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Lu, Zhiwei; Li, Xiaoling; Wei, Jinxiu ; Cai, Miao; Yang, Daoguo; Zhang, Guoqi

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Inactivation of Escherichia coli and Staphylococcus aureus by using a UVC-LED module with a multi-wavelength setting

Zhiwei Lu
School of Mechanical and Electrical Engineering, Guilin University of Electronic Technology
Guilin, China
1198942999@qq.com

Xiaoling Li
School of Mechanical and Electrical Engineering, Guilin University of Electronic Technology
Guilin, China
240288797@qq.com

Jinxiu Wei
School of Mechanical and Electrical Engineering, Guilin University of Electronic Technology
Guilin, China
1300967296@qq.cpm

Miao Cai*
School of Mechanical and Electrical Engineering, Guilin University of Electronic Technology
Guilin, China
caimiao105@163.com

Daoguo Yang
School of Mechanical and Electrical Engineering, Guilin University of Electronic Technology
Guilin, China
daoguo_yang@163.com

Guoqi Zhang
Shenzhen Institute of Wide-Bandgap Semiconductors
Delft Institute of Microsystems and Nanoelectronics(Dimes), Delft University of Technology
Shenzhen, China
g.q.zhang@tudelft.nl

Abstract—**UVC-LED is known as a deep ultraviolet LED. The application development and disinfection efficiency of UVC-LED modules are important problems encountered when UVC-LED products are rushed into commercialization. In this article, a specific disinfection experiment with a UVC-LED module was combined to analyze the disinfection efficiency. UVC-LEDs with wavelengths of 260 and 280 nm were used and supplemented with UVA-LEDs with wavelengths of 360 and 390 nm. The module was packaged to investigate the inactivation of Escherichia coli and Staphylococcus aureus. Two new findings were obtained through the analysis and comparison of the experiments. First, the short wavelength from UVA might have an enhanced destructive effect on microorganisms when the radiation intensity of UVA-LED was sufficient with coupling UVA and UVC. Second, 260 nm UVC-LED lamp beads might have a shorter response time to inactivate microorganisms than 280 nm UVC-LED lamp beads. Bactericidal experiments near the surface and different radiation distances showed that the inactivation rate reached 99.9% after 1 min of exposure when the UVC-LED module was set at 260 or 280 nm wavelength lamp beads for disinfection. The disinfection efficiency of 280 nm UVC-LED lamp beads was higher than that of 260 nm UVC-LED lamp beads because of the increased UV intensity. The radiation distance was within 7.5 cm range, the exposure time was 60 s, the inactivation rate was over 99.9%, and the disinfection effect was remarkable. For current UVC-LED applications, such as near-surface UVC-LED, disinfection and air purification products have a high value.**

Keywords—UVC-LED, disinfection experiment; inactivation (key words)

I. Introduction

Infection has become a common mode of disease transmission. An increase in mortality and hospitalization due to infections and diseases has led to a sharp increase in hospitalization expenses (1,2). Escherichia coli and Staphylococcus aureus are typical representatives of infectious pathogenic bacteria. E. coli ubiquitously exists and rapidly grows, easily causing infection among immunocompromised individuals, infants, and elderly. E. coli also causes intestinal infections, extra-intestinal infections, and acute diarrhea. S. aureus can be found in air, water, dust, and human and animal waste. According to the US Centers for Disease Control, infections caused by S. aureus are only second to E. coli. Food poisoning by S. aureus enterotoxin in the United States accounts for 33% of all bacterial food poisoning cases; in Canada, 45% cases have been recorded, while this condition occurs from time to time in China (3). S. aureus breeds easily on the skin, resulting in pus and sore, which cause infections such as pneumonia and sepsis. Surface or airborne bacterial infections are harmful. In this study, detailed experiments on the inactivation of E. coli and S. aureus, which are two common bacterial species, were conducted to develop effective disinfection methods that could inhibit their reproduction.

In this study, new generation of green light source named UV-LED for disinfection was mainly used. The UV-LED device has three wavelengths of emission: long-wavelength UVA (315–400 nm), medium-wavelength UVB (280–315 nm), and short-wavelength UVC (200–280 nm). The disinfecting light is primarily short-wavelength UVC, which is also known as deep ultraviolet. The disinfection mechanism mostly involves the generation of cyclobutane pyrimidine dimers (CPDs) by UV-induced DNA or RNA, thereby inhibiting replication and proliferation (4). UV light with a wavelength of approximately 260 nm on the DNA absorption effect curve is optimum for bacterial inactivation (5,6). However, in the current market, approximately 280 nm may have better disinfection effect. Various studies have determined that higher-wavelength UVC light rapidly destroys bacteria because of its high power. Although low wavelengths are more effective than high wavelengths, their optical power is lower than that of high wavelengths. A high-

wavelength UVC light, which is closer to the maximum absorption peak of proteins, is not only efficient for sterilization but also for protein inactivation; as such, high-wavelength UVC light is a better choice (7-12). Coupling UVA and UVC fully utilizes the disinfection effect of UVC and the strong penetrating ability of UVA, thereby causing an irreversible damage to bacteria and improving the inactivation efficiency of microorganisms (12,13-17). Therefore, this article combined UVC and UVA in experiments and focused on the disinfection effect of UVC near the surface and at different radiation distances to solve the pathogen inactivation of disease infection. In 2015 in Italy, Gabriele Messina examined the bacterial growth on stethoscopes and inactivated 85.5% of Enterococcus faecalis, 87.5% of S. aureus, 94.3% of E. coli, and 94.9% of Pseudomonas aeruginosa through UV-LED exposure; they also invented a UVC-LED device that automatically sterilizes stethoscope membranes (18-20). However, the effect of this device should be improved. The number of manufacturers of UVC-LED products has increased, but the disinfection effect of many UVC-LED products remains unclear. The specific UV dose from UVC-LED products that can inactivate microorganisms, explanations on specific irradiation time, and radiation distance are also unclear.

This study aimed to analyze the application efficiency of UVC-LED modules in disinfection and to propose an efficient UVC-LED disinfection module that could address vague and non-uniform standards of existing product applications, immature issues of product designs and development technology, and other problems.

II. MATERIALS AND METHODS

A. Experimental system

The UVC-LED module used in this study was composed of six UV-LED lamp beads (LG Innotek Co., Seoul, Korea) corresponding to four wavelengths (260, 280, 360, and 390 nm). This module was connected to an electronic printed circuit board (PCB) as shown in Fig. 1. Fig. 1 (a) presents the UV-LED experimental system, and Fig. 1 (b) illustrates the wavelength of UV-LED lamp bead setting. The work mode from different UVC-LED lamp beads was controled by an independent switch module (Fig. 1 (a)). The wavelength combination of the work modes included five modes: 260 nm*2, 280 nm*2, 280 nm*2+360 nm*1, 280 nm*2+390 nm*1, 280 nm*1+360 nm*1, and 280 nm*1+390 nm*1 (Fig. 1 (b)). Among the work modes, 260 and 280 nm wavelengths are the most effective for disinfection, and they were also the main research object. The UVC-LED lamp beads with wavelengths of 260 and 280 nm were composed of four UVC-LED chips. In this system, an adjustable power supply was used, and the limit voltage from the UVC-LED lamp bead was 28 V. As such, currents at an appropriate voltage (25 or 27 V) of approximately 88 or 140 mA were applied, and the electric power was 2.2 or 3.78 W. The six UV-LEDs were arranged in an array with a 15 mm distance between two 260 or 280 nm LED lamp beads and a 35 mm distance from 260 nm LED lamp beads to 280 nm LED lamp beads. The radiation distance between the PCB and a Petri dish based on our laboratory requirements (from 1.5 mm to 9.5 mm at 2 mm per interval) could be adjusted through the fixture. The detailed settings were described in the experimental operation process.
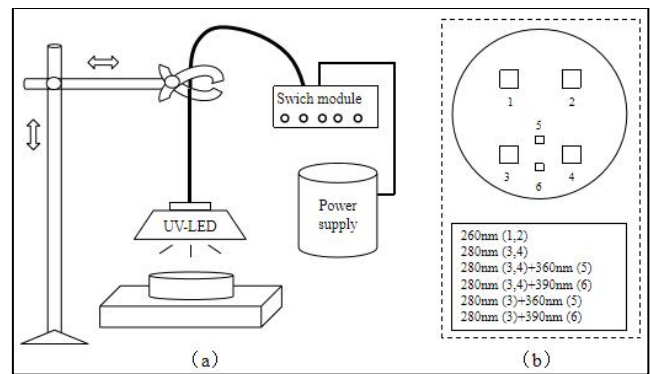

Fig. 1. UV-LED experimental system (a) and wavelength of UV-LED lamp bead setting (b)

B. Irradiance measurements

The irradiance intensity of the UV-LED modules was measured with a UV radiometer (LS125 host + UVC-LED quartz probe, Linshang; Shengzhen, China) calibrated within a range of 200 – 320 nm, which included the entire UV wavelength spectrum. For sample treatment, the radiation distance or radiation height between the UV-LEDs and an optical quartz probe could be adjusted using the fixture based on our laboratory requirements (from 1.5 mm to 9.5 mm at 2 mm per interval), and the irradiance value at the peak wavelength was measured. Irradiance was determined every 5 mm of area corresponding to the dimensions of a Petri dish. Each measured intensity was divided by the maximum irradiance value and averaged to obtain the Petri factor. The final irradiance value was normalized by multiplying the maximum intensity by the Petri factor.

C. Bacterial strains and growth conditions

Two different bacterial strains, namely, E. coli and S. aureus, which are standard laboratory microorganisms, were used in the experiment. E. coli is responsible for many diarrheal diseases, and S. aureus is the most common pathogen in human purulent infections and localized purulent infections. Therefore, the two bacterial strains are suitable as reference isolates for external environment disinfection (21).

The following growth conditions were set. All bacterial strains were cultivated aerobically in Luria – Bertani broth containing distilled water, 5 g/L NaCl, 5 g/L yeast extract, and 10 g/L tryptone. The growth phase was set using a TH30 incubator (Edmund Buehler GmbH, Hechingen, Germany) at 37 ° C and 180 rpm for 10 h until the stationary phase was reached. For the dilution stage, 100 μL of the initial solution of E. coli or S. aureus was diluted six times with 900 μL of 0.9% saline solution. The final bacterial dilution concentrations were approximately 10-6 CFU/mL E. coli and 10-7 CFU/mL S. aureus.

D. Experimental process

Before the UV-LED module was used, the module was covered with a black cover, and the switch module was placed on the external control lamp bead. Three batches of experiments were prepared:

In Part 1, 260 nm*2, 280 nm*2, and 280 nm*2+360 nm*1 UV-LED lamp beads were examined to inactivate E. coli at 1 and 2 min exposure. The specific steps were as follows: we placed the coated E. coli plates directly under UV-LED lamp beads of the corresponding wavelength, and set the radiation distance at 1.5 cm. A set of E. coli plates

were prepared with tin foil for the shading treatment, while the blank groups were examined under the same operating environment. Then, the corresponding switch was operated on the switch module to open the lamp bead. After radiation was administered, the UV-LED lamp beads were switched off. Each experiment was repeated three times, and the final result was calculated as an average.

In Part 2, 280 nm*2, 280 nm*2+360 nm*1, 280 nm*2+390 nm*1, 280 nm*1+360 nm*1, and 280 nm*1+390 nm*1 UV-LED lamp beads were examined to inactivate E. coli at corresponding exposure times and radiation distance settings. The specific steps were as follows: the coated E. coli plates were placed directly under the UV-LED lamp beads at the corresponding wavelength, and the radiation distance was set at 1.5 cm. Another set of E. coli plates was wrapped in tin foil for shading treatment and placed in the same operating environment as the blank control group. Then, the corresponding UV-LED lamp bead was switched on with the switch module, and exposure was performed at different durations (5, 10, 20, 30, 40, 50, 60, 80, and 120 s). After the exposure time was terminated, the UV-LED lamp beads were turned off, and each experiment was repeated twice. The final result was calculated as an average.

In Part 3, 260 nm*2 and 280 nm*2 UVC-LED lamp beads were used to inactivate E. coli and S. aureus at corresponding exposure times and radiation distance settings. The specific steps were as follows: the coated plates of E. coli and the S. aureus were placed directly under the corresponding UVC-LED lamp bead, and the different radiation distances were examined. Another group of E. coli and S. aureus plates was covered with tin foil for shading treatment and placed in the same operating environment as the blank control group. Next, the corresponding switch on the switch module was operated to open the corresponding UVC-LED lamp bead. Different exposure durations (15, 30, 60, and 120 s) and different radiation distances (3.5, 5.5, 7.5, and 9.5 cm) were set. The specific settings of exposure time and radiation distance are shown in Table 4 and 6. After disinfection was completed, the UVC-LED lamp was turned off, and each experiment was repeated twice. The final result was calculated as an average.

E. Bacterial enumeration

Bacteria usually exist in different forms, such as single, double paired, and clustered. No single medium meets the physiological requirements of all bacterial samples. Therefore, the number of colonies measured may be lower than the number of viable bacteria that actually exist. In our study, the bacterial plates of E. coli and S. aureus were irradiated with the UV-LED system and incubated at 37 ℃ for 24h. Afterward, typical colonies were counted through a plate colony counting method in accordance with the principle that each living bacterial cell would grow one colony corresponding. Then, 1mL of the diluted bacterial liquid sample was poured into the nutrient agar medium and stored at 37 ℃. After the bacterial cells were grown in an incubator at constant temperature overnight for 24h, the number of bacterial colonies cultured was obtained.

F. Statistical analysis

Data were processed separately on the basis of the data of each batch of experiments because of the difference in the colony growth of different experimental batches. Data analysis and treatment were then summarized. The number

of colonies grown in the bacterial culture after exposure and the number of bacteria were determined. The percentage of bacterial reduction (inactivation rate) was also analyzed. The whole analysis was based on multiple experimental results as compared with blank experiments to make insurance estimates. The experimental process strictly required operators to follow standards and specifications. The percentage of bacterial reduction could be expressed using the following formula:

$$\text{Percentage of bacteria reduction} = \frac{N_0 - N_t}{N_0} * 100\% ,$$

where N_0 is the number of cells without UV radiation (CFU/mL), and N_t is the average number of cells after UV irradiation (CFU/mL). The experiment was repeated two or three times to ensure the reliability of the data source.

III. RESULTS

A. Part 1

The 260 nm*2, 280 nm*2, and 280 nm*2+360 nm*1 UV-LED lamp beads were examined to inactivate E. coli during exposure for 1 and 2 min. First, the average colony value of the E. coli blank group without UV radiation was 320 CFU/mL. The colony value of the E. coli experiment group with UV radiation is shown in Table 1. Figs. 2 and 3 illustrate the specific inactivation of E. coli at 260 nm*2 and 280 nm*2+360 nm*1 UV-LED radiation on culture dishes, respectively. In Table 2, E. coli still lived after UV-LED radiation in each experiment repeated three times; the number of E. coli remained at 12 CFU/mL for 1 min of exposure and at 1 and 7 CFU/mL for 2 min of exposure. Table 3 shows the number of E. coli remained at 8 CFU/mL for 1 min of exposure, and 1 CFU/mL for 2 min of exposure. Moreover, five blank control plates were cultured for 24h. The average number of experiment group colonies is presented in Table 1. The average number of the blank group colonies on each dish was 320 CFU/mL. Then, the inactivation rate was computed using the obtained value.

Table I. UV-LED parameters, condition setting, and experimental results.
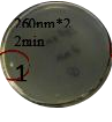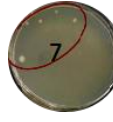
UV-LED lamp beads	1 min				2 min			
	E_n (CFU/mL)			A_n (CFU/mL)	R_a (%)	E_n (CFU/mL)			A_n (CFU/mL)	R_a (%)

UV-LED lamp beads	E_n (CFU/mL)			A_n (CFU/mL)	R_a (%)	E_n (CFU/mL)			A_n (CFU/mL)	R_a (%)
260 nm*2	0	0	12	4	98.750%	0	1	7	3	99.062%
280 nm*2	0	0	0	0	100%	0	0	1	1	99.688%
280 nm*2+ 360 nm*1	0	0	8	3	99.062%	0	0	1	1	99.688%

En: colony value of the E. coli experiment group.
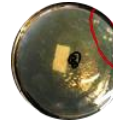An: average colony value.
Ra: percentage of bacterial reduction.

Table II. Inactivation of E. coli by exposure to 260 nm*2 UVC-LED lamp beads for 1 and 2 min at a radiation distance of 1.5 cm.

	260 nm*2			An (CFU/mL)	Ra (%)
	1st	*2nd*	*3rd*		
1 min	1min		12	4	98.75
2 min		260nm*2 2min	7	3	99.062

An: average colony value.

Ra: percentage of bacterial reduction.

Table III. Inactivation of E. coli by exposure to two 280 nm UVC-LED lamp beads and one 360 nm UVC-LED lamp bead for 1 and 2 min at a radiation distance of 1.5 cm.

	280 nm*2+360 nm*1			An (CFU/mL)	Ra (%)
	1st	*2nd*	*3rd*		
1 min				3	99.062
2 min				1	99.688

A_n: average colony value.

R_a: percentage of bacterial reduction.

B. Part 2

The 280 nm*2, 280 nm*2+360 nm*1, 280 nm*2+390 nm*1, 280 nm*1+360 nm*1, and 280 nm*1+390 nm*1 UV-LED lamp beads were examined to inactivate E. coli at corresponding exposure times and radiation distance settings. Fig. 2 shows the results of E. coli inactivation by five wavelength combinations from UV-LED lamp beads with exposure durations of 5, 10, 20, 30, 40, 50, 60, 80, and 120 s and a radiation distance was 1.5 cm. The average number of blank group colonies on each dish was 47 CFU/mL. Although the average number of the colonies of the blank group was low, the results of E. coli inactivation under the five conditions were clear (Fig. 2).
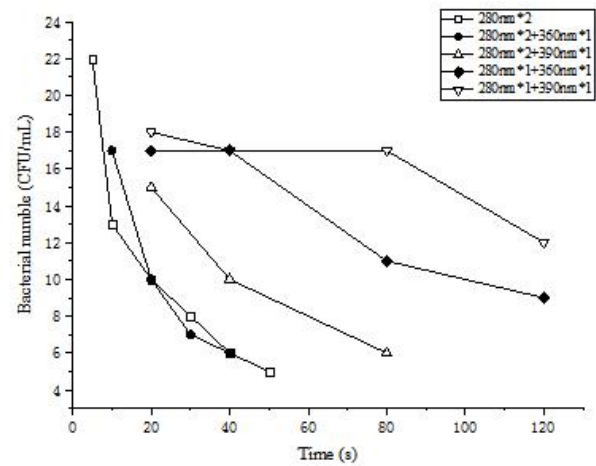

Fig.2 .E. coli inactivation in five wavelength combinations under different exposure times under radiation distance of 1.5 cm.

C. Part 3

The 260 nm*2 and 280 nm*2 UVC-LED lamp beads were examined to inactivate E. coli and S. aureus at corresponding exposure durations and radiation distance settings, and the inactivation rate of E. coli and S. aureus under different exposure durations was determined (Table 4 and Fig. 3). The average number of the colonies on each dish in the blank group was 4,765 CFU/mL for E. coli and 3,916 CFU/mL for S. aureus; thus, the bacterial inactivation rate was calculated. The inactivation of S. aureus from the Petri dish is presented in Table 5. The different radiation distance is also illustrated in Table 6 with 280 nm*2 UVC-LED lamp beads to inactivate S. aureus for 60 s of exposure and radiation distances of 9.5, 7.5, 5.5, and 3.5 cm (from left to right). Each vertical represented two parallel experiments (1st and 2nd). In Table 6, the inactivation rates of the colonies in the experimental groups were 99.949%, 99.974%, 99.949%, and 99.745% that corresponded to the radiation distances of 3.5, 5.5, 7.5, and 9.5 cm, respectively.

Table IV. Inactivation of E. coli and S. aureus by the UVC-LED module.

Time (s)	E. coli		S. aureus			
	260 nm*2		260 nm*2		280 nm*2	
	A_n (CFU/mL)	R_a (% reduction)	A_n (CFU/mL)	R_a (% reduction)	A_n (CFU/mL)	R_a (% reduction)
15	35	99.265%	800	79.57%	/	
30	17	99.643%	350	91.062%	191	95.123%
60	6	99.874%	140	96.425%	9	99.77%
120	0	100%	17	99.566%	2	99.949%

A_n: average colony value.

R_a: percentage of bacterial reduction.

Table V. Inactivation of *S. aureus* in 260 nm*2 and 280 nm*2 UVC-LED lamp beads for 5, 10, 20, 30, 40, 50, 60, 80, and 120 s of exposure at a radiation distance of 1.5 cm.

	260 nm*2		280 nm*2	
	1st	*2nd*	*1st*	*2nd*
Blank group				
15s				
30s				
60s				
120s				

Table VI. Inactivation of S. aureus in 280 nm*2 UVC-LED lamp beads with exposure for 60 s and radiation distances of 9.5, 7.5, 5.5, and 3.5 cm

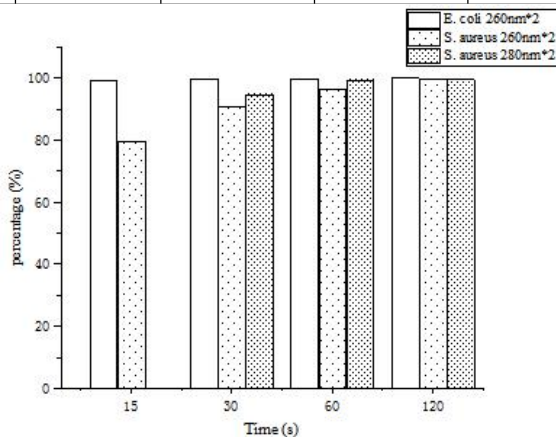|	9.5cm	7.5cm	5.5cm	3.5cm
1st				
2nd				

Fig.3.Inactivation rate of E. coli and S. aureus under different exposure times by UVC-LED modules at a radiation distance of 1.5 cm.

IV. DISCUSSION

In Tables 1 and 2, the inactivation effect of 280 nm UVC-LED was apparently better than that of 260 nm under the same voltage supply. Bowker, Beck, Oguma et al. found that 280 nm with a low cost for a high light power may be preferable (10-12,22). Moreover, coupling UVA and UVC can be effective because of the disinfection effect of UVC and the great penetrating capability of UVA, thereby increasing the efficiency of microbial inactivation (13,14,17,23-24). However, the results of this study were inconsistent with previous findings. The disinfection effect of one or two 280 nm UVC-LED lamp beads was better than that of coupling UVA and UVC (Table 1). The 280 nm*2 UVC-LED lamp beads could yield approximately 100% and 99.688% inactivation rates with 1 and 2 min of exposure, respectively. Furthermore, 280 nm*2+360 nm*1 UV-LED lamp beads obtained approximately 99.062% and 99.688% inactivation rates. Moreover, Fig. 2 shows five wavelength settings of UV-LED lamp beads for E. coli inactivation: 280 nm*2, 280 nm*2+360 nm*1, 280 nm*2+390 nm*1, 280 nm*1+360 nm*1, and 280 nm*1+390 nm*1 for coupling 280 and 360 or 390 nm inactivated E. coli. The number of remaining colonies were not less than that when 280 nm alone was used, so the inactivation effect of 280 nm was better than that of coupling UVA and UVC. The coupling of 280 nm and 360 nm exhibited better inactivation effect than coupling 280 nm and 390 nm. Thus, the shorter wavelength UVA may have better effect on bacterial inactivation when coupling UVA and UVC. From the photoreactivation of DNA repair, photoenzymes are specifically-identified enzymes that repair CPD dimers by absorbing light energy in the 310-480 nm wavelength (UVA or partial visible light) (25). Also, the destructive effect for microorganism, UVA radiation destroys the cell membrane and other cellular components by producing hydroxyl and oxygen radicals to damage proteins (13). Thus, shorter wavelength UVA may have better destructive effect for microorganisms if radiation intensity for UVA-LED is sufficient.

The inactivation of E. coli by 260 nm*2 and 280 nm*2+360 nm*1 UVC-LED lamp beads in 1 min and 2 min exposure, and the three horizontal plates repeated three times are presented in Table 2 and 3. The disinfection effect for E. coli was apparent, only the bacteria colonies on the outer edges were not inactivated. As for the inactivation rate of E. coli and S. aureus in Table 4 and Fig. 3, data analysis shows that when 260 nm*2 UVC-LED lamp beads were used for 15 s exposure of E. coli inactivation, the irradiance average and exposure time can be estimated as a UV dose of 13.5 mJ/cm2 with an inactivation rate of over 99%, whereas the inactivation rate of S. aureus was only 79.57% under the same conditions; however, the inactivation rate of S. aureus was >99% that required exposure of 120 s and 108 mJ/cm2 UV dose. When 280 nm*2 UVC-LED lamp beads were used, the inactivation rate of S. aureus was >99%, the exposure time was 60 s, and the UV dose was approximately 105.18 mJ/cm2. Therefore, the inactivation rate of S. aureus was lesser than that of E. coli. With less exposure time, the stronger the ability of S. aureus to resist UV. In Figs. 5 and 6, the inactivation effect of S. aureus could be compared using 260 nm*2 and 280 nm*2 UVC-LED lamp beads with a set of exposure times. The longer exposure times gained a closer disinfection effect between 260 and 280 nm. The 260 nm UVC-LED lamp beads had more advantage, which might have a shorter response time to inactivate S. aureus. When

the exposure time was at 15 s in similar conditions using 260 nm*2 and 280 nm*2 UVC-LED lamp beads, the inactivation of S. aureus had remarkable result in 260 nm UVC-LED; however, lesser inactivation effect in 280 nm UVC-LED. Still, at exposure times greater than or equal to 30 s, the 280 nm UVC-LED provided inactivation effect than 260 nm. When the exposure time was 60 s, the bacteria on the outer edge of petri dishes were clearly lesser using 280 nm UVC-LED as compared with 260 nm.

Our experiments confirmed that the wavelengths of 260 and 280 nm were effective in inactivating E. coli and S. aureus. The inactivation rate reached 99.9% in 260 or 280 nm UVC-LED module at a 3.5 cm radiation distance. The disinfection effect of the 280 nm UVC-LED lamp bead, also enhanced as shown in Table 6; the radiation distances from left to right were set to 9.5, 7.5, 5.5, and 3.5 cm. When 280 nm*2 UVC-LED lamp beads were used to inactivate the anti-UV ability of S. aureus at a radiation distance of 7.5 cm for 60 s exposure and a UV dose of about 17.82 mJ/cm2, the inactivation rate could reach over 99.9%, and the disinfection effect was remarkable.

In summary, the UVC-LED module design had a strong disinfection effect and efficiency for 260 and 280 nm UVC-LED lamp beads. Although our data were analyzed on the basis of the application mechanism, 260 nm, as the wavelength for the maximum absorption peak of DNA, elicited the strongest bactericidal effect on the DNA of pathogenic bacteria. However, UVC-LED lamp beads with 280 nm wavelength might have more advantages. The 280 nm lamp bead in the UVC-LED module had higher irradiance than the 260 nm lamp bead in a same exposure time, and the UV dose received by the pathogen at 280 nm was stronger than that at 260 nm. The inactivation rate of E. coli and S. aureus reached >99.9% in the radiation distance range of 7.5 cm after the two 280 nm UVC-LED lamp beads were used for 60 s of exposure. Therefore, 280 nm UVC-LED lamp beads could be used as a surface disinfection for various materials, such as applying in tableware surfaces, cosmetics, stethoscopes. It could also be designed into a small air purifier for air disinfection. Moreover, two new findings were observed in our experiments.

(1) UVA at a short wavelength may have a strong destructive effect on microorganisms when the radiation intensity of UVA-LED is sufficient with coupling UVA and UVC.

(2) UVC-LED lamp beads with a wavelength of 260 nm may have a shorter response time to inactivate microorganisms than those with a wavelength of 280 nm.

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