Inactivation of health-related microorganisms in water using UV light-emitting diodes

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ABSTRACT

UV light-emitting diodes (UV-LEDs) offer various wavelength options, while microorganisms have spectral sensitivity, or so-called action spectra, which can be different among species. Accordingly, matching properly the emission spectra of UV-LEDs and the spectral sensitivity of microorganisms is a reasonable strategy to enhance inactivation. In this study, UV-LEDs with nominal peak emissions at 265, 280 and 300 nm were applied to pathogens including *Legionella pneumophila, Pseudomonas aeruginosa, Vibrio parahaemolyticus* and feline calicivirus, in comparison with indicator species including *Escherichia coli, Bacillus subtilis* spores, bacteriophage Qβ and MS2. The results indicated that, for all species tested, 265 nm UV-LED was highest in the fluence-based inactivation rate constant *k,* followed by 280 nm and 300 nm was much lower. The *k* value at 280 nm was close to that at 265 nm for feline calicivirus and MS2, suggesting that 280 nm UV-LED can be as good an option as 265 nm UV-LED to inactivate these viruses. Bacteria tended to show fluence-response curves with shoulder and tailing, while viruses followed log-linear profiles at all wavelengths tested. This study indicates the fluence-response profiles and the fluence required for a target inactivation of microorganisms, which would serve as reference data for future study and applications of UV-LEDs.

Key words | disinfection, light-emitting diode (LED), pathogen, ultraviolet (UV) radiation, water treatment

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INTRODUCTION

The light-emitting diode (LED) with germicidal ultraviolet (UV) emissions, noted UV-LED hereafter, is an emerging source of UV radiation that can bring innovation to water treatment systems. For example, UV-LEDs are mercury-free, tiny in size, flexible in the reactor design, and quick to start without warming-up. UV-LEDs offer variety in emission wavelengths, thus, how to pick up the right one is a question. On the other hand, microorganisms are known to have spectral sensitivity, so-called action spectra, which greatly affects the inactivation efficiency at different wavelengths. Namely, matching properly the emission spectra of UV-LEDs and the spectral sensitivity of target microorganisms can enhance inactivation efficiency.

Health-related microorganisms in water include pathogens and indicators. Pathogens cause diseases in humans and animals through infection while indicators are ideally non-pathogenic but behave similar to or in a way indicative of pathogens, and are thus to be monitored in water treatment processes and in the water environment. As indicators, *Escherichia coli* and bacteriophages have been used commonly in general, while *Bacillus subtilis* spores are used as a challenge organism for validation of UV disinfection systems in Europe (ÖNORM 2001; DVGW 2006). *Legionella* spp. and *Pseudomonas* spp. are opportunistic pathogens which are important in public health due to concerns over susceptible populations and healthcare-associated infections (HAIs). *Pseudomonas aeruginosa* have been listed as a pathogen with critical priority (World Health Organization 2017), while *L. pneumophila* are known for the large-scale

doi: 10.2166/ws.2019.022

outbreak through the public water supply in Flint, Michigan (Zahran *et al.* 2018).

Vibrio parahaemolyticus is known to cause gastrointestinal disease through drinking water and food. As this species is ubiquitous in seawater, seafood-to-human is a common route of infection which is associated with water contamination. In aquaculture industries, mercury UV lamp systems are common in disinfecting water, thus *V. parahaemolyticus* is one of the key targets for UV disinfection. *V. parahaemolyticus* is also known to cause a fatal infectious disease in farmed shrimp, called Early Mortality Syndrome/Acute Hepatopancreatic Necrosis Disease, or EMS/AHPHD (Lightner *et al.* 2012) and thus the significance of this bacterium is not only for human health but for the farming industry and food security.

Among pathogenic viruses, adenovirus has already been tested with UV-LEDs as an important waterborne human pathogen (Oguma *et al.* 2016b), while feline calicivirus (FCV) is another key target to control in veterinary medicine as it causes respiratory infections in felids. Moreover, FCV is a single-strand RNA virus and phylogenetically related to human norovirus (HNV), and thus has been used as a surrogate for HNV historically. The appropriateness of FCV as a HNV surrogate is noted as 'questionable' nowadays (Bae & Schwab 2008; Park *et al.* 2011), but in general, it is worthwhile to diversify viral species in UV-LED studies.

It is needed to develop a dataset on fluence-responses of various microorganisms under UV-LED exposures at different wavelengths, which would serve as reference data for future research and development. A few reviews have made such efforts (Malayeri *et al.* 2016; Song *et al.* 2016), but comparing inactivation profiles reported in different studies can mislead the results because, while UV-LEDs offer variety in the emission wavelengths and high flexibility in the reactor design, no standardized protocol is available yet to test UV-LED apparatus. Namely, each study adopts a setup with a unique design concept and the way to determine the fluence in the systems is different among studies (for example, Bowker *et al.* 2011; Würtele *et al.* 2011; Oguma *et al.* 2013; Oguma *et al.* 2016a). Standardization of a UV-LED test protocol is inevitable to allow comparison among studies in a scientifically correct way. For now without such a protocol, it would be a reasonable and reliable option to compare the fluence-response profiles obtained using an identical exposure setup and the same method to determine the fluence.

Based on the background, the objective of this study is to summarize the fluence-response profiles of various microorganisms obtained using an identical UV-LED setup and the same protocol for fluence measurement. The target microbial species were the above-mentioned bacteria and viruses, and UV-LEDs emitting at 265, 280 and 300 nm were used for comparison. By doing so, we aim to offer reference data for future study and applications of UV-LEDs for disinfection.

MATERIALS AND METHODS

Cultivation and enumeration of microorganisms

Pure cultures of *E. coli* K12 (IFO 3301, Institute for Fermentation, Osaka, Japan), *L. pneumophila* (ATCC® 33152™, American Type Culture Collection, VA, USA), *P. aeruginosa* (ATCC® 10145™), *B. subtilis* spores (ATCC® 6633™) and bacteriophage Qβ (ATCC® 23631-B1™) were cultivated and enumerated as detailed in Rattanakul & Oguma (2018). Bacteriophage MS2 (ATCC® 15597-B1™) was tested as described earlier (Oguma *et al.* 2016b).

A pure culture of *V. parahaemolyticus* (NBRC 12711, Biological Resource Center in the National Institute of Technology and Evaluation, Chiba, Japan) was incubated at 37 °C for 16 h in a sterilized agar medium including 10 g of hipolypeptone, 2 g of yeast extract, 0.5 g of $MgSO_4{\cdot}H_2O$ and 15 g of bacteriological agar in 750 mL of artificial seawater and 250 mL of distilled water.

Feline calicivirus (ATCC® VR-782™) was propagated as detailed elsewhere (Oguma 2018). Briefly, the strain was propagated in Crandell Rees feline kidney cells (CRFK, ATCC® CCL-94™) which were cultured in complete Eagle's minimum essential medium (MEM) supplemented with 5% fetal bovine serum, 100 U/mL of penicillin and 0.1 mg/mL of streptomycin. FCV were cultivated and enumerated at Kitasato Research Center for Environmental Science, Kanagawa, Japan.

For UV-LED exposure, pre-cultured and purified microorganisms were suspended in phosphate-buffered solution at pH 7.2 at the initial concentration of 10^6 in colony-forming

units (CFU) or plaque-forming units (PFU) per 1 mL of sample. The sample was placed in a Petri dish with a pre-sterilized magnetic spin bar and exposed to UV-LEDs as detailed below.

UV-LED setup and exposure

UV-LED exposure was conducted using a setup illustrated in Figure 1, and more details with dimensions are available in Rattanakul & Oguma (2018). The number of UV-LED packages mounted on one circuit board was eight for all species except *V. parahaemolyticus*, FCV and MS2, while these three species were tested using four UV-LED packages on a board as detailed elsewhere (Oguma 2018). The difference in the number of UV-LEDs (either four or eight packages), and therefore the difference in the fluence rate on the sample dish, was properly adjusted at the fluence rate determination as detailed below. UV-LEDs (Nikkiso Giken, Ishikawa, Japan) with nominal peak emissions at 265, 280 and 300 nm (full width at half maximum, FWHM, of about 10–12 nm) were used, and the emission spectra are depicted in Figure 2. The UV-LED packages mounted on one circuit board were identical in the emission, and the combination of different wavelengths was not tested.

The fluence rate was determined by ferrioxalate actinometry (Bolton *et al.* 2011) with correction using the Beer–Lambert Law or so-called Water Factor (Bolton & Linden 2003). The average fluence rate was multiplied by the exposure time to determine the fluence. Details are available in Rattanakul & Oguma (2018). Exposure time varied from about 2 to 2,000 seconds depending on the microbial species and emission wavelengths, and the microbial suspensions were mixed continuously throughout the experiment, including during sampling, at a constant temperature of 20 °C.

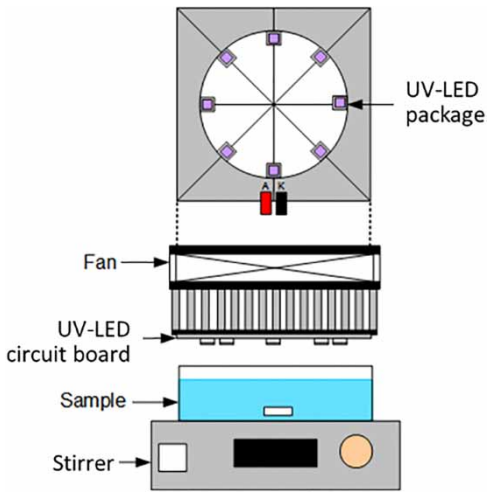

Figure 1 | UV-LED setup (modified from Rattanakul & Oguma 2018).

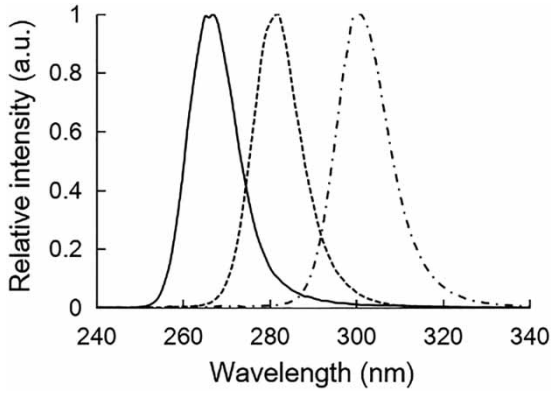

Figure 2 | Emission spectra of UV-LEDs with nominal peak emissions at 265, 280 and 300 nm.

Inactivation kinetics and data analysis

The fluence-response profiles were fitted to a log-linear regression line by the least squares method, and the $\log_{10}$-based inactivation rate constant k (cm^2/mJ) was defined as follows:

$$\log \text{inactivation} = \log (N_0/N_t) = kF + b$$

where N_0 and N_t are the number of colonies (CFU/mL) or plaques (PFU/mL) at time 0 and t of UV-LED exposure, respectively, F (mJ/cm^2) is the fluence, and b is the y-axis intercept of the regression line. Details are available in Hijnen *et al.* (2006). Data in shoulder and tailing regions were excluded in the log-linear fitting. The fluence required for a target level of inactivation was calculated based on the regression line.

RESULTS AND DISCUSSION

Fluence-response profiles for all species tested are shown in Figure 3. It is notable that bacteria, particularly bacterial

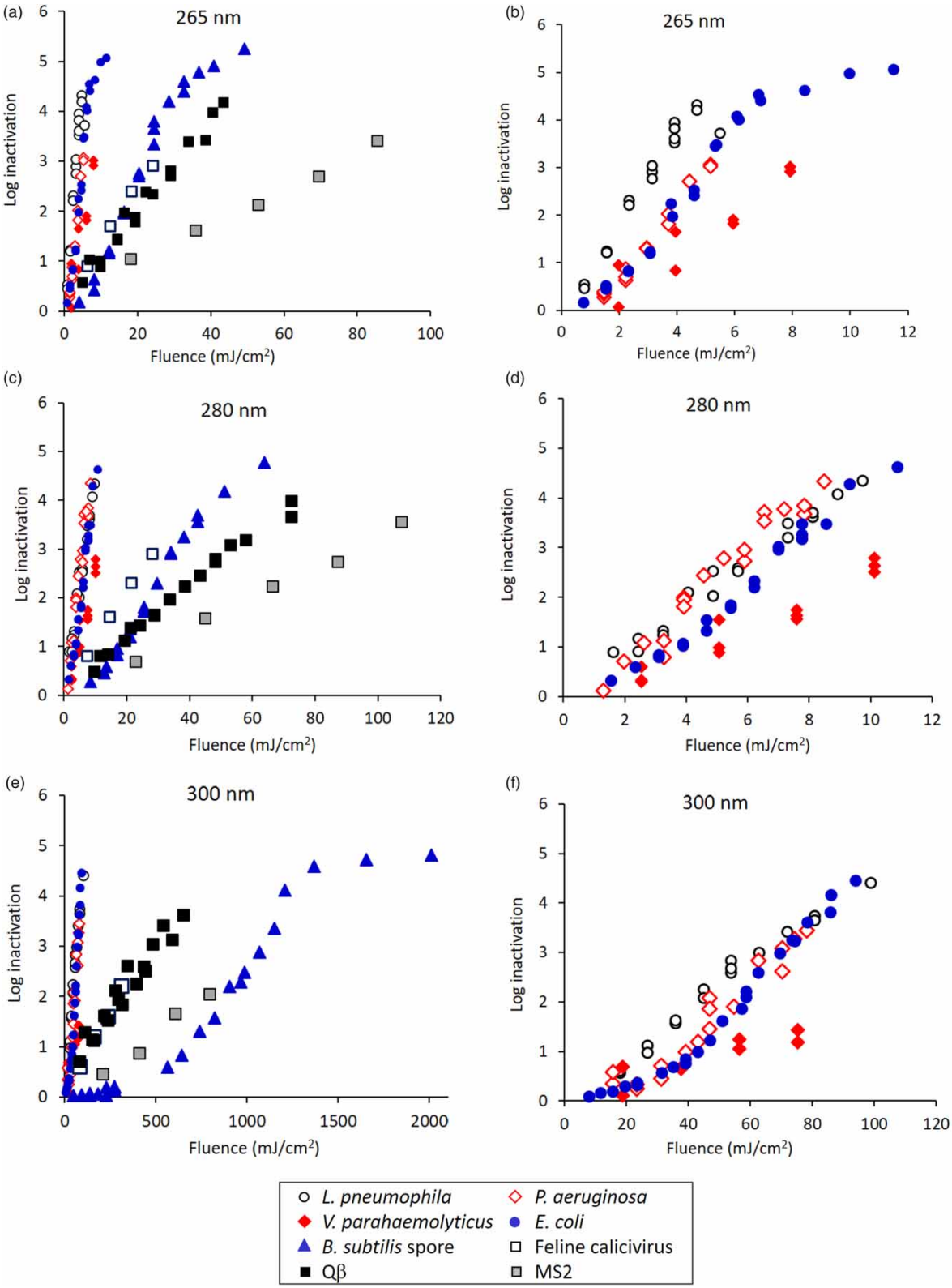

Figure 3 | Fluence-response profiles for microorganisms at 265, 280 and 300 nm UV-LED exposures. Figures on the right are the magnification at low fluences for bacteria.

spores, tended to show shoulder and tailing in the profile. Thus, for bacteria, shoulder was defined based on the apparent curve-shaped profile at low fluences while all data exceeding 4-log inactivation were defined in tailing, and the data in shoulder and tailing regions were eliminated in the log-linear fitting for the regression lines. For viruses, all data were used for the log-linear fitting because no apparent shoulder and tailing were observed with either virus. This is in accordance with the case when using conventional mercury lamps that viruses typically do not show apparent shoulder and tailing in UV disinfection (Hijnen *et al.* 2006). Many studies (for example, Severin *et al.* 1983; Pennell *et al.* 2008; Mbonimpa *et al.* 2018) have challenged the mechanistic explanation for the fact that some species (mostly bacteria and spores) show shoulder and/or tailing in UV inactivation kinetics while others (typically viruses) do not, and the most fundamental and widely accepted interpretation for shoulders is the multi-target model (Severin *et al.* 1983). The multi-target model assumes that photons must hit multiple critical targets in bacteria and spores to complete inactivation while a single hit is critical enough to inactivate viruses. Meanwhile, clumping or aggregation of microorganisms as well as the presence of subpopulations with higher resistance have been proposed as probable causes for tailing phenomena (Cerf 1977; Geeraerd *et al.* 2000; Mbonimpa *et al.* 2018). It would be scientifically reasonable to assume more targets in bacteria and spores than in simple viral particles. No apparent spectral effects were observed regarding the presence of shoulder and tailing, namely, species with shoulder and tailing showed such curves at all wavelengths tested.

Based on the regression lines for the inactivation profiles, the slope factor k and the intercept b were determined for all species as summarized in Table 1. As was partially reported earlier (Rattanakul & Oguma 2018), the 265 nm UV-LED was most efficient for all species tested in the fluence-based inactivation rate constant, followed by 280 nm, and the much lower 300 nm. This is in good agreement with the order of photon absorption efficiency of the pyrimidine base in the genome, the main target biomolecule for UV radiation (Harm 1980). Interestingly, the k value at 280 nm was close to that at 265 nm for FCV and MS2, although the values at 265 nm and 280 nm were still statistically different ($p < 0.05$, one-way analysis of variance (ANOVA)). In our previous study examining human adenovirus serotype 5, we found that the k value for a 285 nm UV-LED was higher than that for a 254 nm low-pressure mercury UV lamp (Oguma *et al.* 2016b). As such, it is suggested that some viruses tend to be sensitive to emissions at around 280 nm. It is known that while photon absorption of DNA and RNA shows a relative peak at 260 ± 5 nm, protein shows a relative peak at around 280 ± 5 nm (Harm 1980). A study demonstrated that UV-induced protein damage played an important role in virus inactivation using low- and medium-pressure mercury UV lamps (Eischeid & Linden 2011). Thinking that some viruses need specific proteins to get into the host cell to cause infection, it is implied that protein damage is critical for some viruses and that can be one of the reasons for the 280 nm UV-LED to be efficient in virus inactivation. More recently, Beck *et al.* (2018) has challenged the quantification of UV-induced protein damage in adenovirus and reported a significant reduction of protein quantities after UV exposure at wavelengths below 240 nm using deuterium lamps, encouraging a growing expectation for the development of short wavelength UV-LEDs in the future.

Viruses were generally more resistant to UV than bacteria, as is the case with conventional mercury UV lamps (Hijnen *et al.* 2006). *V. parahaemolyticus* was less sensitive to UV compared with other bacterial species except *B. subtilis* spore. This is to be taken into account when UV-LEDs are applied to aquaculture and food industries where *V. parahaemolyticus* is an important target to inactivate. The k value of *B. subtilis* spores dropped significantly from 280 nm to 300 nm, suggesting the drastic change in action spectra of this species at this wavelength band. This is supported by a previous study showing action spectra of *B. subtilis* spores (Mamane-Gravetz *et al.* 2005). The b value is the y-axis intercept, thus showing a negative value if the regression line crosses the x-axis (fluence) where the log-linear relationship starts. This was true in most cases with bacteria, and positive b values for viruses at some wavelengths may be partially due to experimental errors in culture assays. A high absolute value for b suggests significant shoulder in the response profile, which was particularly the case with *E. coli* and *B. subtilis* spores. *P. aeruginosa*, *E. coli* and *B. subtilis* spores showed increased absolute value for b at 300 nm, suggesting more apparent shoulder at this wavelength. Namely, more

Table 1 | Responses of bacteria and viruses under UV-LED exposures at different wavelengths

					Fluence [mJ/cm^2] for log inactivation of:			
	n[a]	k[b] [cm^2/mJ]	± SD[c] for k	b[b]	1	2	3	4
Bacteria								
Legionella pneumophila								
265 nm	14	1.039	0.122	−0.34	1.3	2.2	3.2	4.2
280 nm	15	0.458	0.048	−0.02	2.2	4.4	6.6	8.8
300 nm	15	0.051	0.006	−0.23	24.2	43.9	63.5	83.2
Pseudomonas aeruginosa								
265 nm	16	0.774	0.136	−0.91	2.5	3.8	5.1	6.3
280 nm	16	0.582	0.084	−0.49	2.6	4.3	6.0	7.7
300 nm	12	0.058	0.008	−1.12	36.3	53.4	70.5	87.6
Vibrio parahaemolyticus								
265 nm	7	0.359	0.079	−0.06	3.0	5.7	8.5	11.3
280 nm	11	0.281	0.035	−0.31	4.7	8.2	11.8	15.3
300 nm	7	0.017	0.005	0.08	54.4	113.3	172.2	231.1
Escherichia coli								
265 nm	13	0.878	0.116	−1.34	2.7	3.8	4.9	6.1
280 nm	15	0.562	0.063	−1.13	3.8	5.6	7.3	9.1
300 nm	16	0.067	0.010	−1.75	41.2	56.1	71.1	86.1
Bacillus subtilis spores								
265 nm	9	0.197	0.019	−1.25	11.4	16.5	21.6	26.7
280 nm	13	0.112	0.013	−1.05	18.3	27.3	36.3	45.2
300 nm	9	0.005	0.001	−2.17	671.4	882.8	1,094.3	1,305.8
Viruses								
Feline calicivirus								
265 nm	4	0.113	0.008	0.24	6.7	15.6	24.5	33.4
280 nm	4	0.101	0.003	0.09	9.0	18.9	28.9	38.8
300 nm	4	0.007	0.0003	0.06	139.1	286.8	434.6	582.3
Bacteriophage Qβ								
265 nm	17	0.091	0.015	0.19	8.9	19.9	31.0	42.0
280 nm	17	0.052	0.004	0.16	16.0	35.1	54.1	73.2
300 nm	17	0.005	0.002	0.51	100.0	304.8	509.7	714.5
Bacteriophage MS2								
265 nm	5	0.034	0.007	0.38	18.1	47.1	76.1	105.2
280 nm	5	0.033	0.002	0.01	30.3	60.9	91.5	122.1
300 nm	4	0.003	0.0003	−0.18	412.8	763.4	1,114.1	1,464.7

[a]n: number of data for regression analysis, exclusive of data in shoulder and tailing regions.
[b]k, b: slope and intercept for log-linear regression, log inactivation = $k \times$ fluence + b.
[c]SD: standard deviation.

'lag' is to be expected if a 300 nm UV-LED is used to inactivate these species.

Action spectra have been reported for some species of microorganisms. For example, Mamane-Gravetz *et al.*

(2005) examined the action spectra of MS2 and *B. subtilis* spores using a xenon lamp with a monochromator (FWHM of about 10 nm), and reported the action spectra of MS2, relative to the efficiency at 254 nm, as 0.96, 0.62 and 0.27 at 265 nm, 280 nm and 293 nm, respectively, while the value for *B. subtilis* spores was 1.05, 0.99 and 0.20 at 265 nm, 280 nm and 293 nm, respectively. Another outstanding study on action spectra was conducted by Beck *et al.* (2015) using a tunable laser with an extremely sharp monochromatic emission (FWHM less than 1 nm). They reported MS2 action spectra, relative to the efficiency at 254 nm, of 1.20, 0.77 and 0.01 at 265 nm, 280 nm and 300 nm, respectively, while the value for Qβ was 1.22, 0.71 and 0.04 at 265 nm, 280 nm and 300 nm, respectively. In comparison with the current study, our data for *B. subtilis* spores and Qβ are acceptably similar to those reported earlier while the MS2 data in this study, showing relatively high efficiency at 280 nm, are not in good agreement with either studies. The discrepancy may arise from multiple factors including the difference in spectral patterns for different UV radiation sources, difference in the fluence determination methods, and the difference in the statistical analyses for data fitting. As such, it is not that simple to compare data among independent studies particularly when different UV sources are adopted.

Although the fluence-based inactivation efficiency was highest for the 265 nm UV-LED with all species tested, the electrical energy consumption required for 3-log inactivation, $E_{E,3}$ (kWh/m^3), was lowest for the 280 nm UV-LED with *E. coli, P. aeruginosa, L. pneumophila, B. subtilis* spores and bacteriophage Qβ, due to the higher wall-plug efficiency of the 280 nm UV-LED than the 265 nm UV-LED (Rattanakul & Oguma 2018). Based on this fact, it is recommended that, with the currently available UV-LED devices that still suffer from relatively low wall-plug efficiency, not only 265 nm but 280 nm UV-LEDs would be a good option for practical applications in industries. Namely, at selecting a particular UV-LED out from many spectral options, not only the action spectra of the target microorganisms but also the integrity of the UV-LED products are to be considered. Therefore, it would be important to keep updating the technology trend of UV-LEDs while conducting fundamental research for 'robust' data on the inactivation kinetics of diverse microorganisms.

CONCLUSIONS

This study summarizes fluence-response profiles for various microorganisms and indicates the fluence required for a target value of inactivation using UV-LEDs at 265, 280 and 300 nm. Results of this study would be practically useful as reference data for future study and development of UV-LED disinfection apparatus.

ACKNOWLEDGEMENTS

This study has been supported by the Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research 17H03329, Japan Science and Technology Agency, and Ministry of Health, Labour and Welfare. The author is grateful to Nikkiso Giken Co. Ltd for providing the UV-LED apparatus and technical support.

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First received 10 October 2018; accepted in revised form 10 January 2019. Available online 29 January 2019