Food Control 90 (2018) 146–151

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Effect of intermittent 222 nm krypton-chlorine excilamp irradiation on microbial inactivation in water

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ARTICLE INFO

Article history:
Received 10 October 2017
Received in revised form
25 January 2018
Accepted 18 February 2018
Available online 21 February 2018

Keywords:
Water
Pathogen
UV-C irradiation
222-nm
Excilamp
Intermittent treatment

ABSTRACT

In order to assure the microbial safety of drinking water, krypton-chlorine (KrCl) excilamp treatment has emerged as a possible technology to replace the use of conventional 254-nm low-pressure mercury UVC lamps. The aim of this study was to evaluate the disinfection efficiency of the KrCl excilamp emitting power narrow-band UVC radiation at 222-nm against a spectrum of bacteria at different initial populations in model contaminated water and to develop a strategy to effectively sterilize water contaminated with high concentrations of microorganisms. Different inoculum levels (10^{4-5}, 10^{5-6}, and 10^{6-7} CFU/ml) of *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* suspended in sterile distilled water were irradiated with a KrCl excilamp. In water of high inoculum density (10^{6-7} CFU/ml), higher resistance to UVC was observed than in samples of medium and low cell density, as a high concentration of suspended cells lowered the transmittance of UVC rays. However, intermittent application of 222-nm UV irradiation in water containing a high inoculum level showed higher inactivation capacity against all three pathogens than continuous irradiation at the same dose. The results of this study provide an interesting insight into the use of an intermittent 222-nm UVC treatment system in batch-type water reservoir facilities to reduce the risk of waterborne disease.

1. Introduction

Contaminated water is one of the major vehicles of pathogenic microorganisms that can cause severe health problem for humans either by direct consumption or through use in washing of food materials (Kim et al., 2013). Water also has been widely associated with the transmission of viral infectious diseases (Calgua et al., 2014). Contaminated drinking water is a frequently underestimated problem in developed countries because of the effective disinfection systems in modern sewage plants. However, nearly 748 million people worldwide still lack sufficient access to improved drinking water sources, accounting for over 1.7 million deaths a year, especially in developing countries (Gross, Stangl, Hoenes, Sift, & Hessling, 2015).

Consequently, effective water disinfection treatment is a key process for preventing the transmission of pathogens on a global scale and is often achieved by chlorine, ultraviolet (UV) radiation or ozone treatment, all of which have advantages and disadvantages (Betancourt & Rose, 2004). In recent years, the use of UV radiation for inactivating microorganisms in water and wastewater treatment has become more and more popular because, unlike ozone or chlorine, it is easy to apply, requires no additional chemical inputs and produces no toxic by-products (Hamamoto et al., 2007).

UV irradiation is divided into 3 regions according to wavelength: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm), of which UVC has the most germicidal effect. In particular, it is well known that UVC irradiation treatment is a very efficient method for water decontamination by directly damaging the genomes of microbial pathogens. Radiation of this wavelength range is able to pass through the cell walls of bacteria and is partly absorbed by the DNA. By this process, thymine dimers are formed, which prevent further replication of the DNA strains (Gross et al., 2015). The use of UVC radiation in water treatment is also effective against entities with acknowledged resistance to chlorination, such as

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https://doi.org/10.1016/j.foodcont.2018.02.025
0956-7135/

Cryptosporidium and *Giardia* oocysts, at doses of less than 200 J/m^2 (Hijnen, Beerendonk, & Medema, 2006).

However, highly toxic and environmentally harmful low- or medium-pressure mercury vapor lamps, which have high optical output powers of several watts and a wavelength peak commonly in the UVC range of 254 nm, have usually been utilized as a light source in commercial UVC disinfection systems (Gross et al., 2015). The cost of a UVC disinfection device equipped with a low-pressure mercury (LP Hg) lamp is comparatively high because the life of the lamp is rather short and its energy consumption is high. Also, a long warm-up time and variability of the radiation intensity according to temperature are major drawbacks of LP Hg lamps (Ha, Lee, & Kang, 2017; Shin, Kim, Kim, & Kang, 2016). Thus, safer and more reliable UVC disinfection devices are required.

In this context, deep UV-LEDs or dielectric barrier discharge (DBD)-driven excimer lamps (excilamps) are a promising development and may be able to function as a substitute for mercury lamps in the near future. However, commercially available UVC-LEDs usually produce severely lower radiant output powers of mostly less than 3 mW (Gross et al., 2015). Whereas, DBD-driven excilamps are regarded as an attractive alternative to conventional mercury lamps due to wavelength-selective applications (from 74 to 354 nm depending on the type of rare gas and halogen used), absence of mercury, fast warm-up, long lifetime, and geometric variability (Kogelschatz, 2004; Matafonova & Batoev, 2012). In our previous study, a 222-nm KrCl excilamp potentially showed that it can be used as an alternative to LP Hg lamps for inactivating foodborne pathogens on real food surfaces (Ha et al., 2017). Although a number of studies of 222-nm excilamp treatment for water disinfection have been reported (Matafonova, Batoev, Astakhova, Gómez, & Christofi, 2008; Rahmani et al., 2010; Wang, Oppenländer, Gamal El-Din, & Bolton, 2010), some limitations such as shielding effect have been pointed out in the context of practical application.

The objectives of this study were to compare the exact bactericidal effect of the 222-nm KrCl excilamp irradiation according to the contamination level of major foodborne pathogens, including *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes*, in water, and to find a way to effectively sterilize water contaminated with high concentrations of microorganisms.

2. Materials and methods

2.1. Bacterial strains and inoculum conditions

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, and ATCC 15313), were obtained from the bacterial culture collection of Seoul National University (Seoul, South Korea) and used in this study. Stock cultures were stored frozen at −80 °C in 0.7 ml of tryptic soy broth (TSB; MB Cell, CA, USA) and 0.3 ml of 50% glycerol. To obtain working cultures, bacteria were streaked onto tryptic soy agar (TSA; MB Cell), incubated at 37 °C for 24 h, stored at 4 °C.

2.2. Culture preparation and inoculation

Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured individually in 5 ml of TSB at 37 °C for 24 h and all pathogen species (nine strains total) were combined to constitute a mixed culture cocktail. The cell pellet was harvested by centrifugation (4000 × g for 20 min at 4 °C) and washed three times with 0.2% sterile peptone water (PW). Subsequently, final pelleted cells were resuspended in 9 ml of sterile distilled water (DW). This inoculum consisting of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* combined was used in this inactivation study at a final concentration of approximately 10^8 CFU/ml.

For water treatment, DW was used. In the case of the high inoculum load (6–7 log CFU/ml) batch water system, 1 ml of mixed culture cocktail was inoculated into 50 ml of DW at room temperature (22 ± 1 °C). For the medium inoculum level (5–6 log CFU/ml), 0.1 ml of culture cocktail was inoculated into 50 ml of DW. One-tenth ml of cocktail was diluted in 500 ml of DW to give populations of 4–5 log CFU/ml (low inoculum load).

2.3. Experimental apparatus and treatment

A 222-nm KrCl excilamp treatment was carried out in a previously described apparatus (Ha et al., 2017). A dielectric barrier discharge (DBD)-driven excilamp (29 by 9 by 8 cm; UNILAM, Ulsan, South Korea) filled with a KrCl gas mixture with a nominal output power of 20 W was used as a 222-nm UV irradiation source. The excilamp was of cylindrical geometry covered by a metal case having an UV exit window with an area of 60 cm^2 (10 by 6 cm). A modulated electrical field was applied to a quartz glass body filled with KrCl gas. The quartz glass served as a dielectric barrier and prevented the forming plasma from short-circuiting the electrodes (inner-outer). The excilamp system was arranged vertically and directly above the water samples (Fig. 1). The vertical distance between the emitter and the sample was 23 cm (9.1 in.). Radiation intensity of the excilamp was measured with a UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands) calibrated to a range of 200- to 400-nm which includes the entire UV wavelength spectrum and the irradiance of the 222-nm KrCl excilamp was 101.61 μW/cm^2 at the sample location.

The inoculated water samples for UVC irradiation were prepared by transferring 10 ml of cell suspension (described previously) into petri dishes (50 mm diameter) and then mixed continuously (300 rpm) with a magnetic stirrer (TM-17R, Jeio Tech; Daejeon, South Korea) to allow even irradiation (Fig. 1). The samples were treated at room temperature (22 ± 1 °C), at dosages of 0.51, 1.02, 1.52, and 2.03 mJ/cm^2; UV doses were calculated by multiplying irradiance values by the treatment times (5–20 s). Treatments comprised of intermittent irradiation cycles were utilized to decontaminate high inoculum load water samples. One irradiation cycle consisted of 5 s excilamp irradiation followed by stirring for 1 min without irradiation. Each dose of 0.51, 1.02, 1.52, or 2.03 mJ/cm^2 had 1, 2, 3, or 4 cycles in intermittent treatment, respectively. A programmable logic controller (PLC; MOACON, Comfile Technology,

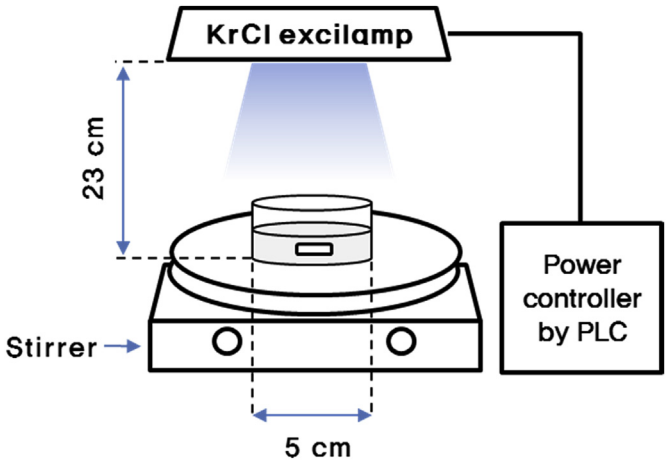

Fig. 1. Schematic diagram of the batch-type water treatment system used in this study.

Seoul, Korea) was built to automatically supply the on-off power into the excilamp system (Fig. 1).

2.4. Bacterial enumeration

After UVC treatment of water, 1 ml sample aliquots were 10-fold serially diluted in 9 ml of 0.2% PW, and 0.1 ml of sample or diluent was spread plated onto selective media to enumerate the three pathogens. Sorbitol MacConkey agar (SMAC; Oxoid, NY, USA), xylose lysine desoxycholate agar (XLD; Oxoid), and Oxford agar base with antimicrobial supplement (OAB; MB Cell) were used as selective media to enumerate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. All selective agar media were incubated at 37 °C for 24–48 h, and typical colonies were counted. To confirm the identity of the pathogens, random colonies were selected from the enumeration plates and subjected to biochemical and serological tests, consisting of the *E. coli* O157:H7 latex agglutination assay (RIM; Remel, KS, USA), the *Salmonella* latex agglutination assay (Oxoid), and the API *Listeria* test (bioMérieux, MO, USA).

2.5. Enumeration of injured cells

To enumerate injured cells, the overlay (OV) method and Phenol Red agar base (Difco) with 1% sorbitol (SPRAB; MB Cell) were used for *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7, respectively (Lee & Kang, 2001; Rhee, Lee, Hillers, McCurdy, & Kang, 2003). For the first step of the overlay method, non-selective TSA medium was used, which enables injured cells to resuscitate. One-tenth-milliliter aliquots of appropriate dilutions were spread plated in duplicate, and the plates were incubated at 37 °C for 2 h to enable injured cells to recover. The plates were then overlaid with 7 ml of the selective medium XLD for *Salmonella* spp. or OAB for *L. monocytogenes*, respectively. Solidified plates were further incubated for an additional 22 h at 37 °C. After incubation, typical black colonies of both pathogens were enumerated. Enumeration of injured *E. coli* O157:H7 on SPRAB was implemented to count typical white colonies after incubation at 37 °C for 24 h, and simultaneously, serological confirmation (*E. coli* O157:H7 latex agglutination test, Remel) was performed on randomly selected white colonies.

2.6. Statistical analysis

All experiments were repeated three times with duplicate samples. Triplicate data were analyzed by the ANOVA procedure of SAS (Version 9.4. SAS Institute Inc., Cary, NC, USA) and Duncan's multiple-range test was used to determine significant differences at a probability level of $P < 0.05$.

3. Results

3.1. Effect of inoculum level on water disinfection by 222-nm KrCl excilamp

The effects of inoculum levels on the log reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in water following 222-nm UVC irradiation are presented in Tables 1–3, respectively. Bactericidal efficacy against all pathogens increased with irradiation dose of the KrCl excilamp. However, the viable-count reduction levels in water containing 10^{4-5} CFU/ml (low-inoculum) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells were significantly higher compared with medium- (10^{5-6} CFU/ml) and high-inoculum (10^{6-7} CFU/ml) samples. Reductions were generally lower as the inoculum level increased. At water samples of an initial population of 10^{6-7} CFU/ml, the lowest reductions of three pathogens were observed (Tables 1–3).

Comparing the quantitative data (except for below the detection limit) at the same dose of 1.02 mJ/cm^2 showed that numbers of *E. coli* O157:H7 were reduced by 3.04, 2.44, and 1.31 log CFU/ml at low-, medium-, and high-inoculum density, respectively. In the case of *S.* Typhimurium, 222-nm UVC irradiation with 1.52 mJ/cm^2 achieved 3.86, 2.94, and 1.84 log reductions in low-, medium-, and high-inoculum load water samples, respectively. Also, reductions of 1.89, 1.56, and 0.91 log CFU/ml in water containing low-, medium-, and high-inoculum concentrations, respectively, were observed after 1.02 mJ/cm^2 222-nm radiation treatment of *L. monocytogenes* (Tables 1–3).

3.2. Effect of intermittent 222-nm UV irradiation on high-inoculated water

Fig. 2 depicts surviving populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells in water (initial population of 10^{6-7} CFU/ml) after continuous or intermittent 222-nm KrCl excilamp treatment. The intermittent 222-nm UVC treatment at a dose of 2.03 mJ/cm^2 (4 cycles) led to mean reductions of 5.18, 3.58, and 3.08 log CFU/ml in *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. The additional reductions were calculated by subtracting the log reductions of continuous 222-nm irradiation from those achieved during intermittent treatment. In the case of *E. coli* O157:H7, additional counts occurring at doses of 1.52 and 2.03 mJ/cm^2 were 1.71 and 2.20 log units, respectively. In *S.* Typhimurium, additional 0.84 and 1.32 log reductions were observed at 1.52 and 2.03 mJ/cm^2 of treatment, respectively. In the case of *L. monocytogenes*, an additional 0.86 and 1.10 log cells were inactivated by intermittent irradiation at 1.52 and 2.03 mJ/cm^2, respectively (Fig. 2).

3.3. Resuscitation of UV-injured cells

Table 4 shows levels of sublethally injured cells of the three pathogens in water of high inoculum load following intermittent 222-nm KrCl excilamp treatment. Determining the difference between inactivation of samples subjected to injured cell recovery methods and those plated directly on selective media revealed the presence of 0.06, 1.33, and 0.01 log units of injured *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells, respectively, after intermittent 222-nm irradiation at maximum treatment dose of 2.03 mJ/cm^2. For *E. coli* O157:H7 and *L. monocytogenes,* there were no significant ($P > 0.05$) differences between the reduction levels enumerated on selective agar versus those on agar used for recovery over the entire range of treatment doses. However, smaller reductions of *S.* Typhimurium were observed by the agar OV method than by direct plating on selective agar, and statistically significant ($P < 0.05$) differences between them were observed at treatment dosages of 1.02, 1.52, and 2.03 mJ/cm^2 (Table 4).

4. Discussion

Several previous studies conducting comparative research on the bactericidal effect between the 222-nm KrCl excilamp and 254-nm LP Hg lamp revealed that KrCl excilamps are superior to conventional Hg lamps, and support the potential utilization of this 222-nm excimer lamp as an alternative UVC disinfection method in the future (Ha et al., 2017; Wang et al., 2010; Yin, Zhu, Koutchma, & Gong, 2015). One of the main reasons given for this superiority is the potential reactivation of pathogenic bacteria after exposure to 254-nm UVC light. Bacteria generally possess a molecular mechanism (photo-reactivation) which uses a single enzyme called DNA-

Table 1
Log reductions[a] of *E. coli* O157:H7 in water following 222-nm KrCl excilamp treatment for various times at an irradiance of 101.61 μW/cm^2.

Treatment time (sec)	Dose (mJ/cm^2)	Initial population (CFU/ml)					
		$10^{4\sim5}$		$10^{5\sim6}$		$10^{6\sim7}$	
0	0	0.00 ± 0.00	A	0.00 ± 0.00	A	0.00 ± 0.00	A
5	0.51	1.62 ± 0.17	B	0.69 ± 0.40	B	0.44 ± 0.32	A
10	1.02	3.04 ± 0.23	C	2.44 ± 0.19	C	1.31 ± 0.36	B
15	1.52	<DL	D	3.92 ± 0.14	D	2.15 ± 0.45	C
20	2.03	<DL	D	<DL	E	2.98 ± 0.06	D

[a] Values are means ± standard deviations from three replications. Values in the same column followed by the same letter are not significantly different ($P > 0.05$). DL, detection limit = 1 log CFU/ml.

Table 2
Log reductions[a] of *S.* Typhimurium in water following 222-nm KrCl excilamp treatment for various times at an irradiance of 101.61 μW/cm^2.

Treatment time (sec)	Dose (mJ/cm^2)	Initial population (CFU/ml)					
		$10^{4\sim5}$		$10^{5\sim6}$		$10^{6\sim7}$	
0	0	0.00 ± 0.00	A	0.00 ± 0.00	A	0.00 ± 0.00	A
5	0.51	1.38 ± 0.14	B	0.72 ± 0.09	B	0.63 ± 0.24	B
10	1.02	2.23 ± 0.27	C	1.92 ± 0.15	C	1.29 ± 0.23	C
15	1.52	3.86 ± 0.76	D	2.94 ± 0.26	D	1.84 ± 0.18	D
20	2.03	<DL	D	4.70 ± 0.44	E	2.26 ± 0.15	E

[a] Values are means ± standard deviations from three replications. Values in the same column followed by the same letter are not significantly different ($P > 0.05$). DL, detection limit = 1 log CFU/ml.

Table 3
Log reductions[a] of *L. monocytogenes* in water following 222-nm KrCl excilamp treatment for various times at an irradiance of 101.61 μW/cm^2.

Treatment time (sec)	Dose (mJ/cm^2)	Initial population (CFU/ml)					
		$10^{4\sim5}$		$10^{5\sim6}$		$10^{6\sim7}$	
0	0	0.00 ± 0.00	A	0.00 ± 0.00	A	0.00 ± 0.00	A
5	0.51	0.92 ± 0.30	B	0.66 ± 0.14	B	0.37 ± 0.14	B
10	1.02	1.89 ± 0.48	C	1.56 ± 0.24	C	0.91 ± 0.24	C
15	1.52	<DL	D	3.03 ± 0.07	D	1.27 ± 0.23	C
20	2.03	<DL	D	<DL	E	1.98 ± 0.26	D

[a] Values are means ± standard deviations from three replications. Values in the same column followed by the same letter are not significantly different ($P > 0.05$). DL, detection limit = 1 log CFU/ml.

photolyase to compensate for DNA damages induced by UVC light. The formation of a pyrimidine dimer can be reversed through this mechanism (Thoma, 1999). Proteins show a strong absorption coefficient at 220 nm (McLean & Giese, 1950) and the KrCl excilamp shows a relatively sharp emission spectrum with a peak at 222-nm targeting the proteins of microorganisms, which is an alternative way to reduce the photo-reactivation mechanism (Ha et al., 2017). Photo-reactivation causes problems especially for large-scale UVC inactivation of microorganisms when treated wastewater, drinking water and sewage are exposed to sunlight (Rahmani et al., 2010). Therefore, the inactivation efficiency of 222-nm UV light can be more significant than that of 254-nm UV light against pathogens in water.

The 222-nm KrCl excilamp has been shown to be effective in the rapid inactivation of bacteria in aqueous suspensions or liquid foods (Matafonova et al., 2008; Orlowska, Koutchma, Kostrzynska, & Tang, 2015; Rahmani et al., 2010; Wang et al., 2010; Yin et al., 2015). Generally, the results demonstrate that narrow-band radiation of 222-nm in the bactericidal region provides a high disinfection effect even at high initial microbial densities. However, excilamps seem to be relatively inferior in their bactericidal effect for water of high turbidity containing suspended scattering particles or UV-absorbing substances that compete with cells. This shielding effect also might result from absorption and light scattering by the microbial cells themselves that have dimensions comparable with the UV wavelength. Our data shows that with all tested bacteria at $10^{6\sim7}$ CFU/ml (the highest initial cell density), there was protection from direct UV as penetration was hindered. To achieve > 4-log reductions (99.99% viability loss), based on the calculated parameters of the Weibull model, treatments for 25.1, 37.8, and 36.5 s (2.54–3.86 mJ/cm^2) would be needed for *E. coli* O157:H7 (scale parameter = 8.07; shape parameter = 1.23; $R^2 = 0.99$; mean squared error [*MSE*] = 0.006), *S.* Typhimurium (scale parameter = 7.58; shape parameter = 0.87; $R^2 = 0.99$; *MSE* = 0.004), and *L. monocytogenes* (scale parameter = 11.63; shape parameter = 1.21; $R^2 = 0.99$; *MSE* = 0.008), respectively, in the high-inoculum concentration water samples (Tables 1–3). Thus, it can be expected that use of 222-nm UVC radiation will be limited to only water of low turbidity and contaminated with relatively low microbial populations (e.g., drinking water). This can be a major hurdle to applying KrCl excilamps for inactivating pathogenic bacteria in wastewater.

The shielding effect as a result of high numbers of suspended cell particles and dissolved constituents of media was also observed

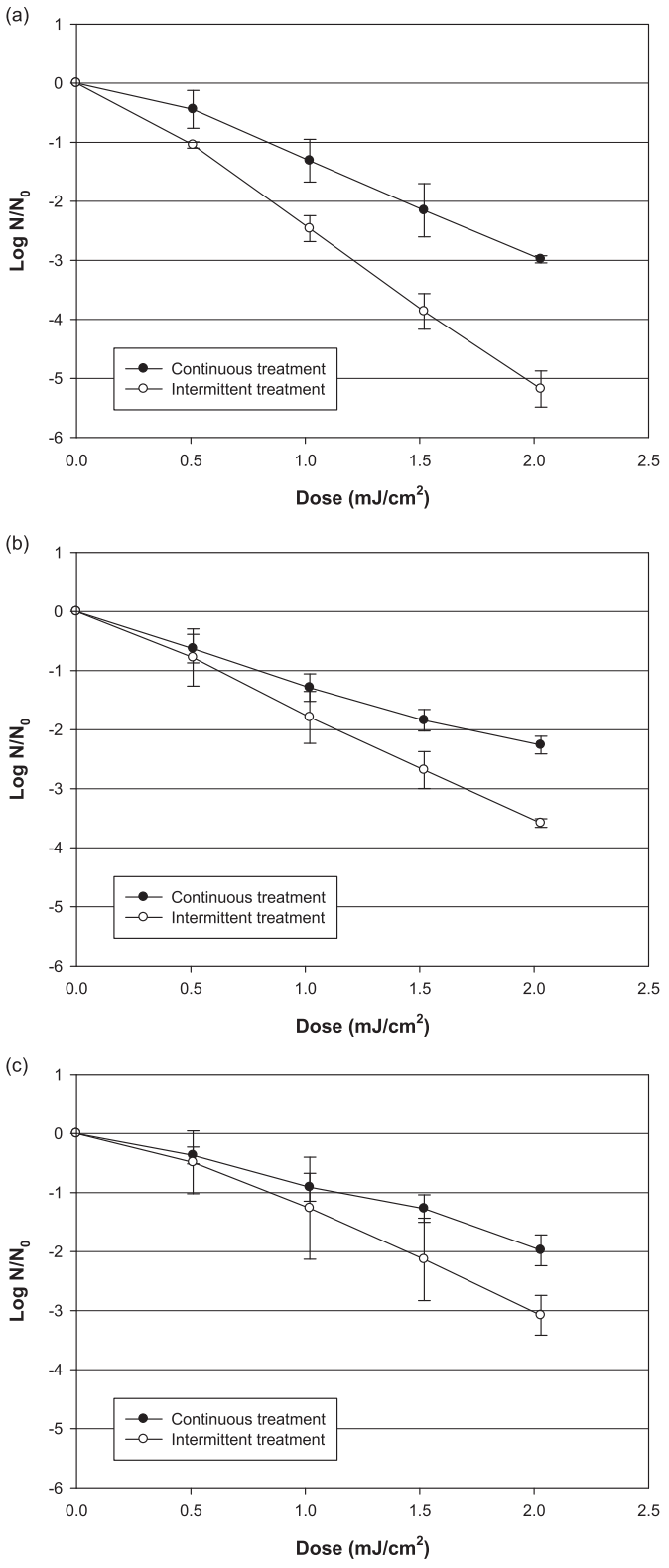

Fig. 2. Log_{10} (CFU/ml) reduction levels of *E. coli* O157:H7 (a), *S.* Typhimurium (b), and *L. monocytogenes* (c) cells in water (initial population of 10^{6-7} CFU/ml) treated with continuous and intermittent 222-nm UV irradiation. The error bars indicate standard deviations calculated from triplicates and the line joining the observations is not a regression line.

in other research studies that used 222-nm excilamps to decontaminate aqueous suspensions. Specifically, at estimated populations of 10^{2-5} CFU/ml, *E. coli* O157:H7 and *Staphylococcus aureus* were the most sensitive and decreased to below the detection limit (1 log CFU/ml) within 15 s of irradiation. While in the case of high initial densities of 10^6–10^7 CFU/ml, killing required extended treatment times of up to 300 s to achieve at least 2.9–3.0 log reductions and 99.9% viability loss (Matafonova et al., 2008). Generally, suspensions with a higher initial cell density yielded a higher tailing plateau and nonlinear survival curves. Rahmani et al. (2010) reported tailing plateaus in the survival curves of *E. coli* due to a significant shielding effect in aqueous media of high initial concentration (10^6 CFU/μl).

For effective inactivation of pathogens in water containing a high-inoculum load, we propose the intermittent application of 222-nm UV irradiation reported in this study. The composition of the irradiation cycle (5 s irradiation followed by a 1 min interval) was chosen based on preliminary experiments. Mercier, Reddy, Corcuff, and Arul (2001) investigated the effect of intermittent 254 nm UV-C treatments (two successive doses of 0.44 kJ/m^2) compared to single standard exposure to 0.88 kJ/m^2 on induction of resistance to *Botrytis cinerea* in bell peppers. Results herein regarding intermittent exposure to UV-C suggest that UV-C doses are additive. There was no added benefit of fragmenting the optimal dose of 0.88 kJ/m^2 into two successive exposures of 0.44 kJ/m^2, which had the same effect as one exposure of 0.88 kJ/m^2 (Mercier et al., 2001). However, as shown in Fig. 2, intermittent irradiation of 222-nm was able to overcome the shielding effect and control pathogens more efficiently. The intermittent treatment at dose of 2.03 mJ/cm^2 (4 cycles) can achieve more than ca. 3–4 log reductions of all pathogens while using the same amount of power (dose based) as that of the continuous treatment. Since the cell suspensions were well mixed during all UV treatments in this study (Fig. 1), it is not clear how intermittent treatment can overcome the shading effect. Intermittent 222-nm irradiation may have caused a difference in degree of cell recovery including photo-reactivation that could be an alternative explanation for the enhanced efficiency of the intermittent treatment.

Even though intermittent 222-nm UV treatment was highly effective, the significance of sublethally injured pathogens in water should be considered. Injured cells are potentially as dangerous as their normal counterparts because they can recover and become functionally normal under favorable conditions (Wu, 2008). In the present study, there were no significant ($P > 0.05$) differences between levels of cells enumerated on SMAC and SPRAB and those on OAB and OV-OAB following intermittent 222-nm excilamp treatment, even at maximum irradiance (2.03 mJ/cm^2). However, S. Typhimurium produced significant numbers of injured cells when inoculated water was treated with high doses (1.02–2.03 mJ/cm^2) of the 222-nm excilamp (Table 4). Similar increases in S. Typhimurium injured cells after UV-C irradiation were also reported by other researchers (Choi, Park, Choi, Kim, & Chun, 2015; Ha et al., 2017; Kim, Kim, & Kang, 2016).

In conclusion, using model aqueous solutions, we have demonstrated ca. 99.99% bacterial disinfection by intermittent 222-nm KrCl excilamp treatment at high initial populations of 10^6–10^7 CFU/ml. This simple approach can be one feasible solution to minimize the shielding effect. However, because the KrCl excilamp water disinfection instrument utilized in this study is a small lab-scale system, there will be some challenges directly applying these results to an industrial-scale water system. Furthermore, in order to attain better understanding of the inactivation mechanisms of microorganisms at intermittent 222-nm UV irradiation more studies have to be conducted.

Table 4
Levels of surviving cells and cells including injured *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in water (initial population of $10^{6\sim7}$ CFU/ml) following intermittent 222-nm UV irradiation.

Treatment type	Dose (mJ/cm^2)	Log reduction [$\log_{10}$ (N_0/N)][a] by organism and selective medium											
		E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
		SMAC		SPRAB		XLD		OV-XLD		OAB		OV-OAB	
KrCl exilamp (intermittent)	0	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa
	0.51	1.05 ± 0.05	Ba	0.62 ± 0.16	Bb	0.78 ± 0.48	Ba	0.26 ± 0.17	Ba	0.49 ± 0.53	ABa	0.45 ± 0.34	Aa
	1.02	2.46 ± 0.22	Ca	1.72 ± 0.11	Cb	1.79 ± 0.44	Ca	0.89 ± 0.07	Cb	1.26 ± 0.86	BCa	1.25 ± 0.44	Ba
	1.52	3.86 ± 0.30	Da	3.48 ± 0.34	Da	2.68 ± 0.31	Da	1.42 ± 0.10	Db	2.13 ± 0.70	CDa	2.10 ± 0.56	Ca
	2.03	5.18 ± 0.31	Ea	5.12 ± 0.42	Ea	3.58 ± 0.07	Ea	2.25 ± 0.03	Eb	3.08 ± 0.34	Da	3.07 ± 0.37	Da

[a] Values are means ± standard deviations from three replications. Values in the same column followed by the same uppercase letter are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row for each pathogen are not significantly different ($P > 0.05$). SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; XLD, xylose lysine desoxycholate agar; OV-XLD, overlay XLD agar on TSA; OAB; Oxford agar base with antimicrobial supplement; OV-OAB, overlay OAB agar on TSA.

Acknowledgments

This research was supported by a grant (NRF-2017R1C1B5018122) from the National Research Foundation of Korea (NRF) funded by the Korea government (Ministry of Science and ICT). This work was also supported by the Agriculture, Food and Rural Affairs Research Center Support Program, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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