Action Spectra of Bacteria and Purification of Pollutant Water at Faucets Using a Water Waveguide Method

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Abstract: Ultraviolet (UV) radiation treatment is an effective method for purifying pollutant water contaminated with bacteria and/or chemicals. As an emerging technology, purification by deep ultraviolet light-emitting diodes (DUV-LEDs) is promising. Few studies have used the point-source characteristics of LEDs and have instead replaced mercury vapor lamps with LEDs. Here, we show our recent progress in the instantaneous purification of contaminated water by combining the point-source characteristics of DUV-LEDs with a water waveguide (WW). Before the demonstration, we determined the efficacy of disinfection as a function of irradiation wavelength (action spectra) by constructing a wavelength tunable DUV light source. We found that, as a function of irradiation wavelength, there is a strong correlation between the dose-based inactivation rate constants and deoxyribonucleic acid (DNA) absorbance. Based on this correlation, the emission wavelength of 265 nm was determined as the most effective wavelength for disinfecting water contaminated with bacteria. Instantaneous 2-log disinfection levels of water contaminated with Escherichia coli O1 or Pseudomonas aeruginosa were demonstrated by using the DUV-LED WW method. We also discuss how far-UVC radiation shorter than 230 nm, which has recently been attracting attention and is known as a safe and effective disinfection wavelength for the human body, cannot give a higher-dose-based inactivation rate constant compared to that of 265 nm irradiation due to the larger absorption coefficient of water with a wavelength shorter than 230 nm.

Keywords: water; organic pollutants; disinfection; inactivation; waveguide; absorption; deep ultraviolet; light-emitting diode; Escherichia coli; Pseudomonas aeruginosa; Staphylococcus epidermidis

1. Introduction

The water we drink has typically gone through various physical and chemical treatment processes to render it safe for human consumption. This “safety”, however, does not equal sterility. Drinking water contains a highly diverse microbiome, albeit in reduced numbers (10^2–10^6 cells/mL). Cells exist in distribution systems under considerable nutrient limitation. The bacterial load and composition are highly dependent on the disinfectant strategy and the infrastructure from pipe to tap. An imbalance or shift in the microbial community can lead to poor water quality (colorimetric and odor) and even the transmission of pathogens. One of the well-known diseases is cryptosporidiosis. In 1993, a waterborne cryptosporidiosis outbreak occurred in Milwaukee, WI, USA. Approximately 400,000 people became ill. Surprisingly, the source of the Cryptosporidium was believed to be the tap water supplied from the Howard Avenue Water Purification Plant. The bacteria-contaminated water was distributed to an estimated 880,000 residents [1,2]. Such infections are still occurring [3]; therefore, it is not an exaggeration to say that even at this stage we need to develop technologies to decontaminate pollutants in water.

UV radiation treatment is an effective method for the disinfection of the bacterial, eukaryotic, and viral contaminants present in water. To inactivate pathogenic bacteria,
viruses, and other microorganisms by UV radiation, low- or medium-pressure mercury vapor lamps have been widely used for many years because they emit high-power UV radiation as high as 20 kW, and the emission wavelength is close to the maximum absorption band of DNA (approximately 260 nm). However, there are many drawbacks to using mercury vapor lamps; for example, the lamps contain highly toxic mercury, require fragile quartz glass tubes to seal in the mercury gas, require a high alternating voltage in the order of 1–10 kV, have a low plug efficiency of approximately 15–35%, and need long warmup times of approximately 1–10 min. Furthermore, the lamps have a lifetime of about 10,000 h [4]. Thus, the lamps need to be replaced about once per year when continuously used.

As an emerging technology, DUV-LEDs may solve these problems with mercury vapor lamps. A DUV-LED is a semiconductor p–n junction device in which the emission occurs as a result of electron–hole injection into the multi quantum well (MQW) semiconductor layer. This device has numerous advantages that may provide solutions to the above drawbacks of UV mercury lamps. DUV-LED devices hold great promise for achieving diverse applications in light of their flexible and adjustable design. Therefore, it is useful to consider them not only as a replacement for mercury vapor lamps but also as suitable for applications that are typical of LEDs [4–7].

This paper focuses on techniques of disinfecting bacteria-contaminated water at the last point of distribution systems: disinfection at a tap using DUV-LEDs. In our previous paper, we demonstrated the instantaneous purification of contaminated water at the tap. The technique is based on the principle of the optical coupling of DUV radiation with the WW [8,9]. That is, the point-source nature of the intense DUV-LED emission can be guided to a confined space of the WW region without reflection and attenuation losses. We demonstrated the effect of the DUV-LED WW disinfection technique by showing the instantaneous purification of water contaminated with *Escherichia coli* (E. coli) DH5α [9]. In this paper, we present our recent progress in the WW disinfection technique by showing the efficacy of disinfecting water contaminated with *E. coli* O1 and *Pseudomonas aeruginosa* (P. aeruginosa), as an example of bacteria typically contaminating water.

We used LEDs with an emission wavelength of 265 nm. Because the 265 nm wavelength matches the absorption spectra of DNA [10–13], the most effective disinfection can be expected by using the emission of a 265 nm wavelength LED. However, a 265 nm emission is strongly absorbed by human cells and protein components, raising concerns about damage to plasma components [14] and platelet aggregation [15]. Thus, a 265 nm radiation is not safe when applied to the human body to inactivate pathogenic bacteria, viruses, and other microorganisms.

In this paper, we also compare the efficacy of far-UVC (FUV) light (200 and 220 nm) and DUV light (240, 260, 280, and 300 nm) against *E. coli* DH5α, *E. coli* O1, *P. aeruginosa*, and *Staphylococcus epidermidis* (S. epidermidis) by using a wavelength-tunable UV light source. FUV light (200–220 nm region) is reported to be both germicidal against some pathogenic bacteria and harmless to mammalian cells [16–20], and 222 nm UVC is well absorbed by peptide bonds and the amino acids [21–24] tryptophan and tyrosine [25–28]. Thus, 222 nm UVC is expected to be absorbed by proteins and/or other biomolecules in the stratum corneum layer before reaching the nuclei within the epidermal cells of mouse skin. This suggests that the FUV light is more suitable than DUV light for use in several applications, especially in the residential setting. Thus, it has been established that FUV light is safe for mammalian cells. However, the efficacy of FUV light on various microorganisms is not clearly understood at present [29], especially from the point of view of microorganisms in water.

The absorption of FUV or DUV radiation in water is strongly influenced by dissolved oxygen, cleaning procedures, and storage methods, and various values of absorption coefficients have been reported [30–34]. However, the tap water we drink is quite different from the high quality purified water prepared for research purposes. Therefore, the values of the absorption coefficient exhibit significantly larger magnitude compared to those...
obtained by previous reports. For example, in our optical absorption measurements, absorption coefficient, $\alpha$ (cm$^{-1}$), at 220 nm was estimated as 0.03–0.04 cm$^{-1}$, and $\alpha$ (cm$^{-1}$) at 265 nm was estimated as 0.01–0.015 cm$^{-1}$. Therefore, the water we drink has a larger absorption coefficient at a wavelength shorter than 230 nm compared to that at 265 nm, and thus there might be a significant reduction in the efficacy of disinfection.

At present, we do not have a high-power FUV-LED [35]; therefore, this reduction cannot be addressed. However, if we have an FUV-LED with a 100 mW emission power (for instance, two times higher than that of the current 265 nm LEDs), we can expect safe disinfection at the tap of water contaminated with these bacteria. To achieve FUV emission easily comparable to the PN-junction-based technologies, we propose here the use of the field emission of electron beams to obtain FUV emission [36,37]. We consider that the combination of the point-source nature of FUV-LED emission and the WW effect will pave the way toward environmentally friendly, portable water purification equipment that instantaneously supplies clean drinking water just when required, making it suitable for emergencies.

2. Materials and Methods

2.1. Culturing and Enumeration of Bacteria

$E. coli$ O1, $P. aeruginosa$, and $S. epidermidis$ were cultured as described previously [8,9]. Then, 50 $\mu$L of the culture was taken and dissolved in 5000 mL of a normal saline solution as contaminated water. After disinfection by the WW method, the degree of purification was analyzed by using 100 $\mu$L of the solution from the disinfected water to coat the nutrient broth agar plates. Colonies were counted after incubation for 24 h at 37 °C. Plates yielding 1 to 5000 CFU were considered for analysis. All experiments were performed at least three times independently. Log inactivation was calculated as $\log (N/N_0)$, where $N$ is the CFU number after irradiation and $N_0$ is the CFU number before irradiation.

2.2. Absorbance Measurements

All absorbance measurements were carried out by a spectrometer through an optical fiber (BIM-6002A, Brolight Technology Corporation, Hangzhou, China) and a spectral calibrated 150 W xenon light source with the emission wavelength from 185 nm to 2000 nm (L7810-03, Hamamatsu Photonics Corporation, Hamamatsu, Japan). A fused silica cell with an optical path length of 1 cm (T-3-ES-10, Toho Quartz Corporation, Tokyo, Japan) was used for the absorbance measurements. The absorbance of tap water was measured by filling the water into the cell, where the absorbance was determined by the ratio of transmission with and without the tap water.

2.3. Wavelength-Tunable DUV Light Source

Figure 1 shows the wavelength-tunable DUV light source to compare the efficacy of the FUV light region (200–230 nm) and the DUV light region (230–300 nm). The Laser-Driven Light Source (LDLS EQ-99X, Energetiq Technology, Inc., Wilmington, NC, USA), which emits a radiation of 170–2100 nm, was used as a broadband emission source. The emission was selected by using a UV band pass filter from 200 to 300 nm (200 nm, 220 nm, 240 nm, 260 nm, 280 nm, and 300 nm) with a 10 nm bandwidth (Edmond Optics Japan, Tokyo, Japan). The emission was weakly focused (almost collimated) and irradiated onto the bacteria by a fused silica lens (diameter of 60 $\varnothing$) of a focal length of 80 mm (SLSQ-60-80P, Sigma Koki Corp., Tokyo, Japan). The bacteria were introduced inside a microplate, and during DUV irradiation, the bacteria were agitated by a microplate shaker (TM-1FN, AS ONE corp., Osaka, Japan). The spectrum of DUV radiation that the bacteria sensed was measured by a spectrometer through an optical fiber. The intensity of DUV radiation that the bacteria sensed was measured using a spectrometer through an optical fiber. For the germicidal fluence estimation (the volume-averaged irradiance in the irradiated volume), we set the water factor, the divergence factor, and the Petri factor to approximately 1, and the reflection factor was estimated based on the Frenel equations.
The reflection factor at each wavelength was estimated as 0.972 at 200 nm, 0.974 at 220 nm, 0.976 at 240 nm, 0.977 at 260 nm, 0.978 at 280 nm, and 0.979 at 300 nm. Based on these values, the fluence at each DUV wavelength was obtained by the UV dose calculation methods [39,40].

![Wavelength-tunable DUV light source](image)

Figure 1. Wavelength-tunable DUV light source. The LDLS, which emits a radiation of 170–2100 nm, was used as a broadband emission source. DUV emission was selected by using a UV band pass filter (Filter) from 200 to 300 nm with a 10 nm bandwidth. The DUV emission (purple arrow) was weakly focused (almost collimated) onto bacteria by a fused silica lens (Lens, diameter of 60 φ m) with a focal length of 80 mm. The bacteria were introduced inside the microplate, and during DUV irradiation, the bacteria were agitated by a microplate shaker.

2.4. Water Waveguide Disinfection System

Figure 2a shows the schematic of the experimental setup for the WW system [8,9]. We used the same schematic again in this study, except that a 280 or 308 nm radiation source (FL-01-G01, Dowa Electronics Materials Co. Ltd., Tokyo, Japan) was also used in addition to the 265 nm radiation source used previously [8,9]. The electrical characteristics, such as current–voltage characteristics, and the emission spectrum are described in Ref. [9]. As an electrical power source, we used a PV cell (GT1618-MF, K-I-S Corporation Ltd., Nagano, Japan) as shown in Figure 2b or a constant current power supply (TP070-1D, Takasago Corporation Ltd., Kanagawa, Japan). Bacteria-contaminated water (CW) was supplied from a water tank (Taper Jar Tank, Nikko Hansen Corporation Ltd., Osaka, Japan). The flow rate of the purified water was maintained at 100–400 mL/min.
Figure 2. WW disinfection system and the enhancement of the DUV light intensity due to internal reflection in the water flow. (a) Schematic of the experimental setup for the WW system [9]. (b) WW disinfection system with a PV cell [9]. Demonstration of the DUV light intensity (c) without and (d) with internal reflection in the water flow by illuminating a phosphor tape [8].

The length of the WW region was approximately 20 cm, and the path length of the DUV light was long. In this case, weak absorption affects the dose of the bacteria. The volume-averaged irradiance in the irradiated volume can be obtained based on considering the absorption effect of the WW region. The exposure time in the WW region can be evaluated by the water flow velocity (cm/s), the cross-sectional area of the WW (cm$^2$) and the flow rate (cm$^3$/s). In this case, the total dose, $D_T$ (mJ/cm$^2$), in the WW region was
calculated by the integration of absorbance for the length of the WW region as described previously [9] and given by the following equation:

\[ D_T = \eta \frac{I_0}{Sv} \int_0^\xi \exp(-\alpha x)dx = \eta \frac{I_0}{\rho \alpha} [1 - \exp(-\alpha \xi)] \]  

where \( \eta \) is the coupling efficiency of the DUV-LED emission into the WW, \( I_0 \) (mW) is the DUV-LED intensity, \( S \) (cm\(^2\)) is the cross-sectional area of the WW, \( v \) (cm/s) is the flow velocity, \( \xi \) (cm) is the integration length of the WW region, \( \alpha \) (cm\(^{-1}\)) is the absorption coefficient of the CW, and \( \rho \) (cm\(^3\)/s) is the flow rate. By substituting the values evaluated in the experiment, the coupling efficiency of \( \eta \) was found to be about 40%, measured by setting an optical fiber into the WW stream; the maximum intensity of DUV-LED, \( I_0 \), was calculated to be 1.8 mW; the flow rate, \( \rho \), was found to be 1.67 cm\(^3\)/s (100 mL/60 s); the cross-sectional area of the WW, \( S \) (cm\(^2\)), was found to be approximately 0.28 cm\(^2\); the length of the WW region, \( \xi \) (cm), was found to be approximately 20 cm; and the absorption coefficient, \( \alpha \) (cm\(^{-1}\)), at 265 nm was estimated as \( \alpha = 0.013 \) cm\(^{-1}\) based on our absorbance measurements. Therefore, the total dose, \( D_T \), in the WW region can be controlled from 0 to 7.6 mJ/cm\(^2\) by changing the emission intensity of the DUV-LED from 0 to 1.8 mW. The CW supplied from the water tank was purified (PW) in the WW region, as shown in Figure 2b.

Another advantage of using the WW is the small absorption coefficient, \( \alpha \), of 0.013 cm\(^{-1}\) at an emission wavelength of 265 nm. To confirm this estimation, the spectral intensity of DUV emission with or without the water stream was evaluated through the optical fiber and a spectrometer. The DUV radiation power estimated by the intensity of the spectrum was 57 \( \mu \)W/cm\(^2\) without the WW and 143 \( \mu \)W/cm\(^2\) with the WW, which is demonstrated by illuminating a phosphor tape (680MSH, 3M Japan Ltd., Tokyo, Japan) as shown in Figure 2c,d [8].

We note here that the dose to evaluate inside the WW region is not an established method compared to that obtained by the established method described in Refs. [39,40]. Therefore, we evaluate the inactivation rate constant of the WW method in comparison with the usual established methods, as described in Figure 1, using the wavelength tunable DUV light source.

3. Results

3.1. Wavelength-Dependent Inactivation Rate Constant for Various Bacteria Using the Wavelength-Tunable DUV Light Source: Microplate Experiments

Before showing the results of the WW methods, we compare the efficacy of inactivation (microplate experiments) of *E. coli* O1 (Figure 3a), *P. aeruginosa* (Figure 3b), and *S. epidermidis* (Figure 3c) by FUV light and DUV light using the wavelength-tunable UV light source shown in Figure 1. The purple circles were obtained by 200 nm irradiation, the blue circles were obtained by 220 nm irradiation, the orange circles were obtained by 240 nm irradiation, the red circles were obtained by 260 nm irradiation, the green circles were obtained by 280 nm irradiation, and the gray circles were obtained by 300 nm irradiation. The dosage
of FUV light or DUV light varied between 0 and 12 mJ/cm² by changing the irradiation duration. We fit curves for these plots using Equation (2).

\[ \kappa(\lambda)D = -\ln\left(\frac{N(D)}{N_0}\right) \]  

Equation (2)

Table 1. Inactivation rate constants as a function of irradiation wavelength for E. coli O1, P. aeruginosa, S. epidermidis, and E. coli DH5α.

| Wavelength (nm) | E. coli O1 | P. aeruginosa | S. epidermidis | E. coli DH5α |
|-----------------|-----------|---------------|----------------|--------------|
| 200             | 0.1       | 0.1           | 0.07           | 0.25         |
| 220             | 0.15      | 0.22          | 0.38           | 1.2          |
| 240             | 0.17      | 0.2           | 0.39           | 1.3          |
| 260             | 0.6       | 0.7           | 0.81           | 3.2          |
| 280             | 0.4       | 0.4           | 0.51           | 2.3          |
| 300             | 0.06      | 0.05          | 0.1            | 0.4          |

Figure 3. DUV dose response of (a) E. coli O1, (b) P. aeruginosa, and (c) S. epidermidis. The purple circles were obtained by 200 nm irradiation, the blue circles were obtained by 220 nm irradiation, the orange circles were obtained by 240 nm irradiation, the red circles were obtained by 260 nm irradiation, the green circles were obtained by 280 nm irradiation, and the gray circles were obtained by 300 nm irradiation. (d) Correlation graph between the inactivation rate constants given in Table 1 and the absorption curve of DNA, where the rate constant of E. coli O1 was multiplied by 1.55 (red circles), the value of P. aeruginosa was multiplied by 1.37 (green circles), the value of S. epidermidis was multiplied by 1.1 (blue circles), and the value of E. coli DH5α was multiplied by 0.275 (red squares) to correlate the absorbance with these rate constants.
We can determine the dose-based inactivation rate constant as a function of irradiation wavelength \( \kappa (\lambda) \) (natural logarithm), where \( D \) is the magnitude of the UV dose (mJ/cm\(^2\)), \( N_0 \) is the number of CFUs on the unirradiated control, and \( N(D) \) is the number of CFUs at a given dose \( D \). The rate constants as a function of irradiation wavelength \( \kappa (\lambda) \) for \( E. coli \) O1, \( P. aeruginosa \), and \( S. epidermidis \) are summarized in Table 1. In this table, the results of the rate constants for \( E. coli \) DH5\(\alpha\) are also described as a comparison. Based on the results of Figure 3a–c, it is found that the most effective wavelength for the inactivation of bacteria is around 260 nm.

The dose-based inactivation rate constant \( (\kappa) \) and the absorption coefficient of DNA \( (\alpha) \) are correlated with each other, and the rate can be linearly proportional to the absorption coefficient as \( \alpha = \mu \kappa \), where \( \mu \) is a proportional constant \([11–13,41–43]\). Figure 3d shows the absorption curve of DNA \([44]\), and the values of the inactivation rate constants for these bacteria are shown in Figure 3a–c, as determined in the experiment, where the rate constant of \( E. coli \) O1 was multiplied by 1.55 (red circles), the value of \( P. aeruginosa \) was multiplied by 1.37 (green circles), the value of \( S. epidermidis \) was multiplied by 1.1 (blue circles), and the value of \( E. coli \) DH5\(\alpha\) was multiplied by 0.275 (red squares) to correlate the absorbance with these rate constants. The facts that (i) the curve of the rate constants as a function of irradiation wavelength does not depend on the bacterial species and (ii) the curve of the rate constants fits well on the absorption curve of DNA strongly suggest that the UV inactivation is based on the unified mechanism where the formation of cyclobutane pyrimidine dimers, pyrimidine 6-4 pyrimidone photoproducts, and their Dewar isomers in DNA is important for the inactivation \([25–28]\). Thus, this result provides direct evidence that the dose-based inactivation rate constant \( (\kappa) \) and the absorption coefficient of DNA \( (\alpha) \) are correlated.

We note here that the action spectrum determined by the values of the inactivation rate constants obtained here agrees well with previously published results \([12,13,45–47]\) but differs from the optical absorption curve of DNA in the shorter wavelength region, below 230 nm. We consider that this difference between the inactivation rate constants and the optical absorption curve is attributable to the absorbance by the peptide bonds of proteins in bacteria. The excitation of peptide bonds is likely to play a minor role in both the modification of DNA and the inactivation of bacteria \([21–24]\).

3.2. Disinfection of Bacteria by Water Waveguide Methods: Water Purification Experiments

3.2.1. \( E. coli \) O1

The results of the efficacy of inactivation for \( E. coli \) O1 using the WW purification system are shown in Figure 4, where Figure 4a,c,e are the control plates (0 mJ/cm\(^2\) DUV dose), Figure 4b is the plate inactivated by a 6.0 mJ/cm\(^2\) dose of 265 nm irradiation, Figure 4d is the plate inactivated by a 6.0 mJ/cm\(^2\) dose of 280 nm irradiation, and Figure 4f is the plate inactivated by a 6.0 mJ/cm\(^2\) dose of 308 nm irradiation. By applying a higher DUV dose, the number of colonies was significantly reduced, from \((2.9 \pm 0.4) \times 10^3\) CFU (control plate, Figure 4a) to \((1.1 \pm 0.2) \times 10^2\) CFU (Figure 4b) for the 6.0 mJ/cm\(^2\) dose of 265 nm irradiation. However, by changing the irradiation wavelength from 265 to 280 or 308 nm, the reduction behavior was less pronounced: \((1.1 \pm 0.2) \times 10^4\) CFU (control plate, Figure 4c) to \((8.5 \pm 3.2) \times 10^2\) CFU for a 6.0 mJ/cm\(^2\) dose of 280 nm irradiation and \((9.8 \pm 1.3) \times 10^3\) CFU (Figure 4e) to \((1.1 \pm 0.2) \times 10^4\) CFU for a 6.0 mJ/cm\(^2\) dose of 308 nm irradiation (Figure 4d). The reduction rate of inactivation as a function of the magnitude of dose for each wavelength is plotted in Figure 4g, where the red circles are obtained by 265 nm irradiation, the green circles are obtained by 280 nm irradiation, and the gray circles are obtained by 308 nm irradiation. Based on these plots, the inactivation rate constant for each wavelength can be determined as \( \kappa_{265} = 0.65, \kappa_{280} = 0.38, \) and \( \kappa_{308} = 0 \). These values agree well with the values described in Table 1 for \( E. coli \) O1, which are obtained by the wavelength-dependent inactivation rate constant experiments.
The results of the efficacy of inactivation for *P. aeruginosa* using the WW purification system are shown in Figure 5, where Figure 5a,c,e are the control plates (0 mJ/cm² DUV dose), Figure 5b is the plate inactivated by a 6.0 mJ/cm² dose of 280 nm irradiation, Figure 5c is the plate inactivated by a 6.0 mJ/cm² dose of 280 nm irradiation, and Figure 5d is the plate inactivated by a 6.0 mJ/cm² dose of 308 nm irradiation. By applying a higher DUV dose, the number of colonies was significantly reduced, from (2.1 ± 0.3) × 10³ CFU (control plate, Figure 4a) to (3.0 ± 0.8) × 10¹ CFU (Figure 4b) for the 6.0 mJ/cm² dose of 265 nm irradiation. However, by changing the irradiation wavelength from 265 to 280 or 308 nm, the reduction behavior was less pronounced: (2.0 ± 0.3) × 10³ CFU (control plate, Figure 4c) to (2.0 ± 0.4) × 10² CFU for a 6.0 mJ/cm² dose of 280 nm irradiation and (2.2 ± 0.1) × 10³ CFU (Figure 4e) to (2.0 ± 0.1) × 10² CFU for a 6.0 mJ/cm² dose of 308 nm irradiation (Figure 4d). The reduction rates of inactivation as a function of the magnitude of dose for each wavelength are plotted in Figure 5g, where the red circles are obtained by 265 nm irradiation, the green circles are obtained by 280 nm irradiation, and the gray circles are obtained by 308 nm irradiation. Based on these plots, the inactivation rate constant for each wavelength can be decided as $\kappa_{265} = 0.65$, $\kappa_{280} = 0.38$, and $\kappa_{308} = 0$. These values agree well with the values described in Table 1, which are obtained by the wavelength-dependent inactivation rate constant experiments.

3.2.2. *P. aeruginosa*

The results of the efficacy of inactivation for *P. aeruginosa* using the WW purification system for *E. coli* O11 are shown in Figure 4, where (a,f) are the results of control plate (0 mJ/cm² DUV dose), (b) is the plate inactivated by a 6.0 mJ/cm² dose of 265 nm irradiation; (c) is the plate inactivated by a 6.0 mJ/cm² dose of 280 nm irradiation, (d) is the plate inactivated by a 6.0 mJ/cm² dose of 308 nm irradiation, and (f) is the plate inactivated by a 6.0 mJ/cm² dose of 308 nm irradiation. The results of control plate (0 mJ/cm² DUV dose), (c) to (8.5 ± 3.2) × 10² CFU for a 6.0 mJ/cm² dose of 280 nm irradiation and (9.8 ± 1.3) × 10³ CFU for a 6.0 mJ/cm² dose of 308 nm irradiation. By applying a higher DUV dose, the number of colonies was significantly reduced, from (2.1 ± 0.3) × 10³ CFU (control plate, Figure 4b) to (1.1 ± 0.2) × 10⁴ CFU for a 6.0 mJ/cm² dose of 308 nm irradiation. However, by changing the irradiation wavelength from 265 to 280 or 308 nm, the reduction behavior was less pronounced: (2.0 ± 0.4) × 10³ CFU for a 6.0 mJ/cm² dose of 280 nm irradiation and (2.2 ± 0.1) × 10³ CFU for a 6.0 mJ/cm² dose of 308 nm irradiation. The reduction curves of inactivation for each irradiation wavelength are fitted by a function of the magnitude of the dose for each wavelength. The red circles are obtained by 265 nm irradiation, the green circles are obtained by 280 nm irradiation, and the gray circles are obtained by 308 nm irradiation. The reduction curves of inactivation for each irradiation wavelength are fitted by a function of the magnitude of the dose for each wavelength. The red circles are obtained by 265 nm irradiation, the green circles are obtained by 280 nm irradiation, and the gray circles are obtained by 308 nm irradiation. Based on these plots, the inactivation rate constant for each wavelength can be decided as $\kappa_{265} = 0.65$, $\kappa_{280} = 0.40$, and $\kappa_{308} = 0.04$. These values agree well with the values described in Table 1 for *P. aeruginosa*, which are obtained by the wavelength-dependent inactivation rate constant experiments.

![Figure 4](image-url)
To obtain a loss-free WW effect, we estimate the reflectivity at the given points of the WW region as shown in Figure 6. Here, all of the reflectivity is calculated using the wavelength of 265 nm, the refractive index of 1.36, and the extinction coefficient of $2.75 \times 10^{-8}$ [30–34]. Figure 6a shows reflectivity as a function of incident angle for the water/air interface. The blue line represents the s-polarized light (perpendicular to the plane of incidence), the green line represents the p-polarized light (parallel to the plane of incidence), and the red line represents the unpolarized light. Due to the low refractive index...
and the extinction coefficient of water, DUV-LEDs with a wide viewing angle (less than 120°) can be efficiently coupled to the WW. Generally, DUV-LEDs emit unpolarized light; however, if we can make and use a p-polarized DUV-LED, the reflection loss at the water/air interface can be completely reduced, especially at the angle of 54° (Brewster angle).

Figure 6. Reflectivity versus incident angle for 265 nm DUV light at the interfaces (a–d) of the WW region. (a) air/water interface, (b) Al-surface/water interface, (c) Fe-surface/water interface, and (d) water/air interface. The blue line shows the s-polarized light (perpendicular to the plane of incidence), the green line shows the p-polarized light (parallel to the plane of incidence), and the red line shows the unpolarized light.

Generally, taps are available in a variety of materials and shapes. When the tap is long and the reflection occurs at the tap-surface/water interface, special care should be taken in terms of the tap material. Figure 6b,c show the difference in reflectivity between the Al-surface/water interface (Figure 6b) and the Fe-surface/water interface (Figure 6c). When we use a tap with an Al surface, due to the low refractive index (0.21) and the high extinction coefficient (3.12) of the Al material [51], a high reflectivity, of more than 90%, can be obtained for all the incident angles (blue line: s-polarized light; green line: p-polarized light; red line: unpolarized light). However, when we use a tap with an Fe surface, due to the high refractive index (1.57) and the high extinction coefficient (1.76) of the Fe material [52], the reflectivity is significantly reduced for all the incident angles, as shown in Figure 6c (blue line: s-polarized light; green line: p-polarized light; red line: unpolarized light). Therefore, when the reflection occurs at the tap-surface/water interface, it is necessary to use Al materials or Al-coated materials inside the surface of the tube. We note here that when we use a fused silica glass tube that is transparent in the FUV-DUV...
regions, Al can be coated on the tube surface. As a reference, Figure 6d shows reflectivity as a function of incident angle at the air/water interface. Total internal reflection occurs at an incident angle larger than 47° (blue line: s-polarized light; green line: p-polarized light; red line: unpolarized light). As there is an abrupt change in reflectivity at this angle, special care should be taken to ensure that the reflection angle inside the water is greater than this angle.

In recent years, FUV radiation with wavelengths shorter than 230 nm has been attracting special interest as a safe and effective disinfection radiation for the human body. This result can be applied to FUV irradiation in air or in vacuum. When we use FUV radiation in water, it is necessary to consider the absorption of FUV radiation in water, because the absorption of water, as shown by the broken blue line in Figure 7, significantly increases when decreasing the FUV wavelength to less than 230 nm. Therefore, the effectiveness of a 220 nm irradiation dose compared to that of a 265 nm irradiation dose is reduced to 50%, which is calculated by using Equation (1). For example, here we consider a 1 m optical path length in water. By considering the absorption effect using Equation (1) ($\xi = 1$ m was used for the integration of Equation (1)), the dispersion curve of the effectiveness of the dose can be obtained, as shown by the orange line in Figure 7, where the effectiveness of the dose is normalized as 1 at a 300 nm DUV wavelength.

Thus, the effectiveness of inactivation of bacteria in water can be determined by both the dispersion curve of the effectiveness of the dose in water (orange curve) and the absorption spectrum of DNA (broken green line). (We note here that in Figure 7, the absorption coefficient of DNA is reduced because it has a large value, in the order of $10^5$ cm$^{-1}$.)

Through Equation (1), the effectiveness of inactivation for the bacteria in water based on the experimentally obtained inactivation rate constants shown in Figure 3d. The red circles in Figure 7 clearly show the modified inactivation rate constants, which consider the absorption of water (here, we consider both the dispersion curve of the effectiveness of the dose in water and the absorption spectrum of DNA. The orange line shows the dispersion curve of the effectiveness of the dose obtained by Equation (1), where a 1 m optical path length in water is considered for the calculation. The red circles signify the modified inactivation rate constants due to the absorption of water. The broken red line shows the interpolation curve of these results.

![Figure 7. Inactivation rate constants modified by the absorption of water in the DUV wavelength region. The broken blue line shows the absorption spectrum of water. The broken green line shows the absorption spectrum of DNA. The orange line shows the dispersion curve of the effectiveness of the dose obtained by Equation (1), where a 1 m optical path length in water is considered for the calculation. The red circles signify the modified inactivation rate constants due to the absorption of water. The broken red line shows the interpolation curve of these results.](image-url)
$10^5$ cm$^{-1}$.) As shown in Figure 3d, we experimentally observed the correlation between the absorption coefficient of DNA and the inactivation rate constants. Here, we estimate the effectiveness of the inactivation for the bacteria in water based on the experimentally obtained inactivation rate constants shown in Figure 3d. The red circles in Figure 7 show the modified inactivation rate constants, which consider the absorption of water (here, we consider a 1 m optical path length in water) obtained by multiplying the dispersion curve of the effectiveness of the dose (orange curve) and the inactivation rate constants shown in Figure 3d. The modified inactivation rate constants in the red circles in Figure 7 clearly show that the effectiveness of inactivation at wavelengths shorter than 230 nm is significantly reduced, to approximately 20%, compared to that at the wavelength of 265 nm. Therefore, it is likely that the emission power of FUV-LEDs with wavelengths shorter than 230 nm must be five times higher compared to that of 265 nm LEDs to obtain the same inactivation efficacy (the same magnitude of log reduction). However, the fabrication of high-power LEDs with wavelengths shorter than 230 nm is extremely difficult at present due to the high resistivity of the p-type AlGaN layer as well as the low hole concentration and high density of threading dislocations in AlGaN materials [36,53].

Several groups have proposed a promising alternative, and one promising approach to avoid these problems associated with the epitaxial growth of the AlGaN layer is to use electron beam (EB) excitation. As reported by Oto et al. [54], DUV emission from EB-pumped AlGaN quantum wells (QWs) resulted in an emission wavelength of 240 nm, a high output power of 100 mW, and a high power efficiency, exceeding 40%. We also constructed a portable EB-pumped DUV emission source [36] by combining an AlGaN QWs DUV emission layer and graphene nanoneedle field emitters of electrons [55–57]. The device has a 20 mW DUV output power and a power efficiency of approximately 4% at the emission wavelength of 240 nm, where $\text{Al}_x\text{Ga}_{1-x}\text{N}/\text{AlN}$ layers with a stoichiometric $x$ of 0.7 were used. By increasing the molar fraction of Al from 0.7 to 0.9, it is possible to obtain FUV radiation shorter than 230 nm. We had some promising results for the fabrication of a high-power and portable EB-pumped FUV emission source; these will be reported elsewhere.

5. Conclusions

In this paper, we showed that the instantaneous disinfection of bacteria-contaminated water is possible by using a low-power DUV-LED because the point-source nature of the intense DUV-LED emission can be guided to a confined space of the WW region without reflection and attenuation losses. Our results show that the WW method presented here can disinfect water contaminated by many types of bacteria, such as Cryptosporidium parvum, Giardia lamblia, and Vibrio cholera, because these pathogenic microorganisms have approximately the same UV sensitivity as that of E. coli and P. aeruginosa presented in this paper [49]. By using a much-higher-power DUV-LED, in the order of 100 mW, it is possible to quickly supply purified water with a higher disinfection level (more than 3-log) and/or instantaneously disinfect water contaminated with more UV-resistive microorganisms, such as Enterovirus [49,58]. To obtain much larger reduction efficacy (more than 3-log level), the use of Al materials is promising, as described in Figure 6b. This is because Al has a high reflectivity of DUV light (more than 90%) over a wide range of reflection angles. This characteristic gives both the longer region and the freedom of shape of the WW.

We discuss the possibility of FUV radiation with wavelengths shorter than 230 nm, because the radiations in this region have been attracting interest as a safe and effective disinfection radiation for the human body. The analysis that considers the absorption of FUV radiation in water shows that the effectiveness of inactivation at wavelengths shorter than 230 nm is significantly reduced, to approximately 20%, compared to that at the wavelength of 265 nm for a 1 m optical path length of the WW. However, to use the WW disinfection system as a consumer product, we consider that the FUV wavelength that can be sterilized without any interference to the human body is a great advantage. We consider that FUV emission devices based on the field emission of electrons are useful at present,
and FUV-LEDs based on PN junctions will be useful in the future for the purification of contaminated water using a WW method. For these DUV and FUV lights to be a feasible option for field implementation, the cost needs to decrease and the power output needs to increase substantially [59].

**Author Contributions:** T.M. is the first author. I.T. and T.M. contributed to the design of the wavelength-tunable DUV light source. T.H. (Tadao Hasegawa) and T.M. contributed to the design of the water waveguide purification system. T.H. (Tsuyoshi Hoshiai) and I.T. completed the inactivation of bacteria and colony-forming experiments. T.H. (Tsuyoshi Hoshiai), I.T. and T.H. (Tadao Hasegawa) performed statistical analyses of the inactivation experiments and deduced inactivation rate constants for various bacteria. T.M. calculated the reflectivity of DUV light at interfaces between water and materials. All authors have read and agreed to the published version of the manuscript.

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