions (especially submerged conditions) is still required to confirm whether this is a feasible option for implementation in humanitarian settings.

Chapter 4: Conclusion

4.1. Summary

This thesis investigated UV light treatment for biofilm control inside secondary storage containers used in humanitarian settings. Planktonic microorganisms in bulk water and surface-attached microorganisms left over on the inner walls of these containers can transition into biofilms, harbouring pathogenic microorganisms that can lead to waterborne disease outbreaks. Therefore, UV light treatment needs to be effective against microorganism in these phases to ensure its applicability for disinfection inside secondary storage containers. To explore this, a scoping literature review was carried out to identify research showcasing the real-world applicability of UV light treatment for bulk water disinfection and biofilm control. These applications included medical device disinfection, underwater antifouling, pilot scale distribution network disinfection, membrane antifouling, various water treatment applications, facial prothesis disinfection, and HVAC cooling coil disinfection. This literature review not only revealed that UV light treatment has germicidal effects beyond controlled laboratory settings, but also that its application in humanitarian settings has not yet been explored. Therefore, design concepts for adapting UV light treatment to humanitarian contexts were developed and the identified literature was used to better inform these ideas.

To translate the design concepts into a practical framework, a digital three-dimensional ray tracing model was constructed to simulate how UV light propagates within a representative secondary storage container. The model included a UVC LED clean light as a UV light source and a jerry can as the representative storage container. These two objects were then used to assemble a physical twin for the purpose of confirming the accuracy of the model through experimental validation. A spectrometer was used to collect UV irradiance readings at 47 locations around the jerry can that may face higher susceptibility to microbial colonization. Using %RMSE as an evaluation metric, the UV irradiances from the ray tracing model were found to be 17% different from the experimental validation measurements. Two locations of interest along the walls of the jerry can were identified from these steps, one representing a "worst-case scenario," where UV irradiance was found to be the lowest out of all the locations

(LIL), and a second location where UV irradiance was orders of magnitude greater than the first location for comparative purposes (MIL).

Non-pathogenic *E. coli* biofilms were grown on polycarbonate coupons inside a CDC Biofilm Reactor to approximate real-world contamination and subsequently underwent UV light treatment at the previously identified locations. Biofilm-bound cell inactivation, quantified as LRV, was found to increase as UV dose increased at both locations. Hypothesis testing revealed that statistically significant differences in LRVs were present between both locations. At MIL, statistically significant differences in LRVs were found between UV doses of 2 and 16 mJ/cm^2, 4 and 16 mJ/cm^2, and 8 and 16 mJ/cm^2, while statistically significant differences were present between 2 and 8 mJ/cm^2, 2 and 16 mJ/cm^2, and 4 and 16 mJ/cm^2 at LIL. These differences created a contradiction of the Bunsen-Roscoe law, which may have been caused by inadequate UV irradiance and treatment time in combination with the short-wavelength UVC LED clean light (254 nm). Alternatively, inconsistent initial biofilm-bound cell concentrations on coupons undergoing UV light treatment may have contributed to the counterintuitive LRV results by influencing relative inactivation, even when absolute inactivation were similar. However, the LRVs from both locations in the jerry can were comparable with LRVs from other UV-biofilm research reported in literature. In some cases, similar LRVs were achieved even when the literature reported significantly larger UV doses delivered to biofilms. To assess the relationship between UV dose and surviving biofilm-bound cells, the data were fitted to the Geeraerd model, Chick-Watson model, and multi-target model. The Geeraerd model is frequently used to describe the non-linear relationship between these variables and, in this study, yielded a better fit (R^2 = 0.76) for the data than the Chick-Watson model and the multi-target model (both R^2 = 0.73).

While this research demonstrates the technical viability of UV light treatment by achieving germicidal thresholds within a secondary storage container, validation under real-world conditions, and especially in submerged conditions, is still needed to determine its feasibility for future implementation in humanitarian settings.

4.2. Limitations

A primary limitation of the ray tracing model was the absence of a spatial gradient to represent the varying intensities along the length of the UV light source. Another limitation included the

absence of detailed material and surface property measurements required by the software to accurately characterize the jerry can. While the model represents a reasonable level of accuracy in predicting real-world UV irradiances, a more refined representation of the UVC LED clean light and detailed property measurements and may have enhanced the precision of the simulation and strengthened the results.

A primary limitation of the UV light treatment experiments was the biofilm characterization approach. It was not possible to directly determine the initial biofilm concentrations on coupons designated for UV exposure; instead, concentrations measured from untreated coupons were used as a representation for the initial values across all coupons in the reactor. Plate counts were selected as the most feasible and accessible method given available resources, while more sophisticated characterization techniques could not be employed due to limited laboratory access. A secondary limitation from this step was conducting the experiments in dry conditions. It was not possible to fill the jerry can with water due to the holes in the jerry can. Any effects of the dry conditions in the absence of UV light were not analyzed (e.g., dark controls) due to restrictions in the number of coupons installed in the CBR.

4.3. Future work

A key aspect of this research project was the development and validation of a digital 3D model to predict UV irradiance at different locations inside the secondary storage container. This modelling and validation approach can be applied to other contexts, including different storage containers and other water and wastewater infrastructure. Problems in pre-established infrastructure or design concepts (such as shadowing, underexposure, spatial variation and inadequate light sources) can be identified and potential solutions can be evaluated by adjusting the digital model.

Future research should explore UV light treatment for biofilm in secondary storage containers under wet conditions. Containers filled with water better represent the conditions encountered in humanitarian contexts. Such studies would allow for the quantification of water-related losses in UV irradiance, leading to a refinement of technical parameters that can optimize the effectiveness of this treatment. In addition to wet conditions, future work should focus on biofilms grown directly on the inner walls of the containers, as this also closely represents field conditions. These biofilms would form under natural conditions as opposed to controlled

laboratory conditions, better capturing the environmental stresses, structural complexity, varying thickness, and resistance characteristics that characterize these real-world microbial structures.

Finally, an interesting finding from this study was that biofilm-bound cells were more readily inactivated when the UV dose was achieved through low irradiance and long exposure times vs. high irradiance for shorter exposure times. Although this was not the primary focus of the research project, it suggests an interesting and important area for future research. Conducting future UV/biofilm research across a wider range of UV irradiances and treatment times would allow the Bunsen-Roscoe law to be evaluated on a larger scale, helping determine whether biofilms respond to UV dose alone or whether irradiance and treatment time play a more integral role than previously thought. This could help clarify the conditions under which the law holds, breaks down, or transitions, ultimately improving the design and operation of UV-based treatment systems intended for biofilm control.

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Appendix A: Raw UV irradiance data

Table 9A: UV irradiance (mJ/cm^2) recorded from the ray tracing model and measured during experimental validation

Hole	Ray tracing model	Experimental validation
1	0.305	0.308
2	0.000	-0.001
3	0.061	0.023
4	0.061	0.073
5	0.487	0.492
6	0.305	0.299
7	0.061	0.068
8	0.061	0.053
9	0.914	0.945
10	0.914	1.157
11	0.426	0.456
12	0.128	0.140
13	0.061	0.087
14	0.609	0.619
15	0.548	0.548
16	0.365	0.352
17	0.128	0.113
18	0.183	0.178
19	0.731	0.760
20	0.853	0.846
21	0.305	0.318
22	0.128	0.104
23	0.183	0.179
24	0.487	0.501
25	0.487	0.554
26	0.365	0.392
27	0.128	0.111
28	0.128	0.140
29	0.128	0.143
30	0.128	0.121
31	0.061	0.045
32	0.061	0.035
33	0.061	0.055
34	0.061	0.092
35	0.061	0.058

36	0.061	0.025
37	0.061	0.017
38	0.061	0.016
39	0.128	0.143
40	0.128	0.149
41	0.183	0.177
42	0.183	0.163
43	0.061	0.058
44	0.061	0.057
45	0.128	0.093
46	0.061	0.035
47	0.061	0.045

Appendix B: UV Irradiance Maps from the Ray Tracing Model

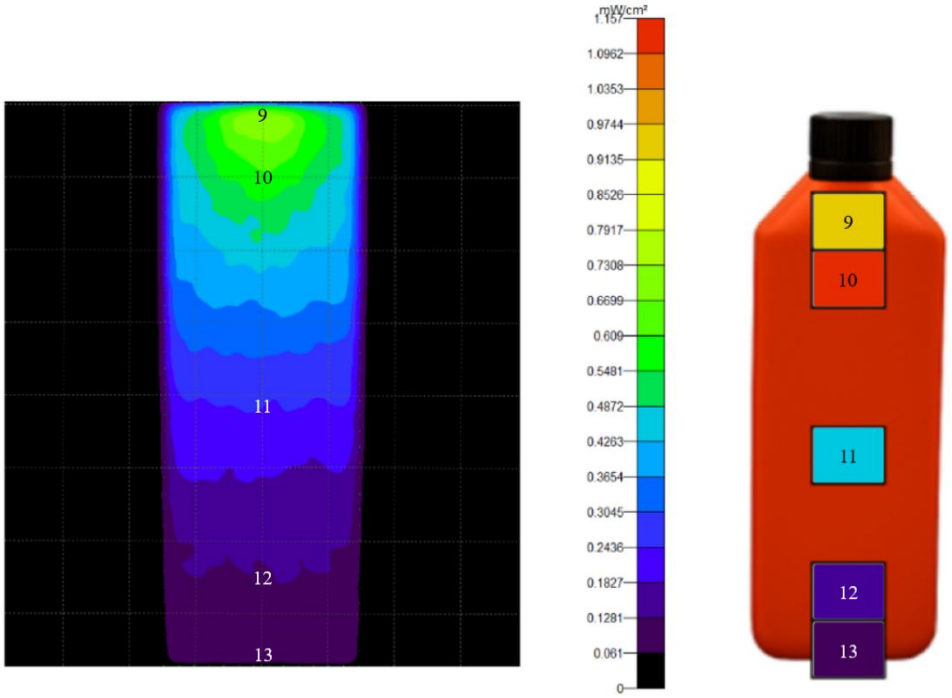

Figure 17B: UV irradiance map from the ray tracing model (left) and measured UV irradiances from the experimental validation (right) taken from the front of the jerry can.

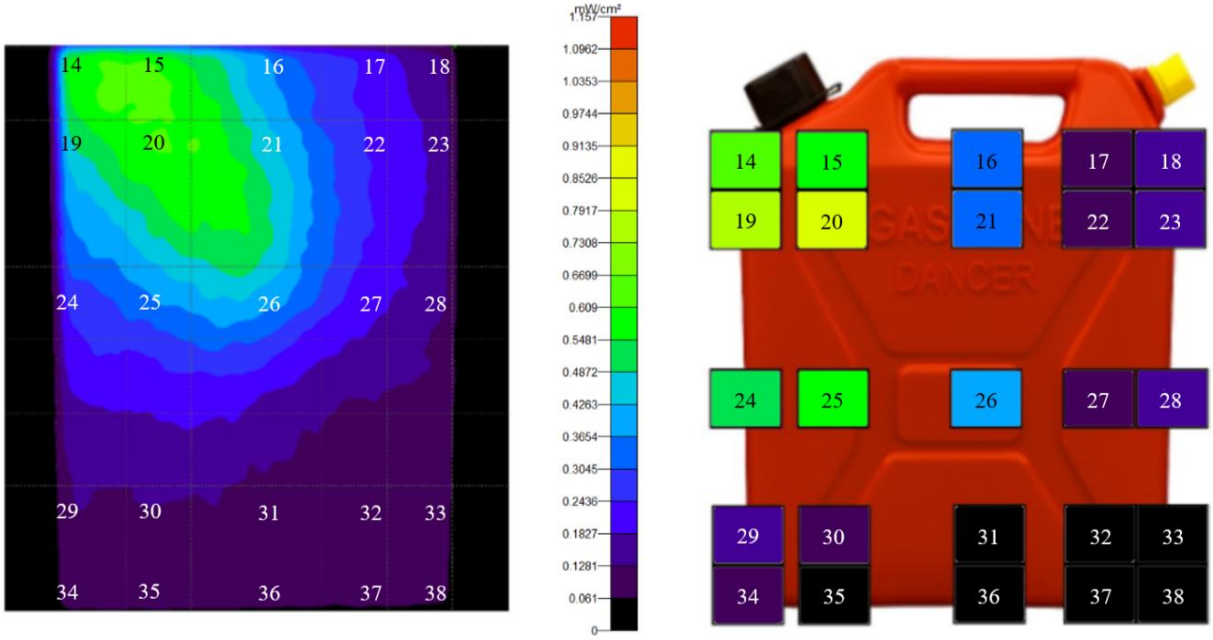

Figure 18B: UV irradiance map from the ray tracing model (left) and measured UV irradiances from the experimental validation (right) taken from the side of the jerry can.

Appendix C: Raw UV light treatment data

Table 10C: LRVs with corresponding UV dose, location, and trial

LRV	UV dose (mJ/cm^2)	Location	Trial
N/A	2	26	7
0.44	2	26	8
0.54	2	26	15
0.04	2	26	16
0.37	4	26	7
0.44	4	26	8
1.43	4	26	15
0.90	4	26	16
1.24	8	26	7
1.00	8	26	8
2.00	8	26	15
0.90	8	26	16
3.15	16	26	7
1.20	16	26	8
2.70	16	26	15
2.82	16	26	16
0.26	2	38	2
1.32	2	38	3
1.36	2	38	11
1.36	2	38	12
1.26	4	38	2
2.12	4	38	3
1.76	4	38	11
1.58	4	38	12
2.26	8	38	2
2.24	8	38	3
2.88	8	38	11
2.66	8	38	12
N/A	16	38	2
3.02	16	38	3
2.88	16	38	11
3.06	16	38	12