ORIGINAL

UVA-LED device to disinfect hydroponic nutrient solution

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Abstract : The number of plant factories in which crops are cultivated in an artificial environment has been increasing every year. In cultivation techniques involving hydroponics, plants are supplied with a circulating nutrient solution, which can become contaminated by pathogens that can propagate and spread throughout plant factories. Therefore, strategies to disinfect hydroponic nutrient solutions are needed. In this study, we developed a new disinfection device equipped with an ultraviolet A (UVA) light emitting diode (LED) that can be used to disinfect hydroponic nutrient solutions in plant factories. We first evaluated the basic disinfection capability of the device and then estimated its bactericidal effect in a small scale model system. The log survival ratio was related to UVA irradiation fluence and the volume of nutrient solution. From the assay results, we devised a kinetics equation to describe the relationship between nutrient solution volume, log survival ratio, and UVA fluence. Together our results show that UVA irradiation could be used to disinfect hydroponic nutrient solutions, and the derived kinetics equations can be used to determine optimal conditions, such as nutrient solution volume, UVA irradiation, and killing activity, to develop devices that disinfect hydroponic nutrient solutions. J. Med. Invest. 65 : 171-176, August, 2018

Keywords **:** *ultraviolet A light emitting diode, disinfection, hydroponic nutrient solutions*

INTRODUCTION

Food safety remains an issue of great concern for the Japanese population (1) and stable food supply systems in Japan that produce high-quality food are needed (2, 3). The number of factories that are devoted to artificial cultivation of plants has been increasing in recent years (4, 5). Many of these factories use hydroponics, in which circulating nutrient solutions instead of soil are used to grow plants in an artificial environment to provide a steady supply of high quality plant materials for food (6, 7). However, contamination of hydroponic nutrient solutions by pathogens that can propagate and spread throughout a plant factory is a significant problem (8, 9). The use of treated wastewater for irrigation is increasing, especially in those areas where water resources are limited (10, 11). Thus, strategies for effective disinfection of hydroponic nutrient solutions are needed.

Given the increased consciousness of the public about food safety and security, newly developed disinfection methods for hydroponic nutrient solutions ideally should : a) avoid the use of chemical disinfectants ; b) have a low environmental impact and no negative effects on human health ; and c) be simple to build and easy to use (12-16). Whereas disinfection devices using ultraviolet-A (UVA) light are effective and avoid the use of chemicals, devices that use low-pressure mercury lamps to generate UVA can be costly due to the short lifespan and high energy consumption of the lamps (12-15). Furthermore, mercury waste generated by spent lamps requires disposal.

In this study, we describe the development of a disinfection device that couples a UVA light-emitting diode (UVA-LED) with a circulation system. The UVA-LED is advantageous in that it has a long life and produces no mercury waste. Moreover, UVA irradiation does not cause significant damage to crops or degrade nutrient solutions. Meanwhile, we adapted batch-process wastewater disinfection methods for the circulation system that allow large amounts of nutrient water to be treated and stored (17, 18). Such a device could be incorporated into existing plant factories without disturbing production lines.

During circulation, the nutrient solution would spend a certain amount of time exposed to the UVA-LED in the disinfection tank and the remaining time circulating through the pipes. As such, the solution would have a UVA irradiation time and non-irradiation time. The timing of UVA irradiation can be related to bacterial growth. In this study, we tested our disinfection device to evaluate its performance under conditions that mimic those seen in an actual plant factory and developed equations to describe the relationship between nutrient solution volume, log survival ratio, and UVA fluence. These equations can be applied in plant factories to optimize disinfection of nutrient solutions.

MATERIAL AND METHODS

Bacterial strains and growth conditions

The *Escherichia coli* strain ATCC25922 was used as a model microorganism for the disinfection experiments (13). The bacteria were cultured in Luria-Bertani (LB) broth (1% tryptone, 1% NaCl, 0.5% yeast extract) at 37°C with rotary shaking for 18 h. Then, 2 mL

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overnight culture was centrifuged at 12,000 rpm for 3 min. The supernatant was discarded and the bacterial pellet was washed three times with sterilized phosphate buffered saline (PBS) (140 mM NaCl, 2.5 mM KCl, 1.6 mM KH_2PO_4, 15 mM Na_2HPO_4) and suspended in PBS at an initial concentration of 10^9 cfu mL^{-1}. After conditioning, the bacterial solution was diluted 100-fold in nutrient solution (see below).

Nutrient solution

For the test nutrient solution we used Amino(OAT) house No. 1 and OAT house No. 2 formulations at 1.5 and 1.0 g L^{-1}, respectively (Otsuka Agri Techno Co., Ltd, Osaka, Japan) (Kawamura *et al*., 2014 ; Hossain *et al*., 2012). We adopted a prescription that is a general-purpose culture solution used in the production of fruits and vegetables, as well as leafy vegetables and flowering plants (13).

Development of a disinfection device

The device has six UVA-LEDs [NC4U134 (T) ; Nichia Corp., Tokushima, Japan] connected in series to a single power source (Fig. 1(a)). The peak wavelength of the diode was 385 nm (Hamamoto *et al*., 2007). The maximum current was 0.5 A, the voltage was 111 V and UVA light intensity was 0.58 kW m^{-2}, which was measured by a spectrophotometer (MCPD-370A ; Otsuka Electronics Corp., Osaka, Japan). The circulating disinfection device we developed included a cylindrical disinfection pipe with a 1 L volume (Fig. 1(b)). For testing, the device was used to irradiate the wells of a sterile, UVA-penetrable 96-well plate with 200 µL of bacterial solution aliquoted to a depth of 5 mm.

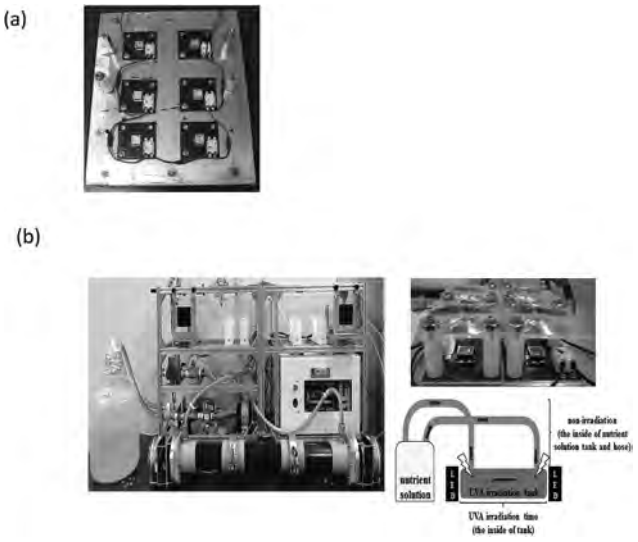


Figure 1. (a) ; Disinfection device. The maximum current for one diode was 0.5 A and the ultraviolet A (UVA) light wavelength was 385 nm. (b) ; Test model for disinfection device. The device is 560 mm x 1200 mm. The cylindrical tank has a 67 mm inner diameter and is 300 mm long. This device has six LEDs.

The model disinfection device was 560 mm long x 1,200 mm wide. The cylindrical tank had a 67 mm inner diameter and a total length of 300 mm. The nutrient solution was disinfected in an external tank while the circulating solution passed through the cylindrical disinfection tank.

Determination of inactivation level

Bacterial inactivation was determined using a colony-forming assay (Hamamoto *et al*., 2007). After UVA irradiation, bacterial suspensions were diluted appropriately, plated on LB agar plates and incubated at 37°C for 18 h. Then, the number of resulting colonies was counted, and the log survival ratio or inactivation percentage was calculated using the following equations :

$$\text{Log survival ratio} = \text{Log}\ (N_t / N_0)$$
$$\text{Inactivation percentage}\ (\%) = 100 - (N_t / N_0 \times 100),$$

where N_t is the colony count of the UVA-irradiated sample, and N_0 is the colony count of the sample before UVA irradiation.

Measurement of 8-OHdG in UVA-irradiated E. coli

The yield of the DNA damage marker 8-OHdG in irradiated *E. coli* suspensions was measured using a competitive enzyme-linked immunosorbent assay (ELISA) kit (High Sensitivity 8-OHdG Check ; Japan Institute for the Control of Aging, Nikken SEIL Corp., Fukuroi, Japan), following the manufacturer's instructions. *E. coli* was cultured in LB broth for 18 h at 37°C until the stationary phase was reached. Cells were centrifuged (5,000 g, 10 min, 4°C), washed three times with sterile PBS and diluted to OD_{600} of 1.5. Exactly 600 µL of the suspension was divided among four wells of a 96-well plate and exposed to 216 kJ m^{-2} UVA-LED light. To evaluate the role of hydrogen peroxide (H_2O_2) or hydroxyl radicals (OH·) in UVA-LED-induced 8-OHdG formation, catalase (2200 U/well) or 10 mmol L^{-1} mannitol, respectively, was added to the bacterial suspension before UVA-LED irradiation. After irradiation, 140 µL of bacterial DNA was extracted with a DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Then, 15 µL 200 mmol L^{-1} sodium acetate buffer (pH 4.8) and 6.0 U nuclease P1 (Wako, Osaka, Japan) were added to 140 µL of the extracted DNA solution. After purging with argon gas, the mixtures were incubated at 37°C for 30 min to digest DNA, followed by the addition of 15 µL 1 mmol L^{-1} Tris-HCl (pH 7.4) and 2.0 U *E. coli* alkaline phosphatase (Takara Bio Inc.). After purging again with argon gas, the mixtures were incubated at 37°C for 1 h to hydrolyze nucleotides to nucleosides. Samples were then deproteinized by ultrafiltration using an Ultrafree-MC 10,000 NMWL filter unit (Millipore, Billerica, MA, USA) at 18,000 g for 10 min. After measuring the DNA concentration, 8-hydroxy-2'-deoxyguanosine (8-OHdH) levels were determined by ELISA.

Calculation of UVA intensity in irradiation pipes

We measured the irradiation intensity in each length of pipe using an accumulated UV meter (MCPD-370A ; Otsuka Electronics Corp., Osaka, Japan). The intensities were 0.262 kW m^{-2}, 0.087 kW m^{-2}, 0.024 kW m^{-2}, 0.007 kW m^{-2}, and 0.005 kW m^{-2}, at 10 cm, 20 cm, 30 cm, 60 cm, and 90 cm, respectively.

STATISTICAL ANALYSIS

Statistical analysis of differences was performed using ANOVA with Bonferroni's multiple comparison tests. Student's *t*-tests were used for paired data when appropriate. A value of $p < 0.05$ was considered statistically significant.

Regression analysis was performed to estimate the relationship between fluence and log survival ratio, and the relationship between the volume of nutrient solution and log survival ratio. An *F*-test was applied for analyzed the significances of the regression analysis. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Inactivation of bacteria by UVA-LED irradiation

We first tested the basic disinfection activity of our newly developed sterilization device by measuring bacterial survival following UVA-LED irradiation of bacterial suspensions at 0.3 kW m^{-2}, 0.6 kW m^{-2}, 0.9 kW m^{-2} and 1.2 kW m^{-2} irradiation intensities (Fig. 2). There was a clear dose-response relationship between log survive ratio and intensity of UVA irradiation.

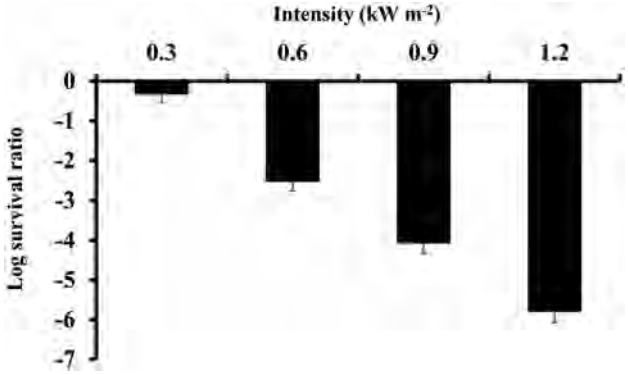


Figure 2. Log survival ratio of *E. coli* as a function of UVA-LED irradiation intensity. The UVA irradiation and non-irradiation time was 15 and 135 sec, respectively. The circulation speed was 4 L min^{-1} and the total volume of nutrient solution was 10 L. The log survival ratio depends on the irradiation intensity. Black columns : 360 sec exposure at each intensity. The data represent means ± SD (n = 3). The relationship between log survive ratio and intensity of UVA analyzed by a multiple linear regression analysis and p value was 0.0001. Because $p <$ 0.05 were considered statistically significant, here was dose-response relation between log survival ratio and intensity of UVA irradiation.

As expected, bacterial survival decreased with increasing UVA intensity. Next, we compared the bacterial inactivation ability using the same fluence (216 kJ m^{-2}) with five different UVA-LED irradiation intensities (0.3 kW m^{-2}, 0.6 kW m^{-2}, 0.9 kW m^{-2}, 1.2 kW m^{-2} and 2.4 kW m^{-2}) (Fig. 3). Surprisingly, bacterial survival at this fluence was similar for all five UVA intensities. There were no significant differences between each of values, respectively. This result indicated that bacterial inactivation promoted by our device was dependent on the fluence of UVA-LED irradiation rather than the intensity.

Effect of circulation time and speed on bacteria inactivation

Nutrient solutions can be sterilized either by batch process or circulation. Our device sterilizes circulating nutrient solutions. During circulation, the solution is not continuously exposed to UVA irradiation. In our device, only the solution in the sterilization pipe was exposed to the UVA-LED. Moreover, when the total volume of the nutrient solution increased concurrently with the circulation speed, the non-irradiation time increased, whereas the radiation time remained the same. Thus, we next varied the non-irradiation time to estimate the total volume dependence of the sterilization activity (Fig. 4). As the total volume of the nutrient solution and in turn the non-irradiation time increased, the bacterial inactivation efficiency decreased.

Changes in circulation speeds also affect the irradiation time and non-irradiation time. For example, as circulation speed increases,

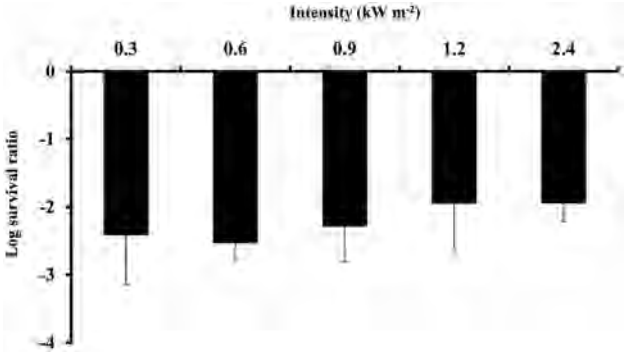


Figure 3. Log survival ratio of *E. coli* as a function of UVA-LED irradiation intensity at constant fluence. The UVA irradiation and non-irradiation time was 15 and 135 sec, respectively. The irradiation fluence was 216 kJ m^{-2} at each intensity. The log survival ratio depends on irradiation strength. The data represent means ± SD (n = 3). There was not significant difference between each of data analyzed by student's t-tests. $p <$ 0.05 were considered statistically significant.

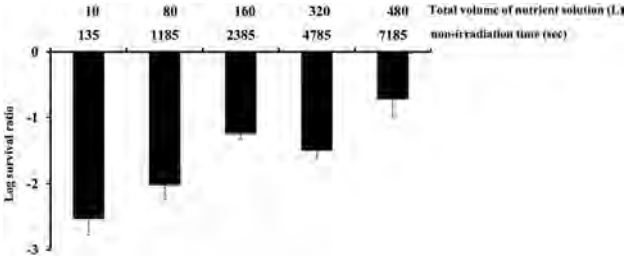


Figure 4. Effect of total volume of nutrient solution and non-irradiation time on the log survival ratio of *E. coli*. The UVA irradiation time was 15 sec and the non-irradiation time varied. The nutrient solution circulated at a rate of 4 L min^{-1} whereas the total volume circulated varied. The fluence was 216 kJ m^{-2}. The data represent means ± SD (n = 3). The relationship between log survive ratio and the total volume of nutrient solution analyzed by a multiple linear regression analysis, and p value was 0.041. Because $p <$ 0.05 were considered statistically significant, there was dose-response relation between log survival ratio and the total volume of nutrient solution.

both the irradiation time and non-irradiation time decrease. Yet, similar to the result obtained for irradiation intensity, as long as the fluence was 216 kJ m^{-2}, there was no significant loss in sterilization activity, even as the circulation speed increased and the irradiation time decreased (Fig. 5).

Comparison between inactivation efficiency of a test model and small-scale model

For application of this disinfection device to actual plant factories, the total volume of the nutrient solution that can be treated must be scalable. To test scalability, we made a smaller model of the sterilization device and used results from this model to derive kinetic equations to describe sterilization effects that can be used on a larger scale. We also compared the inactivation activity of the small-scale model of the sterilization device and compared its inactivation efficiency with that of the test model (Appendix Fig. 1). We observed no significant differences in the course of disinfection between the test model of development and the small-scale model, indicating that the inactivation efficiency of a test model of development can be tested.

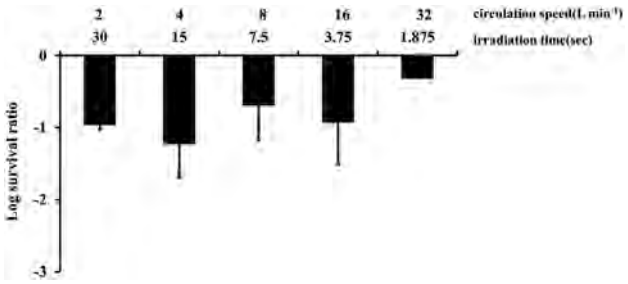
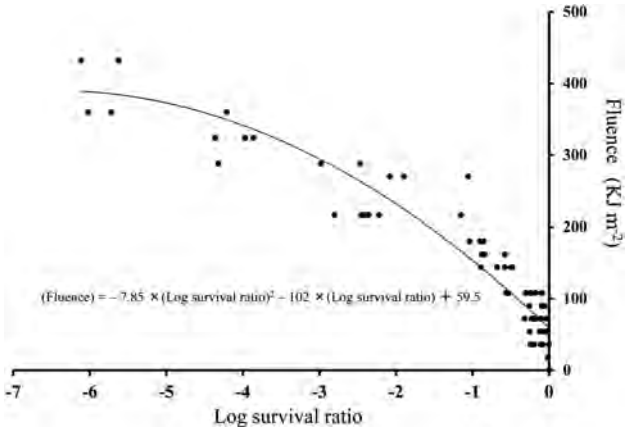


Figure 5. Inactivation of *E. coli* by UVA-LED irradiation. Both the UVA irradiation time and non-irradiation time varied such that circulation speed varied but the total volume circulation was constant at 10 L. The fluence was 216 kJ m^{-2}. The data represent means ± SD (n = 3). The relationship between log survive ratio and the circulation speed analyzed by a multiple linear regression analysis, and p value was 0.045. Because $p < 0.05$ were considered statistically significant, there was dose-response relation between log survival ratio and the volume of nutrient solution.



Appendix Fig 1.

Correlation of log survival ratio and fluence. The y-axis indicates fluence delivered to the bacterial solution and the y-axis indicates the log survival ratio of the bacteria in hydroponic nutrient solution. Regression analysis was performed to estimate the relationship between fluence and log survival ratio, and regression equation was indicated in the figure. A coefficient of determination was 0.909 indicating good fit of the equation. An F-test was applied and p value was lower than 0.001 and F values was 36.57.

Formation of 8-OHdG by UVA-LED

We also used ELISA to measure the formation of 8-OHdG, a compound that arises from oxidative DNA damage, by *E. coli* exposed to UVA-LED irradiation to examine potential oxidative DNA damage induced by UVA-LED (Fig. 6a, b). 8-OHdG levels in UVA-LED-exposed *E. coli* cells were 1.5-fold higher than those of unexposed controls and the levels were related to the UVA-LED fluence rather than intensity. Meanwhile, there were no significant differences in 8-OHdG levels as a function of circulation volume, which is inversely related to the non-irradiation time, suggesting that fluence is the main parameter that affects oxidative DNA damage. Alternatively, at increased non-irradiation times, 8-OHdG could be stored for later release.

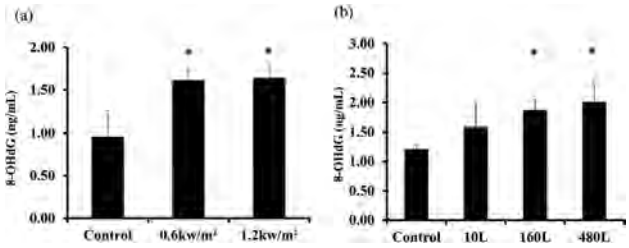


Figure 6. Effect of UVA-LED irradiation and nutrient solution circulation volume on 8-OHdG levels. Bacterial suspensions in 96-well plates were exposed to (a) the indicated UVA-LED irradiation intensity at a fluence of 216 kJ m^{-2} and (b) increasing volumes of circulating at UVA-LED intensity of 0.6 kw/m^2 and a fluence of 216 kJ m^{-2}. 8-OHdG levels were measured by ELISA. *Significant difference at $p < 0.05$ (student's t-test) *vs* control.

Inactivation of bacteria by a test model of the disinfection device

The volume of the tank exposed to UVA-LED irradiation could also affect the disinfection capability. We thus tested devices with varying sizes of storage tanks and found that the disinfection activity remained largely unchanged as the tank volume of the test model device increased from 1 L to 3 L (Fig. 7). Furthermore, when the volume of circulating nutrient solution increased from 5 L to 20 L in the test model, we found that 10 L of circulating nutrient solution had the best disinfection efficiency (Fig. 8). In terms of nutrient solution circulation speed, 4 L min^{-1} had the highest bactericidal effect (Fig. 9).

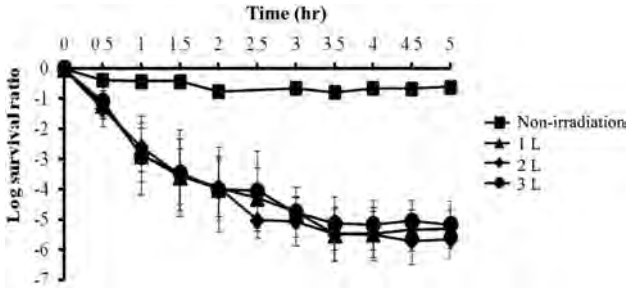


Figure 7. The relationship between log survival ratio and irradiation time. UVA irradiation time was 15 sec and the non-irradiation time was 135 sec. (*circle*) ; Test model of development inactivates *E. coli*. (*square*) ; Small scale model also inactivated *E. coli*.

Effect of increasing time and nutrient solution circulating volume on log survival ratio of *E. coli*. The UVA irradiation tank volume was changed from 1 L to 3 L. The non-irradiated control samples are displayed as *square*. The data represent mean ± SD (n=3). There are not significant differences between 1L, 2L and 3L analyzed by ANOVA. $p <$ 0.05 were considered statistically significant.

The relationship between log survival ratio and irradiation conditions

The above results showed that the log survival ratio of bacteria was significantly related to fluence as well as circulation speed and volume (Appendix Fig. 1). Based on the results for the small-scale model, we derived the following kinetics equation to express the

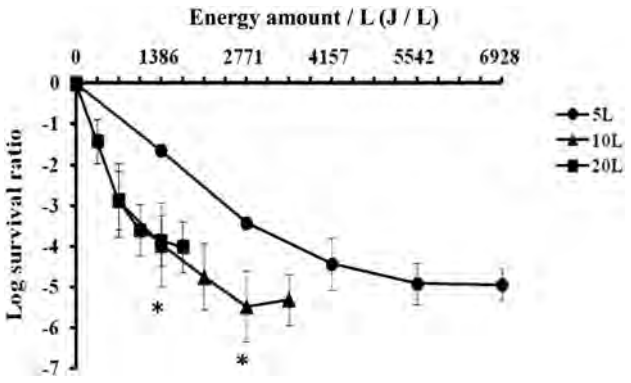


Figure 8. Effect of increasing time and volume of circulating nutrient solution on the log survival ratio of *E. coli*. The volume of the circulating nutrient solution was increased from 5 L to 20 L. The data represent mean ± SD (n = 3). *Significant difference at $p < 0.05$ (ANOVA). F values were 27.6 and 306.4 at 1386 J/L and 2771 J/L, respectively.

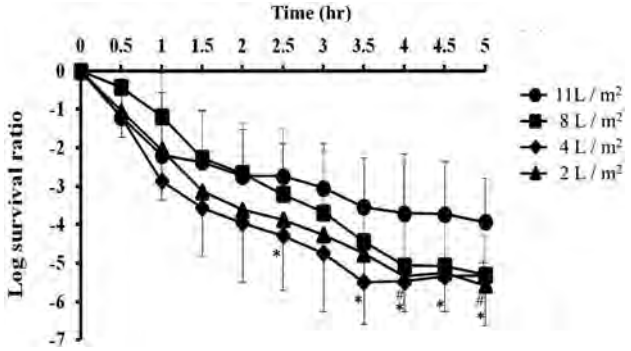


Figure 9. Log survival ratio of *E.coli* with increasing time and nutrient solution circulating speed. The nutrient solution circulating speed was increased from 2 L min^{-1} to 11 L min^{-1}. The data represent mean ± SD (n = 3). There was significant different analyzed by ANOVA between every nutrient solution circulating speed. p value was 0.009 and F value was 6.584, indicating log survival ratio were not same between every nutrient solution circulating speed. #Significant difference at $p < 0.05$ (student's t-test) 2 L min^{-1} *vs* 11 L min^{-1}. *Significant difference at $p < 0.05$ (student's t-test) 4 L min^{-1} *vs* 11 L min^{-1}. +Significant difference at $p < 0.05$ (student's t-test) 8 L min^{-1} *vs* 11 L min^{-1}.

relationship between nutrient solution volume and log survival ratio (Appendix Fig. 2) :

$$T(V)=2.22\times10^{-6}\times V^2-2.28\times10^{-3}\times V+0.975$$

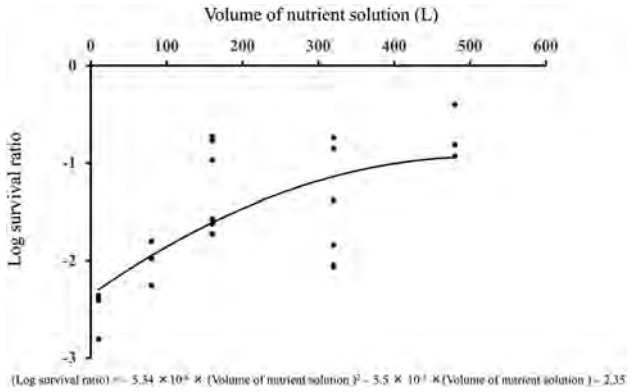
where T(V) is the log survival ratio and V is the nutrient solution volume.

Next, we modified the above equation and input values from our experimental results :

$$E(V)=-7.85\times\frac{S^2}{T(V)^2}-102\times\frac{S}{T(V)}+59.5$$

where S is the desired log survival ratio ; E(V) is the fluence energy ; and T(V) is the observed log survival ratio.

Once a desired log survival ratio is defined, this equation can be used to estimate the required fluence.



Appendix Fig 2.
Correlation of log survival ratio and nutrient solution volume. The y-axis indicates the log survival ratio of the bacteria in hydroponic nutrient solution and the x-axis indicates the hydroponic nutrient solution volume. Regression analysis was performed to estimate the relationship between the volume of the nutrient solution and log survival ratio. Regression analysis was performed to estimate the relationship between fluence and log survival ratio, and regression equation was indicated in the figure. A coefficient of determination was 0.458. An F-test was applied and p value was lower than 0.001 and F values was 7.60.

DISCUSSION

In this study, we tested the ability of UVA-LED irradiation to inactivate bacteria in hydroponic nutrient solutions using a new nutrient solution disinfection device that we developed. Effective water disinfection is often achieved by chlorine, ultraviolet (UV) light or ozone treatment (13, 16), which each have advantages and disadvantages. UV irradiation is a particularly effective disinfection method because it is easy to apply, requires no additional chemical input and produces no hazardous by-products (13). Typically, low-pressure mercury lamps that emit effective germicidal UVC irradiation are used as light sources for UV disinfection. However, these lamps have a relatively high cost due to a short lamp life and high energy consumption. Furthermore, spent lamps are a source of mercury waste that requires specific disposal methods. In contrast, the UVA-LED we used for our device is a safer and less expense alternative to generate UV irradiation due to its long life and lack of mercury. Moreover, LEDs can be miniaturized to allow field applications. Our device disinfects nutrient solutions held in nutrient solution storage tanks, which would allow the device to be installed in existing plant factories without disturbing production lines.

Our disinfection device has several advantages over previous disinfection approaches. First, the device realized a bacteria inactivation efficiency of up to 3 $\log_{10}$, which is sufficient to disinfect hydroponic nutrient solutions. The current device is 560 mm long and 1,200 mm wide with a depth of 300 mm. The small dimensions of the device should not disturb ongoing cultivations (19), but will still deliver effective disinfection.

From our experimental results we also derived a kinetics equation based on the model of Chick-Watson (20-22) :

$$dN/dt=-KC^nN$$

where N is the number of microorganisms ; C is the concentration of residual free chlorine ; n is the dilution coefficient ; and K is the coefficient for translating the disinfecting power. Although the kinetics equation we derived (see Results) is slightly more complicated than the simple equation above, our equation is required to fit numerical values to the desired log survival ratio.

In conclusion, we showed that our device that produces UVA irradiation generated from LEDs can effectively disinfect hydroponic nutrient solutions. We also defined the conditions needed for optimal disinfection of hydroponic nutrient solutions. If a desired log survival ratio goal is defined, the equations we derived can be used to estimate the required UVA fluence. Using these results, similar devices could be customized for installation in plant factories to achieve economical disinfection of hydroponic nutrient solutions without chemicals or generation of hazardous waste.

ACKNOWLEDGEMENTS

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