Research Paper

Comparison of pulsed light inactivation kinetics and modeling of *Escherichia coli* (ATCC-29055), *Clostridium sporogenes* (ATCC-7955) and *Geobacillus stearothermophilus* (ATCC-10149)

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ABSTRACT

Pulsed light (PL) inactivation kinetics of *Escherichia coli* K-12, *Clostridium sporogenes* and *Geobacillus stearothermophilus* were evaluated under different treatment conditions. The PL system was factory set to operate at three pulses per second with a pulse width of 360 μs exposing samples placed on one of the 9 trays on a rack. Two PL parameters were evaluated in the study: number of pulses (a time factor) and the tray position (a spatial distance factor) both influencing the amount of light energy absorbed. As expected, the level of microbial inactivation increased with an increase in the number of pulses (from 1 to 15) and decreased with an increase in the Spatial distance (Tray # 1 to 9) away from the light source. Both the number of pulses and spatial distance as well as their interactions were found to have a significant effect ($P < 0.05$) on the extent of microbial inactivation. Vegetative cells of *E. coli* were most sensitive to PL treatment with a maximum 5 logarithmic reductions on Tray 1 after a 12-pulse treatment (4 s). *G. stearothermophilus* was more resistant to PL than *C. sporogenes*. Overall, the PL treatments (12–15 pulses) achieved a minimum four logarithmic reductions in the populations of all three microorganisms on the top tray at doses still below 12 J/cm^2, the FDA-approved limit.

1. Introduction

Each year there is an estimated 4.0 million episodes of domestically acquired foodborne illness occurs in Canada. Approximately one in eight Canadians experience an episode of domestically acquired foodborne illness each year. There are several viruses and microorganisms of concern which include Norovirus, *Clostridium perfringens*, *Campylobacter* spp., *E. coli* O15:H7, *Listeria monocytogenes*, and nontyphoidal *Salmonella* spp. as the leading pathogens and account for over 90% of the pathogen-specific total (Thomas et al., 2013). With the growing global consumer demand for safe and high-quality products, new and emerging technologies in food processing are being explored and evaluated for ensuring the safety and integrity of food products. These concepts include novelties in existing processes (like the use of high temperature short time treatment, agitation processing, aseptic processing or thin profile processing for commercially sterile products) as well as novel heating alternatives (like the use of microwave, radio frequency and ohmic heating) or the use of non-thermal processing (such as high pressure and pulsed electric field applications) in different areas of food processing. One such new concept that has been recognized to be effective for surface

microbial decontamination of foods is the pulsed light (PL) or UVC treatment.

PL treatment involves exposing of food contact surfaces to short duration, high power pulses of broad-spectrum light (100–1100 nm), several times per second, typically emitted by a Xenon lamp. This is also true for solid foods like fruits and vegetables, meat fish and poultry, as well as on different food packaging materials like low density polyethylene surfaces. It has been commercially successful for liquid foods like juices, but clarity, turbidity and penetration depth have been recognized as important factors.

Pulsed light treatment has been reported to be more effective than the conventionally used UV light treatment due to the associated greater and instantaneous energy impact. The germicidal action of PL has been attributed to the combination of the rich broad spectrum UV content which is responsible for the formation of lethal thymine dimers within the bacterial DNA, which can block DNA transcription and replication, and ultimately leading to cell death (Wang et al., 2005; Elmnasser et al., 2007; Woodling and Moraru, 2007; Kramer and Muranyi, 2013), and as well as to the localized elevation of temperature due to absorption of UV and IR radiations (which accelerate the UV effects) leading to bacterial

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disruption (Dunn et al., 1995; Wekhof, 2000; Takeshita et al., 2003). The pulsed light treatment has been approved by the US Food and Drug Administration as a processing aid for the decontamination of food and food contact surfaces since 1996 (US FDA, 1996).

Several studies have shown that PL treatment can effectively inactivate different strains of microorganisms on various foods and food contact surfaces, as reported for example, 2-log reduction of *Listeria innocua* on fresh-cut mushrooms (Ramos-Villarroel et al., 2012), 2 to 4.5-log reduction of *E. coli* in apple juice (Sauer and Moraru, 2009; Palgan et al., 2011), 1-log reduction of *Salmonella Typhimurium* on beef and tuna carpaccio (Hierro et al., 2012), and > 7-log reduction of *L. innocua* on low density polyethylene surfaces (Ringus and Moraru, 2013). Some studies have also reported the efficacy of UV (in addition to PL) against a broad spectrum of food-related microorganisms including bacteria (Rowan et al., 1999), some viruses (Lamont et al., 2007; Roberts and Hope, 2003; Eischeid et al., 2009), yeasts, conidia (Gómez-López et al., 2005b), parasites (Hijnen et al., 2006) and bacterial spores (Jun et al., 2003). The effectiveness of PL treatment on the inactivation of several food related or food-poisoning bacteria have been evaluated on agar media surface (Anderson et al., 2000; Gomez-Lopez et al., 2005 a,b; MacGregor et al., 1998; Rowan et al., 1999; Takeshita et al., 2002) as well as in liquid environments, such as water, apple juice, orange juice and milk (Huffman et al., 2000; Palgan et al., 2011; Sauer and Moraru, 2009). While a majority of these studies involve clear liquid products, PL is getting recognized as a useful technique for surface decontamination of solid food particles and food contact surfaces as well.

A number of PL equipment have been developed for the food and pharmaceutical industries such as the PureBright™ system (PurePulse Technologies, Inc., San Diego, CA) for biopharmaceutical manufacturers, the Robotic Pulsed Light Sterilizer (RPLS1) and Robotic Tub Decontamination System RTDS2 uses pulsed light technology from Steriline (Steriline S.r.l., Como, Italy) for the pharmaceutical industry market, Claranor Pulsed Light Sterilization Systems (Claranor, Cedex, France) for diversified food processing applications, SteriPulse™ - XLR Pulsed Light Systems (Xenon Corp., Waltham, MA) of different series (S-, RC-, Z-, X-) for disinfection purposes mainly for food industries. They differ in various factors like radiant energy density per pulse characteristics, pulse rate, pulse width, etc. Hence the results obtained by different studies differ as well. Some PL treatments requires more than 50 flashes to achieve a significant inactivation of *L. monocytogenes* and *E. coli* O157:H7 which others can do with only a few pulses flashes (Rajkovic et al., 2010). Consequently, it is necessary to establish a standardized approach and quantification procedure so results can be compared.

The objective of this work were (a) to determine the inactivation kinetics of three microorganisms in the form of cell/spore suspension (a vegetative bacterial strain *Escherichia coli* K-12, a spore forming mesophilic bacteria, *Clostridium sporogenes* and a spore forming thermophilic bacteria, *Geobacillus stearothermophilus*) with the two important PL process parameters: treatment time (number of pulses) and special distance between the sample and the flash lamp, (b) to evaluate the appropriateness traditional log-linear (first order) and the alternative Weibull models for the inactivation kinetics and (c) finally to compare the effectiveness (accuracy) of the two models for describing the quantitative inactivation of the three microorganisms under the treatment conditions employed.

2. Materials and methods

2.1. Preparation of cell cultures and spore suspensions

Escherichia coli: Escherichia coli K-12 (ATCC-29055), which has been used in earlier studies as a surrogate for *E. coli* O157:H7 in PL treatments (Bialka et al., 2008) was used. The culture was maintained at - 40 °C in 20% glycerol solution for long-term storage. To prepare the inoculum, the culture was grown in 500 mL of tryptic soy broth (Sigma-Aldrich, Difco, MO, USA) for 24 h at 37 °C. The culture was then centrifuged for

15 min at 4000 g and 4 °C and re-suspended in sterile distilled water and centrifuged again. The washing-centrifugation procedure was done three times by discarding the supernatant after each centrifugation and re-suspending the pellet in sterile distilled water. The final inoculum stock solution contained approximately 10^7 CFU/mL bacterial cell population and was stored in the freezer until required for treatment.

Clostridium sporogenes: Clostridium sporogenes (ATCC-7955), an anaerobic and mesophilic spore former with high heat resistance has been traditionally used in thermal processing studies as surrogate for *Clostridium botulinum* because of its non-pathogenic nature and similar growth characteristics For the preparation of *C. sporogenes* spores, a loop full of the stock culture of bacterial cells stored at −40 °C in a 30% (v/v) glycerol solution was inoculated into 10 mL of Reinforced Clostridium Medium (RCM) broth (Thermo Scientific, Oxoid, Hants, UK) and incubated at 37 °C for 24 h under anaerobic conditions. Following this, 200 μL of this culture was spread on Campden Sporulation Agar plates, and then incubated at 37 °C for 7 days under anaerobic conditions. After 7 days, spores were detached from agar plates using sterile distilled water. The suspension was centrifuged for 15 min at 4000 g at 4 °C. The pellet was suspended again in sterile distilled water. This operation was repeated twice. After the last wash, the pellet obtained was re-suspended in sterile distilled water. This final suspension was distributed in to several test tubes and heated at 80 °C for 10 min in order to inactivate any vegetative cells, and stored at 4 °C. The spore suspensions contained approximately 10^6–10^7 CFU/mL.

Geobacillus stearothermophilus: Another microbial spore frequently used in thermal processing studies for validating the more severe thermal treatments is *Geobacillus stearothermophilus* which is a thermophilic bacteria nearly five times more resistant than *C. sporogenes. The spores of Geobacillus stearothermophilus* (ATCC-10149) were used in the study. For the preparation of *G. stearothermophilus* spores, a loop full of the stock culture stored at −40 °C in a 30% (v/v) glycerol solution was inoculated into 10 mL of Tryptone Glucose Yeast (TYG) broth and incubated at 55 °C for 24 h under aerobic conditions. 200 μL of this culture was spread on sporulation agar plates, and then incubated at 55 °C for 7 days under aerobic conditions. After 7 days, spores were detached from agar plates and centrifuged according to the same procedure mentioned above for *C. sporogenes* and the spore suspension was stored at 4 °C after heat shock treatment at 80 °C for 10 min in order to inactivate any vegetative cells. The spore suspensions contained approximately 10^7–10^8 CFU/mL.

2.2. Preparation of sample inoculums

1:10 dilution of microbial suspensions were made using 0.1% sterile peptone water (Oxoid, Difco, Hants, UK). 5 mL each diluted suspension (1:10 dilution; approximately 10^7 CFU/mL) was transferred to 50 mm sterile polystyrene petri dishes (VWR), spread over evenly which results in a thickness of ~2.5 mm of inoculum and subjected to various PL treatments.

2.3. Pulsed light treatment

Pulsed light treatment was given in a R&D Benchtop 3800 V Pulsed-Light System- Steripulse-XL (RS-3000C, Xenon Corp., Wilmington, Mass., U.S.A.). The system was factory calibrated to generate an intensity of 1.27 J/cm^2 per pulse at the distance 1.9 cm from the light source quartz window which is at the top tray position. The pulse rate was also factory set at 3 pulses per second. The pulse fluence depended on the intensity of the pulsed light and the distance from the xenon lamp as indicated by the manufacturer. The lamp produced polychromatic radiation in the wavelength range of 200–1000 nm with both continuous and timed mode of operation. The Steripulse-XL sterilization chamber is equipped with a tray rack with 11 different specific distances from the source as can be seen in Fig. 1, ranging from 1.9 cm to 14.6 cm to place the sample to be PL treated. However, at the distance of 1.9 cm (top location possible), the system could not accommodate the placement of a
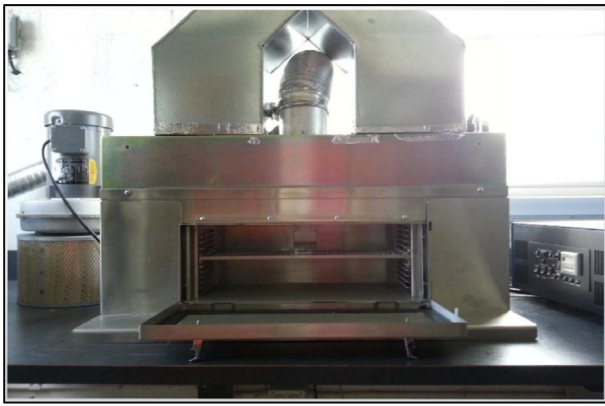
Fig. 1. Steripulse-XL sterilization chamber.

petridish containing the sample. Hence the treatment commenced at the distance of 3.2 cm (labeled Tray 1) followed by alternate distances of 5.7 (Tray 3), 8.3 (Tray 5), 10.8 (Tray 7) and 13.3 cm (Tray 9) to ensure that the treatment of the sample was done at near the top, middle and bottom areas of the chamber. The pulse rate of 3 pulses per second was preset by the factory. The pulse treatment interval (not pulse width) used was therefore 1/3 s (1 pulse) as the minimum to 5 s for the 15 pulses as the maximum. These were used as apparent times for characterization and quantification purposes. However, since the treatment duration (although in milliseconds) was pulse frequency dependent and specific to this unit, they could be converted back to number of pulses by multiplying the accumulated time by 3 for comparative purposes.

In PL treatment, the stress factor on the microorganisms (cell suspension) is the total energy dose ED (J/cm^2) which can be calculated by the modified equation (Luksiene et al., 2013):

$$ED = Ep * t* f \tag{1}$$

where Ep is the energy of one pulse (J/cm^2), t is the treatment duration (s) and f is the pulse frequency (Hz or 1/s). The energy dose varies along the tray distance from the light source and the treatment time (number of pulses) (detailed later).

2.4. Microbial enumeration

After the treatment, the treated suspensions were swirled for few minutes and appropriate serial dilutions ware prepared with 0.1% sterile peptone water (DIFCO) and plated on tryptic soy agar (DIFCO). The

plates were then incubated at conditions appropriate for the test microorganisms (24 h at 37 °C for *E. coli* K-12 and 37 °C for 48 h for *C. sporogenes* under anaerobic conditions, and 55 °C for 24 h under aerobic conditions for *G. stearothermophilus*) and then enumerated.

2.5. Statistical analysis

All experiments were replicated 3 times and SPSS (IBM SPSS Statistics for Windows, Version 22.0) statistical software was used to analyze the variations in mean logarithmic reductions. A full factorial two-way analysis of variance (ANOVA) using the General Linear Model procedure with a 95% confidence level was used to evaluate the significance of the effect of the process parameters: number of pulses (treatment time), special distance of test samples from the flash lamp and their interactions on the microbial inactivation.

2.6. Inactivation modeling

2.6.1. Log-linear model
The log-linear model (D-value model) follows the first order kinetic behavior where it is assumed that the vegetative cells or spores within a population have a similar resistance to applied PL treatment and the number of survivors decrease semi-logarithmically over treatment time. The model is represented as:

$$Log \frac{N_t}{N_0} = \frac{-t}{D} ; (t \geq 0); \ slope = \frac{-1}{D} \tag{2}$$

where N_0 (CFU/mL) is the initial viable count, N_t (CFU/mL) is the number of survivors after exposure to a PL treatment for a specific time t (s) (i.e., 3 × t pulses) and D is the PL treatment time (s) required to destroy 90% of the microbial population, estimated from $\log_{10}$ (N/N$_0$) vs. treatment time (s).

2.6.2. Two parameter Weibull model (van Boekel, 2002)
The Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFiT) (Geeraerd et al., 2005), a freeware add-in for Microsoft Excel, was used to assess non-log-linear microbial survivor curves.

Weibull model is based on the assumption that different fractions of cells or spores in a microbial population may have different resistances to treatment conditions and therefore the survival of their population to a lethal agent represents a cumulative exponential distribution. It is represented as:

$$Log_{10}\left(\frac{N_t}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \tag{3}$$

where N_t (CFU/mL), N_0 (CFU/mL) and t (s) are defined as in the earlier model (1), α is the time required for first decimal reduction (s) and β is a fitting parameter that defines the shape of the curve. In a semi-log plot the Weibull distribution corresponds to a concave upward survivor curve when β < 1, concave downward curve if β > 1, and is linear if β = 1. α is distinguished from the conventional D value, which is derived from the first-order kinetic model and which represents the time of decimal reduction, regardless of the time of heating. The significance of α value is restricted to first decimal reduction of surviving spores or cells from N_0 to $N_0/10$.

The 90% percentile of the failure time distribution is termed the reliable life (t_R) and can be calculated from the parameters α and β (Eq. (3)). In this case the t_R is analogous to the classic D-value when a one $\log_{10}$ reduction is considered (van Boekel, 2002).

$$t_R = \alpha \times (\ln 10)^{\frac{1}{\beta}} \tag{4}$$

Therefore, for a certain number of log reduction, Eq. (4) can be modified as:

$$t_d = \alpha \times \left(-ln\left(10^{-d}\right)\right)^{\frac{1}{\beta}} \tag{5}$$

where d is the number of decimal reductions; hence, d = 5 gives the time required for five log reduction and d = 12 gives a twelve log reduction as in the traditional thermal processes based on the criterion of inactivation (eg., *C. botulinum* spores).

2.7. Model evaluation

The goodness of the fit of the models was assessed using higher regression coefficient (R^2), and lower root mean square error (RMSE).

3. Results and discussion

The treatments given are indicated in time (1–5 s) and/or number of pulses (1–15 pulses) and the position of the Petri dish placed is indicated by the tray numbers (1–9). Tray #1 is the second tray slot and Tray #9 is the 10th tray slot in the treatment chamber. The treatment severity increases with time and decreases with distance away from the light source (increase in designated tray numbers).

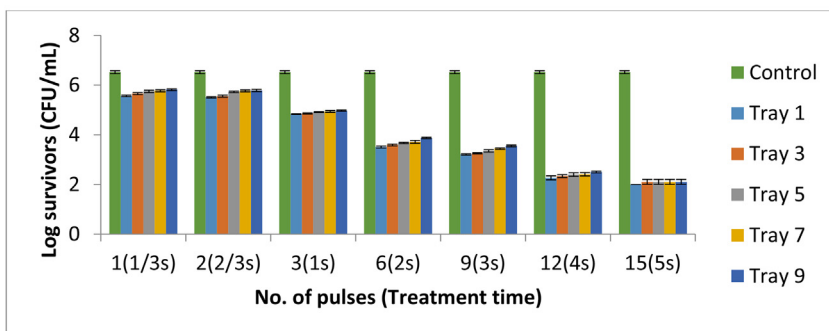### 3.1. Effect of number of pulses (treatment time)

Fig. 2 shows the effect of number of pulses (treatment time) and the distance between the sample and the flash lamp (tray number) on the inactivation of (a) *E. coli* K-12, (b) *C. sporogenes* and c) *G.*
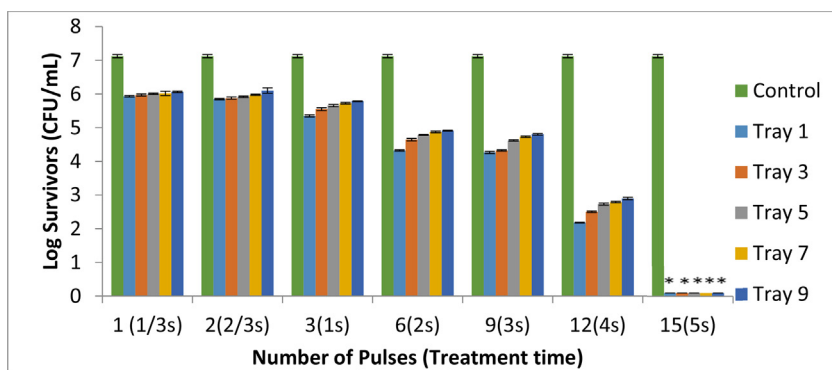
(a) *E. coli* K-12
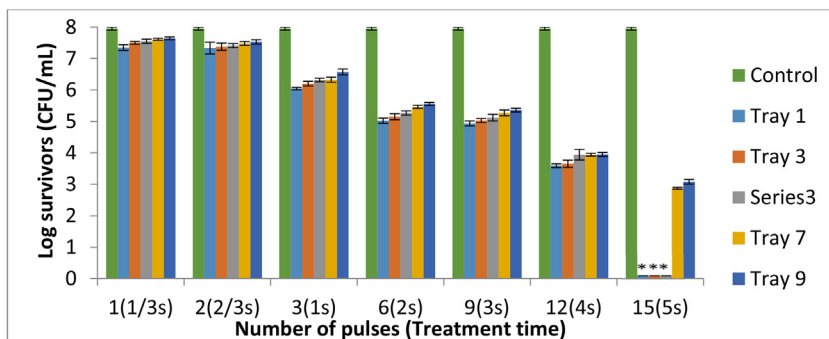
(b) *C. sporogenes*

(c) *G. stearothermophilus*

Fig. 2. Impact of number of pulses (treatment time) and the distance between the sample and the flashlamp in inactivation of (a) *E. coli* K-12, (b) *Clostridium sporogenes* and (c) *Geobacillus stearothermophilus*. *Indicates complete inactivation of the microorganism (no colony observed on the agar plate) after PL treatment on the individual trays.

stearothermophilus. The lethal effect contributed by PL treatment on the different microorganisms was found to be directly proportional to the number of pulses applied and inversely proportional to the sample distance from the flash lamp. This general behavior has also been reported in several other studies (Demirci and Panico, 2008; Gomez-Lopez et al., 2005b; Krishnamurthy et al., 2004; Hillegas and Demirci, 2003; Sharma and Demirci, 2003; Jun et al., 2003; Karaoglan et al., 2017). At least 3-log reduction in microbial population were achieved for all microorganisms studied. A dose level of 12 J/cm^2 has been set as the maximum for PL by the Food and Drug Administration (US FDA, 1996) and the treatments given were within this limit of for all cases except for 12 pulses on Tray #1; 15 pulses on Tray #1 and Tray #3.

Vegetative cells of *E. coli* K-12 were more sensitive to pulse light inactivation as compared to the spores of *C. sporogenes*. and *G. stearothermophilus*. This is in accordance with the early work done by Farkas (2007) where it was stated that bacterial spores require more intense heat, PL, continuous UV or irradiation treatments than vegetative cells. Statistical analysis showed significantly different (P < 0.05) levels of reduction in the number of microbial population after PL treatment with 1–15 pulses. In the case of *E. coli* K-12, the logarithmic reductions reached 5.0 on Tray 1 after treatment with 12 pulses. Further increasing the number of pulses to 15 pulses resulted in lower than the detection limits for the vegetative cells (likely more than 7 logarithmic reductions, since initial concentration was ~10^7 CFU/ml). However, beyond 12 pulses on Tray 1 and 15 pulses on Tray 2, the treatment would exceed the FDA limit for maximum PL exposure. At all tray levels studied, 1 to 2 logarithmic reductions in microbial population were observed with <10 pulse treatments. The spores of *G. stearothermophilus* were more resistant than *C. sporogenes* to PL treatment. The inactivation levels were generally lower as the distance from the light source increased and hence they decreased as the tray numbers increased from 1 to 9. Inactivation of *C. sporogenes* ranged from about 0.62 log$_{10}$ CFU/mL (1 pulse) to no detection levels with increasing the treatment time (12 pulses) on the first tray. Increasing the treatment time to 15 pulses on all but the last two trays also resulted in about 5 log$_{10}$ reduction in spore populations.

3.2. Effect of the spatial distance between the sample and the flash lamp

For any given number of high energy incident light pulses, the microbial inactivation is expected to be higher when the sample is closest to the light source (Ryer, 1997) and this has been clearly demonstrated as shown in Fig. 2. The decreased energy intensity obviously results in lower count reductions. Statistical analysis showed significant differences (P < 0.05) in count reductions in microbial population when different distances (tray numbers) were compared. At 8.3 cm (Tray #5) from the flash lamp, only 2.8 log$_{10}$ reduction was observed in the population of *E. coli* K-12 after a 6-pulse treatment. For similar conditions, Muñoz et al. (2012) and Birmpa et al. (2014) reported 3.6 log$_{10}$ reductions in apple juice and 3.1 log$_{10}$ reductions after 5 s treatment in a liquid matrix, respectively. Similarly, a reduction of 4.5 log$_{10}$ cycles was observed for *G. stearothermophilus* after a 15-pulse treatment on tray 9. This is quantitatively similar to log$_{10}$ reductions of about 6.5 for *G. stearothermophilus* reported by Artíguez and de Marañón (2015). In the case of spores of *Clostridium sporogenes*, no direct comparison of our data could be made with literature since inactivation studies of these spores in liquid medium have not yet been reported.

Further, a general comparison could be made with respect to the relative influence of number of pulses (time effect) vs distance away from the source light (distance effect). Within the framework of the experimental range, the number of pulses varied from 1 to 15 constituting a pulse range of "15" in terms of treatment severity. The microbial inactivation varied from about one log$_{10}$ after one pulse to 5 log$_{10}$ after 15 pulse depending on the type of bacterial source representing a 5 fold increase in inactivation as the treatment increased from one pulse to 15 pulses. Based on the intensity of light (factory calibrated) within the treatment chamber, intensity or flux increased from bottom (Tray 9) to top (Tray 1) by a factor of 3 at any given treatment pulse. The tray-wise

difference in level of bacterial inactivation achieved was generally within one log$_{10}$ cycle at any given treatment pulse level. Time-wise inactivation thus amounted to one log$_{10}$ for every 3 pulses. One log$_{10}$ reduction was also observed tray-wise when the flux was increased by a factor of 3 (tray 9 to tray 1). Hence, the two are somewhat quantitively similar. Within the small test chamber, the space effect was significant probably because of some reflecting or systemic effects. But, the relatively lower tray level variations within the chamber may prove to be an advantage from application standpoint of view because the inactivation effect would then be somewhat more uniform.

3.3. Microbial inactivation curves according to log linear and Weibull model

Figs. 3 and 4 illustrate the survival curves of *E. coli* K-12, *C. sporogenes* and *G. stearothermophilus* fitted to the log linear and Weibull models, respectively. The log-linear model demonstrated a good fit for the data (Fig. 3). The inactivation curves of *E. coli* K-12 and the two spore forming bacteria from the Weibull model (Fig. 4(a), (b) and (c)) demonstrated a slightly upward concavity of survivor plots. Similar trend in the upward concavity of the PL inactivation curves of total aerobic count on different food surfaces has been observed in earlier studies: Iceberg lettuce, white cabbage and cut carrots (Izquier and Gómez-López, 2011) and *Candida inconspicua* isolated from turnip juice Karaoglan et al. (2017).

Till date, the exact mechanisms by which the light pulses cause cellular inactivation are not completely understood. Currently, the most accepted hypothesis is that a combination of a photochemical mechanism (which is contributed by the UV light fraction of the PL spectrum on some constituents of microbial cells) and a photothermal mechanism (which involves heat dissipation by the energy of light pulses resulting in lethal increase in temperature during the treatment) are involved (Takeshita et al., 2003; Wuytack et al., 2003; Cacace and Palmieri and Cacace, 2001). This is commonly also referred to as "multi-hit target theory" (Cheigh et al., 2012, 2013) according to which the shoulders of survivor curves are related to DNA damage and repair phenomena (Jagger, 1967). Microbial DNA repair systems can repair the damage encountered up to certain UV doses (lower doses) which can result in shoulders. Once the maximum DNA repair capability is surpassed, additional higher UV exposure turns out to become lethal for the microorganisms, and lead to exponential decline in the survivors (López-Malo and Palou,2005). The more resistant fraction contributes to any tail in the survivor curve.

3.4. Effect of process parameters on log-linear model parameters

Table 1 summarizes the inactivation kinetic parameter (D value) for the three microorganisms at each spatial (radial) distance from the flash lamp (as denoted by tray numbers, with Tray #1 being the closest and #9 farthest) determined according to conventional log-linear model. D values varied from 0.83 s to 0.997 s for *Escherichia coli K*-12 at different tray levels showing the lowest values on Tray 1 with slightly lower (but statistically significant, P < 0.05) values at farther tray levels. This also supports the earlier observation that the spatial influence is relatively small (with an overall count reduction difference of less than one log$_{10}$ cycle) and hence their relatively lower influence on the inactivation rates. In the case of spores, relatively higher D values of 0.88 s–1.01 s were observed for *Clostridium sporogenes* and further higher values of 0.96 s–1.02 s for *G. stearothermophilus*. Results showed that the spatial distance influence on microbial inactivation was relatively small. On the top tray, statistically significant differences were observed for D values for the three microorganisms: 0.83 s for *E. coli* K-12, 0.88 for *C. sporogenes* and 0.96 for *G. stearothermophilus* in the same order of their thermal resistance (*E. coli* a vegetative bacteria, *C. sporogenes* a mesophilic spore former and *G. stearothermophilus* a thermophilic spore former). In terms of pulses, these would translate to 5 pulses for *E. coli* K-12 and 6 pulses for the other two microorganisms in order to achieve a two logarithmic cycle reduction in their population.
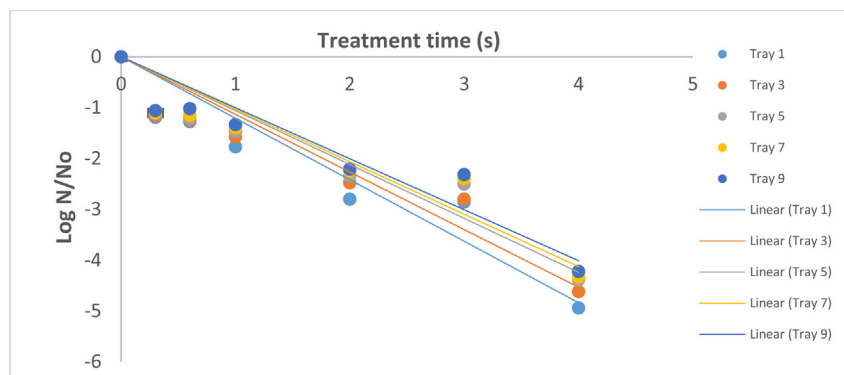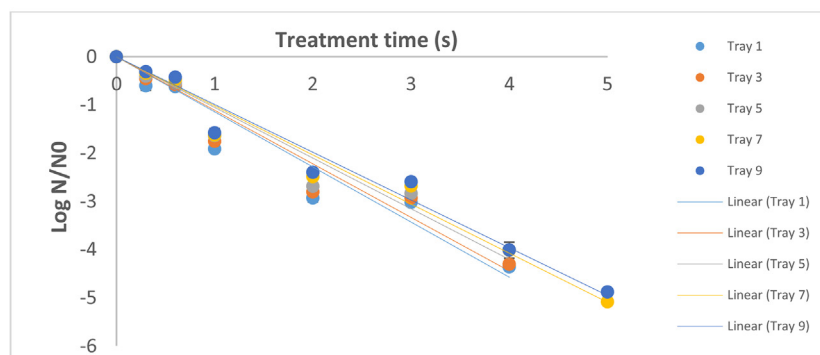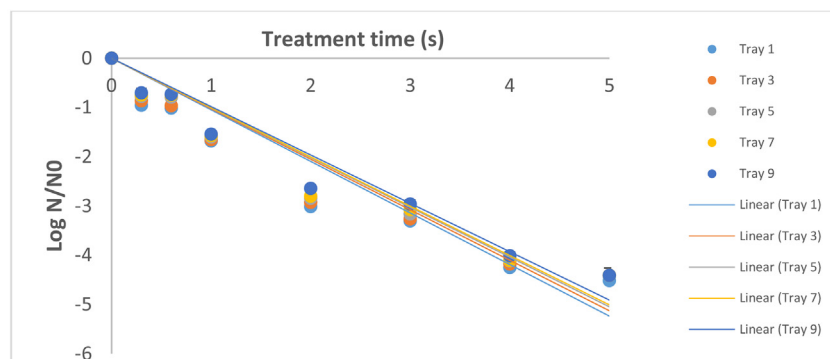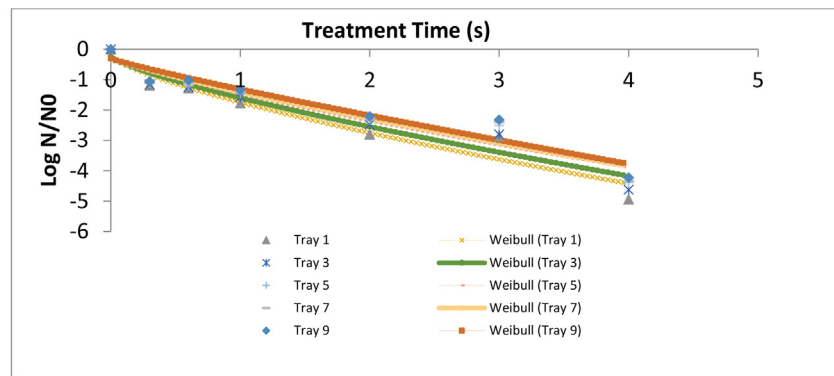
(a) *E. coli* K-12

(b) *C. sporogenes*

(c) *G. stearothermophilus*

Fig. 3. Survivor curves of (a) *Escherichia coli K-12*, (b) *Clostridium sporogenes* and (c) *Geobacillus stearothermophilus* using log-linear model.

Overall, differences were observed in D values both with respect to microbial species and with respect to spatial distance (time effect is already incorporated in the D value). However, the differences observed in D values of different microbial strains at any exposure level were insignificant when compared with their respective large thermal inactivation D values. *E. coli* K-12, a vegetative bacteria, is very sensitive to heat and can be destroyed at temperatures in the 60–100 °C range while the D value of *C. sporogenes* is about 1 min at 121 °C and that of *G. stearothermophilus*, a thermophilic bacteria, is nearly five times higher (~5 min at 121 °C). These extreme differences in thermal and pulsed light D values of different strains arises from the differences in the mechanism of action of microbial inactivation for the two agents (heat and light), and perhaps could be taken as an advantage and used to more effectively kill these thermo-resistant mesophilic and thermophilic spore forming bacteria.

3.5. Effect of process variables on Weibull model parameters

The characteristic time or scale parameter α and the shape parameter β are the paired parameters necessary for the Weibull model. These are detailed in Table 2 for the three microorganisms at each spatial distance from the flash lamp (as denoted by tray numbers, with 1 being the closest and 9 farthest) as determined for the Weibull model. At all the distances studied, the α values of vegetative cells of *E. coli* K-12 were found to be lower than the α values for the sporulating bacteria and the α values of *C. sporogenes* were lower than those of *G. stearothermophilus*. This is consistent with the observations with their thermal D values which show similar trends. The rate scale parameter α in Weibull model corresponds to the rate parameter D value in the first order model, and they generally match when the β parameter is equal to 1. For all the three microorganisms at each distance from the flash lamp, the β values were observed

(a) *E.coli* K-12
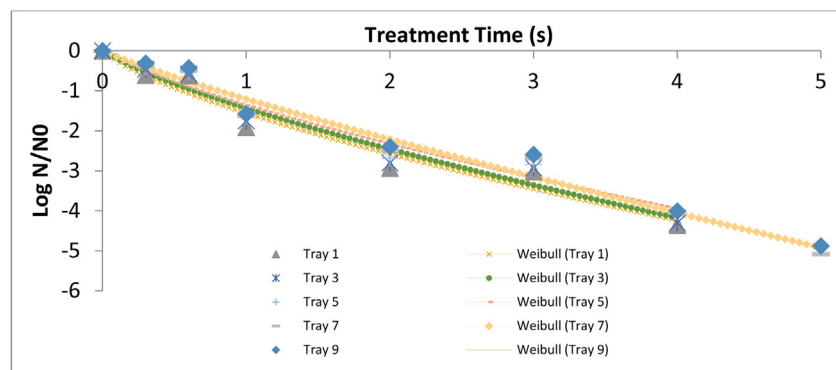
(b) *C. sporogenes*
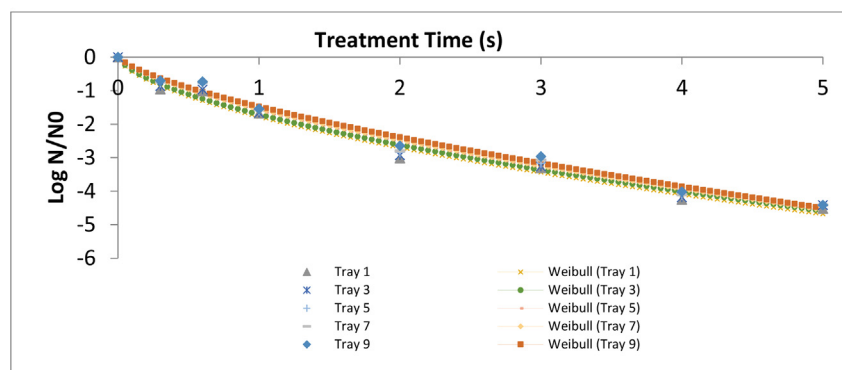
(c) *G. stearothermophilus*

Fig. 4. Survivor curves of (a) Escherichia coli K-12, (b) Clostridium sporogenes and (c) Geobacillus stearothermophilus using Weibull model.

Table 1
D values determined for different trays on basis of log-linear models (a) *Escherichia coli K-12*, (b) *Clostridium sporogenes* and (c) *Geobacillus stearothermophilus*.

Distance from flash lamp	(a) *Escherichia coli K-12* ((s)	(b) *Clostridium sporogenes (s)*	(c) *Geobacillus stearothermophilus (s)*
Tray 1	0.83 ± 0.01[a]	0.88 ± 0.09	0.96 ± 0.01
Tray 3	0.88 ± 0.02	0.90 ± 0.05	0.98 ± 0.02
Tray 5	0.94 ± 0.05	0.95 ± 0.03	0.99 ± 0.03
Tray 7	0.97 ± 0.02	0.98 ± 0.01	1.00 ± 0.01
Tray 9	1.00 ± 0.10	1.01 ± 0.01	1.02 ± 0.02

[a] Standard deviation.

to be < 1 which would be represented by a concave upward inactivation curves for the microorganisms. Karaoglan et al. (2017) also observed upward concavity of survival curves during PL treatment of *Candida*

inconspicua in turnip juice and Bialka et al. (2008) for *E. coli* O157:H7 and *Salmonella enterica* during PL treatment to *E. coli* O157:H7 and *Salmonella* inoculated onto raspberries and strawberries. This trend also shows that there may be a more rapid rate of decline with the initial exposure to PL treatment followed by a subsequent slower rate of inactivation. This behavior has also been observed during the inactivation of *Bacillus licheniformis* spores in carrot juice while studying the combined effects of high pressure, moderate heat and pH by Tola and Ramaswamy (2014) and could result from the assumption that the spore population could have a mixed resistance to thermal/pressure inactivation or the stress adaptability of a sub-population of spores (Peleg and Cole, 1998). Using both the α and β parameters, the reliable life t_R is computed using Eq. (4) and these are also listed in Table 3 for the three microorganisms at each tray level of PL treatment. The t_R values of *E. coli* K-12 (varied from 0.29 – 0.42 s) on different trays and were lower than that determined for both the spores. The t_R values of *G. stearothermophilus* were higher (varied

Table 2

Weibull model parameter α (s), β and calculated t_r (s) values as influenced by treatment time and distance from the flash lamp for the inactivation of (a) *Escherichia coli K-12*, (b) *Clostridium sporogenes* and (c) *Geobacillus stearothermophilus*.

Distance from flash lamp	(a) *Escherichia coli* K-12			(b) Clostridium sporogenes			(c) Geobacillus stearothermophilus		
	α	β	t_r	α	β	t_r	α	β	t_r
Tray 1	0.059 ± 0.04^a	0.53 ± 0.04	0.289 ± 0.04	0.11 ± 0.05	0.62 ± 0.22	0.41 ± 0.18	0.23 ± 0.01	0.81 ± 0.02	0.64 ± 0.04
Tray 3	0.063 ± 0.06	0.52 ± 0.07	0.32 ± 0.06	0.12 ± 0.00	0.64 ± 0.04	0.46 ± 0.01	0.30 ± 0.02	0.90 ± 0.05	0.77 ± 0.07
Tray 5	0.064 ± 0.05	0.50 ± 0.07	0.34 ± 0.04	0.16 ± 0.12	0.69 ± 0.14	0.55 ± 0.21	0.34 ± 0.01	0.93 ± 0.03	0.83 ± 0.05
Tray 7	0.067 ± 0.05	0.50 ± 0.07	0.36 ± 0.05	0.18 ± 0.06	0.71 ± 0.06	0.58 ± 0.12	0.39 ± 0.02	0.97 ± 0.03	0.92 ± 0.06
Tray 9	0.082 ± 0.08	0.51 ± 0.11	0.42 ± 0.07	0.19 ± 0.04	0.72 ± 0.04	0.61 ± 0.09	0.43 ± 0.03	0.10 ± 0.04	0.98 ± 0.08

[a] Standard deviation.

Table 3

Comparison of log-linear and Weibull models for the survivor curves of (a) *Escherichia coli K-12*, (b) *Clostridium sporogenes* and (c) *Geobacillus stearothermophilus* treated at different times (number of pulses) and distance from the flash lamp.

Distance from flash lamp	(a) *Escherichia coli K-12*		(b) *Clostridium sporogenes*		(c) *Geobacillus stearothermophilus*	
	RMSE[a](R^2)[a]		RMSE[a](R^2)[a]		RMSE[a](R^2)[a]	
	Log-Linear	Weibull	Log-Linear	Weibull	Log-Linear	Weibull
Tray 1	0.46 (0.93)	0.51 (0.93)	0.46 (0.96)	0.52 (0.93)	0.27 (0.96)	0.31 (0.96)
Tray 3	0.34 (0.96)	0.55 (0.93)	0.56 (0.94)	0.50 (0.95)	0.42 (0.95)	0.24 (0.97)
Tray 5	0.36 (0.95)	0.62 (0.91)	0.39 (0.97)	0.45 (0.96)	0.44 (0.95)	0.32 (0.96)
Tray 7	0.38 (0.95)	0.71 (0.90)	0.40 (0.96)	0.48 (0.95)	0.36 (0.96)	0.43 (0.95)
Tray 9	0.37 (0.95)	0.76 (0.89)	0.40 (0.96)	0.49 (0.95)	0.25 (0.97)	0.35 (0.96)

[a] Results calculated from average values of three independent replicates.

from 0.64 s–0.98 s) than spores of *C. sporogenes* (varied from 0.41 s–0.61 s) at all distances from the flash lamp studied.

3.6. Model performance comparison through kinetic data

Weibull model scale parameter α represents the first reduction in time (s) that would represent a decimal reduction in the population of surviving microbial population. Like the D value, the scale parameter α slightly increased with an increase in distance between the sample and the flash lamp (increase in tray number) for all the three microorganisms as can be seen in Table 2. As with the D values, the lowest values (more rapid inactivation rate) of α were observed on Tray 1 (closest to the flash lamp) and reached the maximum (slower inactivation) on Tray 9 (farthest from the flash lamp).

From Tables 1 and 2, it can be observed that the reliable life t_R values also increased slightly with an increase in distance between the sample and the flash lamp. The t_R value represent the D value equivalent of Weibull model, for multiple logarithmic reductions. It is the time that gives an effective one decimal reduction in population by taking in to account the shape of the curve. For all the three microorganisms the associated t_R values were found to be lower than the corresponding D values. This trend where the associated t_R values are lower than corresponding D values results from the fact that the survivor curves are slightly concave upwards indicting a slightly more rapid inactivation at the beginning there by giving an effective reduction greater than the straight line representing the D value curve. However, this approach should be viewed with caution because similar trend may not continue and one must not use a multiple of t_R to predict the required number of logarithmic reductions as is commonly practiced with D values. This also might give an incorrect impression that the inactivation rate of spores computed from the Weibull model is higher than those from the log

linear model. This was explained in detail by Tola and Ramaswamy (2014) as to occur mostly when $\beta < 1$ in the Weibull model with a characteristic concave shoulder and the t_R values were overly influenced by the steeper decline in the early phase for the computation of the one log kill. On the other hand, D values are based on the average rate of inactivation over the whole duration. Further, in the log-linear model the extent of inactivation is simply a multiple of D, while with Weibull model it cannot be computed from t_R (both α and β are needed for such computation).

The goodness-of-fit of the models were compared using the R^2 and RMSE values which has been seen in Table 3. The goodness-of-fit of a model increases when the calculated R^2 value approaches 1 and RMSE value is closer to 0. Though log-linear model was slightly better, both models demonstrated good fit for all the studied distances of the sample from the flash lamp.

3.7. Model comparison for 2D, 3D and 4D processes

PL technology is one of the emerging technologies among the different non-thermal processes as an alternative to the traditional thermal treatments. The microorganisms selected for this study involves surrogate microorganisms to represent food-borne pathogens like *E. coli* (*E. coli* K-12) and spore forming bacteria (the mesophilic *C. sporogenes* and thermophilic *G. stearothermophilus*) of concern to the food processing industry due to their ability to form highly resistant spores and their spoilage potential. Hence to determine if the PL treatments performed in this study ensures product safety by achieving a target lethality (equivalent of 2D, 3D or 4D based on a target or reference microbial spore), thermal process calculations can be done for all three microorganisms at all the spatial distances studied. This is done with the help of using D value which is already obtained from the log linear model. The process time required to achieve 2D is equivalent to 2 X D value in the first order model. Similarly, this will be equal to the Weibull model t_{2d} (time required to achieve 2 log reduction using Weibull model) and can be calculated using Eq. (5) with the corresponding α and β values. The times computed in this model represent the duration of PL treatment and not the actual PL exposure time. It is set at 3 pulses per second and hence the number of pulses can be obtained by multiplying the time by 3 and rounded it to the next integer. It should be remembered that the actual PL exposure time is very small, each pulse representing an exposure time of 360 μs (pulse width). Therefore, in the prediction table (Table 4), the corresponding number of pulses is also shown for each of the treatment times by multiplying time by 3 and rounding it. 2D, 3D and 4D values (s) for the log linear model and t_{2d}, t_{3d}, t_{4d} values (s) for the Weibull model along with the corresponding number of pulses are tabulated at each tray level for the three different microorganisms. The predicted times for the 2D-4D reductions were generally close between the first order and Weibull models, except for 4D predictions with *E coli* where somewhat erratic results were found and the required number of pulses with Weibull predictions significantly deviated to much larger exposure levels. The reason for this unusual behavior with Weibull model for higher decimal reductions is not clear, but might have resulted from experimental variations and prediction accuracy of Weibull parameters. In general, however, the required number of pulses were generally

Table 4
2D, 3D, 4D; t_d (d = 2, 3, 4) values in s and corresponding number of pulses (N_p) for (a) *Escherichia coli K-12*, (b) *Clostridium sporogenes* and (c) *Geobacillus stearothermophilus* based on log-linear and Weibull models.

Distance from flash lamp	2D	N_p	t_{d2} (D = 2)	N_p	3D	N_p	t_{d3} (D = 3)	N_p	4D	N_p	t_{d4} (D = 4)	N_p
(a) *Escherichia coli* K-12												
Tray 1	1.65 ± 0.11	5	1.08 ± 0.09	4	2.48 ± 0.28	8	2.33 ± 1.02	8	3.31 ± 0.47	10	4.04 ± 2.30	13
Tray 3	1.76 ± 0.05	6	1.22 ± 0.17	4	2.65 ± 0.14	8	2.70 ± 0.22	9	3.53 ± 0.23	11	4.72 ± 1.23	15
Tray 5	1.89 ± 0.06	6	1.39 ± 0.05	5	2.83 ± 0.10	9	3.14 ± 0.24	10	3.77 ± 0.17	12	5.60 ± 0.03	17
Tray 7	1.94 ± 0.03	6	1.47 ± 0.14	5	2.91 ± 0.03	9	3.32 ± 0.21	10	3.87 ± 0.05	12	5.94 ± 0.18	18
Tray 9	1.99 ± 0.12	6	1.60 ± 0.02	5	2.99 ± 0.03	9	3.52 ± 0.19	11	3.99 ± 0.05	12	6.15 ± 0.22	19
(b) *Clostridium sporogenes*												
Tray 1	1.57 ± 0.04	6	1.27 ± 0.06	4	2.35 ± 0.02	8	2.44 ± 0.02	7	3.14 ± 0.03	11	3.90 ± 0.04	12
Tray 3	1.74 ± 0.10	6	1.35 ± 0.12	4	2.61 ± 0.06	9	2.54 ± 0.02	8	3.48 ± 0.09	11	4.00 ± 0.05	12
Tray 5	1.80 ± 0.15	6	1.49 ± 0.03	4	2.70 ± 0.01	9	2.67 ± 0.01	8	3.60 ± 0.02	12	4.05 ± 0.05	12
Tray 7	1.95 ± 0.02	6	1.53 ± 0.15	5	2.93 ± 0.06	9	2.72 ± 0.02	8	3.91 ± 0.10	12	4.08 ± 0.02	12
Tray 9	2.03 ± 0.11	7	1.61 ± 0.04	5	3.04 ± 0.01	10	2.83 ± 0.01	8	4.05 ± 0.02	13	4.22 ± 0.07	13
(c) *Geobacillus stearothermophilus*												
Tray 1	1.91 ± 0.05	6	1.51 ± 0.08	5	2.87 ± 0.03	9	2.50 ± 0.18	8	3.82 ± 0.04	12	3.57 ± 0.32	11
Tray 3	1.95 ± 0.01	6	1.65 ± 0.11	5	2.93 ± 0.07	9	2.58 ± 0.31	8	3.90 ± 0.11	12	3.55 ± 0.43	11
Tray 5	1.98 ± 0.04	6	1.75 ± 0.02	6	2.97 ± 0.10	9	2.70 ± 0.23	9	3.96 ± 0.17	12	3.68 ± 0.35	12
Tray 7	2.00 ± 0.16	6	1.87 ± 0.06	6	3.00 ± 0.04	9	2.84 ± 0.18	9	4.00 ± 0.07	12	3.83 ± 0.16	12
Tray 9	2.04 ± 0.08	7	1.96 ± 0.15	6	3.05 ± 0.05	10	2.94 ± 0.36	9	4.07 ± 0.09	13	3.92 ± 0.70	12

either same or one pulse more with D value concept as compared to Weibull model, indicating the former to be slightly more conservative. Weibull model is mostly used to describe the PL microbial inactivation kinetics by different researchers, but it is based on two parameters rather than one with the first order approach. The scale and shape parameters, which are determined from regression analysis, deemed to be strongly inter-dependent (van Boekel, 2002) and sometime can lead to significant errors (Tola and Ramaswamy, 2014).

4. Conclusions

Pulsed light treatment can be efficient for the inactivation of a wide range of microorganisms including highly heat resistant species such as spore forming bacteria, inactivation of which is one of the major challenges for the application of non-thermal technologies in a food industry. Two and three pulses of high intensity light were sufficient to cause one logarithmic reduction of *E. coli* K-12 and the spore forming bacteria, respectively. The PL inactivation of microorganisms were dependent on the microbial species, exposure time (number of pulses) as well as the distance from the light source (tray level). The variation in light sensitivity of the microorganisms found in this study for different microorganisms may be due to structural/ compositional differences in the cell walls and membranes due to the presence of a thicker peptidoglycan cell wall in Gram-positive microorganisms like *G. stearothermophilus* and *C. sporogenes* compared to Gram negative organisms like *E. coli* K-12. Both log-linear model and Weibull model demonstrated good fit for the inactivation kinetics and the log-linear model appeared to be slightly more conservative. Several critical parameters are suggested by several researchers which should be considered when designing the experiments to assess the suitability of PL, such as the number of pulses, transparency of the medium, distance from the flash lamp and the depth of the samples. Establishment of standardized treatments and protocols in compliance with the legal requirements for specific food products with specific PL equipment would be required for successful applications of the PL process.

The current study was focused on the application and evaluation of inactivation kinetics of selected microorganisms in liquid media. Such studies are currently being evaluated for surface decontamination of both fruit, vegetable and meat surfaces with both non-pathogenic and pathogenic strains, and will become part of future publications.

Author contribution

Dalia John: Dalia John was responsible for designing the methodology, carrying out experiments, formal investigation and analysis, validation, draft preparation, data curation.

Hosahalli Ramaswamy: Hosahalli Ramaswamy war responsible for conceptualization, supervision, project administration, writing, review and editing and preparation of the manuscript for final submission.

Conflict of interest

The authors declare that they have no known conflict of interest.

Acknowledgements

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