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Master's Thesis of Science in Agricultural Biotechnology

**Application of 222 nm KrCl excilamp and 280 nm UVC
LED for water disinfection**

222 nm KrCl 엑실램프와 280 nm 자외선 발광 다이오드를 이용한 물살균의 적용

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ABSTRACT

Applicability of the 222 nm krypton-chlorine (KrCl) excilamp and 280 nm UVC light-emitting diode (UVC LED) for water disinfection was identified in this study. *Salmonella* Typhimurium and *L. monocytogenes* were inactivated by the KrCl excilamp and UVC LED treatments in water samples with varying turbidities and water samples which spindled with inoculated seafoods. When applied to oyster or flatfish spindled samples, the UVC LED 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 experiments to treat turbid water samples, 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 UVC LED 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.

In the scale-up experiment, the survival curve obtained through the experiment comparing the bactericidal effect between the KrCl excilamp and UVC LED was analyzed by the Weibull model, and the D_{5d} value was calculated. As a result, when the volume of water to be treated is increased by 100 times, it was confirmed that the D_{5d} value of the KrCl excilamp was approximately 5 times and the UVC LED was 3 times higher in two pathogens. This shows that water disinfection effect through the UVC LED was better than excilamp in scale-up environment.

Keywords: Water disinfection, KrCl excilamp, UVC LED, turbidity, scale-up

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I. INTRODUCTION

In the food industry, cross-contamination, which can occur through post-harvest, process, and storage, is an important part of the food safety (Chen et al. 2001). Especially cross-contamination caused by contaminated water is more important when processing raw foods, such as fresh products and seafoods. 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. 2019, Hassard et al. 2017) but have also acknowledged the risk of *Salmonella* and *Listeria* spp. contamination in seafood (Feldhusen 2000). (Amagliani et al. 2012) indicated that *Salmonella* has caused seafood-related outbreaks worldwide. The outbreaks are attributed to 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.

These outbreaks show that the safety of water used for processing and washing is critical. It is obvious that disinfection is one of the most critical processing steps in fresh-cut vegetable production, affecting the quality, safety, and shelf-life of the end product (Gil et al. 2009). As mentioned above, water serves as a source of cross-contamination as re-using processing water may result in the build-up of microbial loads. Therefore, it is important to maintain the safety of wash water.

Water disinfection with chlorine is a usual method in industrial, which is inexpensive and convenient to introduce it. However, there is a trend in avoiding chlorine from disinfection process because of the concern about its efficiency and more about the environmental and health risk associated with the formation of carcinogenic halogenated disinfection by products (Ölmez and Kretzschmar 2009).

For these reasons, alternative methods for disinfecting water have been proposed and the most promising one is UVC irradiation. Among the ultraviolet (UV) ranges, the UV-C (200-280 nm) wavelength having the most effective bactericidal ability is widely used as antibacterial agent in water and air treatments using Hg germicidal lamps (254 nm) (Chang et al. 1985). Mercury-based UV lamps have been widely used.

However, under the Minamata Convention, which came into force in August 2017 in South Korea, the manufacturing and export of germicidal lamps using mercury for industrial use will be phased out from 2020. The Minamata Convention on Mercury is dedicated to the life-cycle management of mercury in order to reduce

the risks to human health and the environment (Selin 2014). 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 et al. 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 et al. 2018), who identified that KrCl excilamp damage not only DNA but also cell membrane. The applicability of UVC LED to inactivate bacteria, yeast, and viruses was also investigated recently (Beck et al. 2017, Kim and Kang 2018, Kim et al. 2017a, b). However, many of these studies were conducted using one UV-C source, and comparative studies between the 222 nm KrCl excilamp and 280 nm UVC LED are limited.

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 (Kim and 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 UVC LED, 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 investigate the influence factor by irradiating UVC with spindle to the seafood samples and 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 UVC LED. Mathematical analysis was used to compare the pathogen inactivation efficacy of the KrCl excilamp and UVC LED. In addition compared the bactericidal efficacy of the KrCl excilamp and UVC LED in scale-up situation.

II. MATERIALS AND METHODS

2.1. Spindle and UVC system

In order to implement the washing system and agitating the sample with UVC irradiation, the spindle and UVC system was developed as shown in Fig 1. Spindle consisted of an electric motor, a time controller and a container to contain food and water samples. The body ($20 \times 21 \times 16 \text{ cm}^3$) consisted of the motor and container ($13 \times 13 \times 13 \text{ cm}^3$) connected vertically. The speed of the spindle's motor was 7000 g. Excilamp and UVC LED were irradiated to the container belong to spindle using iron structure. Placing the excilamp and UVC LED on the structure results in a distance of 20 cm between the UVC and the sample being treated.

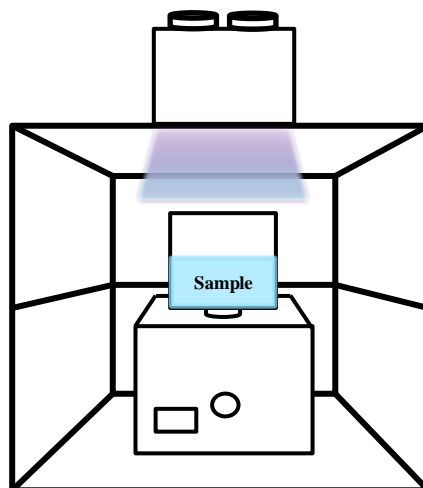


Fig 1. Schematic diagram of UVC spindle system

2.2 Bacterial cell suspension preparation

Three strains of *Salmonella* Typhimurium (DT 104, ATCC 19585, and ATCC 43971) and *Listeria monocytogenes* (ATCC 15313, ATCC 19111, and ATCC 19115) were obtained from Seoul National University (Seoul, South Korea). Stock cultures were grown in tryptic soy broth (TSB; Difco, Becton, Dickinson and Company, Sparks, MD, USA) and stored at -80°C in 0.7 ml TSB and 0.3 ml of 50% glycerol. Working cultures were gained by streaking bacteria in stock into tryptic soy agar (TSA) and incubated at 37°C for 24 h, stored at 4°C. Each strain of *S. Typhimurium* and *L. monocytogenes* was cultivated in 5 ml of TBS at 37°C for 24 in the shaking incubator. The pellet of bacteria was collected by centrifugation ($4000 \times g$ at 4°C for 20 min) and then resuspended by 9 ml of 0.2% peptone water (PW; Bacto, Becton, Dickinson and Company, Sparks, MD, USA). Resuspended pellets were used in the form of cocktail mixed culture of two pathogen, and the final concentration of each strain was approximately 10^9 CFU/ml.

2.3. Wash water of seafoods disinfection using 222 nm KrCl excilamp and 280 nm UVC LED

2.3.1. Sample preparation and inoculation

In this study, oysters and flatfish were used as a seafood sample and those were purchased at a local market (Seoul, South Korea) for implementation of washing process of food industry. A mixed cultured cocktail (9 ml) prepared was combined with 291 ml of distilled water (DW) to make a bacterial solution. The oysters and flatfish were inoculated with the bacterial solution by the dipping methods for 5 min and then dry the samples for 30 min.

2.3.2. UVC treatment

In the case of UVC irradiation, the concept of dosage is usually used, which is the concept of intensity multiplied by time. In order to obtain the UVC dose, the intensity of spindle UVC system were measured as described previously (Kim et al. 2016, Shin et al. 2016) Briefly, the irradiation intensity of 7 points in the container area was recorded using a UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands). Petri factor, which indicates the intensity variation across the UVC treated 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 and UVC LED at the sample location was 1210 and 434 $\mu\text{W}/\text{cm}^2$ each. Inoculated oysters and flatfish were immersed in 250 ml of Distilled water (DW) in the container of spindle. The seafood sample and wash water were treated by excilamp and UVC LED with spindle operating. The dose values of excilamp and UVC LED were 0, 72.60, 145.19, 217.79, 290.39, 362.98 and 0, 70.67, 141.33, 212.00, 282.66, 353.32 mJ/cm^2 each.

2.3.3. Bacterial enumeration

For bacterial enumeration, 3 ml of combination system treated water was obtained each dosage. After then, 1ml of treated and untreated water sample were 10-fold serially diluted in 9 ml of 0.2% PW and 0.1 ml of undiluted water sample or diluents were spread-plated onto selective media. Xylose lysine deoxycholate (XLD) agar (Difco) and Oxford agar base with antimicrobial supplement (Bacto Oxford antimicrobial supplement, Difco) were used as selective media for *S. Typhimurium* and *L. monocytogenes*, respectively. All plates were incubated at 37°C for 24- 48 h before counting the number of typical colonies.

2.3.4. Mathematical modeling analysis

The disinfection trend was analyzed by the Weibull model, and D_{3d} values were calculated. The equation for the Weibull model and D_{3d} is as follows:

$$\log_{10}(N) = \log_{10}(N_0) - x \left(\frac{t}{D_{3d}} \right)^p \quad (1)$$

Where N (CFU/ml) is the population of the microorganisms, N_0 is the initial population, p is the parameter related to the scale and shape of the survival curve, x is the number of the decimal reduction. The Weibull distribution corresponds to a concave downward survival curve if $p > 1$ and upward if $p < 1$.

2.4. Turbidity water sample disinfection using 222 nm KrCl excilamp and 280 nm UVC LED

2.4.1. Turbidity water sample preparation and inoculation

For obtaining water sample which has turbidity, distilled water was manufactured by using bentonite (Sigma-Aldrich, St. Louis, MO) and adjusted to 0, 40, 80, and 120 nephelometric turbidity units (NTU) and sterilized. The turbidity of samples was measured using turbidity meter (TU-2016, Lutron Electron, Taiwan). The sample container of the spindle was covered with a sterile plastic bag, which was filled with 250 ml of turbidity-adjusted water sample. A mixed of *S. Typhimurium* and *L. monocytogenes* cultured cocktail (1 ml) was inoculated into the sample

before UVC treatment and homogenized for more than 1 min with the spindle system.

2.4.2. UVC treatment

In order to obtain the UVC dose, the intensity of spindle UVC system were measured as described previously written in this thesis 2.3.2. The dose values of excilamp and UVC LED were 0 – 40 and 0 - 25 mJ/cm² each.

2.4.3. Bacterial enumeration

For bacterial enumeration, 3 ml of combination system treated water was obtained each dosage. After then, 1ml of treated and untreated water sample were 10-fold serially diluted in 9 ml of 0.2% PW and 0.1 ml of undiluted water sample or diluents were spread-plated onto selective media. Xylose lysine deoxycholate (XLD) agar (Difco) and Oxford agar base with antimicrobial supplement (Bacto Oxford antimicrobial supplement, Difco) were used as selective media for *S. Typhimurium* and *L. monocytogenes*, respectively. All plates were incubated at 37°C for 24- 48 h before counting the number of typical colonies.

2.4.4. Mathematical modeling analysis

Obtain the inactivation and establish the model of turbidity and inactivation rate constant (k) The survival curve of *S. Typhimurium* and *L. monocytogenes* were analyzed using GinaFit, a freeware tool used to assess microbial survivor curves (Geeraerd et al. 2005). The survival curves were fitted well with log linear model (Eq 2.), and the inactivation rate constant (k) was calculated (Eq 3.).

$$\log_{10}(N) = \log_{10}(N_0) - \frac{k_{max}}{\ln(10)} d \quad (2)$$

$$k = \frac{k_{max}}{\ln(10)} \quad (3)$$

where N (CFU/ml) is the population of the microorganisms, N_0 (CFU/ml) is the initial population, k_{max} is the first-order inactivation constant, and d (mJ/cm²) 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 4.) 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.

$$k = (k_0 - \text{plateau}) * \exp(-a * T_b) + \text{plateau} \quad (4)$$

where k is the inactivation rate constant, T_b (NTU) is the turbidity of the sample, k_0 is the inactivation rate at 0 NTU turbidity, a is the constant, and plateau is the k value at infinite turbidity.

Calculate the predictive values 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* by KrCl excilamp,

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

For *L. monocytogenes* by KrCl excilamp,

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

For *S. Typhimurium* by UVC LED treatment,

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

For *L. monocytogenes* by UVC LED 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_0 (CFU/ml) is the initial population, T_b (NTU) is the water turbidity, and d (mJ/cm²) is the treatment dose.

2.4.5. Validation of the predictive model using A_f and B_f

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)

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

$$B_f = 10^{\frac{\sum \log(\text{predicted}/\text{observed})}{n}} \quad (6)$$

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 indicate by how much, on average, a model overpredicts or under predicts the observed data. Perfect agreement between predictions and observations will lead to an accuracy factor and a bias factor of 1.

2.5. Comparison of disinfection effect of 222 nm KrCl excilamp and 280 nm UVC LED in the scale-up situation

2.5.1. UVC treatment after sample preparation and inoculation in a scale-up situation

For establishing a scale-up situation, an experiment was conducted after preparing distilled water samples in 10 ml, 100 ml, and 1000 ml volumes. 10 ml of water samples were put in petri dishes (60 × 15 mm, SPL, Gyeonggi, South Korea) and 100 ml and 1000 ml of water samples were put in 1000 ml beaker. A mixed culture cocktail was inoculated 0.1 ml, 1 ml, and 10 ml in 10ml, 100ml, and 1000 ml respectively.

Three volume scale were irradiated by excilamp and UVC LED and homogenized at 200 rpm using a stir bar (0.2 × 0.2 × 1 cm), 100 ml and 1000 ml of water samples using stir bar (1.5 cm).

The dose values of UVC treatment to water sample were 0 -15 mJ/cm² in 10 ml, 0 – 25 mJ/cm² in 100 ml, and 0 – 40 mJ/cm² in 1000 ml.

2.5.2. Bacterial enumeration

For bacterial enumeration, treated water samples of combination system treated water was obtained each dosage. After then, 1ml of treated and untreated water sample were 10-fold serially diluted in 9 ml of 0.2% PW and 0.1 ml of undiluted water sample or diluents were spread-plated onto selective media. Xylose lysine deoxycholate (XLD) agar (Difco) and Oxford agar base with antimicrobial supplement (Bacto Oxford antimicrobial supplement, Difco) were used as selective media for *S. Typhimurium* and *L. monocytogenes*, respectively. All plates were incubated at 37°C for 24- 48 h before counting the number of typical colonies.

2.5.3. Mathematical modeling analysis

The disinfection trend was analyzed by the Weibull model, and D_{5d} values were calculated. The equation for the Weibull model and D_{5d} is as follows:

$$\log_{10}(N) = \log_{10}(N_0) - x \left(\frac{t}{D_{xd}} \right)^p$$

Where N (CFU/ml) is the population of the microorganisms, N_0 is the initial population, p is the parameter related to the scale and shape of the survival curve, x is the number of the decimal reduction. The Weibull distribution corresponds to a concave downward survival curve if $p > 1$ and upward if $p < 1$.

2.6. 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 $p = 0.05$.

III. RESULTS

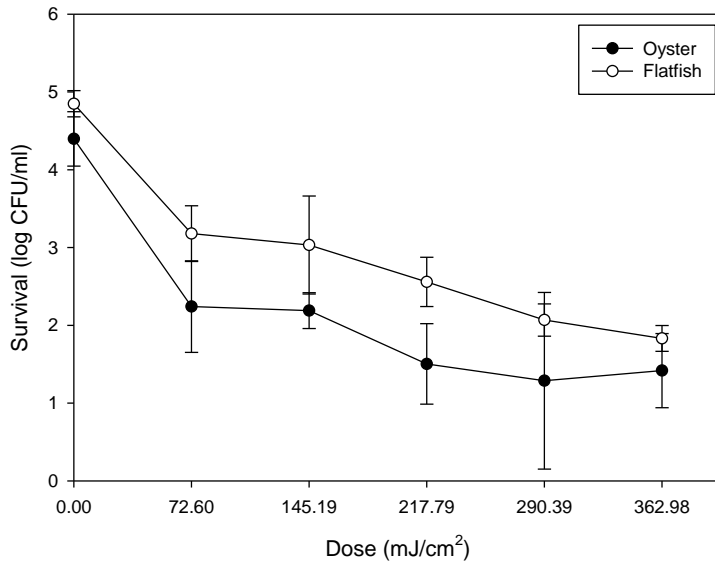
3.1. Inactivation of foodborne pathogen on the wash water of seafoods by 222 nm KrCl excilamp and 280 nm UVC LED

3.1.1. 222 nm KrCl excilamp and 280 nm UVC LED treatment on *S.*

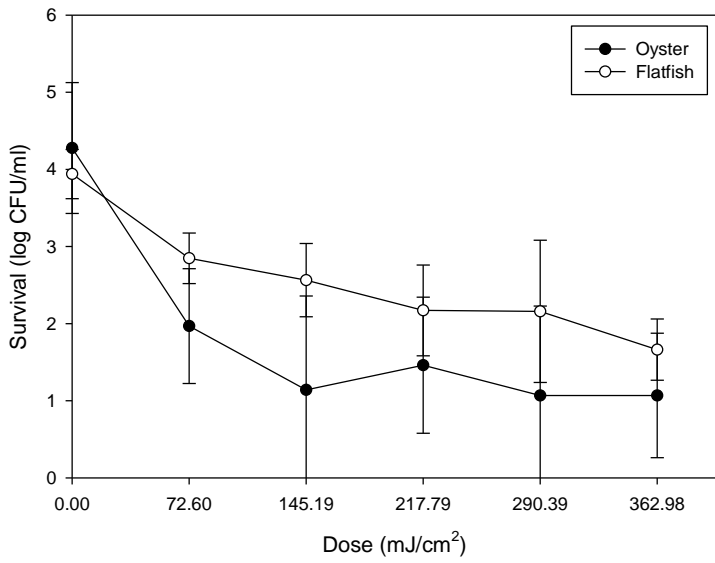
Typhimurium and *L. monocytogenes*

We assumed that pathogens inoculated into flatfish would be inactivated much more rapidly by both treatment than those inoculated into oysters because oyster make the sample more turbid than flatfish do. The expected results were obtained with the UVC LED treatment (Fig 2.), but opposite results were obtained with the KrCl excilamp (Fig 2.). Pathogens inoculated into oyster were inactivated more rapidly by the KrCl excilamp than those inoculated into flatfish.

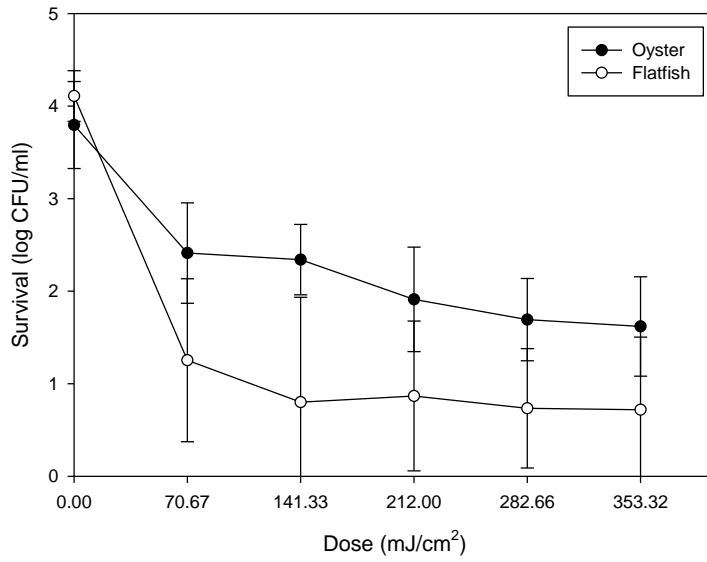
(A)



(B)



(C)



(D)

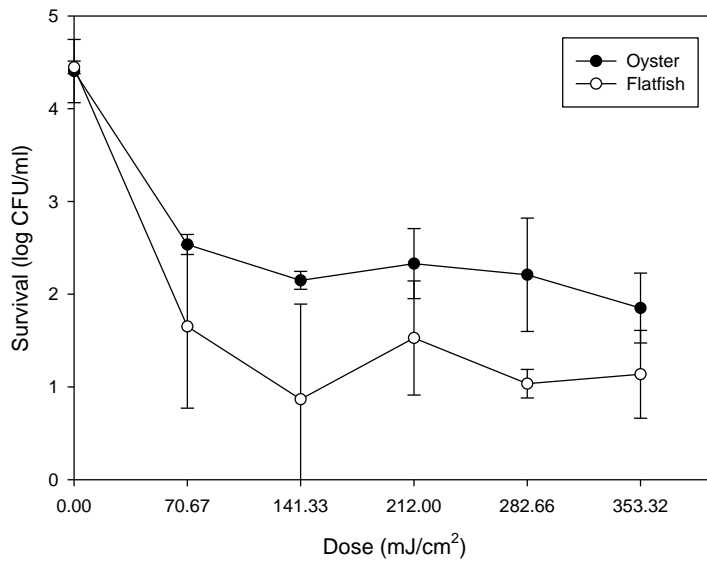


Fig 2. Survival curves of *S. Typhimurium* (A) and *L. monocytogenes* (B) by the KrCl excilamp and *S. Typhimurium* (C) and *L. monocytogenes* (D) by UVC LED inoculated into oysters and flatfish and detached by the spindle system.

3.1.2. Analysis of inactivation results using the Weibull model equation

As shown in Fig 3., inactivation results did not show tendency by UVC sources. In this regard, the D_{3d} values by UVC LED were smaller than those by KrCl excilamp in flatfish, while opposite trends were obtained in oyster when analyzed by the Weibull model (Table 1.). 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 UVC LED treatments when applied to food samples.

Table 1. Parameters of the Weibull model, used to analyze the inactivation trend obtained from the 222 nm KrCl excilamp (Excilamp) and 280 nm UVC LED (LED), and the dose required to achieve a 3-log reduction (D_{3d}) in the spindle combination system with oysters or flatfish.

Sample	Type of Pathogen	Treatment	δ (min) \pm SE	$p \pm$ SE	R^2	D_{3d} (mJ/cm ²)
Oyster	<i>S. Typhimurium</i>	Excilamp	10.06 \pm 22.08	0.26 \pm 0.16	0.94	688
		LED	33.52 \pm 18.64	0.33 \pm 0.07	0.98	936
	<i>L. monocytogenes</i>	Excilamp	7.67 \pm 11.48	0.30 \pm 0.12	0.96	299
		LED	12.35 \pm 33.64	0.20 \pm 0.16	0.93	3001
Flatfish	<i>S. Typhimurium</i>	Excilamp	29.68 \pm 16.00	0.44 \pm 0.09	0.98	360
		LED	1.40 \pm 2.85	0.23 \pm 0.09	0.97	166
	<i>L. monocytogenes</i>	Excilamp	68.27 \pm 24.90	0.47 \pm 0.09	0.98	707
		LED	1.28 \pm 4.53	0.21 \pm 0.14	0.94	239

3.2. Inactivation of foodborne pathogen on the turbidity water sample by 222 nm KrCl excilamp and 280 nm UVC LED

3.2.1. Effect of turbidity on the pathogen inactivation by the 222 nm KrCl excilamp or UVC LED treatment

Reduction levels of *S. Typhimurium* and *L. monocytogenes* by the KrCl excilamp decreased with increasing turbidity (Table 2.). 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 20mJ/cm² of the KrCl excilamp were 0.00, 2.94, 3.55, and 4.24 in the 4, 40, 80, and 120 NTU water samples, respectively. The same trend was observed for *L. monocytogenes* with a higher resistance than *S. Typhimurium*. A similar result was reported by (Kim et al. 2017a), indicating that the resistance of *L. monocytogenes* is higher than *S. Typhimurium* in water with an initial population of 10⁶-10⁷, 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 UVC LED treatment than by the KrCl excilamp. For example, the survived populations (log CFU/ml) of *S. Typhimurium* after 10 mJ/cm² UVC LED 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/ cm² KrCl excilamp treatment (Table 3.). The different bactericidal efficacies between the KrCl excilamp and UVC LED 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 UVC LED might be higher than that of the KrCl excilamp because the wavelength of UVC LED (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 UVC LED 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 UVC LED 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 UVC LED is DNA damage rather than membrane damage (Kim et al. 2017a). In this regard, further study is needed to identify DNA, RNA and enzymes damages of the pathogen by KrCl excilamp and UVC LED.

Table 2. Populations (log CFU/ml) of *S. Typhimurium* (*S*) and *L. monocytogenes* (*L*) on the distilled water with various turbidity subjected to 222-nm krypton-chlorine excilamp

	Turbidity (NTU)	Dose (mJ/cm ²)					
		0	10	20	30	40	50
<i>S</i>	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 BC	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
<i>L</i>	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 3. Populations (log CFU/ml) of *S. Typhimurium* (*S*) and *L. monocytogenes* (*L*) on the distilled water with various turbidity subjected to UVC LED treatment

	Turbidity (NTU)	Dose (mJ/cm ²)					
		0	5	10	15	20	25
<i>S</i>	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
<i>L</i>	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 ($p > 0.05$).

3.2.2 Modeling the effect of sample turbidity on pathogen inactivation by the KrCl excilamp and UVC LED treatment

From the inactivation curves, it was determined that increased turbidity decreased the disinfection efficacy of alternative UVC irradiation treatments, but it remains unclear how to control the UVC 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 UVC irradiation and is also used to compare the resistance of pathogens by UVC irradiation. For example, the inactivation rate constants of viruses are significantly lower than those of bacteria, which means higher resistance to UVC 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 4), decreased exponentially as the turbidity increased, regardless of the type of pathogen and treatment device (Fig 3.). 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

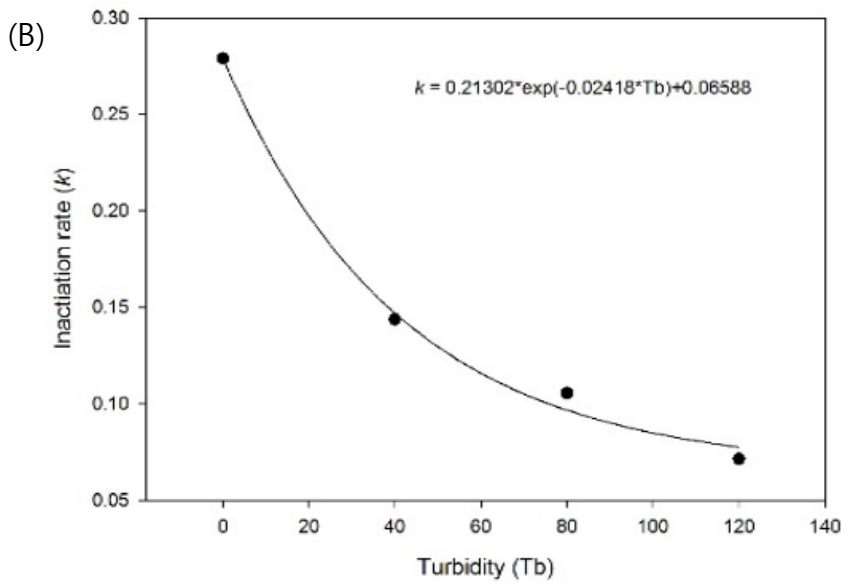
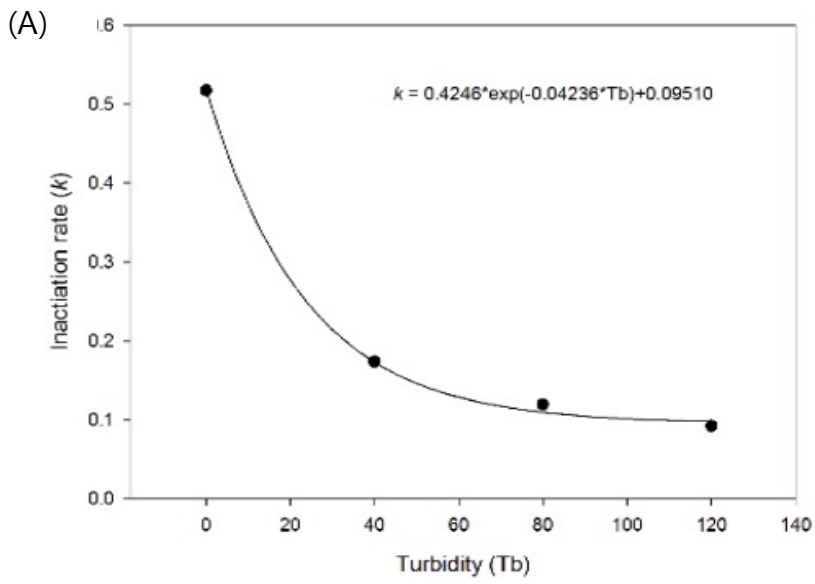
were 0.28, 0.14, 0.11, and 0.07 for 0, 40, 80, and 120 NTU water samples, respectively. The same trends were observed for UVC LED treatments. When the relationship between the sample turbidity and inactivation rate (k) was analyzed by several mathematical models, it fit well with the one-phase exponential decay model (Table 5.). From the developed model, we can adjust the treatment time of the KrCl excilamp and UVC LED. 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 UVC LED 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.

Table 4. Inactivation rate (k) of *S. Typhimurium* and *L. monocytogenes* subjected to 222 nm excilamp and UVC LED treatments at each turbidity

	Turbidity	Excilamp	UVC LED
<i>S. Typhimurium</i>	0	0.52	0.78
	40	0.17	0.44
	80	0.12	0.40
	120	0.09	0.25
<i>L. monocytogenes</i>	0	0.28	0.69
	40	0.14	0.33
	80	0.11	0.30
	120	0.07	0.24

Table 5. Parameters of exponential one phase decay model for inactivation of *S. Typhimurium* and *L. monocytogenes* subjected to 222 nm excilamp and UVC LED treatments at each turbidity

Treatment	Pathogens	k_0	Plateau	a	R ²
Excilamp	<i>S. Typhimurium</i>	0.5197	0.0951	0.0424	0.99
	<i>L. monocytogenes</i>	0.2789	0.0659	0.0242	0.99
UVC LED	<i>S. Typhimurium</i>	0.7738	0.2337	0.0202	0.96
	<i>L. monocytogenes</i>	0.6893	0.2572	0.0419	0.99



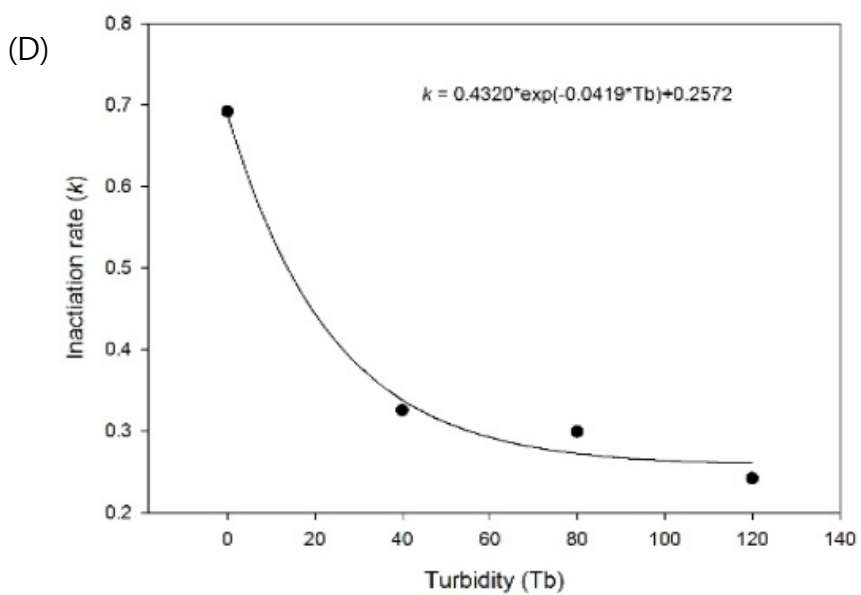
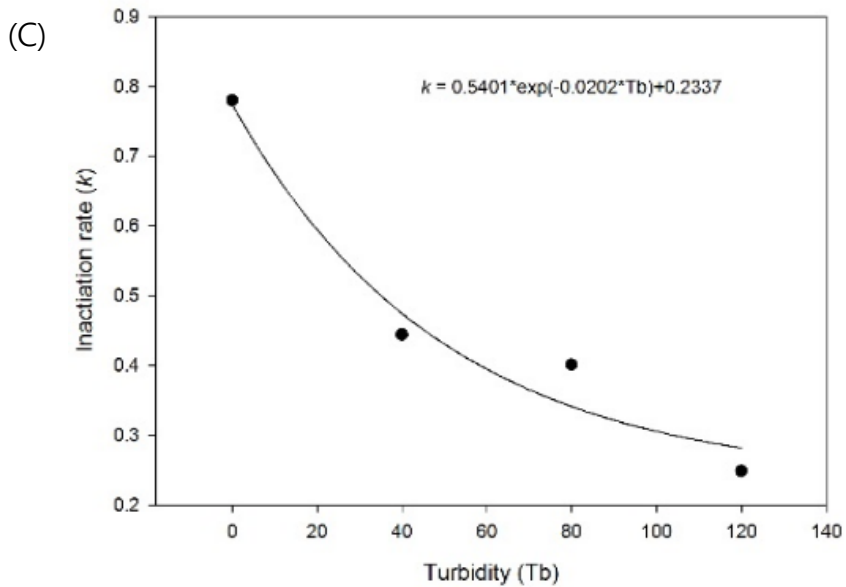


Fig 3. Relationship between inactivation rate (k) values and turbidity. Symbol means *S. Typhimurium* by KrCl excilamp (A), *L. monocytogenes* by KrCl excilamp (B), *S. Typhimurium* by UVC LED (C), and *L. monocytogenes* by UVC LED (D).

3.2.3 Validate the predictive value

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 results, producing 1.13–1.36 A_f values and 0.74–0.90 B_f values (Table 6.). In the case of UVC LED treatment, the predicted values were lower than the experimental results regardless of the type of pathogen (Table 7.).

Table 6. Predicted and observed values of pathogens *S. Typhimurium* (*S*) and *L. monocytogenes* (*L*) subjected to 222 nm excilamp at 20, 60, and 100 NTU.

		Dose (mJ/cm ²)						A _f	B _f
		10		20		30			
	Turbidity	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental		
<i>S</i>	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
<i>L</i>	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 7. Predicted and observed values of pathogens *S. Typhimurium* (*S*) and *L. monocytogenes* (*L*) subjected to UVC LED at 20, 60, and 100 NTU.

		Dose (mJ/cm ²)						A _f	B _f
		5		10		15			
	Turbidity	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental		
<i>S</i>	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
<i>L</i>	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

3.4. Comparison of inactivation effect of 222 nm KrCl excilamp and 280 nm UVC LED in the scale-up situation

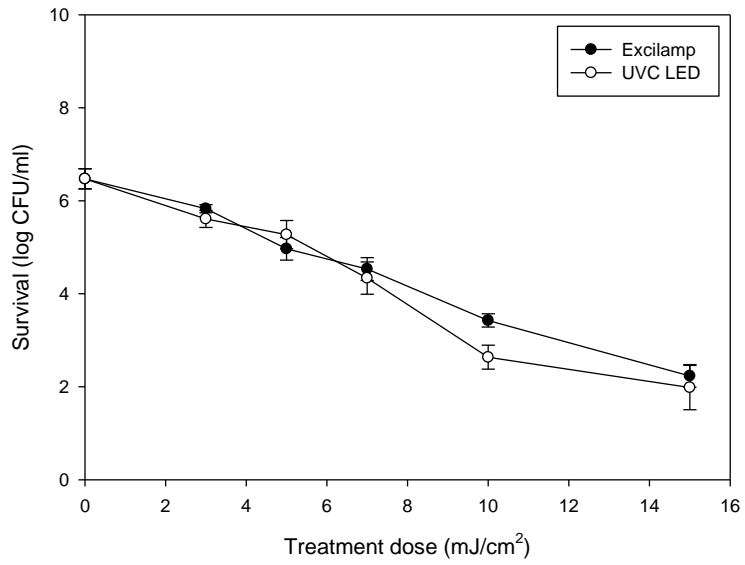
3.4.1. Inactivation of *S. Typhimurium* and *L. monocytogenes* in water in scale-up situation

Inactivation effects of pathogens by KrCl excilamp and UVC LED were decreased with the scale-up situation. It was easily predicted because a penetration of UVC light was decreased in volume-up condition. In this study, the survival population of *S. Typhimurium* (log CFU/ml) after 15 mJ/cm² with KrCl excilamp was under detection limit and 2.23 in 10 ml and 100 ml of water sample, respectively. Similar inactivation tendency was observed in *S. Typhimurium* in 10 and 100 ml treated by UVC LED. On the contrary, when the volume was 1000 ml, the inactivation effect treated by the excilamp and the UVC LED showed a significant difference, and when the reduction of *S. Typhimurium* (log CFU/ml) after 40 mJ/cm² with excilamp and UVC LED was 1.88 and 5.56, respectively.

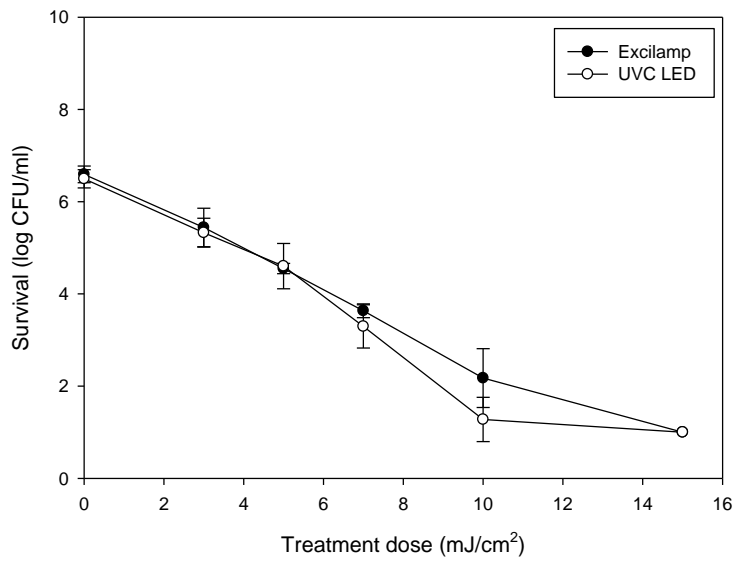
The difference in the inactivation effects between the excilamp and the UVC LED tends to be slightly different from that of *S. Typhimurium* in *L. monocytogenes*. Reduction (log CFU/ml) showed a difference between the excilamp and UVC LED of 1.38 in 10 ml and 2.05 in 100 ml after 10 mJ/cm² irradiation. Survival population (log CFU/ml) of 7.56 and 3.70, respectively, were treated with excilamp and UVC LED in the *L. monocytogenes* at 1000 ml, and the

difference of reduction was 3.86 log CFU/ml. Since gram-positive bacteria have more resistance to the UVC than gram-negative bacteria (Kim et al. 2017a), different tendency may have occurred in *L. monocytogenes*.

(A)



(B)



(C)

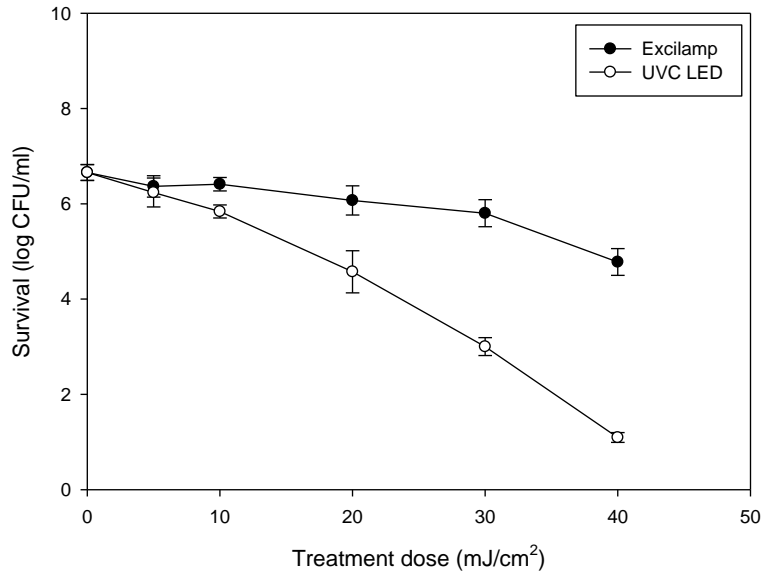
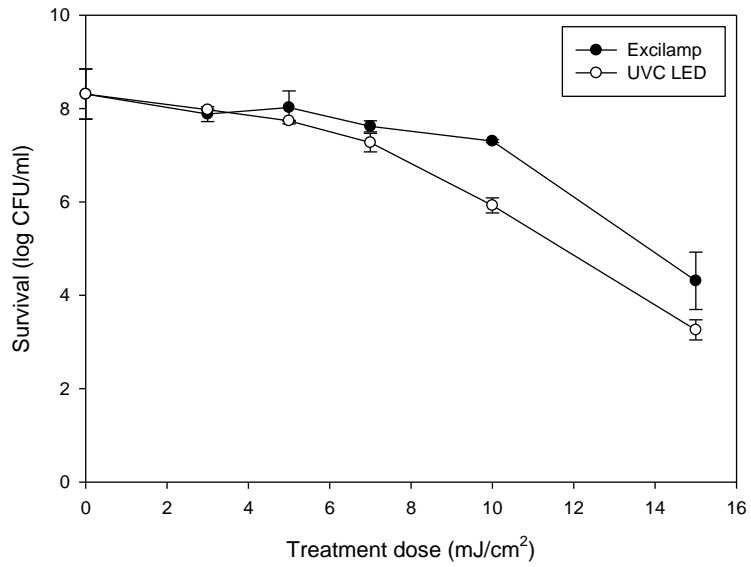
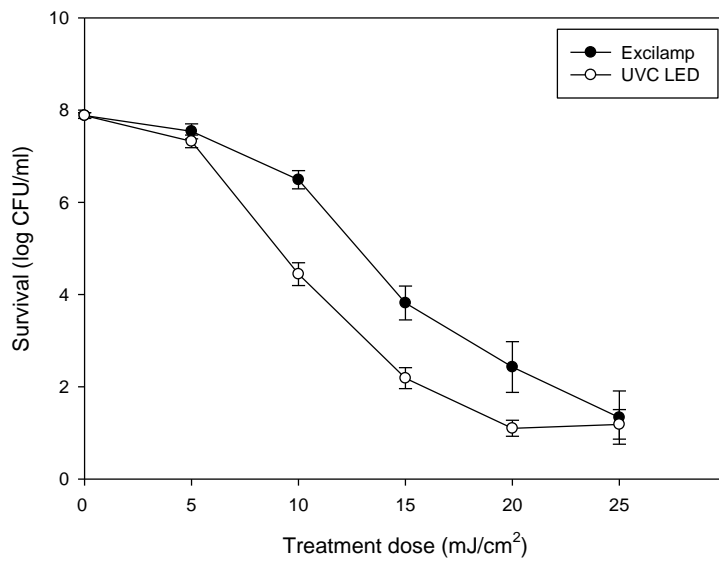


Fig 4. Survival population (log CFU/ml) of *S. Typhimurium* treated by 222 nm KrCl excilamp and 280 nm UVC LED at 10 ml (A), 100 ml (B), and 1000 ml (C).

(A)



(B)



(C)

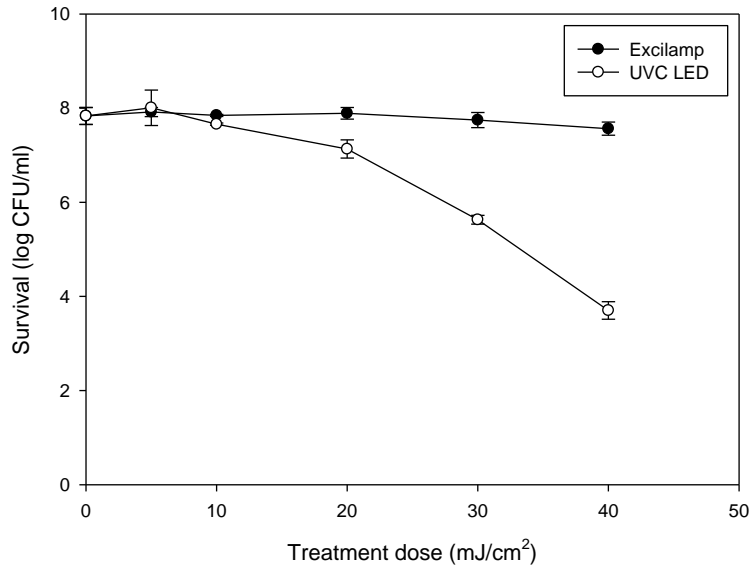


Fig 5. Survival population (log CFU/ml) of *L. monocytogenes* treated by 222 nm KrCl excilamp and 280 nm UVC LED at 10 ml (A), 100 ml (B), and 1000 ml (C).

3.4.2. Modeling the effect of scale-up and turbidity on pathogen inactivation by the KrCl excilamp or UVC LED treatment

The parameters of the Weibull modeling were shown in Table 6. When 0 water sample was treated by UVC, the overall R square values were over 0.95 except the case that *L. monocytogenes* in 1000 ml by excilamp and in 10 ml treated by UVC LED. D_{5d} values that representing the dosage when the 5 log reduction was achieved were calculated and compared at each cases. The D_{5d} values of *S. Typhimurium* and *L. monocytogenes* treated by excilamp and UVC LED in scaling up situation were increased. As an example of that, the D_{5d} values of *S. Typhimurium* treated with UVC LED were 11.68 (10 ml), 15, 43(100 ml), and 37.52 (1000 ml) mJ/cm^2 . When comparing the effect of excilamp and UVC LED, D_{5d} values of UVC LED were lager than excilamp in all case of pathogens, volumes, and UVC. Especially D_{5d} values of *L. monocytogenes* in 1000 ml were 84. 60 mJ/cm^2 treated with excilamp which was more than three times that of UVC LED (26.33 mJ/cm^2).

Table 6. Parameters of Weibull models for inactivation of pathogens on water sample with scale-up subjected to excilamp and UVC LED

Treatment	Pathogen	Scale	delta \pm SE	p \pm SE	R ²	D _{5d}
Excilamp	<i>S. Typhimurium</i>	10	2.00 \pm 0.55	0.88 \pm 0.11	0.993	12.49
		100	3.38 \pm 0.57	0.99 \pm 0.10	0.967	17.13
		1000	32.66 \pm 2.23	2.53 \pm 0.68	0.988	61.68
	<i>L. monocytogenes</i>	10	5.39 \pm 2.05	1.28 \pm 0.30	0.972	16.26
		100	10.41 \pm 0.81	3.61 \pm 0.72	0.987	18.87
		1000	54.71 \pm 7.81	3.69 \pm 1.60	0.924	84.60
UVC LED	<i>S. Typhimurium</i>	10	1.66 \pm 1.18	0.83 \pm 0.25	0.939	11.68
		100	2.96 \pm 1.33	0.98 \pm 0.25	0.957	15.43
		1000	12.19 \pm 0.51	1.43 \pm 0.05	0.999	37.52
	<i>L. monocytogenes</i>	10	3.69 \pm 3.30	1.10 \pm 0.49	0.889	14.94
		100	6.60 \pm 0.34	1.97 \pm 0.12	0.998	15.98
		1000	21.10 \pm 1.15	2.26 \pm 0.19	0.997	43.02

IV. DISCUSSION

To applicate the 222 nm KrCl excilamp and 280 nm UVC LED for the water disinfection, three experiment were conducted in this thesis.

In the first part, the reduction of the treated water was investigated by treating the seafood samples which were inoculated by *S. Typhimurium* and *L. monocytogenes* and immersed in sterilized distilled water into a system combining the spindle and UVC through excilamp and UVC LED. According the previous study, *Salmonella* infection from consumption of seafood product are commonly associated with raw and cross-contamination of seafood during processing and storage (Elbashir et al. 2018). Because *L. monocytogenes* can survive in vast rage of temperature (1-45°C), so this pathogen cannot be overlooked in the processing and storage of seafoods. The results of this experiment showed a slight tendency. As the UVC dosage treated to samples increased, the inactivation effect increased, but there was no difference in tendency between the excilamp and UVC LED. In experiments with oyster samples the D_{3d} values for the *S. Typhimurium* and *L. monocytogenes* with excilamp were smaller than those for UVC LED. In other words, the reduction effect of the excilamp was better than the UVC LED. However, experiments with flatfish samples showed opposite trends, indicating better UVC LED inactivation effects than excilamp. In the flatfish used in this experiment, the

turbidity of the water was not affected during the spindle, but the turbidity of the oyster increased as the spindle was treated. As a result of the turbidity effect, it can be considered that the bactericidal effect of the excilamp and the UVC LED varies depending on the samples. The fact that turbidity is an important factor in sterilization using light has been mentioned in various studies (Song et al. 2016), and it was confirmed through the first part of this thesis when applying UVC for water disinfection.

Therefore, the second experiment was conceived based on the results of the first part. In fact, it is easy to predict that the inactivation effect will decrease as turbidity increase. In this study, however, we wanted to analyze more quantitatively how much the inactivation effect decreased when the turbidity increased. We believe that this research could provide a database for the industrial application of excilamp and UVC LED for water disinfection. As mentioned in the results section, in terms of the inactivation rate constant (k), the UVC LED had better bactericidal ability against turbidity than the excilamp. The predicted value calculated based on the Exponential one phase decay model equation and the value obtained from the experiment were analyzed using A_f and B_f . These results of validation 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 et al. 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.

The third part was experiment that comparing the inactivation effects of excilamp and UVC LED in situation of scale up. Although the experiment was conducted in lab scale, it is a necessary process to check the effect through scale-up if the goal is industrialization. Compared with the D_{5d} value obtained from the modeling results, the D_{5d} value increased 4.9 times and 5.2 times, respectively, when the volume increased from 10 to 1000 ml in both *S. Typhimurium* and *L. monocytogenes* during the excilamp treatment, and increased 3.21 times and 2.88 times in the same situation treated by UVC LED. As the D value increased about 5 times for excilamp and 3 times for UVC LED, the microbial inactivation effect

of the UVC LED is more efficient when scaling up. In each case, the D_{5d} value increased from 1.07 to 1.4 times at 10 to 100 ml, and increase in *S. Typhimurium* and *L. monocytogenes* increased 2.43, 2.69 times for UVC LED and 3.6, 4.48 times for excilamp at 100 to 1000 ml. And these results suggest further quantitative information that may be useful when industrial scale is to be scaled up for further application.

According to previous research, the mechanism of bactericidal effect using UVC is known to induce the formation of photoproducts due to the direct absorption of photons by pyrimidine and purine nucleic acid bases and then synthesize the thymine dimer which interrupts the normal transcription and translation (Gayán et al. 2014). Kang observed that 280 nm KrCl excilamp inactivates respiratory chain dehydrogenase in the cell and induced lipid peroxidation of the cell membrane, leading to the formation of pores (Kang and Kang 2019). In the aspects of inactivation mechanism, since DNA repair is undesirable for microorganism inactivation, and it is necessary to weaken or prevent the DNA repair process. Inhibiting enzymes involved in DNA repair may be one way to increase the inactivation effect of UVC. According to Kalisvaart, UVC that has wavelengths nearing 280 nm mainly was absorbed by protein and might help damage repair enzymes and prevent DNA repair (Kalisvaart 2004). There may be a difference in the inactivation tendency due to the difference in the mechanism resulted from the wavelength difference between the excilamp and the UVC LED.

However, in the experiments conducted in this study, the inactivation pattern between the two devices would be due to the light penetration depth rather than the mechanism. In water disinfection through the light, penetrability is an important factor, and turbidity is directly connected to the factor influencing this penetrability. UVA (wavelength of 320 to 400 nm) radiation had higher penetrability to turbid water than UVC (wavelength of 200 to 280 nm) (Chevremont et al. 2012). In the same way, since 280 nm UVC LED had better penetrability than 222 nm KrCl excilamp, which led to better the bactericidal effect of UVC LED in second experiment with turbid water samples and the third experiment with increasing scale.

Through a series of experiments, we have been able to obtain the experimental results of applying the 222 nm KrCl excilamp and UVC LED for water disinfection. As demonstrated in this study, turbidity was an important factor in water disinfection, and through the predictive model, the dosage can be calculated to reach a certain level of reduction under specific turbidity conditions. It was also examined the inactivation effects of the excilamp and UVC LED in scale-up condition. In both experiments, the bactericidal effect of UVC LED was better than that of excilamp, thus UVC LED would be more useful for industrial application.

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I. 국문초록

222nm KrCl 엑실램프 및 280 nm 자외선 발광 다이오드의 물살균에 대한 적용을 위한 연구를 진행하였다. *Salmonella Typhimurium* 과 *Listeria monocytogenes* 는 다양한 탁도를 갖는 물 샘플과 접종된 해산물에 스피들이 처리되는 물샘플에서 KrCl 엑실램프와 자외선 발광 다이오드 처리에 의해 저감화 되었다. 해산물 적용하는 실험의 샘플은 굴과 광어를 이용했는데, 굴의 경우 스피들 처리 시에 물의 탁도가 증가하는 현상이 발생하였다. 이때 자외선 발광 다이오드 조사시 예상되는 바와 같이 굴 보다 광어 처리수에서 저감화 효과가 좋았지만, KrCl 엑실램프에서는 반대의 경향성이 나타났다. 이러한 결과는 샘플의 종류와 병원성균과 같이 탁도 이외의 요소 역시 고려해야 한다는 점을 시사한다.

탁도를 가진 물에 자외선을 처리하는 실험에서, 생존곡선을 로그 선형 모델을 사용하여 분석하여 유도된 불활성화 속도 상수 (k)를 구하였으며, Exponential one phase decay model 을 통해서 불활성화 속도 상수와 탁도 간의 예측모델을 얻을 수 있었다. 이러한 예측 모델은 실제 실험을 진행하지 않은 탁도에서 얻은 실험 결과를 이용하여 검증되었다. KrCl 엑실램프 및 자외선 발광 다이오드 처리에 의한 물살균 효과는 탁도가 증가함에 따라 감소하였으며, 개발된 예측 모델은 처리하는 장치, 자외선 조사량, 샘플 탁도에 따라 두 병원균의 저감화 수준을 잘 예측하였다.

스케일 업 상황에서 KrCl 엑실램프 및 자외선 발광 다이오드의 저감화 효과를 비교한 실험 결과를 Weibull 모델을 통해 분석하였으며 계산을 통해서 D_{5d} 값을 얻었다. 처리되는 물의 부피가 100 배 증가할 때, 두 병원균 모두에서 D_{5d} 값이 대략 KrCl 엑실램프에서는 5 배, 자외선 발광 다이오드에서는 대략 3 배 증가하였다. 따라서 스케일 업

환경에서 KrCl 엑실램프보다 자외선 발광 다이오드를 통한 물살균이

더욱 효과적이었다.

주요어: 물살균, 엑실램프, 자외선 발광다이오드, 탁도, 스케일 업

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