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Application of the 222 nm krypton-chlorine excilamp and 280 nm UVC light-emitting diode for the inactivation of Listeria monocytogenes and Salmonella Typhimurium in water with various turbidities

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ABSTRACT

Applicability of the 222 nm krypton-chlorine (KrCl) excilamp and 280 nm UVC light-emitting diode (LED-UVC) for water disinfection was identified in this study. *Listeria monocytogenes* and *Salmonella* Typhimurium were inactivated by the KrCl excilamp and LED-UVC treatments in water samples with varying turbidities. Inactivation curves were analyzed using the log linear model, and the derived inactivation rate constant (k) was approached as a function of turbidity using the exponential one-phase decay model. The integrated inactivation model was verified with turbidities other than those used at the experimental levels. Water disinfection efficacy by both the KrCl excilamp and LED-UVC treatments decreased with increasing turbidity, and the developed model predicted well the inactivation levels of both pathogens depending on the type treatment device, treatment dose, and sample turbidity. When applied to oyster or flatfish spindled samples, the LED-UVC showed higher pathogen inactivation efficacy in flatfish than oysters, as expected due to the lower turbidity of the flatfish spindled samples, but opposite results were observed with the KrCl excilamp. This result indicates that factors other than turbidity such as type of food and pathogen also should be considered. In this regard, further study is needed to identify the bactericidal mechanisms of these alternative UV-C irradiation technologies in water and food products.

1. Introduction

Ultraviolet (UV) irradiation has been widely used for pathogen inactivation. Although the mercury lamp has been a common source of UV-C light to date, the regulation of mercury use is becoming stricter. For example, many countries have signed the Minamata Convention, which aimed to reduce mercury use in many products (Matafonova & Batoev, 2018). Therefore, many researchers have interest in alternative light sources, such as the excimer lamp and light emitting diode (LED). The wavelengths of the excilamp vary depending on the type of rare gas and halogen used inside of the lamp. A typical example is the krypton chlorine (KrCl) excilamp that emits 222 nm UV-C light, and studies on pathogen inactivation with the KrCl excilamp have been reported recently. Ha, Lee, and Kang (2017) identified that the bactericidal effect of the KrCl excilamp was more significant than the conventional mercury lamp. Pathogen inactivation mechanism by KrCl excilamp was investigated by Kang, Kim, and Kang (2018), who identified that KrCl excilamp damage not only DNA but also cell membrane. The applicability of LED-UVC to inactivate bacteria, yeast, and viruses was also investigated recently (Beck et al., 2017; Kim & Kang, 2018; Kim, Kim, & Kang, 2017a, 2017b). However, many of these studies were conducted using one UV-C source, and comparative studies between the 222 nm KrCl excilamp and 280 nm LED-UVC are limited.

Seafood-borne illnesses, which are a major public health problem, have been continuously reported and have increased in recent decades worldwide (Elbashir et al., 2018). Among the hazards of seafood, researchers have not only focused on norovirus, *Vibrio parahaemolyticus*, *V. cholera*, and *V. vulnificus* (Bonnin-Jusserand et al., 2017; Hassard et al., 2017) but have also acknowledged the risk of *Salmonella* and *Listeria* spp. contamination in seafood (Feldhusen, 2000). Amagliani, Brandi, and Schiavano (2012) indicated that *Salmonella* has caused seafood-related outbreaks worldwide. The outbreaks are attributed to

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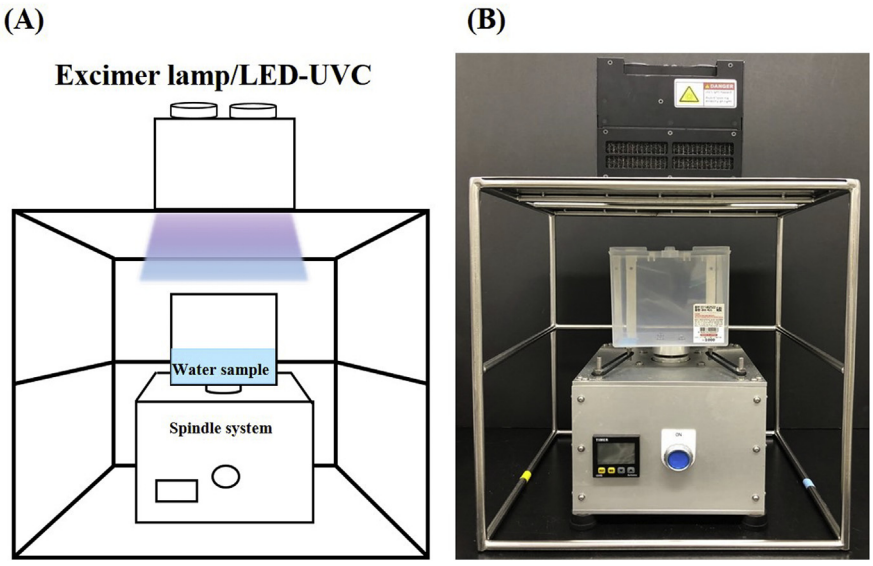

Fig. 1. Schematic diagram (A) and picture (B) of the UV-C spindle combination system at Seoul National University.

the survival ability of *Salmonella* under frozen, dried and high-salt conditions. Feldhusen (2000) indicated that 4.40% of 2453 and 14.84% of 1321 investigated samples were positive for *Listeria monocytogenes,* a psychrotrophic pathogen that can grow at refrigerator temperature (4 °C). Therefore, it is crucial to inactivate *Salmonella* and *Listeria* spp. in seafood contaminated naturally from polluted water or cross-contaminated during the processing stage.

One of major applications of UV-C irradiation application is water disinfection. The inactivation trend by UV-C irradiation in water usually follows first-order kinetics, from which the inactivation rate constant (k) is calculated. The inactivation rate constant (k) not only can be used to compare the susceptibility of a pathogen to UV-C irradiation (D.-Kim & Kang, 2018) but can also be used to analyze the effect of intrinsic or extrinsic factors on the inactivation of foodborne pathogens. There are many factors affecting the inactivation of pathogens by UV-C irradiation, but turbidity is one of the most important intrinsic factors affecting the inactivation efficacy in water. When we applied alternative UV-C irradiation, such as the KrCl excilamp and LED-UVC, simultaneously with the spindle system, which is used for pathogen detachment and inactivation, the turbidity of water can be varied depending on the seafood samples. For example, oysters can make water significantly turbid, while flatfish make water less turbid. Therefore, we investigated the effect of turbidity on the inactivation of pathogens by combination treatment of the spindle system with the 222 nm KrCl excilamp or 280 nm LED-UVC. Mathematical analysis was used to comparethe pathogen inactivation efficacy of the KrCl excilamp and LED-UVC. Oysters and flatfish, which are classified as the highest risk category by Huss, Reilly, and Embarek (2000), were used for food application.

2. Materials and methods

2.1. Bacterial cell suspension preparation

Three strains each of *L. monocytogenes* (ATCC 15313, ATCC 19111, and ATCC 19115) and *S.* Typhimurium (DT 104, ATCC 19585, and ATCC 43971) were obtained from Seoul National University (Seoul, Republic of Korea). Stock and working cultures were prepared as previously reported (S. S. Kim & Kang, 2015). A single colony cultivated on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37 °C for 24 h, and collected by centrifugation at 10 000 × g for 10 min at 4 °C. The final pellets were resuspended in 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). Thereafter, the suspended pellets of the two pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *L. monocytogenes* (10^9 CFU/ml) and *S.* Typhimurium (10^9 CFU/ml).

2.2. Sample preparation and inoculation

Distilled water samples, the turbidity of which was adjusted to 0, 40, 80, and 120 nephelometric turbidity units (NTU) and sterilized, were used in this experiment. The turbidities of samples were adjusted with bentonite (Sigma-Aldrich, St. Louis, MO) and were measured using a turbidity meter (TU-2016, Lutron Electronic, Taiwan). Spindle-UV combination system, developed by Kang and Kang (2019), was used to inactivate detached pathogens from food samples. The spindle system consisted of an electric motor, a timer and a tray to contain the water sample. The drive motor vibrated the tray connected to the motor, and the speed of the motor was 7000 × g. The sample tray of the spindle system was covered with a sterile plastic bag, which was filled with 250 ml of turbidity-adjusted water sample. A mixed of cultured cocktail (1 ml) was inoculated into the sample before treatment and homogenized for more than 1 min with the spindle system.

2.3. Bactericidal treatments

Combination treatments of alternative UV-C irradiation and the spindle system were used to inactivate foodborne pathogens in the prepared water sample. A schematic diagram (A) and picture (B) of the combination treatment are shown in Fig. 1. A 222 nm KrCl excimer lamp, of which wavelength spectrum was identified in a previous study (Ha et al., 2017), was used for excilamp combination. The irradiation intensity of the LED-UVC and KrCl excilamp was evaluated as described previously (Kim, Kim, & Kang, 2016; Shin, Kim, Kim, & Kang, 2016). The distance between the lamp and the bottom of water sample was 19 cm. The irradiation intensity of 7 points in the tray area was recorded using a UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands). Petri factor, which indicates the intensity variation across the sample area, was calculated by dividing the measured intensities with the maximum intensity and average the divided values. The modified intensity was obtained by multiplying the Petri factor and maximum intensity so that the actual intensity values were normalized. The modified radiation intensity of the excilamp at the sample location was 1210 μW/cm^2. For LED-UVC combination

treatment, 14 100-mW LEDs (280 nm; LG Innotek Co., Republic of Korea) were used. The modified radiation intensity of the LEDs was 434 μW/cm^2 at each sample location. These intensity values were utilized to acquire the treatment time when the dosage values of the KrCl excilamp and LED-UVC were 0–50 and 0–25 mJ/cm^2, respectively.

2.4. Bacterial enumeration

For microbial enumeration, homogenized 1-ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone water, and 0.1 ml of stomached or diluted samples was spread plated onto each selective medium. Oxford agar base (OAB; Difco) with antimicrobial supplement (Bacto Oxford antimicrobial supplement; Difco) and xylose lysine deoxycholate (XLD) agar (Difco) were used as selective media for *L. monocytogenes* and *S.* Typhimurium, respectively. All plates were incubated at 37 °C for 24–48 h before counting colonies characteristic of the pathogens.

2.5. Mathematical modeling

The survival curves of *L. monocytogenes* and *S.* Typhimurium were analyzed using GInaFit, a freeware tool used to assess microbial survivor curves (Geeraerd, Valdramidis, & Van Impe, 2005). The survival curves were fitted well with log linear model (Eq. (1)), and the inactivation rate (k) was calculated (Eq. (2)).

$$\log_{10}(N) = \log_{10}(N_0) - \frac{k_{max}}{\ln(10)}d \quad (1)$$

$$k = \frac{k_{max}}{\ln(10)} \quad (2)$$

where N (CFU/ml) is the population of the microorganisms, N_o (CFU/ml) is the initial population, k_{max} is the first-order inactivation constant, and d (mJ/cm^2) is the treatment dose.

The derived inactivation rate (k) was approached as a function of turbidity using GraphPad PRISM (GraphPad Software, Inc., San Diego, CA, USA). The exponential one-phase decay model (Eq. (3)) was used to fit the relationship between the inactivation rate and sample turbidity. The developed function was combined with the log linear model to predict pathogen inactivation as a function of the treatment dose and turbidity.

$$k = (k_0 - \text{plateau})*\exp(-a*T_b) + \text{plateau} \quad (3)$$

where k is the inactivation rate, T_b (NTU) is the turbidity of the sample, k_o is the inactivation rate at 0 NTU turbidity, a is the rate constant, and plateau is the k value at infinite turbidity.

From these results above, the following equations predicting pathogen inactivation as a function of the treatment dose (d) and water turbidity (T_b) were deduced.

For *S.* Typhimurium with the KrCl excilamp,

$$\log_{10}(N) = \log_{10}(N_0) - \{0.4246\cdot\exp(-0.04236*T_b) + 0.09510\}d$$

For L. *monocytogenes* with the KrCl excilamp,

$$\log_{10}(N) = \log_{10}(N_0) - \{0.2130\cdot\exp(-0.02418*T_b) + 0.06588\}d$$

For *S.* Typhimurium with LED-UV treatment,

$$\log_{10}(N) = \log_{10}(N_0) - \{0.5401\cdot\exp(-0.02020*T_b) + 0.23370\}d$$

For L. *monocytogenes* with LED-UV treatment,

$$\log_{10}(N) = \log_{10}(N_0) - \{0.4320\cdot\exp(-0.04190*T_b) + 0.25720\}d$$

where N (CFU/ml) is the population of the microorganisms, N_o (CFU/ml) is the initial population, T_b (NTU) is the water turbidity, and d (mJ/cm^2) is the treatment dose.

2.6. Validation of the predictive model

Turbidities other than those used in the experimental levels—20, 60, and 100 NTU—were used to determine whether the developed model could predict the survival of pathogens. These turbidities were within the range used previously to develop the model. The populations of the pathogens obtained by the developed model were compared with those values experimentally enumerated on selective media. The accuracy factor (A_f) and bias factor (B_f) were used for validation, which are calculated by following equations (Ross, 1996)

$$A_f = 10^{\frac{\sum |\log(predicted/observed))|}{n}} \quad (4)$$

$$B_f = 10^{\frac{\sum \log(predicted/observed)}{n}} \quad (5)$$

where n is the number of observations, A_f represents how absolutely close, on average, the predictions are to the observations. The larger the value is, the less accurate the average estimate is. B_f indicates by how much, on average, a model overpredicts or underpredicts the observed data. Perfect agreement between predictions and observations will lead to an accuracy factor and a bias factor of 1.

2.7. Application for food samples

Oysters and flatfish were purchased at a local market (Seoul, Republic of Korea) for food application. A mixed cultured cocktail (9 ml) prepared as that in the water disinfection experiment was combined with 291 ml of DW to make a bacterial solution. The oysters and flat fish were inoculated with the bacterial solution by the dipping method for 5 min. After drying with fan for 30 min, the inoculated samples were subjected to the combination treatment of the spindle system with the excilamp or LED-UVC. During the treatments, 3 ml of sample was collected, and the bacterial enumeration experiment was conducted. The inactivation trend was analyzed by using the Weibull model, and D_3d values were calculated. The equation for the Weibull model and D_3d is as follows:

$$\log_{10}(N) = \log_{10}(N_0) - \mathrm{x}\cdot\left(\frac{t}{D_x d}\right)^p \quad (6)$$

where N (CFU/g) is the population of the microorganisms, N_o is the initial population, p is the parameter related to the scale and shape of the survival curve, x is the number of decimal reductions, and $D_x d$ is the dose (mJ/cm^2) required to achieve a x-log reduction (22). The Weibull distribution corresponds to a concave downward survival curve if $p > 1$ and upward if $p < 1$.

2.8. Statistical analysis

All experiments were replicated three times. The data were analyzed by the analysis of variance (ANOVA) procedure of the Statistical Analysis System (version 9.3, SAS Institute, Cary, NC), and mean values were separated using Duncan's multiple-range test. Significant differences were determined at a significance level of $p = 0.05$.

3. Results and discussion

3.1. Effect of turbidity on the pathogen inactivation by the KrCl excilamp or LED-UVC treatments

Reduction levels of *S.* Typhimurium and L. *monocytogenes* by the KrCl excilamp decreased with increasing turbidity (Table 1). It was easily predicted because suspended solids can protect the pathogens from UV radiation. In the present study, the survived populations of *S.* Typhimurium (log CFU/ml) after 20 mJ/cm^2 of the KrCl excilamp were 0.00, 2.94, 3.55, and 4.24 in the 0, 40, 80, and 120 NTU water samples, respectively. The same trend was observed for *L. monocytogenes* with a

Table 1
Populations (log CFU/ml) of *S*. Typhimurium (*S*) and *L. monocytogenes* (*L*) in distilled water with various turbidities subjected to the 222 nm krypton-chlorine excilamp[a].

	Turbidity (NTU)	Dose (mJ/cm^2)					
		0	10	20	30	40	50
S	0	5.37 ± 0.70 A	0.20 ± 0.35 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A
	40	6.13 ± 0.04 A	3.94 ± 0.75 B	2.94 ± 0.28 B	0.69 ± 1.20 A	0.00 ± 0.00 A	0.00 ± 0.00 A
	80	6.01 ± 0.59 A	5.32 ± 0.21 C	3.55 ± 0.61 BCE	2.91 ± 0.67 B	0.68 ± 0.59 A	0.57 ± 0.99 AB
	120	6.26 ± 0.06 A	5.41 ± 0.22 C	4.25 ± 0.47 C	3.24 ± 0.47 B	2.99 ± 0.79 B	1.48 ± 0.81 B
L	0	6.98 ± 0.13 A	4.19 ± 0.76 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A
	40	6.87 ± 0.09 A	6.50 ± 0.15 B	4.87 ± 0.39 B	2.63 ± 0.45 B	0.00 ± 0.00 A	0.00 ± 0.00 A
	80	6.80 ± 0.23 A	6.31 ± 0.63 B	5.71 ± 0.29 C	5.45 ± 0.13 C	3.72 ± 0.66 B	1.02 ± 0.89 B
	120	6.97 ± 0.05 A	6.51 ± 0.42 B	6.42 ± 0.12 D	5.65 ± 0.66 C	4.49 ± 0.60 B	3.34 ± 0.21 C

Mean values ± standard deviation.
[a] Values in the same column followed by the same upper-case letter are not significantly different for each pathogen ($p > 0.05$).

Table 2
Populations (log CFU/ml) of *S*. Typhimurium (*S*) and *L. monocytogenes* (*L*) in distilled water with various turbidities subjected to LED-UV treatment[a].

	Turbidity (NTU)	Dose (mJ/cm^2)					
		0	5	10	15	20	25
S	0	6.48 ± 0.29 A	2.58 ± 0.38 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A
	40	6.46 ± 0.25 A	4.74 ± 0.47 B	2.02 ± 0.62 B	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A
	80	6.34 ± 0.27 A	5.19 ± 0.57 B	3.27 ± 0.35 C	0.30 ± 0.52 A	0.00 ± 0.00 A	0.00 ± 0.00 A
	120	6.44 ± 0.08 A	5.56 ± 0.50 B	4.38 ± 0.53 D	2.69 ± 0.35 B	0.00 ± 0.00 A	0.00 ± 0.00 A
L	0	6.92 ± 0.22 A	5.39 ± 0.20 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A
	40	6.90 ± 0.11 A	6.65 ± 0.19 B	5.22 ± 0.49 B	2.75 ± 0.55 B	0.00 ± 0.00 A	0.00 ± 0.00 A
	80	6.97 ± 0.10 A	6.68 ± 0.40 B	5.94 ± 0.18 C	4.07 ± 0.35 C	1.48 ± 0.21 B	0.00 ± 0.00 A
	120	7.08 ± 0.24 A	6.77 ± 0.24 B	6.45 ± 0.20 C	5.22 ± 0.42 D	3.59 ± 0.49 C	1.03 ± 0.30 B

Mean values ± standard deviation.
[a] Values in the same column followed by the same upper-case letter are not significantly different for each pathogen ($p > 0.05$).

higher resistance than *S*. Typhimurium. A similar result was reported by Ha and Kang (2018), indicating that the resistance of *L. monocytogenes* is higher than *S*. Typhimurium in water with an initial population of 10^6-10^7, but the comparative resistance was different with a lower initial population. In this regard, the treatment condition of the KrCl excilamp for water disinfection should be decided carefully considering the type of pathogen and initial concentration of the bacterial pathogen. The light source of UV-C irradiation also affects the bactericidal efficacy. The levels of pathogen inactivation were more significant by LED-UV treatment than by the KrCl excilamp. For example, the survived populations (log CFU/ml) of *S*. Typhimurium after 10-mJ/cm^2 LED-UV treatment were 0.00, 2.02, 3.27, and 4.38 in the 0, 40, 80, and 120 NTU water samples, respectively, which were similar to those after 30-mJ/cm^2 KrCl excilamp treatment (Table 2). The different bactericidal efficacies between the KrCl excilamp and LED-UVC could be attributed to the different wavelengths. The penetration depth of UV is known to be a function of wavelength for a given material. The penetration depth of LED-UVC might be higher than that of the KrCl excilamp because the wavelength of LED-UVC (280 nm) is higher than that of KrCl excilamp (222 nm) In this regard, pathogens located at the lower part of the treatment chamber would be treated by LED-UVC while only pathogens located at the higher part of chamber could be treated by the KrCl excilamp. Additionally, different bactericidal mechanisms between the KrCl excilamp and LED-UVC might result in the different bactericidal efficacies. The primary target of the 222 nm KrCl excilamp is damage to the cellular membranes and intracellular enzyme rather than DNA (Ha et al., 2017) while that of LED-UVC is DNA damage rather than membrane damage (D.-K. Kim et al., 2017a). However, cell membrane damages by KrCl excilamp were not significantly different with those by LED-UVC (Table S1) in the present study when compared by propidium iodide (PI) uptake test according to the previously reported method (Park & Kang, 2013). In this regard, further study is needed to identify DNA, RNA and enzymes damages of the pathogen by KrCl excilamp and LED-UVC.

3.2. Modeling the effect of sample turbidity on pathogen inactivation by the KrCl excilamp or LED-UV treatment

From the inactivation curves, it was determined that increased turbidity decreased the disinfection efficacy of alternative UV-C irradiation treatments, but it remains unclear how to control the UV-C dose numerically with increasing turbidity. In this regard, inactivation curves were analyzed mathematically. The inactivation rate constant (k) is a widely used parameter to describe pathogen inactivation by UV-C irradiation and is also used to compare the resistance of pathogens by UV-C irradiation. For example, the inactivation rate constants of viruses are significantly lower than those of bacteria, which means higher resistance to UV-C irradiation. The inactivation rate constant (k) was calculated by the log linear model in the present study, and it was carefully decided to include the data of the detection limit. In some cases, including the detection limit makes the inactivation trend abnormal, but this method can make the inactivation trend more precise in other cases. Further study is needed to identify how to evaluate the detection limit values for inactivation trend analysis. The parameters, calculated by the log linear model in the present study (Table 3), decreased exponentially as the turbidity increased, regardless of the type of pathogen and treatment device (Fig. 2). For example, the inactivation rates (k) of *S*. Typhimurium subjected to the KrCl excilamp were 0.52, 0.17, 0.12, and 0.09 for 0, 40, 80, and 120 NTU water samples, respectively. The inactivation rates of *L. monocytogenes* by the KrCl excilamp were 0.28, 0.14, 0.11, and 0.07 for 0, 40, 80, and 120 NTU water samples, respectively. The same trends were observed for LED-

Table 3
Inactivation rate (k) of *S*. Typhimurium (S) and *L. monocytogenes* (L) subjected to the 222 nm excilamp and LED-UV treatments at each turbidity.

	Turbidity	Excilamp	LED UV
S	0	0.52	0.78
	40	0.17	0.44
	80	0.12	0.40
	120	0.09	0.25
L	0	0.28	0.69
	40	0.14	0.33
	80	0.11	0.30
	120	0.07	0.24

Table 4
Parameters of the exponential one-phase decay model for inactivation of *S*. Typhimurium (*S*) and *L. monocytogenes* (*L*) subjected to the 222 nm excilamp and LED-UV treatments at each turbidity.

		k_0	Plateau	a	R^2
Excilamp	*S*	0.5197	0.0951	0.0424	0.99
	L	0.2789	0.0659	0.0242	0.99
LED UV	*S*	0.7738	0.2337	0.0202	0.96
	L	0.6893	0.2572	0.0419	0.99

UVC treatments. When the relationship between the sample turbidity and inactivation rate (k) was analyzed by several mathematical models, it fit well with the one-phase exponential decay model (Table 4).

From the developed model, we can adjust the treatment time of the KrCl excilamp and LED-UVC. If the KrCl excilamp was used to inactivate *S*. Typhimurium in water, the treatment time should be adjusted to be 5.13 times longer when the turbidity changes from 0 to 100 NTU. On the other hand, the treatment time can be adjusted to 2.53 times longer for LED-UVC treatment under the same situation. In this regard, the developed model can be applied to adjust the processing conditions considering the sample turbidity, type of treatment and pathogens. Because the conducting experiments under each treatment condition is very time consuming and much labor is needed, we can reduce the effort by using mathematical modeling.

3.3. Validation of the developed model to predict pathogen inactivation by the KrCl excilamp or LED-UV treatment

The developed model was validated with water turbidities that are not used in the previous experiments. The predicted values of *S*. Typhimurium by using the KrCl excilamp were slightly higher than the experimental results, producing 1.09–1.33 A_f values and 1.07–1.33 B_f values. On the other hand, the predicted values of *L. monocytogenes* by using the KrCl excilamp were slightly lower than the experimental

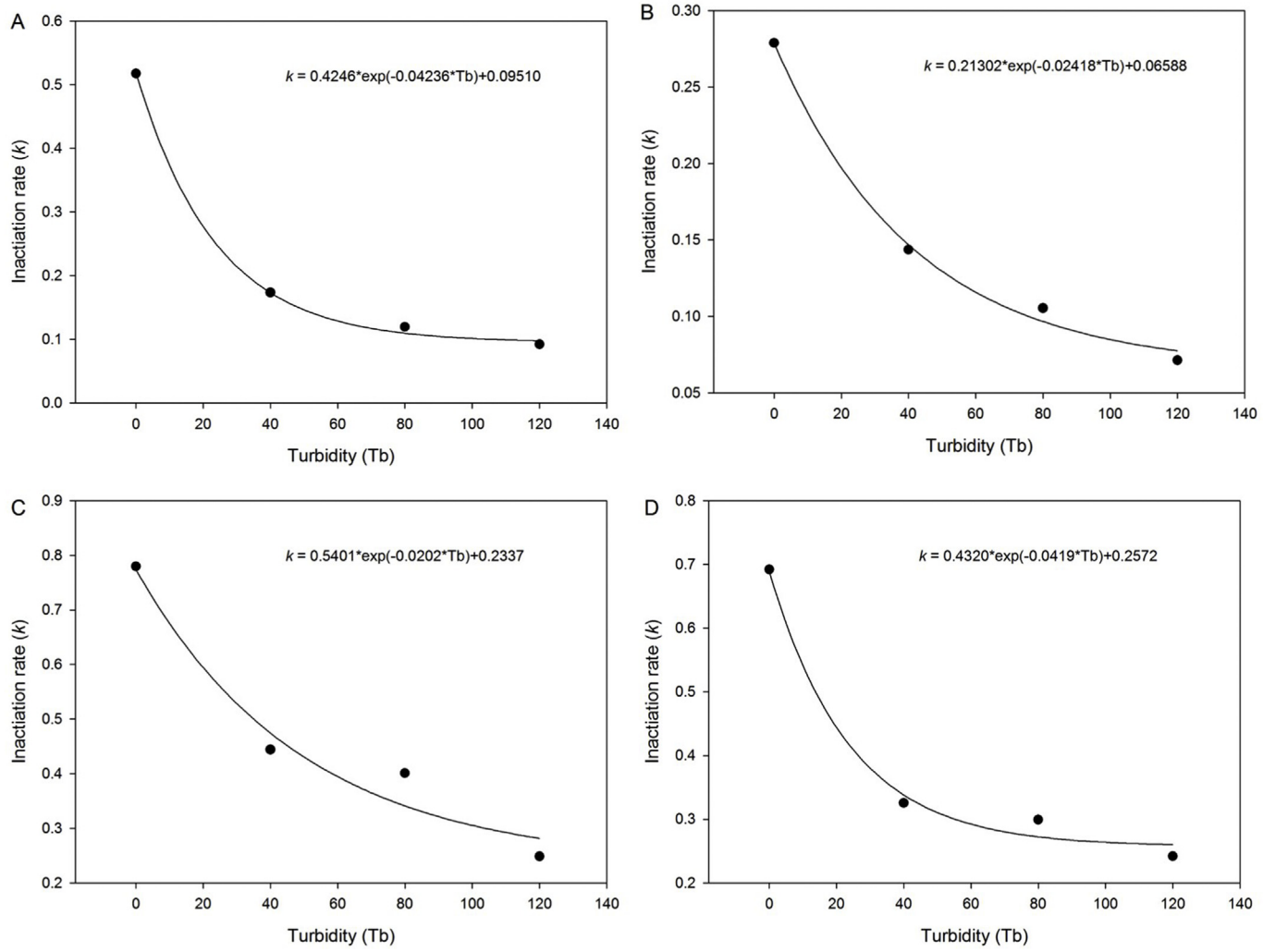

Fig. 2. Relationship between the inactivation rate (k) values and turbidity. Symbol means *S*. Typhimurium by the KrCl excilamp (A), *L. monocytogenes* by the KrCl excilamp (B), *S*. Typhimurium by LED-UV (C), and *L. monocytogenes* by LED-UV (D).

Table 5
Predicted and observed values of the pathogens *S*. Typhimurium (*S*) and *L. monocytogenes* (*L*) subjected to the 222 nm excilamp at 20, 60, and 100 NTU.

		Dose (mJ/cm^2)							
		10		20		30			
	Turbidity	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	A_f	B_f
S	20	3.11	3.29 ± 0.58	0.34	0.26 ± 0.45	0.00	0.00 ± 0.00	1.11	1.07
	60	4.77	4.78 ± 0.06	3.48	2.98 ± 0.11	2.20	1.09 ± 0.44	1.33	1.33
	100	5.09	4.86 ± 0.39	4.08	3.72 ± 0.16	3.06	2.69 ± 0.53	1.09	1.09
L	20	4.80	6.12 ± 0.28	2.83	3.61 ± 0.77	0.85	1.31 ± 0.15	1.36	0.74
	60	5.83	6.70 ± 0.08	4.67	5.66 ± 0.12	3.52	3.42 ± 0.62	1.13	0.90
	100	6.08	6.50 ± 0.20	5.23	6.20 ± 0.17	4.39	5.37 ± 0.47	1.16	0.86

Table 6
Predicted and observed values of the pathogens *S*. Typhimurium (*S*) and *L. monocytogenes* (*L*) subjected to LED-UV at 20, 60, and 100 NTU.

		Dose (mJ/cm^2)							
		5		10		15			
	Turbidity	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	A_f	B_f
S	20	2.67	3.73 ± 0.34	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	1.12	0.89
	60	3.89	4.54 ± 0.32	1.90	2.11 ± 1.00	0.00	0.00 ± 0.00	1.09	0.92
	100	4.40	4.81 ± 0.14	2.86	3.52 ± 0.31	1.33	1.18 ± 0.16	1.15	0.94
L	20	4.78	6.46 ± 0.57	2.56	2.71 ± 1.65	0.34	0.39 ± 0.68	1.18	0.85
	60	5.35	6.59 ± 0.02	3.89	4.57 ± 0.73	2.43	2.90 ± 1.07	1.20	0.83
	100	5.60	6.60 ± 0.19	4.28	5.99 ± 0.23	2.96	3.93 ± 0.99	1.30	0.77

results, producing 1.13–1.36 Af values and 0.74–0.90 Bf values (Table 5). In the case of LED-UV treatment, the predicted values were lower than the experimental results regardless of the type of pathogen (Table 6). These results indicate that the treatment conditions should be established conservatively to inactivate the pathogens completely. There are several factors to consider. First, microorganisms are living things that show fundamental variation. Second, the resistance of microorganisms can be changed depending on the growth condition. In the present study, inactivation experiments were conducted with the pathogens grown under ideal growth conditions; however, usually, the resistance of pathogen is higher than that in the laboratory (Hijnen, Beerendonk, & Medema, 2006). Under growth conditions in which resistance would increase, the treatment dosage should be adjusted to be stronger. In this regard, Hijnen et al. (2006) insists on including correction factors 2–7 considering this factor. Finally, some microorganisms acquire resistance during bactericidal treatment, resulting in a tailing effect. In this regard, bactericidal treatment should be applied sufficiently to ensure pathogen-free water, but a prolonged treatment time is energy consuming. The mathematical modeling represented here can be used to identify treatment conditions roughly at the first stage to solve the dilemma. Thereafter, we recommend modifying the conditions precisely with a validation experiment to ensure the water safety.

3.4. Application of the KrCl excilamp and LED-UVC treatment for inactivation of pathogens inoculated in food samples

For food application, pathogens were inoculated into oysters or flatfish. After drying, the inoculated pathogens were simultaneously detached by the spindle system and subjected to the KrCl excilamp or LED-UVC. We assumed that pathogens inoculated into flatfish would be inactivated much more rapidly by both treatments than those inoculated into oysters because oysters make the sample more turbid than flatfish do. The expected results were obtained with the LED-UVC treatment (Fig. 3C and D), but opposite results were obtained with the KrCl excilamp (Fig. 3A and B). Pathogens inoculated into oysters were inactivated more rapidly by the KrCl excilamp than those inoculated into flatfish. In this regard, the D_3d values by LED-UVC were smaller than those by the KrCl excilamp in flatfish, while opposite trends were obtained in oysters when analyzed by the Weibull model (Table 7). These results indicated that not only the turbidity of the sample but also other factors can affect the inactivation efficacy of KrCl excilamp treatment. Further study is needed to identify the factors affecting the pathogen inactivation by the KrCl excilamp and LED-UVC treatments when applied to food samples.

4. Conclusion

Inactivation of *S*. Typhimurium and L. *monocytogenes* by KrCl excilamp or LED-UVC in water was identified in the present study. Effect of turbidity on the inactivation of pathogens was mathematically analyzed and inactivation rate (k) was used to compare the inactivation rate by UV-C treatments. LED-UVC was more effective to inactivate pathogens than KrCl excilamp, and the inhibitory effect by increased turbidity was less significant. Predictive model was suggested for each UV-C treatment and validated. When UV-C treatments was applied for pathogen inactivation inoculated into oyster or flatfish, pathogens in flatfish was inactivated more rapidly than that in oyster by LED-UVC treatment. The opposite trend was observed by KrCl excilamp, which implicates not only turbidity but also other factor can affect the inactivation efficacy in seafood processing. The results indicated in the present study would be fundamental data for water disinfection or seafood processing using KrCl excilamp or LED-UVC.

Conflicts of interest

No conflicts of interest to declare.

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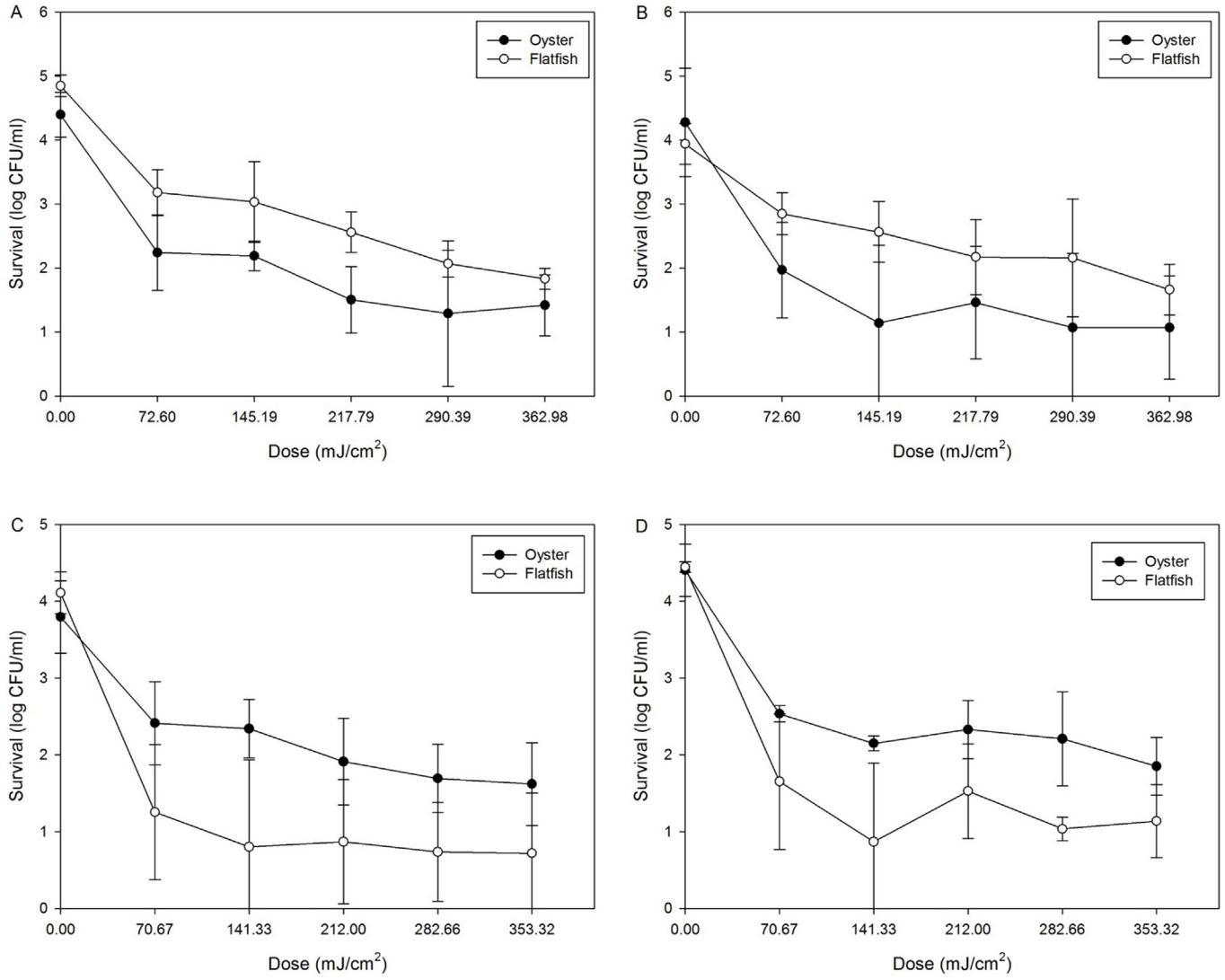

Fig. 3. Survival curves of *S*. Typhimurium (A) and *L. monocytogenes* (B) by the KrCl excilamp and *S*. Typhimurium (C) and *L. monocytogenes* (D) by LED-UVC inoculated into oysters and flatfish and detached by the spindle system.

Table 7
Parameters of the Weibull model, used to analyze the inactivation trend obtained from the 222 nm KrCl excilamp (Excilamp) and 280 nm LED-UVC (LED), and the dose required to achieve a 3-log reduction (D3d) in the spindle combination system with oysters or flatfish.

Sample	Type of Pathogen	Treatment	δ (min) ± SE	p ± SE	R^2	D_3d (mJ/cm^2)
Oyster	*S*. Typhimurium	Excilamp	10.06 ± 22.08	0.26 ± 0.16	0.94	688
		LED	33.52 ± 18.64	0.33 ± 0.07	0.98	936
	L. monocytogenes	Excilamp	7.67 ± 11.48	0.30 ± 0.12	0.96	299
		LED	12.35 ± 33.64	0.20 ± 0.16	0.93	3001
Flatfish	*S*. Typhimurium	Excilamp	29.68 ± 16.00	0.44 ± 0.09	0.98	360
		LED	1.40 ± 2.85	0.23 ± 0.09	0.97	166
	L. monocytogenes	Excilamp	68.27 ± 24.90	0.47 ± 0.09	0.98	707
		LED	1.28 ± 4.53	0.21 ± 0.14	0.94	239

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.108458.

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