Inactivation kinetics and efficiencies of UV-LEDs against *Pseudomonas aeruginosa*, *Legionella pneumophila*, and surrogate microorganisms

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**Abstract**

To demonstrate the effectiveness of UV light-emitting diodes (UV-LEDs) to disinfect water, UV-LEDs at peak emission wavelengths of 265, 280, and 300 nm were adopted to inactivate pathogenic species, including *Pseudomonas aeruginosa* and *Legionella pneumophila*, and surrogate species, including *Escherichia coli*, *Bacillus subtilis* spores, and bacteriophage Q3 in water, compared to conventional low-pressure UV lamp emitting at 254 nm. The inactivation profiles of each species showed either a linear or sigmoidal survival curve, which both fit well with the Geeraerd's model. Based on the inactivation rate constant, the 265-nm UV-LED showed most effective influence, except for with *E. coli* which showed similar inactivation rates at 265 and 254 nm. Electrical energy consumption required for 3-log10 inactivation (*E*3) was lowest for the 280-nm UV-LED for all microbial species tested. Taken together, the findings of this study determined the inactivation profiles and kinetics of both pathogenic bacteria and surrogate species under UV-LED exposure at different wavelengths. We also demonstrated that not only inactivation rate constants, but also energy efficiency should be considered when selecting an emission wavelength for UV-LEDs.

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1. Introduction

Ultraviolet light-emitting diodes (UV-LEDs) are small, mercury-free devices with a flexible and adjustable design. UV-LEDs can be used without a warming up period, enabling diverse application of this device such as on-demand operation. The effectiveness of UV-LEDs at various wavelengths for water disinfection has been demonstrated in many studies, with most studies investigating surrogate microorganisms such as the indicator bacterium *Escherichia coli*; indicator viruses such as bacteriophage MS2, Q3, and T7; and aerobic spore-forming bacteria (Bowker et al., 2011; Oguma et al., 2015; Beck et al., 2017).

For example, *E. coli* inactivation by UV-LEDs with wavelengths varying from 255 to 280 nm exhibit a range of inactivation rate constants between 0.29 and 0.42 cm²/mJ, which are comparable to the values found at 254 nm (Chatterley and Linden, 2010; Bowker et al., 2011; Oguma et al., 2013). In contrast, MS2 as a common surrogate for enteric viruses showed different sensitivities to different UV-LED wavelengths, and the effectiveness at 280 nm was better than that using a 254-nm low-pressure UV lamp (LPUV) at the same dose (Beck et al., 2017). Although MS2 is commonly used in North America for UV system validation in drinking water treatment plants and UV studies (USEPA, 2006), *Bacillus subtilis* spores are more widely used in Europe (ONORM, 2001). Moreover, because similar behaviors of aerobic spores (e.g. *B. subtilis* spores) and *Cryptosporidium* oocysts such as resistance and removal in water treatment processes have been observed (Facile et al., 2000; Muhammad et al., 2008), *B. subtilis* spores has been proposed to be as a conservative surrogate of *Cryptosporidium* to identify drinking water contamination. Although UV-LEDs were shown to be effective against surrogate microorganisms, information regarding the effectiveness of UV-LEDs against pathogenic microorganisms is very limited. Only two recent studies have reported the sensitivity of UV-LEDs on a pathogenic virus, an adenovirus known to be the most UV-resistant species (Oguma et al., 2015; Beck et al., 2017), but there have been no studies on pathogenic bacteria or protozoa in water. Because of health risk to humans posed by these microorganisms, it is necessary to examine the effectiveness of UV-LEDs against pathogenic bacteria and protozoa.

*Pseudomonas aeruginosa*, an opportunistic bacterium, is sometimes detected in drinking water pipeline systems because of its ability to form biofilms with extracellular polymeric substances.
Biofilm formation makes it difficult for residual chlorine to diffuse and inactivate microorganisms in inner layers, and can result in re-contamination of drinking water (Meena and Gerba, 2009). Infection by P. aeruginosa has been reported following intake of contaminated drinking water, and serious infection cases are predominantly found in hospitals, resulting in pneumonia, bloodstream infections, urinary tract infections, and surgical site infections. The Centers for Disease Control and Prevention (CDC) in the United States reported 51,000 infections of P. aeruginosa per year with approximately 6700 cases of multi-drug resistance (Meena and Gerba, 2009). The purity of the spore solution according to phase-contrast microscopy was up to 90%. The number of active spores was determined by CFU assays with nutrient agar at 37 °C after 24 h incubation. 

**Bacteriophage Qβ**. An aliquot (100 μL) of bacteriophage Qβ had been purified and characterized by Buse et al. (2015) for cultivation and determination of L. pneumophila was performed as follows. A stock solution of L. pneumophila was cultivated in buffered yeast extract medium at 37 °C for 48 h and then washed 3 times with PBS before use. The enumeration method was performed using a CFU assay with buffered charcoal yeast extract (BCYE-α) as a host applied to Qβ-containing samples, which were evaluated as plaque-forming units (PFU) per mL (Adam, 1959).

For pathogenic microorganisms, including *L. pneumophila* ATCC 33152 and *P. aeruginosa* ATCC 10145, the details of cultivation were as follows.

**Legionella pneumophila**. A modified method of Buse et al. (2015) for cultivation and determination of *L. pneumophila* was performed as follows. A stock solution of *L. pneumophila* was cultivated in buffered yeast extract medium at 37 °C for 48 h and then washed 3 times with PBS before use. The enumeration method was performed using a CFU assay with buffered charcoal yeast extract (BCYE-α) as a host applied to Qβ-containing samples, which were evaluated as plaque-forming units (PFU) per mL (Adam, 1959).

**Escherichia coli**. A pure culture of *E. coli* was incubated at 37 °C overnight in Luria-Bertani broth and subsequently washed with phosphate-buffered solution (PBS, pH 7.2) 3 times before the UV exposure experiments. The number of *E. coli* was determined in a colony-forming unit (CFU) assay with Chromocult agar according to the method of manufacturer (Merck, Darmstadt, Germany).

**Bacillus subtilis** spores. Cultivation, harvesting and determination technique of *B. subtilis* spores were modified from the methods of Nicholson and Setlow (1990) in which a pure culture stock of *B. subtilis* was cultivated in Trypticase soy broth at 37 °C for 6 h, transferred into liquid enrichment medium, 2 × 5G medium, and then incubated at 37 °C with vigorous aeration for 7–8 days for sporulation. Subsequently, *B. subtilis* spores were harvested by washing with cold sterile water three times, heating at 80 °C for 12 min to inactivate vegetative and germinating cells, and washing again with cold sterile water 6 times sequentially. The purity of the spore solution according to phase-contrast microscopy was up to 90%. The number of active spores was determined by CFU assays with nutrient agar at 37 °C after 24 h incubation.
A single target model, if necessary, was applied to compare with the multi-target model, and Geeraerd’s model is derived from Eq. (3).

\[
\frac{N_t}{N_0} = 10^{-kF} \left( 1 - \frac{N_{res}}{N_0} \right) \left( 1 + \frac{10^{kF}}{1 + (10^{kF} - 1) - 10^{-kF}} \right) + \frac{N_{res}}{N_0}
\]  

where \(N_{res}\) is the microbial concentration of a specific subpopulation, either more resistant or appearing as a result of experimental artifacts (CFU or PFU/mL), \(k\) is the maximum inactivation rate constant of critical components, given as the slope of the linear part (cm²/mJ), \(t\) is the shoulder length (mJ/cm²), which can be obtained by dividing the value of \(y\)-intercept of the linear part with the \(k\).

### 2.5. Electrical energy efficiency

The electrical energy per order \((E_{EO})\) is a parameter used to assess the performance of different UV disinfection systems (i.e. UV-LEDs and LPUV) based on electrical energy consumption, which is defined as the amount of electrical energy required to lower the concentration of microbes by one order of magnitude in a specific volume of water. If the linear fluence-response profile is observed, \(E_{EO}\) can be derived as follows (Sharpless and Linden, 2005):

\[
E_{EO} = \frac{A}{3.6 \times 10^3 \times V \times k \times C \times WF}
\]  

where \(E_{EO}\) is the electrical energy per order of magnitude (kWh/m³), \(A\) is the irradiant surface area (cm²), \(V\) is the volume of sample (mL), \(k\) is the fluence-based inactivation rate constant (cm²/mJ), \(WF\) is the water factor, the value of 3.6 \times 10^3 is a unit conversion constant for mW and kW, sec and hr, and mL and m³, and \(C\) is the wall plug efficiency which can be calculated based on Eq. (5).

\[
C = \frac{P_{output}}{P_{input}} = \frac{F_A}{I_A \times V_A}
\]

where \(P_{output}\) is the UV-LEDs optical power (mW), \(P_{input}\) is the applied electrical power (mW), \(I_A\) is the applied current (mA), \(V_A\) is the applied voltage (V), and \(F_A\) is the radiant flux (mW). Information regarding radiant flux per UV-LED chip provided by the manufacturer (Nikkiso Giken Co. Ltd.) was 2.01 mW for 265-nm, 1.82 mW for 280-nm, and 0.026 for the 300-nm UV-LEDs, respectively.

In cases where fluence-response curves showed non-linear log reduction (e.g. shoulder, tailing or sigmoidal curve), the electrical energy per specific \(n\)-log reduction, \((E_{E,n},\text{kWh/m}^3/\text{n-log reduction})\) was calculated as follows (Beck et al., 2017):

\[
E_{E,n} = \frac{A \times F_n}{3.6 \times 10^3 \times V \times C \times WF}
\]

where \(F_n\) is the fluence required for \(n\)-log reduction (mJ/cm²).

### 2.6. Model fitting and statistical analysis

Fit testing of the inactivation kinetic models for each microorganism was conducted using Microsoft Excel and either root mean square error (RMSE) or the coefficient of determination \((R^2)\) was determined to assess the goodness of fit with the observed data. For non-linear fluence-response curves; such as a curve with shoulder and tailing, \(R^2\) was not applicable because of invalidation of the \(R^2\) assumption, and a higher \(R^2\) indicated a better predicted value (model) fitted with the observed data. In contrast, a lower value for RMSE indicated a better fit. Analysis of covariance was introduced to indicate a difference in the inactivation rate in the linear part of
the inactivation profile at different UV wavelengths and p-values less than 0.05 indicated a significant difference.

3. Results and discussion

3.1. Inactivation kinetic modeling

Fluence-response curves or survival curves of target microorganisms inactivated by different UV wavelengths are shown in Fig. 2. Two types of survival curve, linear and sigmoidal curves (a curve with a shoulder and tailing), were observed in this study. The linear curve was observed for _L. pneumophila_ and bacteriophage Qβ, whereas a sigmoidal curve was found for _E. coli_, _P. aeruginosa_, and _B. subtilis_ spores. In sigmoidal curves, the shoulder may indicate the resynthesis rate of vital components, which reached a level higher than the destruction rate (Mossel et al., 1995), while tailing may have resulted from multiple-hit lowering inactivation efficiency and the existence of a small number of resistant sub-populations, including UV-induced and adapted resistance (Cerf, 1997). In the present study, the length of the shoulder on fluence-response curves as shown in Table 1 differed based on the microbial species and emission wavelengths, indicating that accumulation of damage or resynthesis of vital component before inactivation may differ among each microorganism, and different wavelengths resulted in different magnitudes of damage affecting the shoulder for the microorganisms. For instance, the shoulder length at 254 nm was longer than that at 265 nm, but there was a clear increasing trend following exposure to longer wavelengths (280 and 300 nm) in _E. coli_ and _B. subtilis_ spores, showing the highest value at 300 nm. This indicates that damage resynthetic ability may be mostly supressed at 265 nm, but other unknown mechanisms may contribute to this phenomenon and it is needed to be examined in further studies. Tailing was observed after microorganisms were inactivated at approximately 4.5-3log inactivation at all wavelengths. These results support that resistant sub-populations were present even in pure cultures because after 4.5-3log inactivation, the microbial concentration remained approximately ~100–300 CFU/ml compared to original number of 10^8–10^9 CFU/ml (Cerf, 1997).

Introduction of the multi-target model to the fluence-response curves of all microorganisms as shown in Fig. 2 suggested that this model fitted well with the observed data, except for cases of _E. coli_ and _B. subtilis_ spores exhibiting sigmoidal curves. Because the multi-target model does not account for tailing, which may over-estimate the effectiveness of UV-LEDs in the tailing region, another model covering both shoulder and tailing effects should be employed. The Geeraerd’s model appeared to be suitable in the present study because this model not only uses a deterministic approach based on fitting, but also is supported by biological mechanisms in the background, such as possible factors affecting the shoulder (microorganisms being clumped, damage re-synthesis ability, or difference in critical components) and tailing (existence of resistant sub-population, including adapted resistance).

As shown in Fig. 2, Geeraerd’s model showed good fitting of not only the sigmoidal curves (_E. coli_ and _B. subtilis_ spores), but also curves with shoulders (_P. aeruginosa_) and linear curves of _L. pneumophila_ and bacteriophage Qβ. Considering the goodness of curve fitting based on RMSE and R² as shown in Table 2 for the comparison between the multi-target and Geeraerd’s model, the RMSE and R² values in linear fluence-response curves of _L. pneumophila_ and bacteriophage Qβ for both models were equivalent at all wavelengths, and the sigmoidal curves for _E. coli_ and _B. subtilis_ spores were better characterized by Geeraerd’s model at all wavelengths. For _P. aeruginosa_ exhibiting fluence-response curves with a shoulder, the RSME of both models was similar. In conclusion, based on the results shown in Table 2, the Geeraerd’s model covered all survival curve situations in this study.

3.2. Inactivation efficiency of UV-LEDs and LPUV

To compare the efficiency of UV inactivation at different wavelengths, inactivation rate constants calculated form a linear part for each microorganism are shown in Table 3.

3.2.1. 254 nm (LPUV)

Inactivation at this wavelength was effective against vegetative bacteria cells, particularly _E. coli_, _P. aeruginosa_, and _L. pneumophila_, with inactivation rate constants (k254 ± 95% confidence interval) of 0.81 ± 0.07, 0.45 ± 0.05, and 0.86 ± 0.03 cm²/ml, respectively. The range of k254 values for different _E. coli_ strains reported in previous studies (Sommer et al., 2000; Hijnen et al., 2006; Rattanakul et al., 2014) was 0.44–0.91 cm²/ml, which is consistent with the results of the present study. Similarly, k254 for _L. pneumophila_ was comparable to those in other studies (~0.7 cm²/ml) (Oguma et al., 2004; Cervero-Aragó et al., 2014), while _P. aeruginosa_ appeared to be

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Table 1

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>UV wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>254 nm</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2.62 ± 0.09</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.52 ± 0.18</td>
</tr>
<tr>
<td><em>L. pneumophila</em></td>
<td>-0</td>
</tr>
<tr>
<td>Bacteriophage Qβ</td>
<td>-0</td>
</tr>
<tr>
<td><em>B. subtilis</em> spores</td>
<td>12.6 ± 0.51</td>
</tr>
</tbody>
</table>

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Fig. 2. Inactivation profiles and model fittings for tested microorganisms under exposures to LPUV and 265, 280-, and 300-nm UV-LEDs. Internal figures show the inactivation profile and model fitting for 300-nm UV-LEDs.
be repaired rapidly via specific rather than cyclobutane pyrimidine dimers and (6,4) photoproducts to the major UV-induced lesions in spores as spore photo-products (260 nm (Harm, 1980). Similarly, the UV absorbance of nucleic acid is highest at approximately 265-nm UV-LED achieved highest bacterial inactivation (Mamane-Gravetz et al., 2005; Rattanakul et al., 2014; Beck et al., 2015) in the present study was higher than in previous studies. There was no significance difference among the wavelengths tested. The UV absorbance of protein showed a relative peak at 280 nm (5.61 ± 0.04 cm2/mJ, respectively; these values are comparable to previously reported values (Mamane-Gravetz et al., 2005; Rattanakul et al., 2014; Beck et al., 2015). The high UV resistance in B. subtilis spores may be related to the major UV-induced lesions in spores as spore photo-products rather than cyclobutane pyrimidine dimers and (6,4) photoproducts, major lesions in bacteria, and that spore photo-products can be repaired rapidly via specific mechanisms (Setlow, 2001). For Qb and many viruses, factors, including their small size and host machinery-involving life cycle, may contribute to their high UV resistance.

3.2.2. 265 nm UV-LEDs

Inactivation rate constants of all tested microorganism were highest by inactivation with 265-nm UV-LEDs, except for E. coli whose rate constant was not significantly different from that at 254 nm (p > 0.05). The observed value of k254 for E. coli in the present study was higher than in previous studies (0.17–0.37 cm2/mJ) (Chatterley and Linden, 2010; Oguma et al., 2013) and differences in experimental conditions and the shape of the inactivation profile may be involved. k265 values for P. aeruginosa and L. pneumophila, which were first reported in the present study, were 0.77 ± 0.05 and 0.86 ± 0.05 cm2/mJ, respectively. There was no significant difference (p > 0.05) in k254 between E. coli and P. aeruginosa, and the k265 values for these species were slightly lower than that of L. pneumophila. Among the wavelengths tested, the 265-nm UV-LED achieved highest bacterial inactivation regardless of the bacterial species. This is because, as well-noted, UV absorbance of nucleic acid is highest at approximately 280 nm (Harm, 1980). Similarly, the k280 for B. subtilis spores and Qb were slightly lower than that of E. coli.

3.2.3. 280 nm UV-LEDs

The UV absorbance of protein showed a relative peak at approximately 280 nm (Harm, 1980), and UV-induced protein damage may enhance microbial inactivation. However, as the UV absorbance of the genome decreases at this wavelength relative to that at 265 nm, k280 values for all microorganisms were also decreased to 0.56 ± 0.04 cm2/mJ for E. coli, 0.51 ± 0.05 cm2/mJ for P. aeruginosa, 0.45 ± 0.01 × 10−1 cm2/mJ for L. pneumophila, 0.6 ± 0.01 cm2/mJ for Qb, and 0.10 ± 0.006 cm2/mJ for B. subtilis spores. At 280 nm, Qb and B. subtilis spores were more UV-resistant than bacteria, as was the case at 265 nm. Furthermore, inactivation by 280-nm UV-LED showed lower effectiveness than that by 254-nm LPUV in E. coli, L. pneumophila and Qb, but remained the same as that at 254 nm, particularly for P. aeruginosa (p < 0.05) and was slightly higher for B. subtilis. For Qb, UV-induced protein damage may not be a dominant cause of inactivation at this wavelength, although a previous study demonstrated that UV-induced protein damage played an important role in virus inactivation (Eischeid and Lindén, 2011). The number of protein components involved in the infection processes of Qb is lower than those of enteric viruses, which may result in a lower contribution of protein damage to inactivation.

3.2.4. 300 nm UV-LEDs

Although UV absorbance of both genome and protein considerably decreases at 300 nm, the inactivation rate constants of tested microorganisms (k300) were still observable. The k300 values for vegetative bacteria cells were lowest among the other wavelengths. Similarly, k300 values for B. subtilis spores and Qb were lowest among the wavelengths tested. These data are consistent with those of a previous study that tested the efficiency of UV-LEDs at 310 nm, demonstrating low but observable inactivation against E. coli (Oguma et al., 2013).

The present study showed that E. coli was more sensitive to UV than were the pathogenic bacteria, P. aeruginosa and L. pneumophila at all wavelengths. This poses a fundamental question regarding relevance of adopting E. coli as a surrogate for water-borne pathogenic bacteria for UV disinfection. This study also revealed that compared to conventional LPUV (254 nm) and other UV-LEDs wavelengths, inactivation of pathogenic and surrogate microorganisms by 265-nm UV-LEDs showed the best results based on the
fluence-based inactivation rate constants.

3.3. Electrical energy efficiency

Selection of UV-LEDs at different wavelengths based solely on inactivation rate constants can generate misleading results. Electrical energy efficiency is another factor involved in making an economically reasonable decision. In this study, electrical energy consumption for 3-log10 inactivation ($E_{3}$) was considered for all tested microorganisms, as the fluence-response curves of E. coli, P. aeruginosa, and B. subtilis spores were non-linear.

Table 4 shows the values of $E_{3}$ for all microorganisms at different UV wavelengths. $E_{3}$ for tested microorganisms at 254 nm (LPUV) were 0.006–0.064 kWh/m², which were lower than those of UV-LEDs at all wavelengths for all species. Notably, $E_{3}$ of LPV for Qβ and B. subtilis spores were approximately 5–10-fold higher than that for bacteria. Although 265-nm UV-LEDs provided higher inactivation rates than 254-nm LPUV, 265-nm UV-LEDs required approximately 25–40-fold more energy consumption for 3-log10 inactivation among all tested microorganisms. The $E_{3}$ of 265-nm UV-LEDs for bacterial species (L. pneumophila, E. coli, and P. aeruginosa) was lower than that for Qβ and B. subtilis spores, as was the case with LPUV at 254 nm. Additionally, 280-nm UV-LEDs consumed approximately half as much energy as 265-nm UV-LEDs for all microorganisms, definitely because of the difference in wall plug efficiency. The $E_{3}$ values of all tested microorganisms at 300 nm was highest compared to other wavelengths, showing the highest wall plug efficiency among the UV-LEDs tested. The low inactivation effectiveness resulted in high energy consumption to achieve a specific level of inactivation.

The $E_{3}$ values observed in this study for E. coli inactivation at 280 nm were lower than the value of 1.04 kWh/m², which was calculated based on the data reported by Beck et al. (2017). The lower energy consumption in the present study was mostly attributable to the difference in wall plug efficiency. Also, our results show that LPUV was more energy efficient than UV-LEDs at all wavelengths, as was reported previously (Austin et al., 2013; Beck et al., 2017). For UV-LEDs, 265 nm showed highest inactivation rate constants, while the 280-nm product was the most energy-efficient among the wavelengths tested. Energy consumption of 300 nm UV-LED was very high for all tested microbial species, particularly for B. subtilis spores requiring 17.4 kWh/m² for 3-log10 inactivation. Thus, energy consumption required for a certain level of inactivation may be a useful indicator for comparing UV light sources, including UV-LEDs.

4. Conclusions

UV-LEDs are effective for inactivating P. aeruginosa, L. pneumophila, and surrogate microorganisms in water. Among UV-LEDs with nominal peak emissions at 265, 280, and 300 nm, the 280-nm UV-LED is a good option for achieving a high inactivation rate constant and showed the lowest energy consumption for achieving 3-log10 inactivation in all microbial species tested. Our results can be used in the development of water disinfection systems with UV-LEDs.

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