

## Article

# Instantaneous Water Purification by Deep Ultraviolet Light in Water Waveguide: *Escherichia Coli* Bacteria Disinfection

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Received: 19 March 2019; Accepted: 5 May 2019; Published: 9 May 2019



**Abstract:** The necessity of small water purification equipment has been increasing in recent years as a result of frequent natural disasters. Ultraviolet (UV) radiation treatment is an effective method for the disinfection of bacterial contaminants in water. As an emerging technology, disinfection 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 demonstrate the instantaneous purification of contaminated water by combining the point source characteristics of DUV-LEDs with a water waveguide (WW). The principle is based on the WW region acting as an effective DUV disinfectant, whereby a high UV dose in a confined WW region can be applied to bacterial contaminants in a short period of time (around one second). We demonstrate the effect of this DUV-LED WW disinfection technique by showing the results of 3-log disinfection levels of water contaminated with *Escherichia coli* bacteria after a short treatment time. We believe that the combination of the point-source nature of DUV-LED emission, the water-waveguide effect, and a small photovoltaic cell paves the way toward environmentally friendly and emergency preparedness portable water purification equipment that instantaneously supplies clean water just before drinking.

**Keywords:** water; disinfection; bacteria; waveguide; deep-ultraviolet; light-emitting diode; *Escherichia coli*

## 1. Introduction

Ultraviolet (UV) radiation treatment is an effective method for the disinfection of bacterial, eukaryotic, and viral contaminants present in water, and it serves as an alternative technology to chemical disinfection techniques [1–3]. The advantages of UV disinfection are: (i) fast treatment; (ii) minimal cost [4] and labor; (iii) no hazards associated with chemical handling and disposal; (iv) eliminates the need for chloramine or hypochlorite chemicals; and (v) no disinfection byproducts produced [5]. The wavelength of UV radiation used for disinfection is generally shorter than 280 nm, which places it in the UVC region (200–280 nm). This wavelength is selected because the UVC region matches the absorption spectra of deoxyribonucleic acid (DNA) [6–9]. UVC radiation absorption causes the inactivation of microbe contaminants by the formation of cyclobutane pyrimidine dimers, pyrimidine 6-4 pyrimidone photoproducts, and their Dewar isomers in DNA [10,11]. This UVC-irradiated DNA becomes irreversibly cross-linked, thereby restricting further replication of the DNA strands without intermediate steps [12,13].

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) [1,6–9]. However, there are many drawbacks to using mercury vapor lamps: the lamps contain highly toxic mercury, require fragile quartz glass tubes to seal in the mercury gas, require a high alternating voltage on 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 [14]. Thus, the lamps need to be replaced about once per year when continuously used.

As an emerging technology, deep-ultraviolet light-emitting diodes (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. Recently, DUV-LEDs based on aluminum gallium nitride (AlGaIn) semiconductor materials [15,16] have led to several advances, such as the achievement of a narrow emission spectrum that can be tuned between 210 (AlN) to 365 nm (GaIn) by changing the stoichiometry between Al and Ga, a low operating voltage on the order of 10 V DC, a small emission area on the order of 1 mm<sup>2</sup>, and instantaneous operation. However, the obtained output power, external quantum efficiency, and lifetime of DUV-LEDs have not yet reached their theoretical maxima at the present stage. Therefore, many studies have been conducted [14,17,18] on how to achieve an output power of 1 W, an external quantum efficiency of 50%, a lifetime of 100,000 h, and a low price, as these characteristics are already available with indium-based nitride semiconductors, such as indium gallium nitride (InGaIn) blue LEDs.

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. Many unique disinfection techniques that use DUV-LEDs have been proposed. Two examples are (i) the search for the effective emission wavelength which inactivates viruses and microorganisms (this emission wavelength was not achievable with mercury vapor lamps) [14,19,20], and (ii) the construction of germicidal DUV-LED lamps by combining multiple wavelength irradiation [21–23] in order to obtain higher pathogen inactivation.

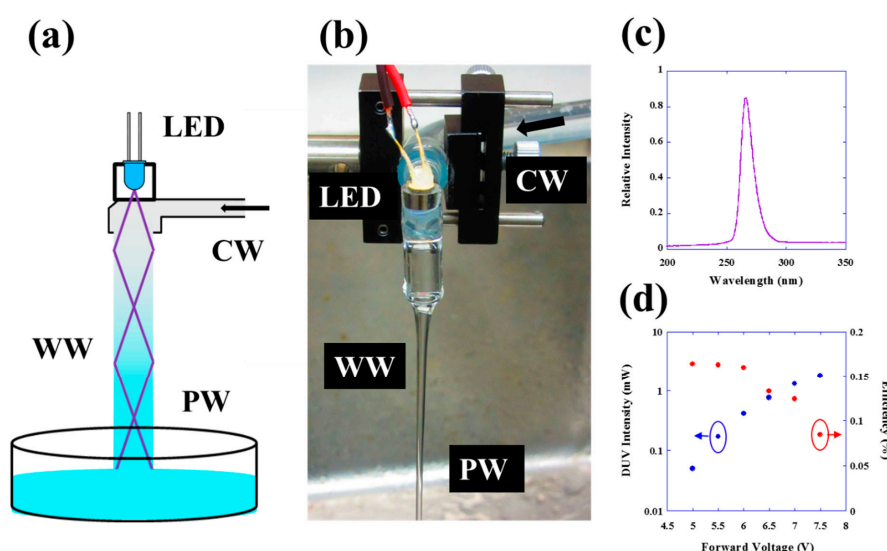
Recently, we proposed a new washing technology involving the use of a small DUV-LED device with 1 mW optical output power and running water [24]. The concept is based on the optical coupling of DUV radiation with a water stream. By using the water-waveguide (WW) effect, both physical cleaning by running water and photochemical inactivation by DUV light can be achieved simultaneously. The well-known WW effect can be seen in many educational and/or artistic video images of visible light traveling along a water stream under internal reflection [25,26]. However, this WW effect is not only suitable for artistic demonstrations; it is also technically useful as an environmental technology. This is because the combination of the point-source nature of DUV-LED emissions and the WW effect has the potential to reduce the large amount of water consumed by washing technologies in a simple manner.

In this research, by applying the recently developed WW method described above, we show another application: the demonstration of the instantaneous purification of contaminated water. The principle is based on the WW region acting as an effective DUV disinfectant, whereby a high DUV dose (approximately 10 mJ/cm<sup>2</sup>) can be applied to bacterial, eukaryotic, and viral contaminants in a short period of time (around one second). This is 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. Therefore, the bacteria inside the WW region receive a high DUV dose on the order of 10 mJ/cm<sup>2</sup>, leading to instantaneous disinfection. We demonstrate the effect of the DUV-LED WW disinfection technique by showing the instantaneous purification of water contaminated with *Escherichia coli* (*E. coli*) bacteria. We believe that the combination of the point-source nature of DUV-LED emission and the WW effect paves the way toward environmentally friendly and emergency preparedness portable water purification equipment that instantaneously supplies clean water just before drinking.

## 2. Materials and Methods

### 2.1. Water Purification by DUV-LED Water Waveguide Method

The main components of the WW purification system, shown in Figure 1a (schematic) and Figure 1b (photo), are a TO-CAN-type DUV-LED capped with a lens cap (VPT731 from Nikkiso Ltd., Tokyo, Japan) and *E. coli* bacteria-contaminated water (CW) supplied from a water tank. To introduce the 265 nm emission into the water stream, we used a T-shaped glass tube with a diameter of 6 mm.



**Figure 1.** (a) Schematic and (b) photograph of the water waveguide purification system. *Escherichia coli* bacteria-contaminated water (CW) is supplied from a water tank reservoir, and the *E. coli* bacteria were disinfected at the water-waveguide (WW) region. To introduce the 265 nm emission from a deep-ultraviolet light-emitting diode (DUV-LED) into the WW region, we used a T-shaped glass tube. The length of the WW region was approximately 20 cm. After disinfection by the WW method, the degree of purification was analyzed by using 100  $\mu$ L of the solution from the purified water (PW) to coat nutrient broth agar plates. (c) Emission spectrum of the DUV-LED. The peak emission wavelength was 265 nm with a spectral width of 12 nm. (d) DUV intensity (blue circles) and wall-plug efficiency (red circles) as a function of forward voltage. DUV-LED intensity varied from 0.05 to 1.8 mW at a forward voltage of 5–7.5 V and a driving current of 20–300 mA. The efficiency gradually dropped beyond an applied voltage of 6 V (rated voltage) because of the thermal quenching effect.

Figure 1c shows the emission spectrum of the DUV-LED. The emission wavelength was 265 nm with a spectral width (full-width at half maximum) of 12 nm, as measured by a spectrometer through an optical fiber (BIM-6002A, Brolight Technology Corporation, Hangzhou, China). As shown by the relation between the forward voltage and DUV emission intensity depicted by blue circles in Figure 1d, the emission intensity,  $I$ , of the LED varied from 0.05 to 1.8 mW at a forward voltage of 5–7.5 V and a driving current of 20–300 mA. The efficiency depicted by red circles gradually dropped beyond the applied voltage of 6 V (rated voltage) because of the thermal quenching effect. Therefore, to prevent the device from heating up, the DUV-LED was carefully operated by blowing air into it when the forward voltage exceeded 6 V.

Total reflection inside the WW occurs at incident angles larger than  $47^\circ$ , as estimated by Snell's law. A refractive index of water,  $n$ , of 1.36 for this wavelength region was used for the estimation [27–29]. Therefore, a DUV-LED with viewing angle of  $6^\circ$ , as shown in Figure 1b, satisfies the total internal reflection condition. Here, we note that special care should be taken so that the lens of the DUV-LED is not immersed in water [24]. This is because the T-shaped glass tube for obtaining the WW effect was made of borosilicate glass material, which has a large (greater than  $10 \text{ cm}^{-1}$ ) absorption coefficient at a wavelength of 265 nm. The CW supplied from the water tank was purified (PW) in the WW region, as

shown in Figure 1a,b. The density of the bacterial cells in the CW was controlled within a range of approximately  $10^3$ – $10^4$  cells/mL, and the CW flow rate was 100 mL/min.

The length of the WW region was approximately 20 cm, and the path length of the DUV light was long [30]. In this case, the weak absorption affects the dose of bacteria. Here, we consider the absorption effect of the WW region. The total dose,  $D_T$  (mJ/cm<sup>2</sup>), in the WW region is given by the following equation:

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

where  $\eta$  is the coupling efficiency of the DUV-LED emission in the WW,  $I_0$  (mW) is the DUV-LED intensity,  $S$  (cm<sup>2</sup>) is the cross-sectional area of the WW,  $v$  (cm/s) is the flow velocity,  $\xi$  (cm) is the length of the WW region,  $\alpha$  (cm<sup>−1</sup>) is the absorption coefficient of the CW at 265 nm, and  $\rho$  (cm<sup>3</sup>/s) is the flow rate. After substituting the variables with the values obtained in the experiment, the coupling efficiency ( $\eta$ ) was about 40% when measured by setting an optical fiber in the WW stream. The maximum intensity of the DUV-LED ( $I_0$ ) was 1.8 mW, the flow rate ( $\rho$ ) was 1.67 cm<sup>3</sup>/s (100 mL/60 s), the length of the WW region ( $\xi$ ) was approximately 20 cm, and the absorption coefficient ( $\alpha$ ) at 265 nm was estimated as  $\alpha = 0.02$  cm<sup>−1</sup>. Therefore, the total dose ( $D_T$ ) in the WW region could be controlled from 0 to 6.0 mJ/cm<sup>2</sup> by changing the emission intensity of the DUV-LED from 0 to 1.8 mW.

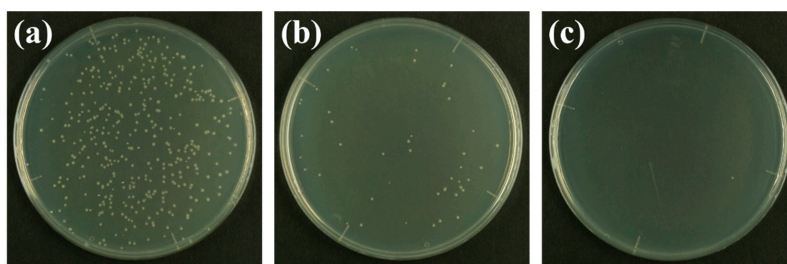
## 2.2. Culturing and Enumeration of Microorganisms

A pure culture of the *E. coli* strain DH5 $\alpha$  was incubated in nutrient broth (E-MC63; EIKEN Chemical Co., Tokyo, Japan) at 37 °C for 20 h. Bacteria at a concentration of  $10^9$ – $10^{10}$  colony-forming units (CFU)/mL were formed and used for the experiments. Then, 10  $\mu$ L of the culture was taken and dissolved in 5 L of normal saline solution as contaminated water. After the disinfection by the WW method, the degree of purification was analyzed by using 100  $\mu$ L of solution from the disinfected water to coat nutrient broth agar plates. The colonies were counted after incubation for 24 h at 37 °C. Plates yielding 1–500 CFU were considered for analysis. All experiments were performed at least three times independently.

## 3. Results and Discussion

### 3.1. Efficacy of Disinfection by DUV-LED Water-Waveguide Method

The results of the efficacy of disinfection by using the WW purification system are shown in Figure 2, where Figure 2a is the control plate (0 mJ/cm<sup>2</sup> DUV dose), Figure 2b is the plate disinfected by a 1.0 mJ/cm<sup>2</sup> DUV dose, and Figure 2c is the plate disinfected by a 6.0 mJ/cm<sup>2</sup> DUV dose. By applying a higher DUV dose, the number of colonies was significantly reduced from  $365 \pm 51$  CFU (control plate, Figure 2a) to  $55 \pm 19$  CFU for the 1.0 mJ/cm<sup>2</sup> DUV-dose plate (Figure 2b) and  $2.0 \pm 0.9$  CFU for the 6.0 mJ/cm<sup>2</sup> DUV-dose plate (Figure 2c). Here, the numbers reported are the means and standard deviations of the number of CFU. These results clearly show that purified clean water with disinfection levels from 2- to 3-log<sub>10</sub> can be available almost instantaneously by using the DUV-LED WW disinfection method.



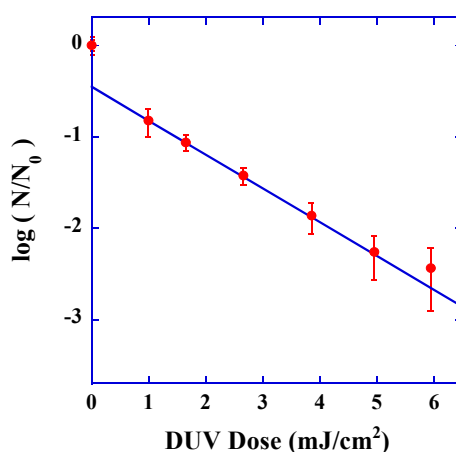
**Figure 2.** Results of the disinfection for *E. coli*. (a) Control plate (0 mJ/cm<sup>2</sup> DUV dose), (b) plate disinfected by 1.0 mJ/cm<sup>2</sup> DUV dose, and (c) plate disinfected by 6.0 mJ/cm<sup>2</sup> DUV dose. The number of colonies counted were  $365 \pm 51$  colony-forming units (CFU) for the control plate (a),  $55 \pm 19$  CFU for the 1.0 mJ/cm<sup>2</sup> DUV-dose plate (b), and  $2.0 \pm 0.9$  CFU for the 6.0 mJ/cm<sup>2</sup> DUV-dose plate (c).

### 3.2. Theoretical Analysis of the Disinfection Rates

To quantitatively investigate the reduction in inactivation rates as a function of the DUV dose, we plotted the CFU response to DUV (disinfected CFU by DUV irradiation,  $N(D)$ ), divided by control CFU,  $N_0$ ) caused by the WW treatment, as shown in Figure 3. The disinfection kinetics as a function of the DUV dose was determined by a single-target model [31]:

$$\text{Log}\left(\frac{N_0}{N(D)}\right) = K_{EC}D \quad (2)$$

where  $K_{EC}$  (cm<sup>2</sup>/mJ) is the log<sub>10</sub> dose-based disinfection rate constant,  $D$  is the magnitude of the DUV dose (mJ/cm<sup>2</sup>),  $N_0$  is the number of CFUs on the unirradiated control (CFU/mL), and  $N(D)$  is the number of CFUs at a given dose  $D$ . The DUV dose–response results can be fitted using a single disinfection constant,  $K_{EC} = 0.37$  (cm<sup>2</sup>/mJ), as shown by the solid line in Figure 3.



**Figure 3.** DUV dose–response of *E. coli* disinfected by water-waveguide treatment. The disinfection kinetics as a function of the DUV dose was determined by a single-target model. The DUV dose–response results could be fitted using a single disinfection constant,  $K_{EC} = 0.37$  (cm<sup>2</sup>/mJ), as shown by the solid line.

There are many different values of UV disinfection constants reported for *E. coli*, with the lowest value equal to 0.16 cm<sup>2</sup>/mJ [32] and the highest value equal to 0.8 cm<sup>2</sup>/mJ [33]. It is difficult to precisely compare UV disinfection constants because the strains of *E. coli*, the source of the UV lamps (low-pressure or medium-pressure mercury lamps or UV-LED), and the irradiation apparatus are different among reports. The DUV-LED WW disinfection method described here offers a unique apparatus configuration, and this apparatus is different from other previously reported UV disinfection procedures, in which contaminated suspensions were placed in Petri dishes containing magnetic spin



bars and rotated during UV exposure [18–23,32,33]. However, the disinfection constant obtained in this study,  $K_{EC} = 0.37$  (cm<sup>2</sup>/mJ), almost coincides with the most frequently reported values, such as 0.29 cm<sup>2</sup>/mJ by using a 260 nm LED [20], 0.37 cm<sup>2</sup>/mJ by using a 265 nm LED [34], and 0.303 cm<sup>2</sup>/mJ by using a 253.7 nm mercury vapor lamp [35]. Therefore, we believe that the DUV-LED WW method offers not only an efficient purification of contaminated water but also a novel and simple method for determining the disinfection constant of various bacteria and viruses.

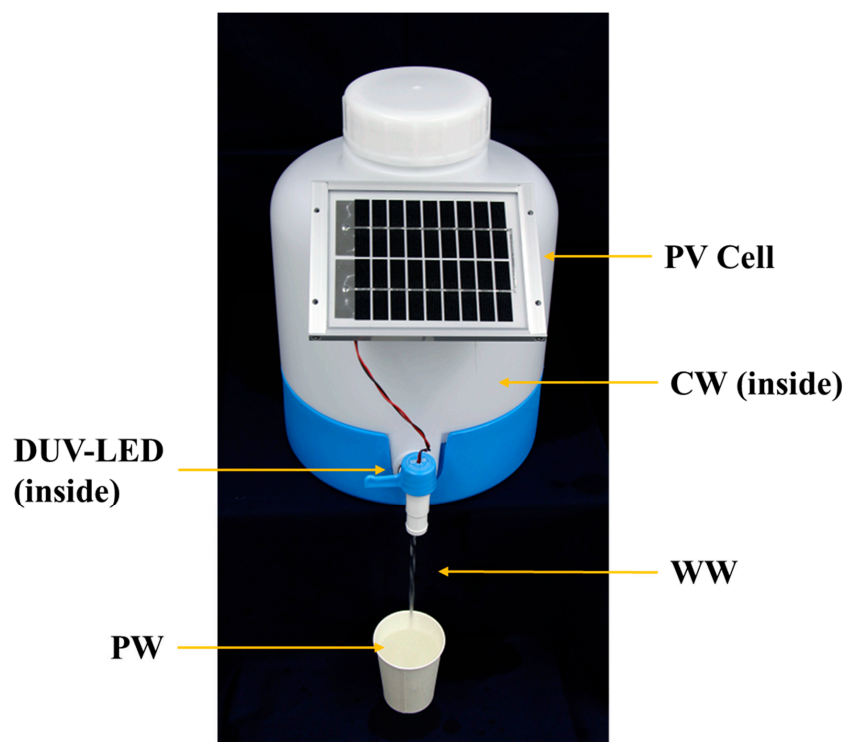
In order to translate the DUV-LED WW disinfection method into practical use, it is necessary to consider the disinfection efficacy not only for *E. coli* but also for other bacteria that cause serious waterborne infections, such as *Vibrio cholerae* (*V. cholerae*) [1,36,37], *Cryptosporidium parvum* oocysts (*C. parvum*) [38–41], *Giardia lamblia* cysts (*G. lamblia*) [42,43], *Legionella pneumophila* (*L. pneumophila*) [33,36,44], and *Pseudomonas aeruginosa* (*P. aeruginosa*) [14,33,35]. We aimed to obtain the UV sensitivity of the 3-log-level reduction in these bacteria by using the DUV-LED WW method shown in Figure 1. The dose for the 3-log-level reduction, disinfection constants (cm<sup>2</sup>/mJ), and the required DUV-LED intensities were evaluated, and the results are presented in Table 1. We note here that the required DUV-LED intensities listed in Table 1 for each bacteria species are the values for obtaining 3-log purified water with a flow rate of 100 mL/min. The magnitude of the dose for 3-log-level reduction in bacteria other than *E. coli* was estimated on the basis of previously reported values [1,33,35–44]. The results presented in Table 1 show that the combination of a DUV intensity of 2.5 mW and the WW method could disinfect almost all of the bacteria, except *P. aeruginosa*, to a 3-log-level reduction at a rate of 100 mL/min. Therefore, by using a DUV-LED with much higher power (on the order of 100 mW), which is available at the present stage, the water flow rate can be increased from 100 to 4000 mL/min, and/or we can instantaneously disinfect more UV-resistant bacteria, such as *P. aeruginosa* and *Bacillus subtilis* spores [33,45,46].

**Table 1.** Dose requirements vs. type of bacteria (first row) to obtain 3-log-level reductions (second row), disinfection constants (third row), and required DUV-LED intensity (fourth row) for *E. coli*, *V. cholerae*, *C. parvum*, *G. lamblia*, *L. pneumophila*, and *P. aeruginosa*. The values of the dose and the disinfection constants for species other than *E. coli* were evaluated using the references reported in the fifth row. The required DUV-LED intensity for each bacteria species was calculated to obtain 3-log-level purified water with a flow rate of 100 mL/min.

Type of Bacteria	Dose for 3-log (mJ/cm <sup>2</sup> )	Disinfection Constant (cm <sup>2</sup> /mJ)	Required DUV LED Intensity (mW)	Reference
<i>E. coli</i>	8.1	0.37	2.43	This study
<i>V. cholerae</i>	2.2–3.0	1.0–1.36	0.66–0.9	[1,36,37]
<i>C. parvum</i>	2.0–6.0	0.5–1.5	0.6–1.8	[38–41]
<i>G. lamblia</i>	2.0–6.0	0.5–1.5	0.6–1.8	[42,43]
<i>L. pneumophila</i>	2.8–6.9	0.43–1.07	0.84–2.07	[33,36,44]
<i>P. aeruginosa</i>	7.0–16.0	0.19–0.43	2.1–4.8	[14,33,35]

### 3.3. Portable Water Purification System with DUV-LED, Water Waveguide, and Photovoltaic Cell

The combination of the DUV-LED WW method and a small photovoltaic (PV) cell, as shown in Figure 4, is currently under investigation for practical use. This apparatus includes a PV cell with an open-circuit voltage of 11 V and short-circuit current of 230 mA (GT1618-MF, Nagano, Japan), which could provide enough electric power to the DUV-LED anywhere. The combination of a small DUV-LED, a small PV cell, and the WW method will offer a new convenient point-of-use faucet for emergency preparedness and disaster relief.



**Figure 4.** New convenient water purification equipment for emergency preparedness and disaster relief. Contaminated water (CW) containing *E. coli* bacteria in a water tank (20 L). CW is instantaneously (approximately 2 s) changed into purified water (PW) by combining the point-source characteristics of the DUV-LED and the water waveguide (WW). The electric power of the DUV-LED is supplied from a small photovoltaic (PV) cell with an open-circuit voltage of 11 V and short-circuit current of 230 mA.

#### 4. Conclusions

We successfully demonstrated a novel instantaneous water purification technique that uses the synergistic effect between DUV-LEDs and the WW method. The instantaneous disinfection of bacteria using a low-power DUV-LED is possible 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 combination of the low-power DUV-LED and the WW method could disinfect many types of bacteria, such as *E. coli*, *V. cholerae*, and *C. parvum*, leading to a 3-log-level purified water supply with a flow rate of 100 mL/min. By using a DUV-LED with much higher power (on 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 more UV-resistant bacteria, such as *P. aeruginosa* and *Bacillus subtilis* spores [33,45,46]. We believe that the combination of the point-source nature of DUV-LED emission, the water-waveguide effect, and a small photovoltaic cell paves the way toward environmentally friendly and emergency preparedness and disaster relief portable water purification equipment that instantaneously supplies clean water just before drinking.

**Author Contributions:** T.M. is the co-first author. T.M., I.T., and T.H. contributed to the design of the water waveguide purification system and completed all the experiments. I.T. and T.H. provided technical support and bacterial expertise for the bacterial growth and colony-forming experiments. T.M., I.T., and T.H. constructed the quantitative model of the water waveguide DUV-LED disinfection results. T.M. and I.T. performed the statistical analysis for the infectivity experiments. All authors read and approved this submitted manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** The authors are grateful to Nikkiso Giken Corporation Ltd. for providing the DUV-LED devices and technical support for this study. The authors would like to thank Tomomi Fukuoka, Rika Kikojima, and Yuji Kohmura for their help with the water purification experiments. The authors would also like to thank Motoaki Iwaya and Sho Iwayama at Meijyo University for information about the present status of DUV-LEDs.

**Conflicts of Interest:** The authors declare no competing interests.

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