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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).