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Inactivation of *Escherichia coli*, *Listeria* and *Salmonella* by single and multiple wavelength ultraviolet-light emitting diodes

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ABSTRACT

This study compared the inactivation efficacy and performance of UV-LEDs emitting at 259, 268, 275, 289, and 370 nm against a low pressure mercury lamp at 253.7 nm for the foodborne pathogens, *E. coli*, *Listeria* and *Salmonella*. Action spectra were determined for three pathogenic and three non-pathogenic strains and compared with UV absorbance of their bacterial DNA. The lethality of UV wavelengths correlated with bacterial DNA absorbance. At an equivalent UV dose (7 $mJ{\cdot}cm^{-2}$), UV-LEDs emitting at 259 and 268 nm achieved the highest log count reductions out of the tested wavelengths. Refrigeration (4 °C) increased irradiance of the 268 nm UV-LEDs while not affecting reduction of *Listeria* compared to 25°C. Combining 259 and 289 nm UV-LED wavelengths at an equivalent UV dose had a synergistic effect on reduction of *E. coli* and *Listeria*, yielding a 1.2 and 0.6 log higher reduction, respectively, than the expected additive effect.

Industrial relevance: UV-LED treatment at 259, 268, and 275 nm can either equal or, in most cases, surpass the inactivation efficacy of traditional LPM lamps at 253.7 nm. Further, the determined action spectra can be used to identify the optimum inactivation wavelength for common foodborne pathogens and hence increase processing efficiency. In some cases, inactivation efficacy can be improved by combining UV wavelengths in order to achieve a synergistic effect. The effectiveness of UV-LED treatment at refrigeration temperatures validates their use in cold environments. Overall, UV-LEDs have strong potential within the food industry due to their advantages and possibilities for incorporation into a wide variety of treatment systems.

1. Introduction

Ultraviolet-C (UV-C) light ranges from 100 to 280 nm and is considered to have the highest microbial inactivation efficacy in the UV portion of the electromagnetic spectrum because its emission aligns with the absorbance of DNA (~260 nm) (Koutchma, Forney, & Moraru, 2009). UV-C light at 253.7 nm has been the most commonly applied wavelength for use in water, air and surface disinfection (Gally & Stevens, 2017; Jay et al., 2007) due to the fact that it can be easily produced by low pressure mercury (LPM) bulbs, a readily available type of continuous UV light source. Microbial inactivation by UV-C light is achieved primarily through DNA absorption of UV photons and subsequent damage to DNA via pyrimidine dimerization (Goodsell, 2001). Among food safety interventions, UV-C light is a technology that can have complementary applications in food surface treatment as well as preservation of beverages. The key drivers of UV-C treatment are its low cost, dry nature, ease of application, high energy efficiency, lack of disinfection by-products, and its non-thermal and non-chemical character.

UV light emitting diodes (UV-LEDs) are an emerging continuous UV light source that have several advantages over LPM lamps as they can emit a wide variety of wavelengths from 210 to 395 nm (Taniyasu, Kasu, & Makimoto, 2006), contain no toxic mercury, do not require warm-up time, and do not suffer degradation in life-time or irradiance from on/off cycles (Ke-Xun et al., 2009). Due to their small size, UV-LEDs lend themselves effectively to a wider variety of disinfection apparatus designs. They can also be used in cold environments as they have shown to increase irradiance as temperatures approach 0 °C (Cao, Leboeuf, Rowland, & Liu, 2003), as opposed to LPM lamps, which decrease in output at low temperatures. This further increases the versatility of UV-LEDs in terms of application. Further, UV-LED systems are capable of emitting multiple wavelengths at the same time. This opens

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the possibility of a synergistic effect in terms of bacterial inactivation (i.e. larger inactivation than expected due to the additive effect between the two individual wavelengths) (Beck et al., 2017; Nakahashi et al., 2014).

The ability of UV-LEDs to be tuned to various wavelengths in the UV range allows for the construction of action spectra, which describe the efficacy of a range of UV wavelengths in the inactivation of a microorganism in a particular medium (Bolton, 2017). These results can be useful in industrial applications where the inactivation of a specific microorganism(s) is desired. Bolton (2017) has shown that lethality for most waterborne microorganisms peaks between 250 and 270 nm. However, the exact UV sensitivity can vary depending on the target species. Currently, complete action spectra for foodborne pathogens are lacking and such information could increase the inactivation efficacy of UV-LEDs in the food processing industry.

The goal of this study was to determine the optimal UV treatment wavelength(s) and conditions for inactivation of the common foodborne pathogens *E. coli*, *Listeria* and *Salmonella*. In order to do so, we investigated the inactivation efficacy of UV-LEDs emitting at various wavelengths (259, 268, 275, 289, and 370 nm) and an LPM lamp at 253.7 nm against these bacteria at an equivalent UV dose. The resulting inactivation at each wavelength was used to construct action spectra for three pathogenic strains of *E. coli*, *Listeria* and *Salmonella* and three non-pathogenic counterparts. The use of combination wavelengths was also explored in order to determine possible synergistic effects. Finally, the impact of UV-LED ambient temperatures on the inactivation of *Listeria* was explored.

2. Materials and methods

2.1. Bacterial strains and growth media

A single pathogenic strain of *E. coli*, *Listeria*, and *Salmonella* was chosen for this study and each was paired with a non-pathogenic counterpart strain which were investigated in previous surrogate studies. *E. coli* O157:H7 (ATCC 700972), *E. coli* ATCC 8739 (*E. coli* O157:H7 surrogate) (Orlowska, Koutchma, Kostrzynska, & Tang, 2015), and *L. innocua* ATCC 51742 (non-pathogenic *Listeria*) were obtained from the American Type Culture Collection (Manassas, VA, USA). *E. coli* ATCC 8739 was originally isolated from feces and *E. coli* O157:H7 was originally isolated from contaminated beef. *L. monocytogenes* and *L. seeligeri* (non-pathogenic *Listeria*) were also obtained from the American Type Culture Collection. All *Listeria* strains were originally isolated from produce (serotypes are unknown). Five pathogenic *Salmonella enterica* subsp. *enterica* serovars: Typhimurium, Tennessee, Heidelberg, Schwarzengrund, and I:6,7:-:1,6 (an unnamed serovar); as well as subspecies *arizonae* sv. 42:z4,z24:- and *diarizonae* sv. 11:k:z53 were obtained from the Public Health Agency of Canada (Guelph, ON, Canada). All strains were originally isolated from produce. *Enterococcus faecium* NRRL-B2354, which has been used as a surrogate for *Salmonella* and other pathogens in thermal processing of dry foods (Kopit, Kim, Siezen, Harris, & Marco, 2014) was obtained from the Agricultural Research Service Culture Collection (Peoria, IL, USA). All isolates were stored in TSB with 20% glycerol at −80 °C.

2.2. Growth of bacterial cultures

Tryptic soy agar (TSA) and tryptone soy broth (TSB) were obtained from Fisher Scientific (Ottawa, ON, Canada). *Listeria* strains were grown overnight at 30 °C in 40 mL of TSB in a 50 mL centrifuge tube. *E. coli* and *Salmonella* strains as well as *E. faecium* NRRL-B2354 were grown in 25 mL of TSB at 37 °C. Growth was stopped once cultures reached stationary phase with a minimum concentration of 10^9 cells·mL^{-1} as determined by measurement of optical density at 600 nm (OD_{600} = 1.25) using a UV–Vis spectrophotometer (Biochrom Ultrospec 3100 Pro; Buckinghamshire, UK). The bacterial cultures were harvested by centrifuging at 4000 x g for 15 min (ThermoFisher Scientific Sorvall ST 16R; Ottawa, ON, Canada). The pellet was washed in 25 mL of 0.9% saline and then re-suspended in 0.9% saline to a final concentration of 10^9 cells·mL^{-1}. These stock suspensions were kept refrigerated at 4 °C for use up to one week as no changes in UV sensitivity or viability were observed for any tested bacterial strains during this period. The use of the same suspensions also reduced the variability of initial viable cell count between similar trials.

2.3. Determination of absorption coefficients of bacterial suspensions

Bacterial suspensions were diluted in 0.9% saline to a concentration of 10^8 cells·mL^{-1} as determined by measurement of OD_{600}. The absorption coefficient of these suspensions at each UV treatment wavelength was measured for each bacterium using the Biochrom Ultrospec 3100 Pro in quartz cuvettes with a 1 cm path length.

2.4. UV treatment setup and irradiance measurements

2.4.1. LPM lamp

A single LPM lamp (R-52G Mineralight, 115 V, UVP, LLC; Cambridge, UK) was used as the source of monochromatic UV-C light at 253.7 nm. The lamp was attached to a post by an adjustable bracket that allowed the irradiance to be altered by changing the distance between the lamp and base plate. The LPM lamp was allowed to warm up for at least 30 min prior to use. The distance between the light source and the sample surface was 19.08 cm.

2.4.2. UV-LED collimated beam unit

The UV-LED collimated beam unit (PearlBeam™, AquiSense Technologies; Erlanger, KY, USA) was used for UV treatments at 259, 268, 289, and 370 nm. This unit consisted of an array containing three LEDs of each wavelength, nine in total. For treatments involving 268 nm light, the 370 nm UV-LEDs were replaced with the 268 nm UV-LEDs. The LED array was attached to a collimator 26.42 cm in length.

2.4.3. Irradiance measurements

The emission spectra of individual UV-LED wavelengths and the LPM lamp were collected as a function of absolute spectral irradiance (i.e. power output per unit area per wavelength) using the USB2000 + spectrometer (Ocean Optics; Largo, FL, USA) equipped with a 2 m fiber optic probe (Ocean Optics; Largo, FL, USA) with a 600 μm core diameter and a 3200 μm collection surface. The probe was calibrated between 200 and 900 nm using the DH2000 UV–Vis-NIR light source (Ocean Optics; Largo, FL, USA). For measurements, the fiber optic probe was placed in the centre of the UV light beam at the same height and location as the surface of the bacterial suspensions. The measured irradiance incident to the surface of the treated sample (i.e. incident irradiance) was calculated by rectangular integration of the peaks using the SpectraSuite software (Ocean Optics; Largo, FL, USA).

2.4.4. Microplate UV-LED unit

The microplate UV-LED unit (Phoseon Technologies Inc.; Hillsboro, OR, USA) was used as the light source for UV treatments at 275 nm. This unit consisted of a fully enclosed 105 × 85 mm UV-LED array, which was centred above a movable sample drawer. UV treatment could only be performed when the sample drawer was in the “closed” position. Due to the closed design of this unit, irradiance could not be measured with a spectrometer. Rather, a standard curve correlating UV degradation of methylene blue (0.25 g·L^{-1} in 0.15% H_2O_2; $A_{663\ nm}$) (Sigma-Aldrich; Oakville, ON, Canada) and average UV dose following exposure to 268 and 289 nm UV-LED light of measured irradiance was used to estimate UV dose at 275 nm (Giannakis et al., 2015).

Table 1
Measured peak emission wavelength, absorption coefficient (α), water factor (WF), incident irradiance (E_0), average irradiance (E_{avg}), treatment time required to reach a UV dose of 7 mJ·cm^{-2}, and LCR achieved for treatment of each tested bacterium at 253.7 nm using the LPM lamp, at 259, 268, 275, 289, and 370 nm.

Bacterium	Peak emission wavelength (nm)	E_0 (mW·cm^{-2})	α (cm^{-1})	WF	E_{avg} (mW·cm^{-2})	Treatment time for 7 mJ·cm^{-2} (s)	LCR ± SD
L. monocytogenes	253.7	0.490	0.821	0.690	0.323	22	4.03 ± 0.32
	259	0.0215	0.817	0.691	0.014	500	3.95 ± 0.38
	268	0.138	0.792	0.699	0.092	76	4.68 ± 0.13
	289	0.221	0.647	0.743	0.157	45	1.05 ± 0.15
	370†	6.25	0.349	0.781	4.66	1	0.18 ± 0.03[g]
L. seeligeri	253.7	0.490	0.924	0.661	0.309	23	1.63 ± 0.10
	259	0.0215	0.920	0.662	0.014	500	3.77 ± 0.22
	268	0.138	0.893	0.670	0.088	80	3.50 ± 0.16
	289	0.221	0.719	0.721	0.152	46	0.50 ± 0.03
	370†	6.25	0.384	0.835	4.99	1	0.12 ± 0.03[g]
L. innocua	259	0.0215	0.939	0.657	0.013	519	4.33 ± 0.61
E. coli ATCC 8739	253.7	0.490	0.855	0.680	0.318	22	0.38 ± 0.10
	259	0.0215	0.856	0.680	0.014	500	1.10 ± 0.12[a]
	268	0.138	0.843	0.684	0.090	78	1.07 ± 0.16[a]
	275	0.70	0.820	0.690	0.483	14	0.67 ± 0.05
	289	0.221	0.735	0.716	0.151	46	0.09 ± 0.11[f]
	370†	6.25	0.479	0.800	4.775	1	0.10 ± 0.07[f]
E. coli O157:H7	253.7	0.490	0.855	0.680	0.318	22	3.24 ± 0.32
	259	0.0215	0.856	0.680	0.014	500	5.21 ± 0.13
	268	0.138	0.847	0.683	0.090	78	4.88 ± 0.18
	289	0.221	0.735	0.716	0.151	46	0.67 ± 0.02
	370†	6.25	0.479	0.800	4.775	1	0.06 ± 0.02[f]
S. enterica subsp. *diarizonae*	253.7	0.490	0.800	0.696	0.326	21	4.00 ± 0.28
	259	0.0215	0.797	0.697	0.014	500	5.32 ± 0.15[b]
	268	0.138	0.781	0.702	0.093	75	4.60 ± 0.15[c]
	289	0.221	0.787	0.700	0.148	47	4.30 ± 0.19[c]
Salmonella cocktail	253.7	0.490	0.825	0.689	0.320	22	4.67 ± 0.18
	259	0.0215	0.823	0.689	0.014	500	4.57 ± 0.12[b,d]
	268	0.138	0.812	0.693	0.091	77	4.72 ± 0.39[d]
	289	0.221	0.709	0.724	0.153	46	1.65 ± 0.14
E. faecium NRRL-B2354	253.7	0.490	0.665	0.738	0.345	20	2.32 ± 0.19
	259	0.0215	0.668	0.737	0.015	467	3.79 ± 0.14[e]
	268	0.138	0.660	0.739	0.097	72	3.89 ± 0.23[e]
	289	0.221	0.552	0.775	0.164	43	0.35 ± 0.13

NOTE: PF, RF, and DF do not depend on absorbance and therefore do not change in a manner dependent on the microorganism or wavelength. These factors were 0.999, 0.975, and 0.980, respectively.
a,b,c,d,e,f,g Figures with the same letter beside them are not significantly different. Statistical significance was assessed for similar LCR values.
† UV-LED treatments at 370 nm were also conducted at a UV dose of 15 mJ cm^{-2} due to the short treatment time required for 7 mJ cm^{-2} and no significant difference in LCR observed.

2.4.5. Determination of average irradiance, treatment time, peak emission wavelength, and full width half maximum

Correction factors, Petri factor (PF), reflection factor (RF) divergence factor (DF), and water factor (WF), were calculated according to Bolton and Linden (2003) using Eqs. (1), (2), (3), and (4):

$$PF = \frac{\sum \frac{E_n}{E_0}}{n} \quad (1)$$

where:

E_n = incident irradiance measured at various distances from the centre of the Petri dish (measured every 5 mm) (mW·cm^{-2})
E_0 = incident irradiance measured at the centre of the Petri dish (mW·cm^{-2})
n = number of measurements taken.

In this study E_n did not differ from E_0, hence from here on out we will refer to E_0 as "incident irradiance".

$$RF = 1 - R \quad (2)$$

where:

R = fraction of UV light reflected by the surface of the treatment medium. For water/0.9% saline, this is approximately 0.025 as given by the Frensel law (Meyer-Arendt, 1984)

$$DF = \frac{L}{L + l} \quad (3)$$

where:

L = distance between the light source and sample surface (cm)
l = sample depth (cm).

$$WF = \frac{1 - 10^{-\alpha \times l}}{\alpha \times l \times ln\,10} \quad (4)$$

where:

α = absorption coefficient (cm^{-1}).

These correction factors were used to determine the *average irradiance* delivered throughout the entire volume of the sample (E_{avg}, mW·cm^{-2}) using Eq. (5):

$$E_{avg} = E_0 \times PF \times RF \times DF \times WF \quad (5)$$

The average irradiance was used to calculate the treatment time required to achieve the desired UV dose using the equation: $t = D/E_{avg}$, where: D = UV dose (mJ·cm^{-2}), and t = treatment time (s). In the case of the microplate UV-LED unit, the time required for a desired UV dose was determined by chemical actinometry as described in Section 2.4.4.

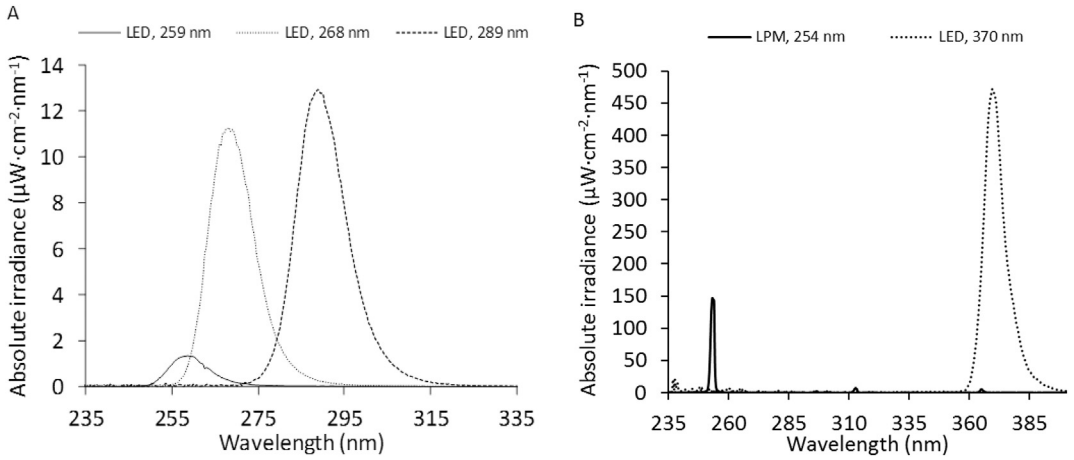

Fig. 1. Emission spectra of the (A) 259, 268, and 289 nm UV-LEDs and the (B) 253.7 nm LPM lamp and the 370 nm UV-LED in terms of absolute spectral irradiance.

2.5. UV treatment of bacterial suspensions

Bacterial suspensions containing 4 mL of 10^8 cells·mL^{-1} in 0.9% saline solution were treated with a UV dose of 7 mJ·cm^{-2} by exposure to individual wavelengths of 259, 268, 289 and 370 nm in a Petri dish (35 × 10 mm) under stirring at 450 rpm (using the LPM unit, the UV-LED collimated beam unit, and the microplate UV-LED unit). The sample depth was 0.42 cm. The difference in incident irradiance values between different wavelengths resulted in variable treatment times required to achieve an equivalent UV dose of 7 mJ·cm^{-2}. *Salmonella* and *E. faecium* NRRL-B2354 were not treated at 370 nm due to the lack of efficacy observed when treating *E. coli* and *Listeria*.

E. coli and *Listeria* strains were treated using 259/289 and 259/370 nm combination wavelengths, where a UV dose of 7 mJ·cm^{-2} at each wavelength was applied simultaneously resulting in a total dose of 14 mJ·cm^{-2} per treatment. The combination treatment using two wavelengths was started simultaneously, and each UV-LED was turned off consecutively once the 7 mJ·cm^{-2} UV dose had been applied at that wavelength (Table 1). UV treatments at 268 nm were also performed at 4 °C with *Listeria* strains where the UV-LED unit was installed in a Freestanding Refrigerator (VWR; Mississauga, ON, Canada).

2.6. Enumeration of surviving bacterial cells

Following UV treatment, control and UV treated bacterial suspensions were serially diluted in sterile 0.9% saline and 100 μL of the appropriate dilutions were spread onto TSA plates in triplicate. The TSA plates were incubated overnight at 37 °C (except *Listeria*, which was incubated for 24 h at 30 °C), and colony-forming units (CFU·mL^{-1}) were determined the following day. Log count reduction (LCR) was determined using the equation: $LCR = \log(N_0/N_1)$; where: N_0 = CFU/mL of the control sample and N_1 = CFU/mL of the UV treated sample. In the case of synergistic wavelength experiments, theoretical additive LCRs were determined by adding the LCRs achieved at each individual wavelength used in the combination treatment. Where applicable, the UV dose required for one LCR (i.e. D value) was calculated using the log-linear portion of the inactivation curve by first applying a linear fit and taking the negative inverse of the slope of those fits.

2.7. Determination of absorbance spectra of bacterial cells and extracted DNA

The extraction of DNA from bacterial cell suspensions (10^8 cells·mL^{-1} in 0.9% saline solution) was performed using the Gentra Puregene Yeast/Bact. Kit (Qiagen; Hilden, Germany) in accordance with the manufacturer's protocol (Qiagen, 2014). The absorbance spectrum of the extracted DNA (approximately 20 μg·mL^{-1} in double distilled water) as well as whole cell suspensions (10^8 cells·mL^{-1} in 0.9% saline solution) were measured using a 1 cm quartz cuvette in a UV–Vis spectrophotometer between 200 and 900 nm in conjunction with the SWIFT II Wavescan software version 2.06 (GE Healthcare Life Sciences; Mississauga, ON).

2.8. Statistical analysis

All trials were repeated a minimum of three times and all samples were plated in triplicate. The statistical differences between mean LCRs of UV treatments were assessed using Welch's *t*-test in Microsoft Excel. Statistical significance was determined at $p < 0.05$. LCRs were reported as average ± standard deviation (SD).

3. Results

3.1. Determination of irradiance, peak emission wavelengths, and treatment times for the UV-LEDs and LPM lamp

The emission spectrum of the UV-LED collimated beam unit at wavelengths of 259, 268, 289, and 370 nm as well as that of the LPM lamp at 253.7 nm is shown in Fig. 1. Peak emission wavelength, cell suspension absorption coefficients and correction factors, incident and average irradiance and treatment times for all tested bacteria are shown in Table 1. There was a considerable difference in incident irradiance between the tested wavelengths, with the 259 nm UV-LED being the lowest at 0.0215 mW·cm^{-2} and the 370 nm UV-LED being the highest at 6.25 mW·cm^{-2}. Each tested strain had a specific absorption coefficient that influenced the value of the calculated water factor and eventually the average irradiance delivered to a sample. This in turn influenced the treatment time required to achieve a UV dose of 7 mJ·cm^{-2} for a given wavelength (Bolton and Linden, 2003).

3.2. Inactivation efficacy of UV-LEDs at multiple wavelengths

The LCR following UV treatments between 259 and 370 nm is shown in Table 1. *S. enterica diarizonae* was chosen for individual treatments because it was found to be the least UV sensitive strain among those constituting the *Salmonella* cocktail at 253.7 nm (data not shown). All tested bacteria exhibited the highest UV sensitivity at either 259 or 268 nm. Among the three individual pathogenic bacteria, *S. enterica diarizonae* and *E. coli* O157:H7 exhibited the highest overall UV sensitivities, which occurred at 259 nm. In contrast, *L. monocytogenes* peaked in UV sensitivity at 268 nm. Overall, *E. coli* ATCC 8739 was shown to be the least UV sensitive bacterium tested in this study,

showing significantly lower LCR values ($p < 0.05$) at all wavelengths between 259 and 289 nm. Also, with the exception of *S. enterica diarizonae* (to be discussed later), all tested bacteria showed a progressive decrease in UV sensitivity at wavelengths beyond 268 nm. Negligible LCRs were observed following UV treatment at 370 nm with all tested bacteria.

The inactivation of *L. monocytogenes* was compared with *L. innocua* and *L. seeligeri* at 259 nm. Between the two non-pathogenic strains, only *L. seeligeri* was significantly less ($p < 0.05$) UV sensitive than *L. monocytogenes*. Therefore, *L. seeligeri* was selected as the non-pathogenic *Listeria* strain for use in further experiments. Similarly, *E. coli* ATCC 8739 and *E. faecium* NRRL-B2354 showed significantly lower ($p < 0.05$) LCR values than their respective pathogens, (*E. coli* O157:H7 and *S. enterica diarizonae*) at all wavelengths between 259 and 289 nm, suggesting that they have potential for use as non-pathogenic UV indicator organisms (Table 1).

3.3. Inactivation efficacy of the LPM lamp

The inactivation of bacterial strains at an equivalent UV dose of 7 mJ·cm^{-2} using an LPM lamp emitting at 253.7 nm was conducted in order to compare its inactivation efficacy against that of the UV-LED wavelengths (Table 1). The *Salmonella* cocktail showed the highest UV sensitivity among all tested strains at 253.7 nm. Among the individual pathogenic strains, *L. monocytogenes* and *S. enterica diarizonae* showed the highest UV sensitivity at 253.7 nm followed by *E. coli* O157:H7. As with UV-LED treatments, the non-pathogenic strains showed lower UV sensitivity at 253.7 nm compared to the pathogenic strains. In addition, *E. coli* ATCC 8739 was shown to be the least UV sensitive bacterium tested at 253.7 nm.

3.4. Inactivation of E. coli ATCC 8739 at 253.7 and 275 nm

The inactivation of *E. coli* ATCC 8739 was measured at UV doses from 0 to 20 mJ·cm^{-2} using both the LPM lamp at 253.7 nm and the UV-LED unit at 275 nm (Fig. 2). The purpose of this experiment was to determine the rate and character of the *E. coli* inactivation, as opposed to merely the UV sensitivity at a single UV dose. UV treatment at 275 nm resulted in a higher D value in comparison to 253.7 nm, indicating a lower rate of inactivation at 275 nm. Despite this, treatment at 275 nm resulted in a larger LCR at every tested UV dose due to a lack of initial shouldering when compared to 253.7 nm.

3.5. Construction of UV action spectra of pathogenic and non-pathogenic bacteria

The inactivation data between 253.7 and 370 nm was used to construct action spectra for tested pathogenic and non-pathogenic strains (Fig. 3). Overall, the action spectra showed that UV-LED treatment in the range between 259 and 268 nm amounted to equivalent or, in most cases, greater inactivation efficacy than the LPM lamp at 253.7 nm (Fig. 3). *L. monocytogenes* and the *Salmonella* cocktail were the only tested bacteria that did not show a significant difference ($p > 0.05$) in LCR between UV treatment at 253.7 and 259 nm. All tested non-pathogenic strains showed maximal UV sensitivity at both 259 and 268 nm with no significant difference ($p > 0.05$) between these two wavelengths. More variability was observed among the pathogenic strains as UV sensitivity for *L. monocytogenes* peaked at 268 nm while *E. coli* O157:H7 and *S. enterica diarizonae* both peaked at 259 nm. The *Salmonella* cocktail showed maximal and non-significantly different ($p > 0.05$) UV sensitivity between 253.7 and 268 nm. Also, a significant decrease ($p < 0.05$) in efficacy following either 268 or 275 nm UV treatment was shown for all tested bacteria except *S. enterica diarizonae*, which showed the broadest action spectrum among the tested bacteria.

Furthermore, we explored the relationship between the action spectrum of each bacterium and the absorbance of their respective DNA and cell suspensions (Fig. 4). The DNA absorbance spectrum for each bacterium was similar, peaking between 255 and 257 nm. The UV action spectra and DNA absorbance follow the same general trend, peaking in the UV-C region, close to 260 nm, and showing a progressive decrease thereafter. Again, the single exception to this trend is *S. enterica diarizonae*, whose action spectrum in the UV-B region does not correlate to its DNA absorbance profile. Also, the UV absorbance of cell suspensions peaked at a similar wavelength range of 254–258 nm, and decreased steadily as wavelength increased (Fig. 4).

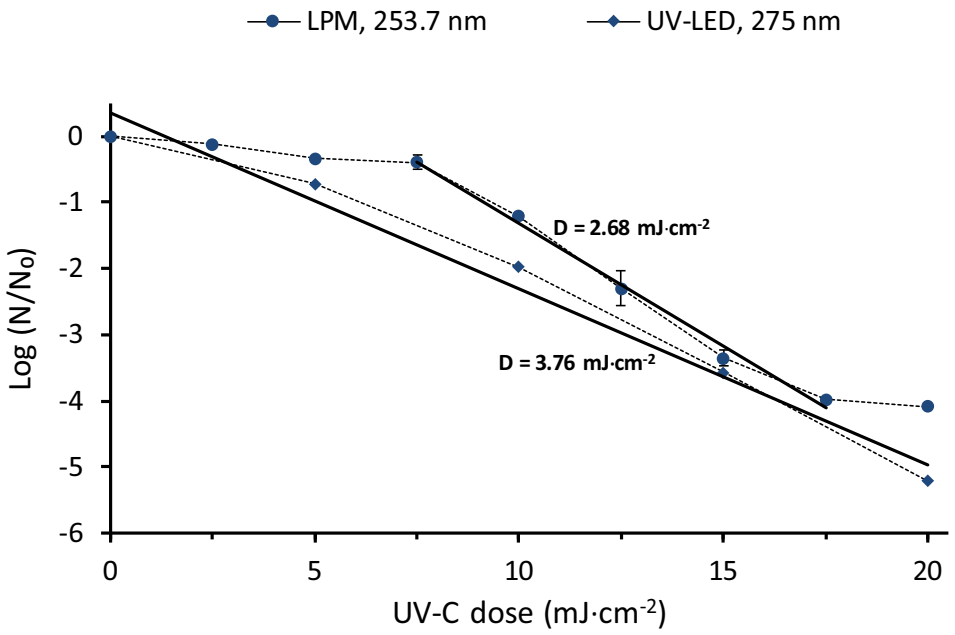

Fig. 2. Inactivation of *E. coli* ATCC 8739 in saline suspension when treated using an LPM lamp at 253.7 nm and UV-LED at 275 nm in comparison to previous results by Bowker, Sain, Shatalov, and Ducoste (2011) using *E. coli* ATCC 11229. Decimal reduction doses (D values) were calculated using the log-linear inactivation phase.

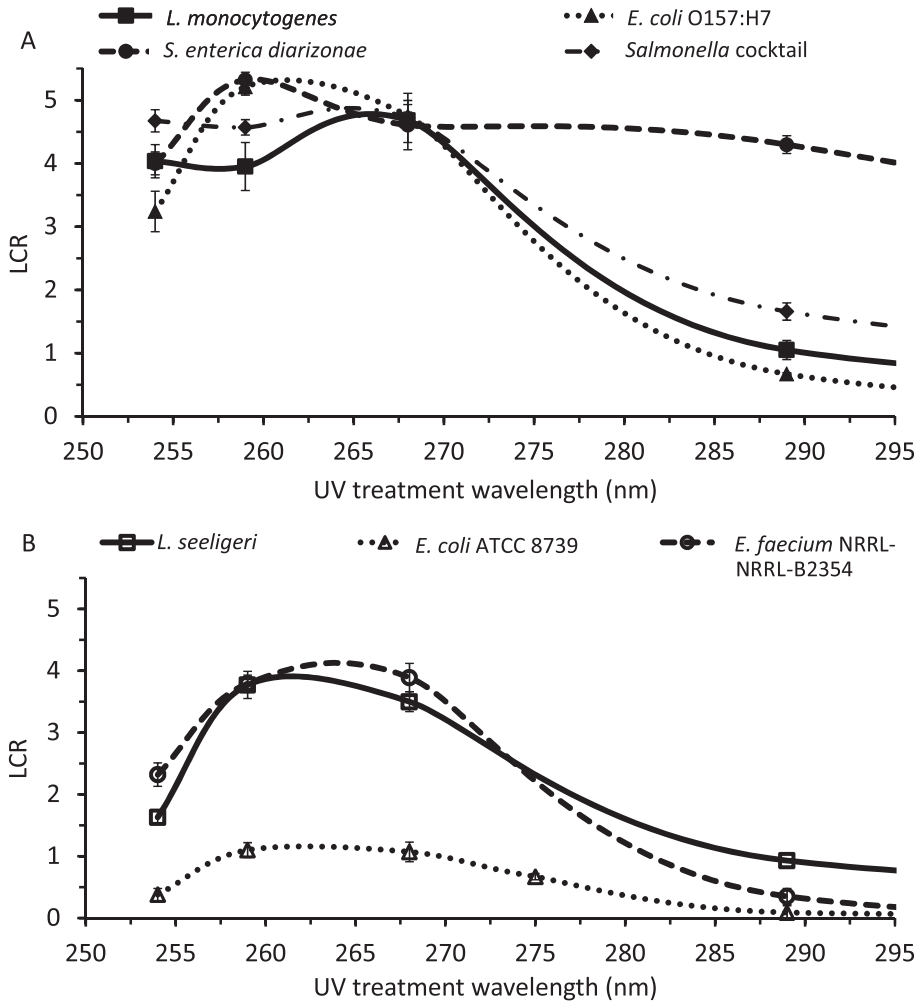

Fig. 3. The UV action spectra of *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica diarizonae* (A); *L. seeligeri*, *E. coli* ATCC 8739, and *E. faecium* NRRL-B2354 (B) following UV treatment in saline suspensions at 253.7 nm using the LPM lamp, at 259, 268, 275, and 289 nm using UV-LEDs.
NOTE: Results at 370 nm were omitted due to negligible LCR.

3.6. Effect of temperature on inactivation of Listeria using a UV-LED

In order to determine the impact of temperature on the effectiveness of UV-LEDs, *L. monocytogenes* and *L. seeligeri* were exposed to a UV dose of 7 mJ·cm^{-2} at 268 nm at both room (25 °C) and refrigeration (4 °C) temperatures. Neither *L. monocytogenes* nor *L. seeligeri* exhibited any significant differences in LCR between UV treatments at 25 °C and 4 °C ($p > 0.05$) (Table 2). However, the treatment times were reduced by 6–7 s because the UV-LEDs exhibited a minor increase in incident irradiance at 4 °C.

3.7. Effect of UV-LED combination wavelengths on bacterial inactivation

Individual pathogenic and non-pathogenic strains were exposed to simultaneous combined UV treatment with wavelength of either 259/289 or 259/370 nm to determine whether a synergistic inactivation effect could be achieved. Following UV treatment of *E. coli* ATCC 8739 with 259/289 nm, an LCR of 2.42 ± 0.31 log was observed, which is significantly greater ($p < 0.05$) than the theoretical additive LCR of 1.20 ± 0.31 log. This also occurred to a lesser extent with *L. seeligeri* where a 4.87 ± 0.07 log reduction was observed following 259/289 nm UV treatment compared to the expected 4.27 ± 0.22 log from the additive effect ($p < 0.05$). No synergistic effect was observed with any other tested bacteria following UV combination treatments with 259/289 nm nor was it observed whatsoever using 259/370 nm.

4. Discussion

The measured UV sensitivity of pathogenic *E. coli*, *Listeria*, and *Salmonella* strains and their non-pathogenic counterparts in the range of 253.7–370 nm was most pronounced at either 259 or 268 nm (Table 1). This is in accordance with several previous findings that have determined action spectra for other bacteria in the approximate UV range between 200 and 300 nm (Bolton, 2017). These results highlight the notion that UV treatment of foodborne pathogens at 253.7 nm is not the most effective option. Given that 259 nm is closer to the bacterial DNA absorbance peak (~260 nm) than 253.7 nm, it is proposed that this 4 nm shift in emission was responsible for the significantly higher observed inactivation, rather than any differences in light emitted by UV-LEDs compared to LPM lamps. As expected, the peak inactivation wavelengths for all tested bacteria aligned fairly well with their respective DNA absorbance profiles. With the exception of *S. enterica diarizonae*, UV sensitivity of all bacteria decreased significantly at wavelengths beyond 268 or 275 nm.

Of particular importance are the observed differences among the action spectra of the tested *E. coli*, *Listeria*, and *Salmonella* strains. Firstly, the overall lower UV sensitivity of tested non-pathogenic strains, including *E. coli* ATCC 8739, *L. seeligeri*, and *E. faecium* NRRL-B2354, in comparison to their pathogenic counterparts suggests that they could potentially serve as surrogates in industrial UV processing experiments. Also, the use of an *E. coli* surrogate with more comparable

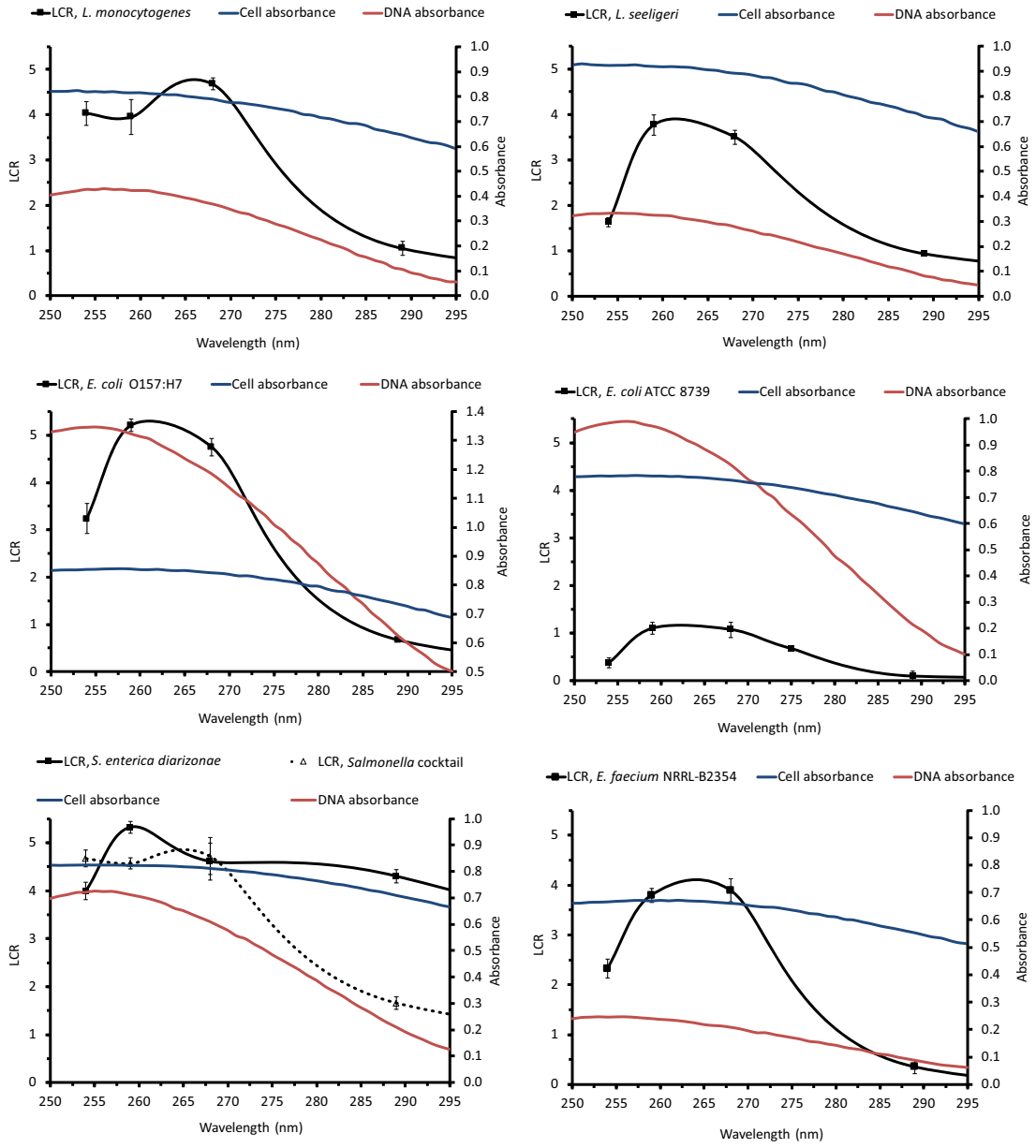

Fig. 4. Action spectrum of pathogenic *L. monocytogenes*, *E. coli* O157:H7, *S. enterica diarizonae* and the *Salmonella* cocktail; and non-pathogenic *L. seeligeri*, *E. coli* ATCC 8739, and *E. faecium* NRRL-B2354 compared to their respective DNA and cellular absorbance spectra.

UV sensitivity should be considered for future applications since *E. coli* ATCC 8739 was far less UV sensitive than *E. coli* O157:H7 at 259 and 268 nm. Further, *L. monocytogenes* and *L. seeligeri* showed significantly different UV sensitivity peaks at 268 and 259 nm, respectively. This demonstrates that even within the same genus bacteria can vary in their UV sensitivity.

We reported a 1.3-fold higher D value in the log-linear inactivation phase of *E. coli* ATCC 8739 following treatment with UV-LED light at 275 nm compared to 253.7 nm. Despite the higher D value, UV treatment at 275 nm showed higher maximal inactivation potential due to the observed shouldering region at 253.7 nm. This is in contrast to a previous study by Bowker et al. (2011), who demonstrated a higher D value at 253.7 nm compared to 275 nm.

Pathogenic *Salmonella* serovars showed the most variable action

Table 2
Comparison of LCR and UV treatment parameters for *L. monocytogenes* and *L. seeligeri* at room (25 °C) and refrigeration (4 °C) temperatures using a 268 nm UV-LED with a UV dose of 7 mJ·cm^{-2}.

Bacterium	25 °C			4 °C		
	E_0 (mW·cm^{-2})	Treatment time (s)	LCR ± SD	E_0 (mW·cm^{-2})	Treatment time (s)	LCR ± SD
L. monocytogenes	0.138	76	4.68 ± 0.13	0.149	70	4.64 ± 0.26
L. seeligeri	0.138	80	3.56 ± 0.16	0.149	73	3.18 ± 0.31

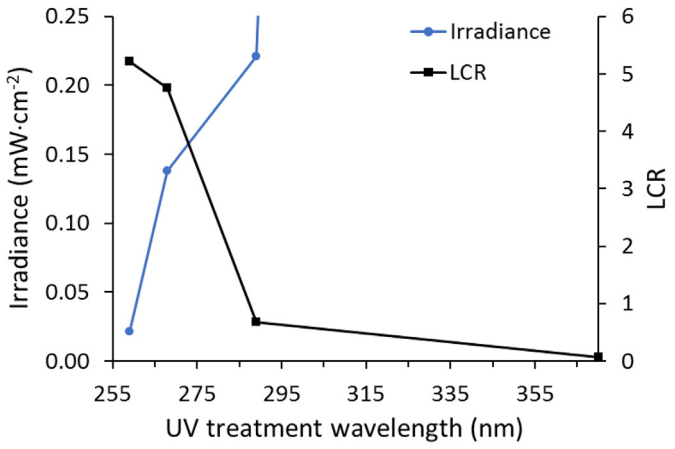

Fig. 5. Relationship between UV treatment wavelength, irradiance, and log count reduction of *E. coli* O157:H7 using UV-LEDs between 259 and 370 nm and a UV dose of 7 mJ·cm^{-2}.

spectra among the tested bacteria. For example, the *Salmonella* cocktail did not show a significant difference in UV sensitivity between treatments at 253.7, 259, and 268 nm. This was not observed with any of the individually tested bacteria. However, it was shown that *S. enterica diarizonae* is significantly more sensitive to UV light at 289 nm compared to all other bacteria including the *Salmonella* cocktail as a whole. This highlighted the variation and broad nature of the *Salmonella* UV dose response among different serovars. This could possibly be due to an alternate mechanism of bacterial inactivation that is not related to DNA damage. Li, Wang, Huo, Lu, and Hu (2017) have examined the effect of UV treatment at different wavelengths (253.7, 265, and 280 nm) on the rate of photo-reactivation and dark repair in *E. coli* CGMCC 1.3373. They reported that there was no difference between these rates at 253.7 and 265 nm; however, UV light at 280 nm caused a significant decrease in both dark repair and photo-reactivation. This indicated that UV inactivation of bacteria at 280 nm was not due to DNA damage. Further, the authors suggested that this inactivation was a consequence of protein damage. The possible differences in the inactivation mechanisms of certain bacteria, such as *S. enterica diarizonae*, using UV-B and UV-C light should be explored in future studies.

The synergistic inactivation following UV treatment at 259/289 nm shown in this study could also be a result of alternative inactivation mechanisms at 289 nm, leading to a hurdle effect. As previously stated, Nakahashi et al. (2014) have found that a combination of UV light at 254 and 365 nm produced a synergistic effect against *V. parahaemolyticus* compared to the pure additive effect of the two individual wavelengths. In contrast, Beck et al. (2017) examined the ability of 260 and 280 nm UV-LEDs to inactivate *E. coli*, MS2, Adenovirus 2 and *B. pumilus* spores, finding no synergistic effect. Similarly, Li et al. (2017) found no synergistic effect between 280 and 265 nm UV-LEDs in the inactivation of *E. coli* CGMCC 1.3373. These mixed results indicated that although synergistic effects can occur in certain situations, they are dependent on the wavelengths used, as well as the microorganism being treated. Future studies should focus on elucidating synergistic effects caused by combination UV treatments.

UV-LED treatment of *Listeria* at refrigeration temperature was more efficient than at room temperature because incident irradiance was increased while bacterial reduction was unaffected, leading to lower treatment times. These results are in agreement with a previous study by Shin, Kim, Kim, and Kang (2016) which reported that the reduction of *L. monocytogenes* was not significantly different at temperatures ranging from 0 to 37 °C.

The trade-off between wavelength, irradiance and inactivation efficacy of UV-LEDs is an important consideration when selecting treatment wavelengths (Fig. 5). For example, the 259 nm UV-LED showed a lower irradiance compared to 268 nm, resulting in longer treatment times to achieve an equivalent UV dose (Table 1), even though the inactivation efficacy at both wavelengths was similar. This means that UV-LED treatment at 268 nm is a better choice than at 259 nm. Inactivation efficacy significantly decreases at wavelengths above 280 nm offsetting any additional increase in irradiance. However, at wavelengths below 280 nm, irradiance and lifetime of UV-LEDs decrease significantly, leading to longer treatment times. The above considerations suggest that treatment at approximately 280 nm could be an optimal choice in the current state of UV-LED development.

5. Conclusions

The advantages of UV-LEDs over LPM lamps have been shown in this study. UV-LEDs in the 259–275 nm range have shown to be either as effective or, in most cases, more effective than LPM lamps emitting at 253.7 nm in the reduction of three common foodborne pathogens at an equivalent UV dose. UV-LEDs at approximately 280 nm were found to be the best choice in terms of bacterial inactivation, life time and irradiance compared to an LPM lamp. The utilization of multiple wavelengths in combination treatments to obtain synergistic germicidal effects against certain microorganisms is possible with UV-LEDs. The action spectra generated for *E. coli*, *Listeria*, and *Salmonella* can be used to optimize UV treatment, improving inactivation efficacy and saving on treatment time and costs.

Conflict of interest

The authors declare no conflict of interest.

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