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Inactivation modeling of human enteric virus surrogates, MS2, Qβ, and ΦX174, in water using UVC-LEDs, a novel disinfecting system

Do-Kyun Kim [a,b], Soo-Ji Kim [a,b], Dong-Hyun Kang [a,b,*]

[a] Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea
[b] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do 25354, Republic of Korea

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ABSTRACT

In order to assure the microbial safety of drinking water, UVC-LED treatment has emerged as a possible technology to replace the use of conventional low pressure (LP) mercury vapor UV lamps. In this investigation, inactivation of Human Enteric Virus (HuEV) surrogates with UVC-LEDs was investigated in a water disinfection system, and kinetic model equations were applied to depict the surviving infectivities of the viruses. MS2, Qβ, and ΦX 174 bacteriophages were inoculated into sterile distilled water (DW) and irradiated with UVC-LED printed circuit boards (PCBs) (266 nm and 279 nm) or conventional LP lamps. Infectivities of bacteriophages were effectively reduced by up to 7-log after 9 mJ/cm^2 treatment for MS2 and Qβ, and 1 mJ/cm^2 for ΦX 174. UVC-LEDs showed a superior viral inactivation effect compared to conventional LP lamps at the same dose (1 mJ/cm^2). Non-log linear plot patterns were observed, so that Weibull, Biphasic, Log linear-tail, and Weibull-tail model equations were used to fit the virus survival curves. For MS2 and Qβ, Weibull and Biphasic models fit well with R^2 values approximately equal to 0.97–0.99, and the Weibull-tail equation accurately described survival of ΦX 174. The level of UV-susceptibility among coliphages measured by the inactivation rate constant, *k*, was statistically different (ΦX 174 (ssDNA) > MS2, Qβ (ssRNA)), and indicated that sensitivity to UV was attributed to viral genetic material.

1. Introduction

Ultraviolet (UV) disinfection has been widely applied to ensure the safety of potable water as well as for performing food surface pasteurization. UV irradiation controls a broad range of microorganisms including bacterial pathogens, yeasts, and molds, because UV induces nucleotides to produce pyrimidine dimers so that microorganisms cannot replicate DNA and reproduce (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Blatchley et al., 2008; Lopez-Malo, Palou, Barbosa-Canovas, Tapia, & Cano, 2005). Furthermore, UV treatment does not generate by-products and barely affects nutritional values of food. Therefore, UV irradiation has caught the attention of not only the field of water disinfection, but also the food industry. In 2000, the US-FDA approved UV disinfection as an effective method for controlling pathogens and spoilage microorganisms in food, water and beverages.

Traditionally, low pressure (LP) mercury vapor UV lamps have been used to inactivate harmful microorganisms in industrial settings. However, because LP lamps contain mercury, their use entails substantial human health and environmental risks (Aoyagi et al., 2011; Bohrerova, Shemer, Lantis, Impellitteri, & Linden, 2008; Hamamoto et al., 2007). Moreover, the warm-up time requirement for maximum irradiance intensity and temperature-dependent changes in fluence rate are some of the disadvantages of LP lamps. UVC-light emitting diodes (UVC-LEDs), which offer potential as an alternative technology, have been developed and studied recently. UVC-LEDs do not contain mercury, thus alleviating this risk, and prompt maximal irradiation is possible with consistent intensity over a broad temperature range. Furthermore, small size UVC-LED modules can be easily incorporated into various shapes of processing devices for the food industry (Kim, Kim, & Kang, 2016; Shin, Kim, Kim, & Kang, 2016). Still, UVC-LEDs have the disadvantage of low irradiance intensity compared to conventional LP lamps, but the development of LEDs has been rapid and LED lighting systems with wavelengths in the visible spectrum have already been utilized in order to support plant growth with less energy consumption (Craig, Yuk, Khoo, & Zhou, 2015).

Human enteric viruses (HuEV) including hepatitis A virus, rotavirus, and norovirus are primarily causal agents of foodborne illnesses, but HuEV may also cause more severe diseases, such as hepatitis, poliomyelitis, meningitis and others (Abbaszadegan, Lechevallier, & Gerba, 2003; Abzug, 2014; Wong et al., 2009). The fecal-oral route is the main mode for transmission of HuEV, especially through consumption of contaminated drinking water (Lopman et al., 2012; Pallansch &

* Corresponding author at: Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

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Ross, 2001). Therefore, the US-EPA emphasizes that water disinfection systems should achieve 4-log reduction of viruses for surface water (US EPA, 2006). Working with pathogenic viruses poses many technical challenges. For example, a complex culturing method using intestinal stem cells exists for norovirus, several weeks are needed for confirmation of hepatitis A, and difficulty surrounds the culturing of rotavirus. For these reasons, surrogate viruses have been used by many researchers (Aoyagi et al., 2011; Bowker, Sain, Shatalov, & Ducoste, 2011; Meng & Gerba, 1996). Bacteriophages are similar to HuEVs in morphological, structural, and genetic material aspects, so that bacteriophages can feasibly be utilized as tractable and safe surrogates for HuEV (Collins et al., 2006; Grabow, 2001; Shirasaki, Matsushita, Matsui, Urasaki, & Ohno, 2009).

The objective of this study was to evaluate the viral inactivation effect of a UVC-LED water disinfection system. Also, predictive model equations were derived in order to calculate certain irradiation dosages for accomplishing expected inactivation levels by water treatment facilities.

2. Materials and methods

2.1. Experimental setup

UVC-LEDs (LG Innotek Co., Seoul, Korea), emitting peak wavelengths of 266 or 279 nm, were connected to electronic printed circuit boards (PCB). The '4 corners' arrangement with 6 cm distance between the LED modules and 4 cm distance between LED PCB and irradiated food surfaces were set up in accordance with prior research (Kim et al., 2016; Shin et al., 2016). Following protocols of previous investigations (Bolton & Linden, 2003; Kim et al., 2016; Shin et al., 2016), the petri factor indicating even distribution of UV irradiation over the surface was calculated. A petri factor over 0.9, which implies 90% uniformity in light distribution, was measured using the 4-corners array. In order to obtain the petri factor, an area one-eighth of the petri dish surface was scanned by an optical spectrometer (AvaSpec-ULS2048-USB2-UA-50, Avantes; Apeldoorn, Netherlands) for every 5 mm. The probe was maintained at four centimeters distance from the UV sources. The fluence rate of each point was divided by the maximum intensity and averaged to calculate the petri factor. Corrected irradiance and adjusted fluence rate value was calculated by multiplying the maximum intensity by the petri factor value. The required constant electric current was provided by a DC power supply (TPM series, Toyotech; Incheon, Korea); 23 mA for 266 nm PCB, 20 mA for 279 nm PCB.

A low pressure UV lamp (TUV 16 W 4P-SE, Philips; AE Eindhoven, Netherlands) was used to perform conventional water disinfection treatment. Because the intensity of the LP lamp was much higher than that of UVC-LEDs, LP lamp intensity was attenuated by covering it with polypropylene (PP) films (thickness 0.05 mm) in order to adjust the irradiation power to a level equivalent to that of UVC-LEDs (Kim et al., 2016). Every variable, such as distance between UV source and petri dish, treatment time, stirring RPM, was equivalent except for the PP film cover in the LP lamp treatment.

The details of a continuous type water disinfection system were described by Shin et al. (2016) and are schematically shown in Fig. 1. For the system, five UVC-LEDs (peak wavelength: 279 nm; each: 10 mW, total: 50 mW) were combined into a single LED unit module, and four of these LED modules were affixed to the four sides of a manufactured quartz pipe (Kum-Kang Quartz, Gyeonggi, Republic of Korea) to expose water passing through the pipe to 200 mW UVC-LED power.

2.2. Intensity measurements

Irradiance intensity of the 2 UV sources (UVC-LEDs and LP lamp) was evaluated with a spectrometer (AvaSpec-ULS2048-USB2-UA-50, Avantes; Apeldoorn, Netherlands) in accordance with previous research (Kim et al., 2016; Shin et al., 2016). Briefly, the probe was placed at 4 cm distance from UVC sources and intensity values of the total area equivalent to the dimensions of a petri dish were scanned at every 5 mm. In order to obtain the petri factor, all measured intensity ratios, which were divided by maximum irradiance value, were averaged. The petri factors were multiplied by maximum intensity, so that the actual intensity values were normalized and utilized to acquire treatment time for dosages of 0.3, 0.5, 0.7, 1, 2, 3, 6, and 9 mJ/cm^2.

2.3. Cultivation and assay of viruses

Bacteriophage MS2 (ATCC 15597-B1), Qβ (ATCC 23631-B1), and ΦX 174 (ATCC 13706-B1), and their host strains, *Escherichia coli* C3000 (ATCC 15597) and *E. coli* CN13 (ATCC 700609), were obtained from the Culture Collection at Seoul National University (Seoul, Republic of Korea). The phages were propagated by inoculating 1 ml of late exponential or early stationary phase host strain (*E. coli* C3000 for MS2 and Qβ; or *E. coli* CN13 for ΦX 174) into 50 ml of tryptic soy broth (TSB) (Difco, Becton Dickinson and Company, Sparks, MD, USA) and incubating overnight at 37 °C. One-hundred microliter of each stock coliphage and 500 μl of host strain were combined and incubated overnight at 37 °C. The cultures were centrifuged for 20 min at 4000 ×*g*, and the supernatant was carefully collected into sterile 15 ml conical disposable centrifuge tubes and stored at −70 °C until investigation.

The phages were assayed using the soft agar overlay (double-agar layer) plaque assay method (Cho, Gandhi, Hwang, Lee, & Kim, 2011; Kropinski, Mazzocco, Waddell, Lingohr, & Johnson, 2009). One hundred microliter of overnight-incubated host strains were inoculated into 5 ml TSB and incubated for 4 h at 37 °C; the resulting early log-phase host strains were plated onto the bottom layer of agar to produce a bacterial lawn. The bottom agar layer contained 1 g/l yeast extract, 1 g/l glucose, 8 g/l sodium chloride, 0.22 g/l calcium chloride, 10 g/l tryptone, and 15 g/l agar. Also, LB broth (Difco) with 1% (w/v) agar (Difco) was used for the soft agar overlay (method described in Section 2.4).

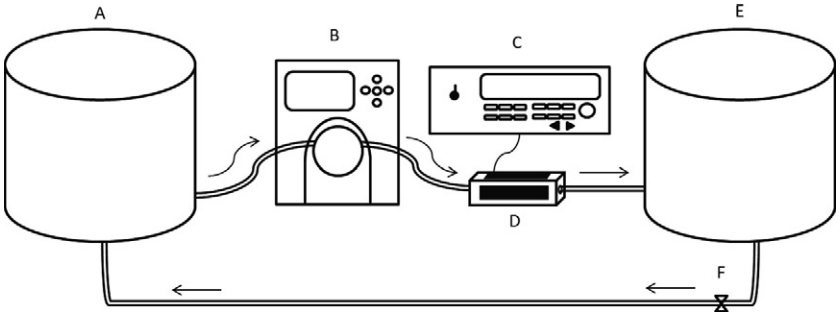

Fig. 1. Schematic depiction of continuous type water disinfection system. Liquid sample flow was adjusted to 100 ml per minute (mLPM) and 300 mLPM with the peristaltic pump. Components: A, viral reservoir before UVC treatment; B, peristaltic pump; C, power supply; D, UVC-LED modules attached to quartz pipe; E, viral reservoir after UVC treatment; F, ball type valve.

2.4. UVC treatment and enumeration of virus

For the batch-type disinfection system, 10 ml of sterile distilled water (DW) (room temperature; 22 ± 1 °C) was transferred into a 90 mm petri dish, and 100 μl of the bacteriophage being evaluated was added (final concentration; 8–9 Log PFU/ml). The suspension was stirred at 300 rpm by a magnetic stirrer (TM-17R, Jeio Tech; Daejeon, Republic of Korea) to facilitate thorough mixing as well as uniform dose distribution. UVC-LED PCBs (266 or 279 nm) or a LP lamp (covered by PP films) was placed at 4 cm distance above the sample and vertically collimated so that UV light could reach the liquid surface perpendicularly. The samples were then treated in a chamber (TH-TG-300, Jeio Tech; Daejeon, Republic of Korea).

Details of the continuous type water disinfection system were described previously by Shin et al. (2016). One liter of sterile DW was inoculated with 1 ml of bacteriophage stock (final concentration; 10^{5-6} PFU/ml), and this virus reservoir (Fig. 1) was transferred by peristaltic pump (JWS600, JenieWell; Seoul, Republic of Korea) through a sterile silicon tube terminating in a manufactured quartz pipe (3 × 3 × 11 mm, 10 mm diameter) (Kum-Kang Quartz, Gyeonggi, Republic of Korea). In the middle of the quartz pipe, LED unit modules (peak wavelength 279 nm; total power 200 mW) or an intensity-attenuated LP lamp was attached such that viral inactivation occurred through the pipe. Because the dosage of UVC treatment of the single-pass system was not enough to inactivate the virus, the inoculated liquid was re-circulated and irradiated for up to 5 times (passes). Theoretically, the retention times of the liquid circulated through the quartz pipe were 5.2 s and 1.7 s for 100 mLPM and 300 mLPM, respectively.

After treatment, samples were 10-fold serially diluted with 0.2% (w/v) peptone water (Bacto, Becton, Dickinson and Company; Sparks, MD, USA). One-hundred microliter of selected diluents were aliquoted to 5 ml soft agar tempered to 50 °C in which 100 μl of the corresponding log-phase host bacterium was added, and the soft agar was gently vortexed and carefully poured onto the bottom agar layer so as not to generate bubbles. After solidification, agar plates were incubated at 37 °C for 24 h, and typical plaques were enumerated.

2.5. Modeling of survival curves

All experiments were conducted with duplicate samples and repeated three times or with three trials, and survival curves from batch-type water disinfection trials were fitted with the 4 typical non-log linear model equations by using GInaFiT (Geeraerd, Valdramidis, & Van Impe, 2005); 1. Weibull model (Eq. (1)), 2. Biphasic model (Eq. (2)), 3. Log linear-tail model (Eq. (3)), 4. Weibull-tail model (Eq. (4))

The Weibull model equation (Van Boekel, 2002) is described as:

$$\log N = \log N_0 - \left(\frac{t}{\alpha}\right)^{\beta} \quad (1)$$

where α value represents the time for achieving 1 log reduction at the first stage of inactivation and β value represents the shape of the line, such as upward concavity of a curve when β < 1, downward concavity when β > 1, and linear curve when β = 1.

The Biphasic model equation (Cerf, 1977) is given by:

$$\log N = \log N_0 + \log\left[f \times e^{-k_{max1} \times t} + (1-f) \times e^{-k_{max2} \times t}\right] \quad (2)$$

where f indicates the fraction of the initial population in a major subpopulation, $(1 - f)$ is the fraction of the initial population in a minor subpopulation. Also, the 2 slopes from a biphasic curve are determined by k_{max1} and k_{max2}, which indicate specific inactivation rates of the 2 populations, respectively.

The log linear-tail model equation (Geeraerd, Herremans, & Van Impe, 2000) is described as:

$$\log N = \log\left[\left(10^{N_0} - 10^{N_{res}}\right) \times e^{-k_{max} \times t} + 10^{\log N_{res}}\right] \quad (3)$$

where k_{max} indicates a specific inactivation rate in the linear fraction, and N_{res} means the remaining population density after treatment.

The Weibull-tail model equation (Albert & Mafart, 2005) is described as:

$$\log N = \log\left[\left(10^{\log N_0} - 10^{\log N_{res}}\right) \times 10^{\left(\frac{-t}{\alpha}\right)^{\beta}} + 10^{\log N_{res}}\right] \quad (4)$$

Because this equation is derived from the Weibull model and represents the tailing effect, the parameters are from Eqs. (1) and (3). In order to estimate the goodness of fit of the 4 modeling equations, the regression coefficient (R^2) and mean square error (MSE) were assessed. Also, D_{3d} and D_{5d} which indicate dosages necessary for achieving 3 log- and 5 log-reductions by UVC-LED irradiation from each modeling equation were analyzed by Microsoft Excel 2010. After describing the suitable equations in terms of independent variables, dose values for the certain log reductions were calculated by the 'Goal seek' function.

The inactivation rate constant, k, which indicates the level of log inactivation when an irradiation dose of 1 mJ/cm^2 is imposed within the linear range of survival curves, were calculated. Based on the survival curves (Biphasic model for MS2 and Qβ, Weibull-tail model for ΦX 174), dosages of 0–1 mJ/cm^2 were established for the linear ranges of all the viruses, and the levels of viral reductions were averaged to draw the inactivation rate constants of the three viruses.

2.6. Statistical analysis

All experiments were conducted with duplicate samples and repeated three times or with three trials. The D_{3d} and D_{5d} values, the continuous-type disinfection trials, and inactivation rate constants, k were analyzed with ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Tukey's multiple range test to determine if there were significant differences ($P < 0.05$) between the mean values.

3. Results

3.1. Inactivation of viruses by batch type and flow type UV treatment

Figs. 2–4 show surviving infectivities of MS2, Qβ, and ΦX 174 after batch-type UV treatment. MS2 and Qβ showed similar inactivation rates, and ΦX 174 was the most sensitive to UVC treatment. Especially, both 266 and 279 nm UVC-LED treatment showed effective viral inactivation. For 266 nm UVC-LEDs, over 7 log reductions were observed in the three HuEV surrogates when exposed to 9 mJ/cm^2 for MS2 and Qβ, and 1 mJ/cm^2 for ΦX 174. And, when using 279 nm UVC-LEDs, viruses were inactivated by up to approximately 6 log after dosages of 9 mJ/cm^2 for MS2 and Qβ, and 2 mJ/cm^2 for ΦX 174. However, the conventional LP lamp showed less virucidal effect compared to UVC-LEDs. The coliphages were reduced by 3.7, 3.9, and 4.2 log for MS2, Qβ, and ΦX 174, respectively, when exposed to 9 mJ/cm^2, and ΦX 174 also showed statistically significant greater sensitivity to UV irradiation among the three viruses ($P < 0.05$).

Inactivation of the three HuEV surrogates by flow-type UVC treatment is presented in Figs. 5 and 6. As the number of both treatments recirculation passes increased, log reductions of viruses also gradually increased. In the UVC-LED system, for MS2, 2.3 log reduction was achieved by 5 repeat passes at a flow rate of 100 mLPM and by <2 log with 5 repeat passes at 300 mLPM. Similar results were observed for Qβ; however ΦX 174, which demonstrated higher sensitivity, was inactivated by 4.6 log with only 3 repeat passes at 300 mLPM. Similar to the batch-type water disinfection system, lower levels of viral

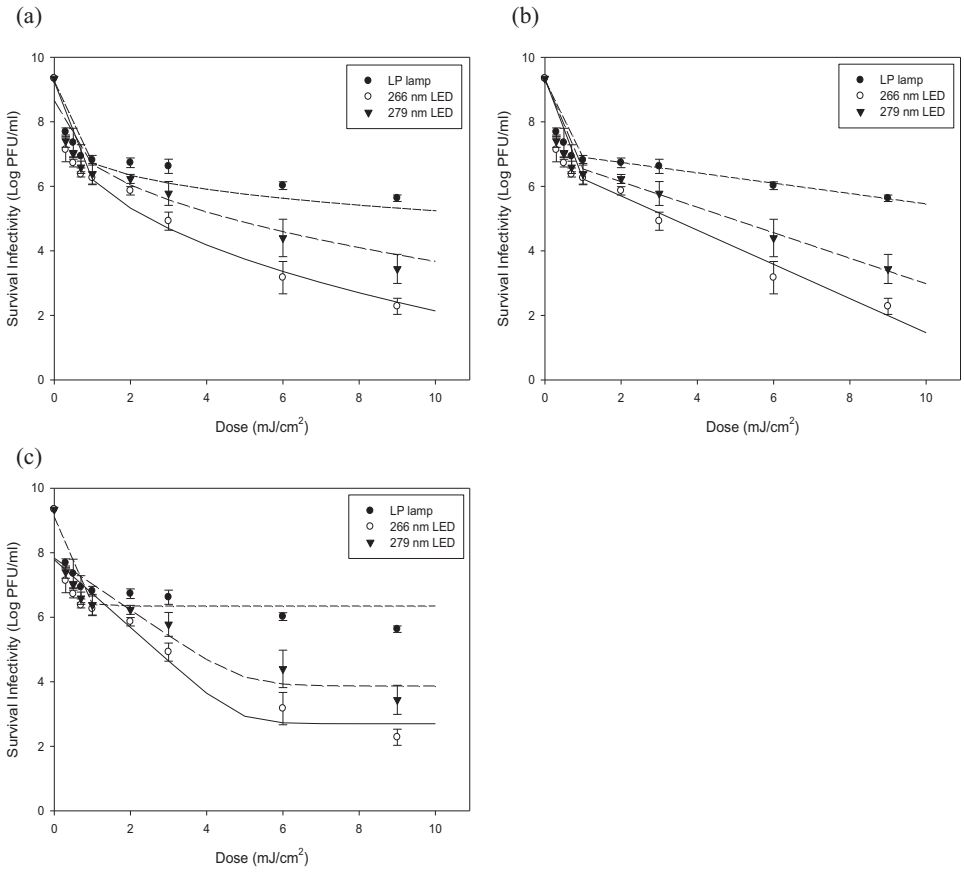

Fig. 2. Plotting and analysis of the surviving infectivity of MS2 after UVC-LED irradiation with model equations: (a) Weibull model, (b) Biphasic model, and (c) Log linear-tail model.

inactivation were achieved with the LP lamp system compared to those levels using the UVC-LED system (Fig. 6). There were statistical differences between the inactivation efficacies of UVC-LED and LP lamp in five-time treatments for both flow rates (100, 300 mLPM) except for 100 mLPM treatment of MS2 (data not shown). Especially, only 2.8 log reduction of ΦX 174 was achieved by the LP lamp after five-time treatment.

3.2. Applying model equation to fit inactivation curves

Four potentially applicable equations such as Weibull, Biphasic, Log linear-tail, and Weibull-tail models were fitted to the viral survival data and the results are shown in Figs. 2–4.

For MS2 and Qβ phages, surviving infectivity data were analyzed by Weibull, Biphasic, and Log linear-tail models. Meanwhile, ΦX 174 data were analyzed by Weibull, Weibull-tail, and Log linear-tail models. Regression lines fitted well based on MSE and R^2 when Weibull and Biphasic models were applied to the surviving infectivities of MS2 (Table 1a), and MSE values of approximately 0.04 for the UV sources were calculated by the Biphasic model. Similarly, Weibull and Biphasic equations depicted the surviving infectivities of Qβ very well (Table 1b). The Weibull model described the results obtained with UVC-LEDs very well, while those of the LP lamp were well characterized by the Biphasic model, as shown by a 0.09 MSE. Because MS2 and Qβ showed insignificant tailing effects, the log linear-tail model was not appropriate for representing the infectivities. On the other hand, ΦX 174 showed a noteworthy tailing effect, so the Weibull-tail equation was applied instead of the Biphasic model. The Weibull-tail model showed a MSE of approximately 0.05, while the Log linear-tail represented the survival curves fairly well (MSE = 0.15–0.20), whereas Weibull did not, especially plots after a dosage of 1 mJ/cm^2.

The D_{3d} and D_{5d} values from the 3 modeling equations are presented in Table 1. For MS2, approximately 2 mJ/cm^2 dosage with 266 nm UVC-LEDs could accomplish a 3-log reduction based on the Weibull and Biphasic models while the LP lamp needed about 7–8 mJ/cm^2 for the same level of disinfection, which demonstrated the different efficacies of UV sources in inactivating viruses. The LP lamp t_{5d} showed a large difference between the Weibull and Biphasic models, so 3–10 times greater irradiance dose was indicated by the Weibull model. Meanwhile, the Log linear-tail model had poor goodness of fit, so that the necessary dose values were not thought to be as reliable as those values obtained from other equations. For Qβ, 1–2 mJ/cm^2 doses were needed to achieve 3-log reduction according to the Weibull and Biphasic models, and 4–7 mJ/cm^2 doses for 5-log reduction. Compared to the other viruses, ΦX 174 showed greater UV-sensitive properties so low doses of UVC irradiation could easily inactivate this coliphage. The LP lamp could not achieve 5-log reduction of ΦX 174 in the Weibull-tail and Log linear-tail equations. Even though there were different viral inactivation effects among the peak wavelengths, based on t_{xd} values, 266 nm and 279 nm UVC-LED showed statistically identical virucidal effects in all the three viruses.

Table 2 shows inactivation rate constants, which indicate UV-susceptibility of the three coliphages in the early stage of linear inactivation. Sensitivity of MS2 and Qβ to UV irradiation were not statistically different ($P > 0.05$), while ΦX 174 had a significantly greater level of susceptibility ($P < 0.05$) to both the LP lamp and UVC-LEDs (266 nm and 279 nm). The k values showed significant differences between the LP lamp and 266 nm UVC-LEDs for Qβ and ΦX 174. For MS2, differences

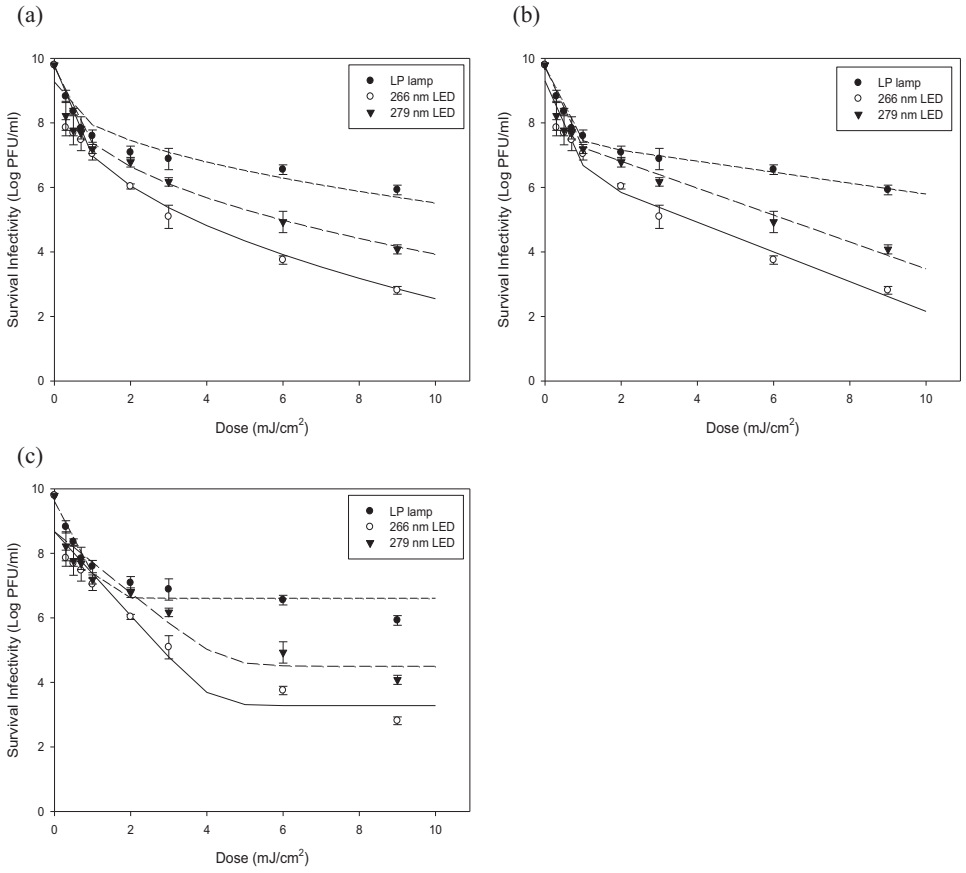

Fig. 3. Plotting and analysis of the surviving infectivity of Qβ after UVC-LED irradiation with model equations: (a) Weibull model, (b) Biphasic model, and (c) Log linear-tail model.

in numerical *k* values between the LP lamp and UVC-LEDs existed but no statistical differences were demonstrated.

4. Discussion

Securement of potable water is considered a significant issue throughout the world. WHO reported that 0.5 million deaths per year were attributed to poor drinking-water and the deaths primarily occur in developing countries (World Health Organization, 2014). In order to ensure safe water, UV disinfection has been investigated and its potential inactivation effect has been demonstrated (Hijnen, Beerendonk, & Medema, 2006). Artificially inoculated water of various types has been treated with UV and chlorine, and approximately 6-log reductions of *E. coli* and *Shigella dysenteriae* were achieved while minimizing photoreactivation (Wang, Hu, Hu, & Wei, 2011). A synergistic effect in microbial inactivation was observed after UV and chlorine treatment followed by addition of H_2O_2 because H_2O_2 helped to degrade organic pollutants (Cho, Cates and Kim, 2011). Also, in 2000, UVC irradiation was approved as one of the effective control methods to ensure food, water and beverage safety by the US-FDA.

In addition, virus inactivating efficacy of UV light has also been studied. The conventional low pressure (LP) UV lamp inactivated mammalian RNA viruses, such as murine norovirus and feline calicivirus, by up to over 4 log PFU/ml at 30–40 mJ/cm^2. MS2 showed relatively higher resistance to UV treatment, so that approximately 2 log reduction was achieved by 40 mJ/cm^2 UVC irradiation using the same system (Park, Linden, & Sobsey, 2011). Influenza viruses H5N1 and H1N1 easily lost their infectivity following LP lamp irradiation at dosages of 25–60 mJ/cm^2. Likewise previous studies have shown that other viruses are more sensitive to UV treatment than is MS2 (2 log reduction at 40 mJ/cm^2) (Lenes et al., 2010). Also, using UVC LEDs (255 and 280 nm), MS2 and Qβ were reduced by 1.5–3 log PFU/ml at 40 mJ/cm2 (Aoyagi et al., 2011; Bowker et al., 2011).

Although conventional UVC LP lamps are used in disinfection systems, especially for water treatment, the potential risks from mercury, the gas used in LP lamps, pose a major hazard to human health as well as to the environment (Chevremont, Boudenne, Coulomb, & Farnet, 2013). Also, the necessity of a long warm-up time for maximum irradiance intensity and poor irradiance at low temperature are critical limitations of commercial LP lamps (Kim et al., 2016; Shin et al., 2016).

LED-based UVC irradiation has been researched recently, with the hope of validating this technology as an alternative to conventional LP lamps, because of its advantages such as selection of emitting wavelengths, higher durability, convenient incorporation into process devices, and environmental friendliness (Bowker et al., 2011; Chatterley & Linden, 2010; Würtele et al., 2011). Lately, UVC-LED treatment was applied as a pasteurization intervention to inactivate major foodborne pathogens, such as *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* inoculated onto sliced cheese surfaces as well as to disinfect pathogens in water (Kim et al., 2016; Shin et al., 2016). A LED type UV system has recently been incorporated into domestic humidifiers to disinfect the water reservoir, because mass production of UVC-LEDs has become possible through increased development of UVC-LED technology.

In this research, we assessed the virucidal effect of UVC-LEDs, a novel inactivation technology, and a conventional LP lamp and applied kinetic model equations to describe the survival infectivities of human enteric virus surrogates. In the present study, water disinfection was carried out using distilled water (DW) that contains no organic materials in order to model ideal conditions. It is thought necessary to establish kinetic model equations under standard conditions by using DW to minimize the interfering effects of other compound such as phosphates and other minerals. Also, it is known that inactivation of microorganisms by UV is not influenced by environmental conditions including pH,

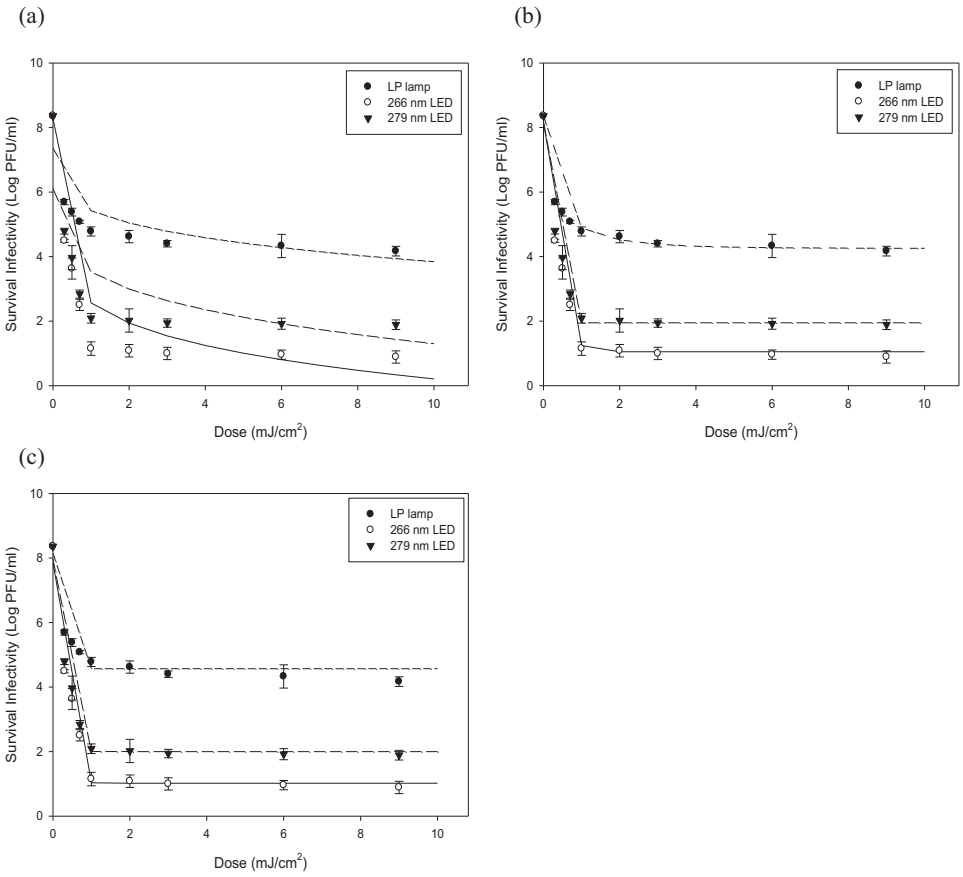

Fig. 4. Plotting and analysis of the surviving infectivity of ΦX 174 after UVC-LED irradiation with model equations: (a) Weibull model, (b) Biphasic model, and (c) Log linear-tail model.

temperature and reactive organic substances (Hijnen et al., 2006), however, UV transmission would be affected by any substances which increasing opaqueness of liquid samples. Different inactivation levels of *E. coli* were observed in products of liquid egg white, liquid whole egg, and liquid egg yolk. Lower inactivation was achieved in higher turbidity egg products such as whole egg and egg yolk (Unluturk, Atilgan, Baysal, & Tari, 2008). Furthermore, Ngadi, Smith, and Cayouette (2003) showed that pH level did not affect the inactivation of *E. coli* O157:H7 in apple juice and egg white, but depth of UV transmission had strongly effected on microbial reduction. Therefore, inconsistent results could be drawn in real water disinfection system dealing with organic-abundant water.

Inactivation of viruses showed a similar trend in that higher dosages of UV treatment led to higher reduction levels in three HuEV surrogates. However, UV susceptibility was distinguished in terms of generic materials that viruses inherit; single stranded DNA (ssDNA) virus ΦX 174 or single stranded RNA (ssRNA) viruses MS2 and Qβ (Table 2). The UV sensitivity of viruses can be evaluated by the inactivation rate constant, k (cm^2/mJ) (Hijnen et al., 2006; Song, Mohseni, & Taghipour, 2016). The k values for MS2 and Qβ were not significantly different regardless of treatment conditions. However, statistically higher UV-sensitivity, namely a higher inactivation rate constant, was observed in ΦX 174. We found susceptibility of the three viruses to UV to be consistent with that reported by other research studies (Aoyagi et al., 2011; Hijnen et al., 2006), although there are differences in numerical values of k because UVC-LED manufacturing technology has improved such that higher irradiance intensity can be generated. However, other researchers only controlled dosage values so that irradiance time, distance, and intensity were different between LP lamps and UVC-LEDs, because of the much higher fluence rates in LP lamps. In this study, we maintained a fixed distance between UV-sources and petri dish and adjusted the irradiance intensity of the conventional LP lamp to a level of similar to that of UVC-LEDs by attenuating the fluence rate with layers of 0.05 mm thickness polypropylene (PP) films.

In many cases, survival curves following UV treatment showed dose-dependent linear regression lines (Aoyagi et al., 2011; Bowker et al., 2011; Mamane-Gravetz, Linden, Cabaj, & Sommer, 2005; Meng & Gerba, 1996). However, this study showed non-linear inactivation curves, so Weibull, Biphasic, Log linear-tail, and Weibull-tail model equations were applied to describe the viral survival patterns. The survival curve after treatment with a conventional LP lamp was located uppermost among the three regression lines. The 279 nm UVC-LEDs treatment curve fell in the middle of the distribution of lines, and the lower lines represented the regression line of 266 nm UVC-LEDs treatment. This demonstrates that the virucidal effect of UVC-LEDs was statistically much greater than that of the LP lamp ($P < 0.05$). There were corresponding results for Qβ and ΦX 174. Similar results were reported indicating slightly higher inactivation for 255 nm compared to 275 nm LEDs for MS2 (Bowker et al., 2011). Maximum spectral sensitivity of MS2 was demonstrated at around 260 nm (Mamane-Gravetz et al., 2005), so 266 nm LEDs used in the present study showed effective disinfection. LP lamps were shown by some researchers to have the least disinfection efficacy (Song et al., 2016), but further research is needed to investigate UV source-dependent inactivation levels.

The doses necessary for achieving 3 log- and 5 log-reductions were calculated by 'Goal seek' function in Excel using each kinetic equation. With 266 nm LEDs, approximately 1 and 5 mJ/cm^2 could accomplish 3 and 5 log- reductions based on the Biphasic model equation, while 5 and 17 mJ/cm^2 were needed for the same level of reduction with the LP lamp. The D_{3d} and D_{5d} values for Qβ were similar to those of MS2 when using the Biphasic model. However, much lower values were

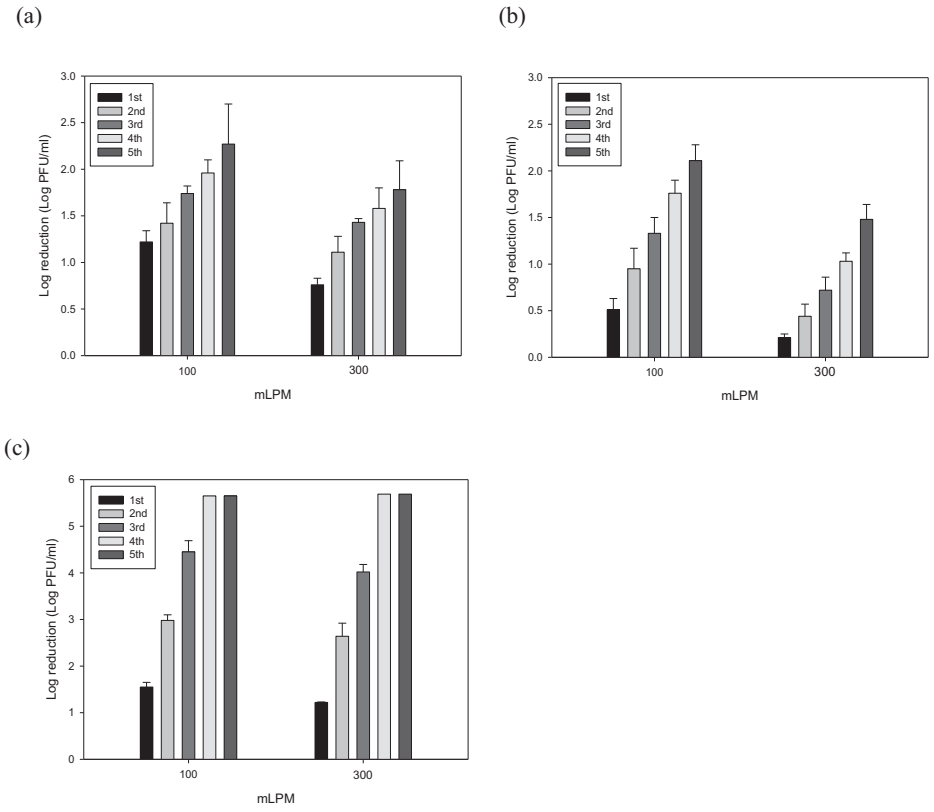

Fig. 5. Accumulative log reduction of MS2 (a), Qβ (b), and ΦX 174 (c) after treatment with a continuous-type UVC-LED water disinfection system for a number of repetitive treatment recirculation passes at two flow rates: 100 mLPM and 300 mLPM.

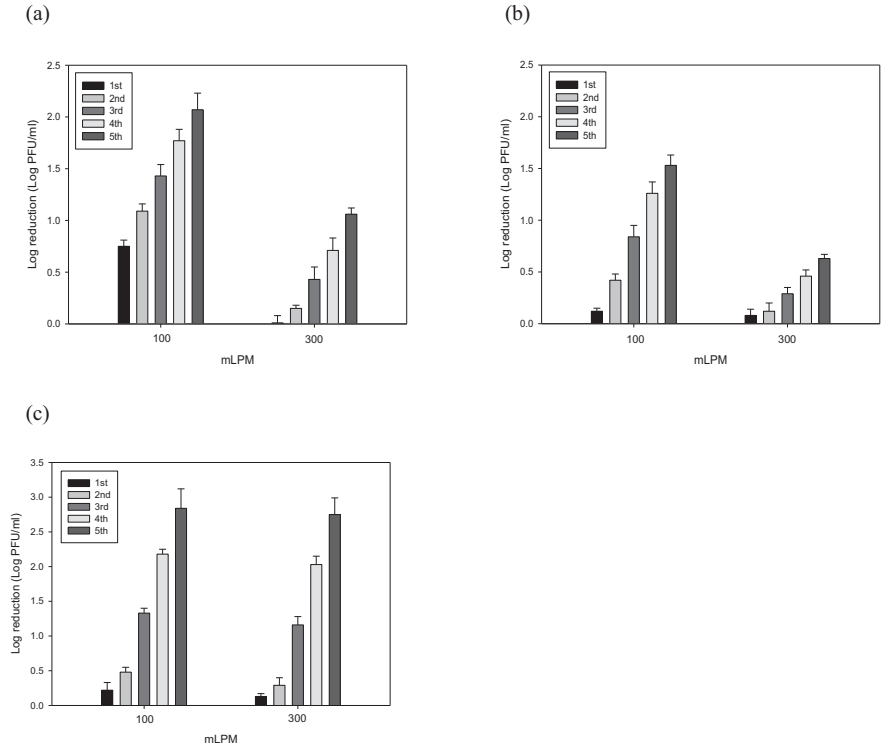

Fig. 6. Accumulative log reduction of MS2 (a), Qβ (b), and ΦX 174 (c) after treatment with a continuous-type low-pressure UV lamp (254 nm) water disinfection system for a number of repetitive treatment recirculation passes at two flow rates: 100 mLPM and 300 mLPM.

Table 1
Comparison of goodness of fit of kinetic model equations for surviving infectivities of MS2 (a), Qβ (b), and ΦX 174 (c) in a batch-type UVC-LED water disinfection system and the calculated D_{3d} and D_{5d} values which indicate UV dosages necessary for 3- or 5- log reduction from each model.

(a)

	Weibull				Biphasic				Log-tail			
	MSE	R^2	D_{3d}	D_{5d}	MSE	R^2	D_{3d}	D_{5d}	MSE	R^2	D_{3d}	D_{5d}
LP lamp	0.0291	0.9809	7.38 ± 7.00 A[a]	75.05 ± 21.94 A	0.0143	0.9922	4.63 ± 0.92 A	17.51 ± 0.80 A	0.2012	0.8681	ND[b]	ND
266 nm	0.0848	0.9858	1.73 ± 0.65 A	5.08 ± 0.82 B	0.0753	0.9895	1.51 ± 1.00 B	5.21 ± 0.61 B	0.6660	0.8883	2.88 ± 0.15 A	ND
279 nm	0.1594	0.9586	1.64 ± 0.53 A	6.91 ± 1.10 B	0.0221	0.9952	2.43 ± 1.14 AB	9.73 ± 5.08 B	0.6499	0.8313	3.85 ± 0.66 A	ND

(b)

	Weibull				Biphasic				Log-tail			
	MSE	R^2	D_{3d}	D_{5d}	MSE	R^2	D_{3d}	D_{5d}	MSE	R^2	D_{3d}	D_{5d}
LP lamp	0.1479	0.9234	5.45 ± 1.61 A	24.93 ± 7.88 A	0.0105	0.9955	4.11 ± 1.58 A	16.77 ± 3.16 A	0.1491	0.9228	ND	ND
266 nm	0.0216	0.9966	1.28 ± 0.10 B	4.03 ± 0.08 B	0.2008	0.9740	1.27 + 0.22 B	4.84 ± 0.69 B	0.3806	0.9408	2.33 ± 0.16 A	4.02 ± 0.35
279 nm	0.0187	0.9953	2.23 ± 0.68 B	7.39 ± 1.48 B	0.0497	0.9896	2.01 ± 0.19 AB	6.95 ± 0.16 B	0.3837	0.9037	3.23 ± 0.50 B	ND

(c)

	Weibull				Weibull-tail				Log-tail			
	MSE	R^2	D_{3d}	D_{5d}	MSE	R^2	D_{3d}	D_{5d}	MSE	R^2	D_{3d}	D_{5d}
LP lamp	0.3861	0.8251	3.69 ± 2.94 A	36.24 ± 16.53 A	0.0080	0.9970	0.63 ± 0.14 A	ND	0.1535	0.9305	0.47 ± 0.01 A	ND
266 nm	0.7663	0.9087	3.30 ± 4.24 A	11.04 ± 14.35 A	0.1174	0.9883	0.22 ± 0.02 B	0.53 ± 0.01 A	0.2061	0.9754	0.36 ± 0.01 A	0.60 ± 0.01 A
279 nm	1.5727	0.7472	4.25 ± 5.26 A	16.57 ± 22.00 A	0.0240	0.9968	0.24 ± 0.08 B	0.53 ± 0.35 A	0.1462	0.9765	0.28 ± 0.09 A	0.68 ± 0.16 A

[a] Data represent means ± standard deviations of three measurements after UV treatment. Values within columns followed by the same upper case letters are not statistically different ($P > 0.05$).
[b] ND; Not determined.

calculated for ΦX 174 with the Weibull-tail model because this coliphage retained the highest UV-susceptibility.

5. Conclusion

Through this study, the efficacy of inactivating human enteric virus surrogates by using UVC-LEDs was assessed. UVC-LED treatment showed a strong virucidal effect compared to conventional LP lamps in a batch-type water disinfection system. However, the flow and circulating disinfection system needed improvement to accomplish higher reduction of HuEVs. By using kinetics modeling, dosages necessary for 3 to 5 log reduction were calculated, and the proper UVC-LED treatment level for sustaining potable water safety can be determined from the data.

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Table 2
Inactivation rate constant, k (cm^2/mJ), from the first linear portion of survival infectivity curves of MS2, Qβ, and ΦX 174 after UV treatments.

	Inactivation rate constant, k (cm^2/mJ)		
	MS2	Qβ	ΦX 174
LP lamp	2.53 ± 0.15 Aa[a]	2.20 ± 0.19 Aa	3.58 ± 0.14 Ba
266 nm LEDs	3.08 ± 0.19 Aa	2.75 ± 0.19 Ab	7.40 ± 0.14 Bc
279 nm LEDs	2.96 ± 0.33 Aa	2.60 ± 0.14 Aab	5.09 ± 0.15 Bb

[a] Data represent means ± standard deviations of three measurements after UV treatment. Values within rows followed by the same upper case letters and values within columns followed by the same lower case letters are not statistically different ($P > 0.05$).

References

Abbaszadegan, M., Lechevallier, M., & Gerba, C. (2003). Occurrence of viruses in US groundwaters. *American Water Works Association Journal*, *95*, 107–120.
Abzug, M. J. (2014). The enteroviruses: Problems in need of treatments. *Journal of Infection*, *68*, S108–S114.
Albert, I., & Mafart, P. (2005). A modified Weibull model for bacterial inactivation. *International Journal of Food Microbiology*, *100*, 197–211.
Aoyagi, Y., Takeuchi, M., Yoshida, K., Kurouchi, M., Yasui, N., Kamiko, N., ... Nanishi, Y. (2011). Inactivation of bacterial viruses in water using deep ultraviolet semiconductor light-emitting diode. *Journal of Environmental Engineering*, *137*, 1215–1218.
Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry - a critical review. *Journal of the Science of Food and Agriculture*, *80*, 637–645.
Blatchley, E. R., Shen, C., Scheible, O. K., Robinson, J. P., Ragheb, K., Bergstrom, D. E., & Rokjer, K. (2008). Validation of large-scale, monochromatic UV disinfection systems for drinking water using dyed microspheres. *Water Research*, *42*, 677–688.
Bohrerova, z., Shemer, H., Lantis, R., Impellitteri, C. A., & Linden, K. G. (2008). Comparative disinfection efficiency of pulse and continuous-wave UV irradiation technologies. *Water Research*, *42*, 2975–2982.
Bolton, J. R., & Linden, K. G. (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering*, *129*, 209–215.
Bowker, C., Sain, A., Shatalov, M., & Ducoste, J. (2011). Microbial UV fluence-response assessment using a novel UV-LED collimated beam system. *Water Research*, *45*, 2011–2019.
Cerf, O. (1977). Tailing of survival curves of bacterial spores. *Journal of Applied Bacteriology*, *42*, 1–19.
Chatterley, C., & Linden, K. (2010). Demonstration and evaluation of germicidal UV-LEDs for point-of-use water disinfection. *Journal of Water and Health*, *8*, 479–486.
Chevremont, A. C., Boudenne, J. L., Coulomb, B., & Farnet, A. M. (2013). Impact of watering with UV-LED-treated wastewater on microbial and physico-chemical parameters of soil. *Water Research*, *47*, 1971–1982.
Cho, M., Cates, E. L., & Kim, J. -H. (2011a). Inactivation and surface interactions of MS-2 bacteriophage in a TiO_2 photoelectrocatalytic reactor. *Water Research*, *45*, 2104–2110.
Cho, M., Gandhi, V., Hwang, T. -M., Lee, S., & Kim, J. -H. (2011b). Investigating synergism during sequential inactivation of MS-2 phage and *Bacillus subtilis* spores with UV/H_2O_2 followed by free chlorine. *Water Research*, *45*, 1063–1070.
Collins, K. E., Cronin, A. A., Rueedi, J., Pedley, S., Joyce, E., Humble, P. J., & Tellam, J. H. (2006). Fate and transport of bacteriophage in UK aquifers as surrogates for pathogenic viruses. *Engineering Geology*, *85*, 33–38.
Craig, D.'. S., Yuk, H. -G., Khoo, G. H., & Zhou, W. (2015). Application of light-emitting diodes in food production, postharvest preservation, and microbiological food safety. *Comprehensive Reviews in Food Science and Food Safety*, *14*, 719–740.
Geeraerd, A. H., Herremans, C. H., & Van Impe, J. F. (2000). Structure model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, *59*, 185–209.
Geeraerd, A. H., Valdramidis, V. P., & Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, *102*, 95–105.

Grabow, W. O. K. (2001). Bacteriophages: Update on application as models for viruses in water. *Water SA*, *27*, 251–268.

Hamamoto, A., Mori, M., Takahashi, A., Nakano, M., Wakikawa, N., Akutagawa, M., ... Kinouchi, Y. (2007). New water disinfection system using UVA light-emitting diodes. *Journal of Applied Microbiology*, *103*, 2291–2298.

Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. F. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, *40*, 3–22.

Kim, S. -J., Kim, D. -K., & Kang, D. -H. (2016). Using UVC light-emitting diodes at wavelengths of 266 to 279 nanometers to inactivate foodborne pathogens and pasteurize sliced cheese. *Applied and Environmental Microbiology*, *82*, 11–17.

Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of bacteriophages by double agar overlay plaque assay. In M. R. J. Clokie, & A. M. Kropinski (Eds.), *Bacteriophages; methods and protocols (Vol 1: Isolation, characterization, and interactions)*. Leicester, UK: Humana Press.

Lenes, D., Deboosere, N., Menard-Szczebara, F., Jossent, J., Alexandre, V., Machinal, C., & Vialette, M. (2010). Assessment of the removal and inactivation of influenza viruses H5N1 and H1N1 by drinking water treatment. *Water Research*, *44*, 2473–2486.

Lopez-Malo, A., Palou, E., Barbosa-Canovas, G. V., Tapia, M. S., & Cano, M. P. (2005). Ultraviolet light and food preservation. *Novel food processing technologies* (pp. 405–422). Madrid, Spain: CRC Press.

Lopman, B., Gastanaduy, P., Park, G. W., Hall, A. J., Parashar, U. D., & Vinje, J. (2012). Environmental transmission of norovirus gastroenteritis. *Current Opinion in Virology*, *2*, 96–102.

Mamane-Gravetz, H., Linden, K. G., Cabaj, A., & Sommer, R. (2005). Spectral sensitivity of *Bacillus subtilis* spores and *MS2 Coliphage* for validation testing of ultraviolet reactors for water disinfection. *Environmental Science and Technology*, *39*, 7845–7852.

Meng, Q. S., & Gerba, C. P. (1996). Comparative inactivation of enteric adenoviruses, poliovirus and coliphages by ultraviolet irradiation. *Water Research*, *30*, 2665–2668.

Ngadi, M., Smith, J. P., & Cayouette, B. (2003). Kinetics of ultraviolet light inactivation of *Escherichia coli* O157:H7 in liquid foods. *Journal of the Science of Food and Agriculture*, *83*, 1551–1555.

Pallansch, M. A., & Ross, R. P. (2001). Enteroviruses: Polioviruses, coxackieviruses, echoviruses, and newer enteroviruses. In D. M. Knipe, P. M. Howley, & D. E. Griffin (Eds.), *Fields virology* (4th ed.). Philadelphia: Lippincott Williams & Wilkins.

Park, G. W., Linden, K. G., & Sobsey, M. D. (2011). Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. *Letters in Applied Microbiology*, *52*, 162–167.

Shin, J. -Y., Kim, S. -J., Kim, D. -K., & Kang, D. -H. (2016). Fundamental characteristics of deep-UV light-emitting diodes and their application to control foodborne pathogens. *Applied and Environmental Microbiology*, *82*, 2–10.

Shirasaki, N., Matsushita, T., Matsui, Y., Urasaki, T., & Ohno, K. (2009). Comparison of behaviors of two surrogates for pathogenic waterborne viruses, bacteriophages Qβ and MS2, during the aluminum coagulation process. *Water Research*, *43*, 605–612.

Song, K., Mohseni, M., & Taghipour, F. (2016). Application of ultraviolet light-emitting diodes (UV-LEDs) for water disinfection: A review. *Water Research*, *94*, 341–349.

Unluturk, S., Atilgan, M. R., Baysal, A. H., & Tari, C. (2008). Use of UV-C radiation as a non-thermal process for liquid egg products (LEP). *Journal of Food Engineering*, *85*, 561–568.

US Environmental Protection Agency (2006). *National Primary Drinking Water Regulation: Long-Term 2 Enhanced Surface Water Treatment Rule; Final Rule. Federal Register (40 CFR 9, 141, and 142)71. (pp. 653–786), 653–786.*

Van Boekel, M. A. J. S. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, *74*, 139–159.

Wang, X., Hu, X., Hu, C., & Wei, D. (2011). Sequential use of ultraviolet light and chlorine for reclaimed water disinfection. *Journal of Environmental Sciences*, *23*, 1605–1610.

Wong, M., Kumar, L., Jenkins, T. M., Xagoraraki, I., Phanikumar, M. S., & Rose, J. B. (2009). Evaluation of public health risks at recreational beaches in Lake Michigan via detection of enteric viruses and a human-specific bacteriological marker. *Water Research*, *43*, 1137–1149.

World Health Organization (2014). *Preventing Diarrhoea Through Better Water, Sanitation and Hygiene: Exposures and impacts in low- andmiddle-income countries.* Available at: http://apps.who.int/iris/bitstream/10665/150112/1/9789241564823_eng.pdf?ua=1/&ua=1. Accessed 2016.07.29.

Würtele, M. A., Kolbe, T., Lipsz, M., Kulberg, A., Weyers, M., Kneissl, M., & Jekel, M. (2011). Application of GaN-based ultraviolet-C light emitting diodes -UV LEDs- for water disinfection. *Water Research*, *45*, 1481–1489.