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UV-C inactivation of microorganisms in droplets on food contact surfaces using UV-C light-emitting diode devices

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The main objective of this study was to investigate the effectiveness of ultraviolet light (UV-C) emitting diodes for the decontamination of stainless steel food contact surfaces. *Listeria monocytogenes* (ATCC 19115), *Escherichia coli* (ATCC 25922), and *Salmonella enterica* serovar Typhimurium (ATCC 700720) were chosen as challenge microorganisms. Target microorganisms were subjected to UV-C dosages of 0, 2, 4, 6, and 8 mJ cm^{-2} at an average fluence of 0.163 mW/cm^2 using a near-collimated beam operating at 279 nm wavelength. *Escherichia coli* showed lower sensitivity to UV-C light compared to *Salmonella* Typhimurium and followed first-order kinetics. *Escherichia coli* and *Salmonella* Typhimurium were reduced by more than 3-log$_{10}$ cycles at the maximum UV dosage of 12 mJ cm^{-2}. In contrast, *Listeria monocytogenes* followed the Weibull model with an apparent shoulder in the initial doses. A maximum reduction of 4.4-log$_{10}$ was achieved at the highest exposure level. This study showed that UV-C LED devices represent an excellent alternative for the inactivation of foodborne microorganisms in droplets. Results clearly demonstrate that UV-C LED devices can serve as an additional sanitation method to routine cleaning practices, which are commonly utilized in the food industry.

KEYWORDS

UV-C LED, food contact surfaces, non-thermal processing, UV light, food and environment safety, food safety
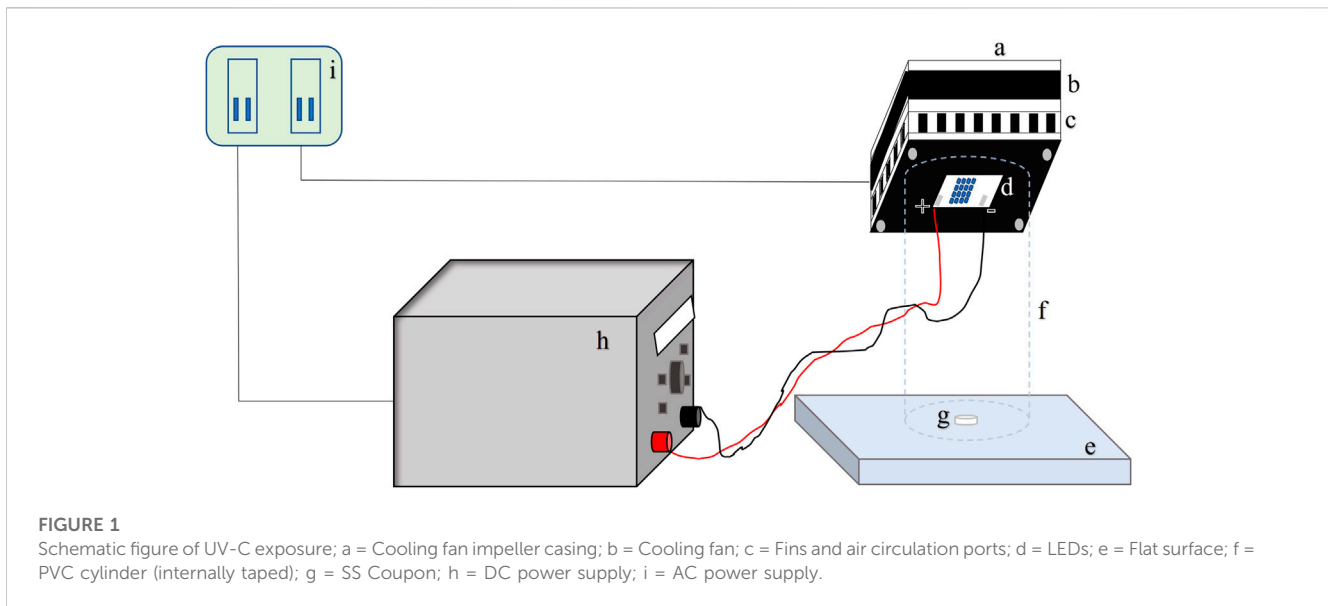
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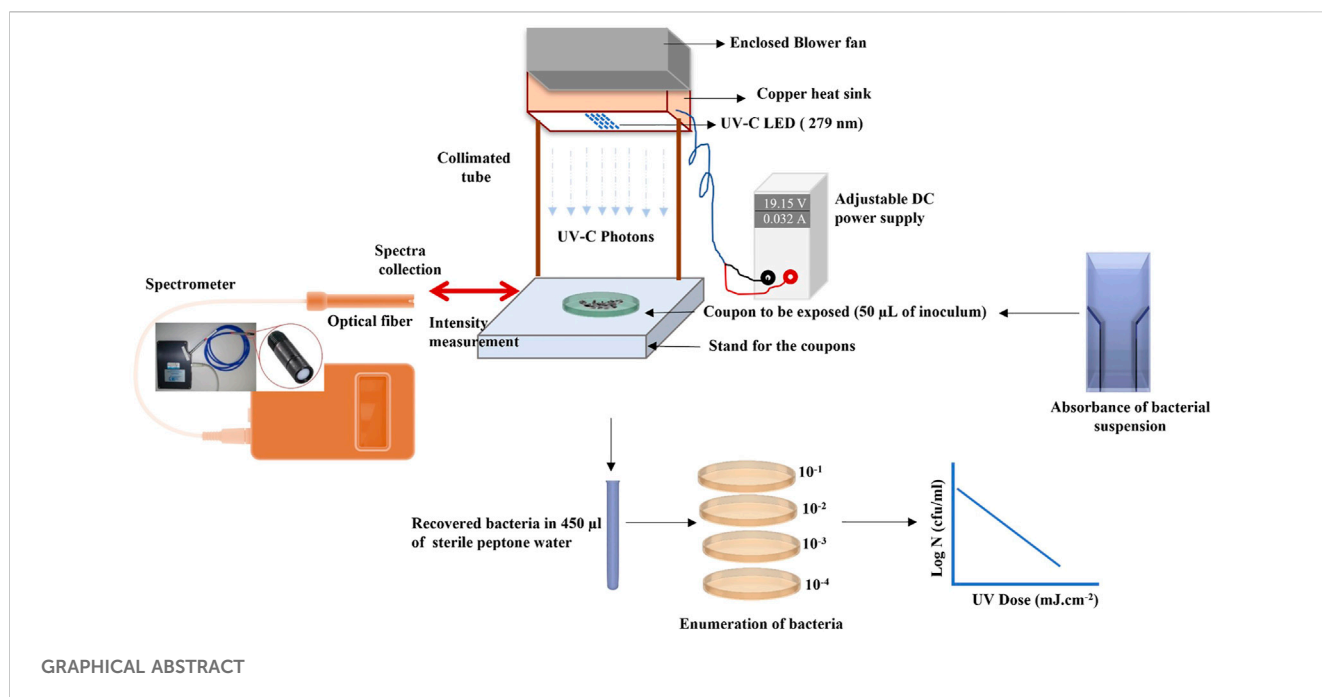
1 Introduction

Microbiological cross-contamination has been a contributing factor to several well-documented outbreaks of foodborne illness in the United States (Jackson et al., 2011; CDC, 2008). The prevalence of microorganisms on food contact surfaces has been linked to their high environmental stability (Kramer and Assadian, 2014). In a food preparation setting, surfaces can be contaminated by various vectors (air and other gases, water and other liquids, and physical objects or people), which can lead to the transfer of pathogens (*Listeria monocytogenes*, *Salmonella* serovars, and *Escherichia coli*) to various food products (D'Souza et al., 2006). In practice, infected droplets with multiple microorganisms can land on food contact surfaces and form biofilms. Biofilms can harbor pathogens and spoilage microorganisms that can lead to food contamination and spoilage if not sanitized or disinfected. Commonly found microbes on food contact surfaces are enterobacteria, lactic acid bacteria, micrococci, streptococci, pseudomonads, and bacilli (Holah et al., 2002). A comprehensive review by Galié et al. (2018) highlights the importance of the development of alternative and sustainable strategies for controlling biofilms in the food industry.

Sources and vectors of pathogens may be associated with a specific process step or may affect the processing line in general. For example, contaminated air in the production environment might affect many processing steps within a production line, whereas vectors associated with a specific line procedure may be associated solely with a specific process step. Cleaning-in-place procedures, if not done correctly, may leave residual droplets contaminated with pathogenic microorganisms leading to matured biofilm formation. The cleanliness of surfaces, good manufacturing, and design practices are important in combating hygiene problems within the food industry (Holah, Lelieveld, and Gabric, 2016). Currently, disinfection after the removal of biofilms, using suitable cleaning procedures, is also required in food plants

where wet surfaces provide favorable conditions for microbial growth. In the food industry, equipment design and choice of surface materials are important in inactivating microbial biofilm formation. The most practical material in processing equipment is steel (Carrascosa et al., 2021), which can be treated with mechanical grinding, brushing, lapping, and electrolytic or mechanical polishing. Dead ends, corners, cracks, crevices, gaskets, valves, and joints are vulnerable points for contaminated water droplets. Therefore, mechanical/chemical cleaning will always be limited, and unless huge attention is paid to details, it is impossible to guarantee the prevention of food contamination and future outbreaks. Therefore, we aim to investigate the efficacy of eliminating microbial contamination with LED UV-C light.

There is limited literature on the application of UV-C LED (279 nm) for inactivating microorganisms in fluid suspensions and surfaces. Kim, Kim, and Kang (2017) investigated the bactericidal effect of 266–279 nm wavelength on vegetative cells. Inoculated media were treated at dosages of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mJ/cm^2. The authors observed that low wavelength (266 nm) resulted in a higher microbial reduction of bacteria and yeasts compared to high wavelength UV-Cs. *E. coli* O157:H7 and *Salmonella* spp. were easily inactivated by UV-C LEDs. It is also worth noting that the applied doses to the microorganism were relatively low, as documented in the literature for 254 nm wavelength. UV-C susceptibility followed the sequence of Gram-negative bacteria > Gram-positive bacteria > yeasts. Green et al. (2018) qualitatively showed that UV LEDs in the 259–275 nm range must be either as effective as or, in most cases, more effective than low-pressure mercury (LMP) lamps emitting at 253.7 nm in the reduction of three common foodborne pathogens. In contrast, according to Kim, Kim, and Kang (2016), 266 nm UV-C LEDs demonstrated over 5-log reductions in *E. coli* O157:H7 and *S.* Typhimurium, whereas 1–2-log reductions were achieved using UV lamps at the same dosages with UV lamp intensity attenuated to the levels of UV-C LED.

FIGURE 1
Schematic figure of UV-C exposure; a = Cooling fan impeller casing; b = Cooling fan; c = Fins and air circulation ports; d = LEDs; e = Flat surface; f = PVC cylinder (internally taped); g = SS Coupon; h = DC power supply; i = AC power supply.

Due to the germicidal effects (direct damage of DNA) of UV-C (200–280 nm) light, it is envisaged that the application of UV energy can be translated for surface sanitation in the food industry to control microorganisms in food environments (Fan et al., 2017). In literature, the UV-C light treatment was successfully applied to the decontamination of various food products, including chicken, raw salmon, hazelnut, tiger nut milk, and tomato (Holck et al., 2018). UV-C light treatment has also been used in the food industry for different purposes, including air sanitation in meat and vegetable processing and the reduction of pathogens in red meat, poultry, and fish (Wong, Linton, and Gerrard, 1998; Liltved and Landfald, 2000). UV-C treatment of heat-resistant yeasts, molds, and *Bacillus* spores are also common applications in orange juice processing (Tran and Farid, 2004). Additionally, UV-C irradiation has been successfully applied to the pasteurization of certain liquid foods, such as milk and fruit juices (Koutchma et al., 2004; Matak et al., 2005; Pendyala et al., 2020), but data on food contact surfaces are limited, especially on droplets, a prerequisite for biofilm formation. Such data are required to evaluate the inactivation of bacterial cells through exposure to UV irradiation. To the best of our knowledge, this study is the first to apply a mathematical modeling approach to investigate the effect of a single UV-C wavelength on microorganisms in droplets.

Our objective in this study was to investigate the efficiency of UV-C LED irradiation as a non-thermal decontamination process for inactivating microorganisms in a droplet on food contact surfaces (*E. coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes*). For this purpose, the effects of 279 nm UV-C LED were investigated due to the already established DNA damage along with less known protein degradation.

2 Materials and methods

2.1 Materials

Peptone water: 20 g of buffered peptone water base (Bio-Rad Laboratories, Inc., Hercules, CA, United States) was added to 1,000 ml of de-ionized water, followed by mixing (500 rpm at 150°C for 5 min) and autoclaving (121°C for 15 min at 15 psi). Phosphate buffered saline (PBS) of 1% was prepared from stock, as mentioned by Maclean et al. (2009).

2.2 Bacterial strain and culture conditions

Escherichia coli ATCC 25922, *Salmonella* Typhimurium ATCC 700720, and *Listeria monocytogenes* ATCC 19115 were obtained from American Type Culture Collection (Manassas, Virginia) in a freeze-dried vial (stored at 4°C till reactivation). *E. coli* and *Salmonella* Typhimurium were propagated in the tryptic soy broth (TSB) (211825, Becton Dickinson and Company, Le Pont de Claix, France) and plated on tryptic soy agar (TSA) (236950, Becton Dickinson and Company, Le Pont de Claix, France). Both cultures were inoculated at 2% in sterile TSB broth, followed by incubation at 37°C for 18 h for propagation. The method for propagation and plating was adapted from Beck et al. (2017) and Vashisht et al. (2021). *Listeria* was propagated and plated using buffered *Listeria* enrichment broth base (CM0897; Oxoid Ltd., Basingstoke, United Kingdom) and *Listeria* selective agar base (CM0856; Oxoid Ltd., Basingstoke, United Kingdom) with *Listeria* selective supplement (Oxoid Ltd., Basingstoke, United Kingdom). *Listeria* was inoculated at 2% in sterile broth, followed by 24 h of incubation at 37°C. Bhullar et al. (2019) was used as reference for *Listeria* propagation and plating. All cultures were stored at 4°C after incubation.

Fresh bacterial cells were harvested by centrifugation (3,000 × g, 5 min) using a high-speed microcentrifuge (VWR International, Radnor, Pennsylvania, United States) at room temperature. Cell pellets were washed twice in 0.1% (w/v) PBS (Becton Dickinson, New Jersey, United States) and re-suspended in 1 ml of PBS. Appropriate dilutions in peptone water (in 0.1% PW) were plated in duplicate onto TSA (Oxoid Ltd., Basingstoke, United Kingdom) plates for *E. coli* and *Salmonella* suspensions and incubated for 24 h at 37°C. *Listeria monocytogenes* suspensions were plated on *Listeria* selective agar base (CM0856; Oxoid Ltd., Basingstoke, United Kingdom) plates with incubation for 24 h at 37°C to enumerate the original population densities in each cell suspension. The suspensions contained
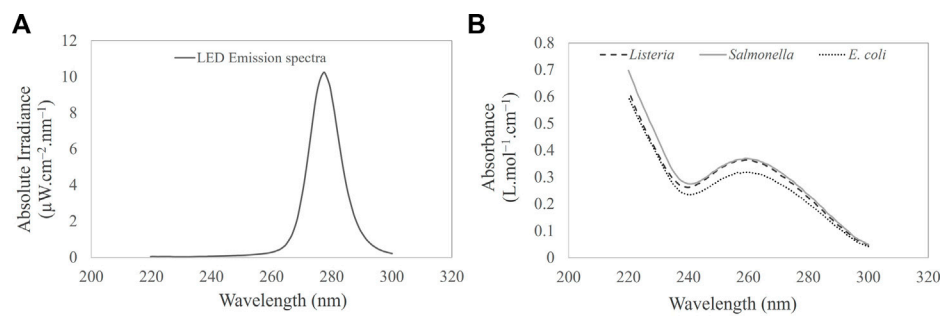
FIGURE 2
(A) UV-C LED emission spectra. **(B)** UV-C (220–300 nm) absorption spectra of *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Escherichia coli* cell suspension in PBS.

approximately 10^9 cfu/ml. Appropriate dilutions were made to reach the target concentration of 10^7 cfu/ml.

2.3 Inoculation and UV-C exposures

The 0.5-inch (12.7-mm) stainless steel circular coupons (grade 316L) were procured from BioSurface Technologies Corporation (Bozeman, MT). The coupons were sterilized using an autoclave to remove any natural flora. Cell suspensions of the test microorganism 7-$\log_{10}$ CFU/ml were inoculated on a coupon as a single 50-µL droplet (see Figure 3 for droplet geometry). The inoculated coupons were exposed to UV-C irradiation at a known height of 4 cm. A UV-C LED (IRTRONIX, Torrance, CA, United States), which emits at a peak wavelength of 279 nm, mounted on top of a quasi-collimated system (Figure 1), was used to perform irradiation experiments. A panel with sixteen UV-C light-emitting diodes was connected to the heat sink to prevent overheating of the LEDs. A PVC pipe (non-reflective black taping inside) was used to maintain the distance between the coupon and LEDs. This study used a DC power source generating 180 W Multirange 60V/8A DC Power Supply (BK Precision, Yorba Linda, CA, United States). Central irradiance incident on the surface of the droplet was measured with a high sensitivity calibrated spectrometer (QE Pro series, Ocean Optics, Dunedin, FL, United States) as absolute spectral irradiance (µw/cm²/nm). The spectrometer is equipped with an irradiance probe, a solarization-resistant optical fiber with a Spectralon cosine corrector of 180° field of view, coupled to its end. Spectralon is a Lambertian diffuse material with a reflectance higher than 95% in the 250–400 nm range. The irradiance of the LED system was measured across the sampling area where the coupon was placed to assess any inconsistency or spectral shift within the exposure area. The system was stable across the sampling area (14.9 cm²). The UV-C dose was calculated by summing the average UV irradiance over the wavelength range. It was calculated as a product of the UV intensity (mW.cm⁻²) and exposure time (sec). The UV-C dose was expressed as mJ.cm⁻². UV dose exposures ranging from 0 to 8 mJ cm⁻² were delivered to the microorganisms. All experiments were conducted in triplicate.

2.4 Enumeration of microorganisms after UV-C treatments

After UV-C treatments, the coupon was washed using a fixed volume of diluent (i.e., suspending coupon in diluent and vortex) followed by decimal dilutions of the treated samples and control using 0.1% peptone water (12013259, Bio-Rad Laboratories, Inc., Hercules, CA, United States). *E. coli*, *Salmonella*, or *L. monocytogenes* inoculated droplets were diluted to between 10^{-2} and 10^{-7}. Serial dilution of the irradiated samples was conducted using peptone water followed by spread plating within 2 h. The TSA plate was spread with a 100-µL diluted irradiated sample in duplicate. For *Listeria* enumeration, *Listeria* selective agar with *Listeria* selective supplement was used. The plates were incubated at 37°C for 18–24 h. Isolated colonies on the plates in the range of 25–250 colonies were counted and expressed as CFU/ml. All experiments were conducted in triplicate, and viable plate counts from three replications are reported as log CFU/ml.

2.5 Optical properties and UV-C spectra of bacterial suspensions

Optical properties and UV spectra of bacterial suspensions were measured in a double-beam Cary 100 spectrophotometer (Varian, United States) equipped with a 6-inch single integrating sphere (Labsphere, DRA-CA-30, United States) to include scattered light. The sphere primarily collects the total transmitted light through a bacterial suspension when the sample is placed at the entrance port and collects the total reflected light (diffuse reflectance) when the bacterial suspension is positioned at the exit port. The UV-C (220–280 nm) absorption spectra were determined for all three bacteria, as illustrated in Figure 2B. The method was adapted from Vashisht et al. (2022). The absorbance was determined using the Beer–Lambert law (Eq. 1):

$$A = \varepsilon c l, \qquad (1)$$

$$A = log_{10}\frac{1}{T} = -log_{10} T, \qquad (2)$$

where `A means absorbance, ε molar absorption coefficient, c molar concentration, l optical path length, and T transmittance.

TABLE 1 Optical properties and calculated UV-C doses for microorganisms.

Bacteria	Absorbance (279 nm) (L mol^{-1} cm^{-1})	[a] Transmittance (%.cm^{-1}) (279 nm)	UV-C dose (mJ. cm^{-2})	Exposure time (seconds)
Escherichia coli ATCC 25922	0.06 ± 0.002	86.23	0	0
			4	25
			6	37.5
			8	50
Salmonella Typhimurium ATCC 700720	0.03 ± 0.001	92.97	0	0
			2	12.5
			4	25
			6	37.5
			8	50
Listeria monocytogenes ATCC 19115	0.02 ± 0.001	95.57	0	0
			2	12.5
			4	25
			6	37.5

[a]Transmittance was determined using the equation ($A = log_{10}\frac{1}{T} = -log_{10} T$). All data are reported as mean ± standard deviation.

2.6 Film geometry analysis

The bacterial suspension droplet on a 1-cm stainless steel coupon was analyzed using a camera of 16 megapixels. A picture was taken with a camera module, which allows for streamlined image processing in Python. The height of the camera sensor and film was aligned with a guided laser (Line Lasers-0109, Dreyoo) while images were captured. Film thickness was quantified using image pixels by Java-based image processing software (ImageJ).

2.7 Statistical analysis and mathematical modeling

A balanced design with three independent biological replicates for each treatment was exposed to the selected UV-C treatment. Each sample was independent and randomly assigned to a treatment. One-way ANOVA with Tukey's HSD test was performed to assess the effects of UV-C on the SAS statistical computing environment (SAS, 2016). Data are presented as means ± standard deviation. Statistical significance was tested at a 5% significance level. All log reductions from the UV-C inactivation treatments were recorded, and log-linear and nonlinear models were fitted in Microsoft Excel and GInaFiT Tool (Excel Add-in). Model fit statistics, including adjusted R^2 and root mean square error (RMSE), were compared among the competing models (Geeraerd, Valdramidis, and Van Impe, 2005).

Log-linear and nonlinear models available in the GInaFiT were used to describe the UV-C inactivation kinetics of target microorganisms. The selected models provide a good fit to microbial inactivation data and goodness of fit parameters were

evaluated (including R^2, RMSE, and rate constants). The linear inactivation model is given in the following equation:

$$C = C_0 e^{-k_{max} \times D}. \qquad (3)$$

For identification purposes, the expression was reformulated as

$$log_{10}(C) = log_{10}(C_0) - \frac{k_{max} \times D}{ln_{10}}, \qquad (4)$$

where C is the initial concentration of microorganisms, C_0 is the concentration of microorganisms at dose D, k is the degradation rate constant, and D is the UV dose delivered.
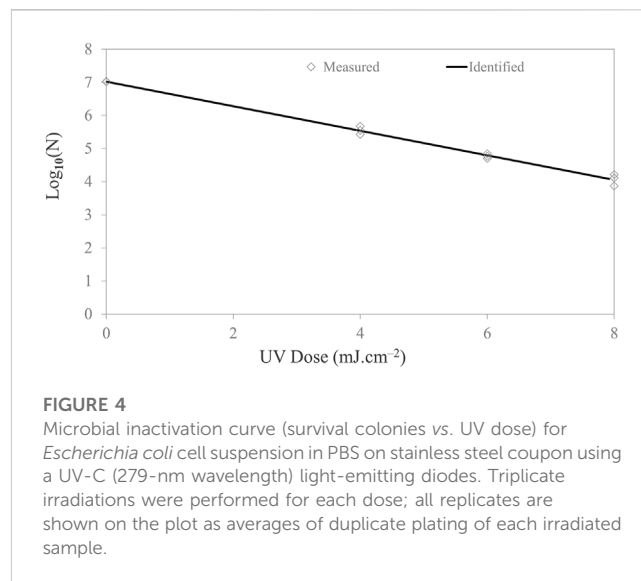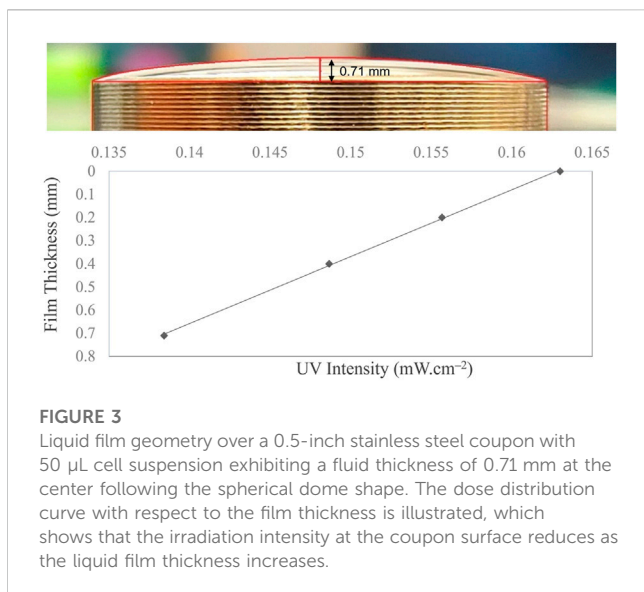
The nonlinear model equation is as follows:

$$\frac{N}{N_0} = 10^{\left(-\left(\left(D/\delta\right)^p\right)\right)}. \qquad (5)$$

For identification purposes, the expression was reformulated as

$$Log_{10} = Log_{10}(N_0) - \left(\left(D/\delta\right)^p\right), \qquad (6)$$

$$t_{xd} = \delta \times (x)^{1/p}, \qquad (7)$$

where N is the initial concentration of the microorganisms, N_0 is the concentration of microorganisms after the dose, p is the Weibull slope (or threshold parameter), δ represents a 90% reduction in the microbial population, and D is exposure dosage. The numerical values for inactivation kinetic parameters, the dosage required to obtain 5-log reduction, and other similar parameters were calculated for the model. The values of δ and p were used to calculate the desired log reduction at a specific dosage. The dose required to obtain an x log reduction (t_{xd}) was calculated using Eq. 7.

FIGURE 3
Liquid film geometry over a 0.5-inch stainless steel coupon with 50 μL cell suspension exhibiting a fluid thickness of 0.71 mm at the center following the spherical dome shape. The dose distribution curve with respect to the film thickness is illustrated, which shows that the irradiation intensity at the coupon surface reduces as the liquid film thickness increases.

FIGURE 4
Microbial inactivation curve (survival colonies *vs*. UV dose) for *Escherichia coli* cell suspension in PBS on stainless steel coupon using a UV-C (279-nm wavelength) light-emitting diodes. Triplicate irradiations were performed for each dose; all replicates are shown on the plot as averages of duplicate plating of each irradiated sample.

3 Results and discussion

3.1 UV-C LED absolute irradiance, emission spectra, and UV-C dose

The absolute irradiance of the LED device was determined using a QEPro high-performance spectrometer (Ocean Optics; Largo, FL, United States), as described briefly in Section 2.3. Figure 2A illustrates the wavelength and corresponding absolute irradiance (power output per unit area per wavelength) of the LED device. The peak emission of the LED device was observed at 279 nm, exhibiting an irradiance or average fluence rate of 0.163 ± 0.003 mJ cm^{-2} at a distance of 4 cm from the optical source. The current (0.032A) and voltage (19.15 V) were optimized to achieve the desired power. The UV dose and corresponding exposure times are shown in Table 1.

3.2 Absorption spectra and optical properties

Figure 2B shows the UV absorption spectra of *E. coli*, *Salmonella*, and *Listeria*. All UV wavelengths cause photochemical effects, and wavelengths in the UV-C range are particularly damaging to bacterial cells as they are absorbed by proteins and DNA (Bolton and Cotton, 2011; Rauth, 1965). A maximum peak at 260 nm was observed, as illustrated in Figure 2B. This corresponds absorption of UV energy by the bacterial DNA. Absorption in the peptide bonds within proteins at wavelengths above 260 nm was also observed. Pyrimidines (thymine, cytosine, and uracil) absorb approximately ten times more UV than purines. As evidenced in the literature, the optimum wavelength for inactivating bacteria, at 265 nm, is approximately 15% more effective than the UV-C peak of 254 nm. Perhaps, the optimum wavelength for inactivating spores is 270 nm, which is approximately 40% more effective than 254 nm (Waites et al., 1988).

A decrease in bacterial absorption was observed between 270 and 300 nm. It is envisaged that at a wavelength of 279 nm, the transmission was higher than that at 254 nm (12%). The increase in the UV transmission allows the photons to penetrate the fluid layers achieving a better dose distribution compared to 254 nm. Perhaps, the fluid region close to the source will achieve a high dosage compared to the bottom fluid layers. The spatial UV fluence rate can be described as a function of the optical transmissivity of the test fluid, the UV output of the optical source, and the distance to the source. The absorbance values of *E. coli, Salmonella* Typhimurium, and *Listeria* suspensions were 0.06 ± 0.002, 0.03 ± 0.001, and 0.02 ± 0.001 L mol^{-1} cm^{-1} corresponding to the UV transmission of the 86.23%, 92.97%, and 95.57% transmittance.

3.3 Film geometry and intensity distribution of droplet

Figure 3 shows the liquid film geometry over the 1-inch stainless steel coupon. The three-dimensional shape was identical to the spherical dome, with the maximum and minimum thicknesses at the center and periphery, respectively. The maximum thickness at the center was calculated as 0.71 mm using ImageJ software, as explained in Section 2.6. Over the thickness of the film, the UV intensity decreased as a function of fluid depth. UV intensity across the film depends on the film thickness and fluid optical properties. Fluid-dependent optical properties [i.e., absorbance (or transmittance), concentration, and scattering] are critical parameters that affect UV delivery and impact microbial inactivation. The UV-C dose is the number of photons absorbed per surface area by an irradiated fluid during a particular exposure time. The gradients in UV intensity as a function of fluid depth are illustrated in Figure 3. Only 84% of UV-C energy reaches the bottom layer of the fluid at the center, where the droplet is 0.71 mm thick.
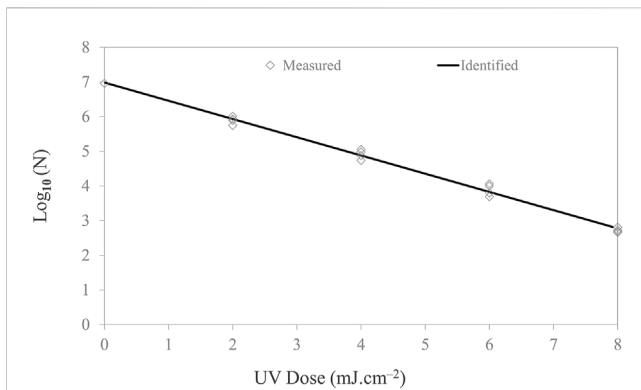
FIGURE 5
Microbial inactivation curve (survival colonies *vs.* UV dose) for *Salmonella* Typhimurium cell suspension in PBS on stainless steel coupon using UV-C (279 nm wavelength). Triplicate irradiations were performed for each dose; all replicates are shown on the plot as averages of duplicate plating of each irradiated sample.

4 UV-C inactivation of *E. coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes*

4.1 UV inactivation of *E. coli* in a droplet

In this investigation, a LED system was tested against a suite of microorganisms in a droplet on the food contact surface. Survival curves were constructed by plotting log concentration *versus* UV doses. In general, the UV-C dose was calculated by multiplying the irradiance (mW/cm^2) by the exposure time. Figure 4 shows the survival curves of *E. coli*. The survival curves followed a logarithmic order of death. With a maximum dosage dose of $8 mJ/cm^2$, a 3-log reduction for *E. coli* was achieved. Microbial inactivation followed the log-linear model with adjusted R^2 values of 99.2% and K_{max} value of 0.85 ± 0.02, with a low RMSE of 0.101.

For 1-, 2-, 3-, and 4-log inactivation of the *Escherichia coli* ATCC 25922 on the stainless steel coupon, the delivered UV doses were 2.70, 5.39, 8.09, $10.79 mJ/cm^2$, with a calculated D_{10} value of $2.70 mJ cm^{-2}$. Survival curves did not show tailing. The dose required for 4-log reduction was extrapolated based on the rate constants. In comparison, Kim and Kang (2020) reported a $0.9–1.44-log_{10}$ reduction of *E. coli* 0157:H7 with a UV-C dose of $2 mJ cm^{-2}$ on different dried food contact surfaces with 280 nm UV-C LEDs. Sommer et al. (1998) reported that UV-C doses of 6.0, 6.5, 7.0, 8.0, 9.0, and $10.0 mJ cm^{-2}$ resulted in 1-, 2-, 3-, 4-, 5-, and $6-log_{10}$ reduction of *Escherichia coli* ATCC 25922, respectively, using 254 nm low-pressure lamp as the UV source. Lu et al. (2021) examined the inactivation effect of UV LEDs with three different wavelengths on solid agar surfaces (mimicking food contact surface) and investigated any occurrence of synergistic effects under the simultaneous operation of any two UV wavebands (i.e., 369/288 nm and 369/271 nm). The authors observed that the 271-nm UV LED had the highest inactivation efficiency among the UV LEDs applied alone. Irradiance by a combination of 288/271-nm UV LEDs had a positive synergistic effect against *E. coli*, *S. marcescens*, and *S.* Typhimurium. The k-values revealed that the inactivation capacities of the 271-nm UV LED for the tested bacteria are in the following order: *E. coli* > *S. marcescens* > *P. alcaligenes* > *S. epidermidis* > *S.* Typhimurium.

4.2 UV-C inactivation of *Salmonella* in a droplet

The *Salmonella* populations were reduced by $4-log_{10}$ at the highest UV-C dose of $8 mJ cm^{-2}$. The first-order inactivation kinetics plotted in Figure 5 shows the inactivation trend. Kinetic rate constants are documented in Table 2. The experimental data were fitted to a log-linear model. The D_{10} ($2.303/K_{max}$) value of *Salmonella* was observed to be $1.90 mJ/cm^2/log$. Figure 5 shows the log reductions obtained for *Salmonella* following exposure to 2, 4, 6,

TABLE 2 Kinetic constants and D_{10} values of microorganisms.

	Log-linear model				Fluence (UV dose) ($mJ.cm^{-2}$) for a given log reduction without photoreactivation			
Microorganism	[a] k_{max}	[b] R^2	[c] R^2 adj	[d] RMSE	1D	2D	3D	4D
Escherichia coli (ATCC 25922)	0.85 ± 0.02	0.993	0.992	0.101	2.70 ± 0.1	5.39 ± 0.2	8.09 ± 0.3	10.79 ± 0.4
Salmonella Typhimurium (ATCC 700720)	1.21 ± 0.03	0.992	0.992	0.123	1.90 ± 0.1	3.81 ± 0.1	5.71 ± 0.2	7.61 ± 0.3
	Weibull model							
Microorganism	[e] delta (Δ)	[f] p	[b] R^2	[c] R^2 adj	[d] RMSE	1D	2D	3D	4D
Listeria monocytogenes ATCC 19115	3.02 ± 0.19	2.15 ± 0.17	0.994	0.993	0.154	3.02 ± 0.1	4.16 ± 0.1	5.02 ± 0.2	5.76 ± 0.2

[a]k_{max}, correlation coefficient.
[b]R^2 represents the variance proportion explained by an independent variable for a dependent variable.
[c]Adjusted R^2 indicates how well data points fit a curve in accordance with the model.
[e]Dose required for 1-log inactivation.
[d]Root mean sum of squared error; 1D, 2D, 3D, 4D represent 90%, 99%, 99.9%, 99.99% reduction.
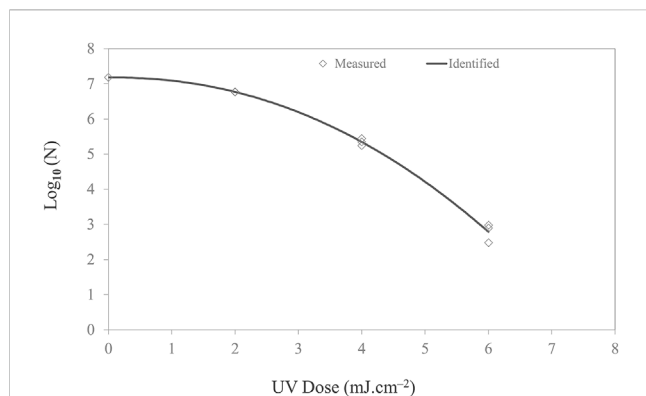[f]*Listeria* inactivation follows the Weibull model with equation [N/N0 = 10*(-((t/Δ)*p))].

FIGURE 6
Microbial inactivation curve (survival colonies *vs.* UV dose) for *Listeria monocytogenes* cell suspension in PBS on stainless steel coupon using UV-C (279 nm wavelength) light-emitting diodes with total irradiance of 0.163 mW/cm². Triplicate irradiations were performed for each dose; all replicates are shown on the plot as averages of duplicate plating of each irradiated sample.

and 8 mJ cm^{-2} of the UV-C dosage. The data show a significant ($p \leq$ 0.05) increase in dose-dependent inactivation.

For the 1-, 2-, 3-, and 4-log inactivation of *Salmonella* on the stainless steel coupon, the UV-C dose required was 1.90 ± 0.06, 3.81 ± 0.12, 5.71 ± 0.19, and 7.61 ± 0.25 mJ cm^{-2}. Kim and Kang (2020) reported a 0.5–1.66-log reduction of *Salmonella* Typhimurium at a dose of 3.0 mJ/cm² for dried food contact surfaces. Chang et al. (1985) reported that 7.00 mJ cm^{-2} UV-C dose inactivated 99.99% *Salmonella* Typhimurium while using a 254-nm low-pressure lamp (254 nm) for liquid suspension. A UV-C dose of 7.7 mJ cm^{-2} is required for 99.99% inactivation of *Salmonella* Typhimurium (Hu et al., 2012). Chen, Craik, and Bolton (2009) also reported that 7.8 mJ/cm² UV-C dose inactivated 3-log$_{10}$ of *Salmonella* Typhimurium. Among the different strains, UV sensitivity varies due to differences in their genetic makeup.

4.3 UV-C inactivation of *Listeria* in a thin-film droplet

The efficacy of UV-C light in inactivating *Listeria* is reported in Figure 6. A nonlinear inactivation trend was observed for *Listeria*. The shoulder curves started with a horizontal slope before developing into full exponential decay. It can be hypothesized that the lag in response to the stimulus implies that either a threshold dose may be necessary before measurable effects occur or repair mechanisms actively deal with low-level damage at low doses (Casarett, 1968). This effect is species-dependent and appears to be a function of light intensity. However, for low irradiance, the shoulder can be significant and prolonged for certain bacteria (Kowalski, 2001).

The inactivation followed the Weibull model with a delta value and *p*-value of 3.02 ± 0.19 and 2.15 ± 0.17, respectively. The *p*-value signifies the shape of the microbial inactivation curve, which was observed to be convex. To describe the survival of *Listeria* in a film, different inactivation models available in the GInaFiT program, namely, the log-linear and Weibull, were fitted to the experimental inactivation data and evaluated for the goodness of fit. The GInaFiT tool was employed to perform regression analysis of microbial inactivation data. Adjusted R^2 and RMSE values were 0.98 and 0.244, respectively. For 1-, 2-, 3-, and 4-log inactivation of *Listeria* on the stainless steel coupon, the UV-C doses required were 3.02 ± 0.06, 4.16 ± 0.12, 5.02 ± 0.18, and 5.76 ± 0.24 mJ cm^{-2}.

The initial resistance toward UV-C inactivation might result from thicker cell walls in Gram-positive microorganisms. Several studies reported that *Listeria* shows increased resistance to any disinfection treatment if it had already been under any type of stress (Bernbom, Vogel, and Gram, 2011; Schaik et al., 1999; McKinney et al., 2009; Gahan, O'Driscoll, and Hill, 1996), for instance, *L. monocytogenes* (Gram positive) stressed with either acid or antibiotic showed a higher resistance to UV-C inactivation (McKinney et al., 2009). These findings indicate that despite the UV-C doses required for log inactivation of *Listeria* being considerably lower than required for 1-log$_{10}$ inactivation of *E. coli* and *Salmonella*, the UV-C survival of *Listeria* was similar to that reported for other Gram-negative zoonotic pathogens, such as *E. coli* O157:H7 and *Salmonella* spp. (Manreet S Bhullar et al., 2018). The aforementioned findings are critical in understanding the inactivation of microorganisms in a thin-film droplet. We hypothesize that diffuse reflectivity spreads photons in all directions, regardless of the incident angle of incoming light rays. Perhaps the back reflection from the stainless steel coupon may explain the lower D value of target microorganisms compared to microbes suspended in clear solutions under stirred conditions. In addition, photons at 279 nm may cause protein damage and form dimers. The usefulness of surface rate constants is critical for equipment sterilization and disinfection of food contact surfaces. The inactivation rates of microbes on surfaces may vary depending on the type of surface used for the studies. Perhaps it is worth noting that the UV-C sensitivity of microorganisms on surfaces may be higher or lower than observed in water, for any given species; the absolute differences do not seem great that it would invalidate the use of water-based rate constants to predict surface disinfection rates.

5 Conclusion

UV-C light effectively inactivated the target microbes as a droplet on food contact surfaces (>3-log$_{10}$ reduction). The optical property of the droplet dictated the inactivation rate. Many published studies on UV inactivation of microbes involved exposure on petri dishes or other solid materials. Therefore, these results can be considered applicable to surface disinfection. These sets of experiments concluded that photons at 279 nm can be used to disinfect food contact surfaces. This information is critical to design

an effective UV-based surface disinfection system. The implementation of this novel technology could be used in combination with current chemical sanitizers to control spoilage and pathogenic microorganisms.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Conflict of interest

AP declared working as an editorial board member at Frontiers at the time of submission. This had no impact on the peer review process and the final decision.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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