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Research Progresses of Ultraviolet Light-Emitting Diodes (UV LEDs) Disinfection Technology in Water Microbial Inactivation

YANG Chengshuang[1,2], WANG Xinchang[1,2], SUN Guanlang[1,2], FAN Bingfeng[1,2], XIE Jianing[1,2]

(1. School of Physics and Optoelectronic Engineering; 2. Guangdong-Hong Kong-Macao Joint Laboratory for Intelligent Micro-Nano Optoelectronic Technol., Foshan University, Foshan 528225, CHN)

Abstract: In this article, first, the technical bottlenecks of ultraviolet light-emitting diodes (UV LEDs) encountered in the current stage of research and development are introduced. Then the inactivation mechanism of UV LEDs to harmful microorganisms in water is analyzed. In the process of microbial inactivation, the different application types of UV LEDs are examined in detail, including the application of UV LEDs in different types of UV reactors, advanced oxidation processes (AOPs) based on UV LEDs, the combined use of UV LEDs with different wavelengths, etc. Finally, the issues of deep UV LEDs in the field of drinking water sterilization and disinfection are summarized and an outlook is put forward.

Key words: UV LEDs; microorganisms; drinking water; sterilization; application

紫外 LED 消毒技术在水体微生物灭活中的研究进展

杨成双[1,2]，王新昌[1,2]，孙观朗[1,2]，范冰丰[1,2]，谢嘉宁[1,2]*

(佛山科学技术学院 1. 物理与光电工程学院；2. 粤港澳智能微纳光电技术联合实验室，广东 佛山 528225)

摘　要：文章首先介绍了紫外发光二极管(UV LED)器件以及目前研发阶段遇到的技术瓶颈。然后分析了 UV LED 对水中有害微生物的灭活机理。在微生物灭活过程中，详细考察了 UV LED 的不同应用类型，包括 UV LED 在不同类型 UV 反应器中的应用、基于 UV LED 的高级氧化工艺(AOPs)、不同波长 UV LED 的组合使用等。最后，总结了深紫外发光二极管在饮用水杀菌消毒领域存在的问题并进行了展望。

关键词：紫外线 LED；微生物；饮用水；灭菌；应用

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0 Introduction

At the end of 2019, the emergence of the new coronavirus (COVID-19) has posed an enormous threat to human life, health, and safety. To effectively prevent the spread of the new coronavirus, various disinfection and sterilization products have received extensive attention. Among the many antivirus methods, ultraviolet disinfection and sterilization are relatively economical, healthy, and environmentally friendly[1-2]. In addition, the advantages of ultraviolet light in drinking water treatment are more prominent, and traditional chemical disinfection methods can no longer meet the needs. Chlorine is the most commonly used disinfectant in water treatment, which can quickly inactivate pathogenic bacteria in water, maintain continuous disinfection capability for a period of time, and inhibit the growth and reproduction of pathogenic bacteria in subsequent water bodies[3-4]. However, the latest researches show that chlorine cannot entirely inactivate some microorganisms in water,

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* **通信作者**：谢嘉宁　**E-mail**：xiejianingfs@126.com

such as Cryptosporidium and Giardia. They will react with organic pollutants in water to produce trihalomethanes and haloacetic acids, which are harmful to human health. Ultraviolet rays are particularly effective against chlorine pathogens[5]. As a kind of green broad-spectrum fungicide, they have no secondary pollution and do not increase the genetic toxicity of pollutants. These advantages make UV radiation a viable method of drinking water disinfection[6-7]. Fig. 1 shows the classification and application of ultraviolet rays by wavelength[8].

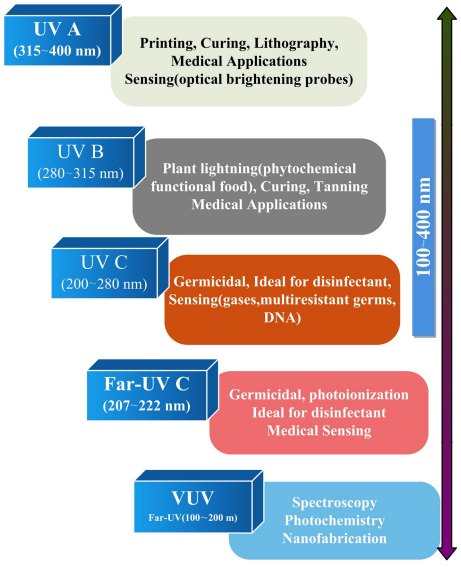

Fig. 1 Classification and application of UV LEDs[8]

Ultraviolet LEDs with emission wavelengths greater than 300 nm and less than 300 nm are called ultraviolet light LEDs and deep ultraviolet LEDs, respectively. The ultraviolet light can be divided into long-wave ultraviolet (315 ~ 400 nm), medium-wave ultraviolet (280 ~ 315 nm), short-wave ultraviolet (200 ~ 280 nm) and vacuum ultraviolet (100 ~ 200 nm) according to the wavelength range, and different wavelengths of ultraviolet light have different application fields[9]. The wavelength of ultraviolet light has a crucial impact on the efficiency of water disinfection. Xiao et al.[10] had proved that the sterilization ability of long-wave ultraviolet is far weaker than that of short-wave ultraviolet. Although UVA has a certain inactivation effect on microorganisms, the effect is not obvious[11]. The primary use for sterilization is 200 ~ 320 nm wavelength of short-wave ultraviolet. In this wavelength range, the corresponding optimal inactivation wavelength for different microorganisms in the water is also different. Its effectiveness may vary depending on the type of microorganisms[12]. The protein and nucleic acid composition are unique, so their response to ultraviolet light is also different, and this concept is called microbial spectrum[13]. Other ultraviolet bands are outside the absorption peak of microorganisms and cannot directly inactivate microorganisms. For example, Hamamoto et al.[14] and Mori et al.[15] proved that 365 nm ultraviolet light-emitting diodes alone could not kill microorganisms in water timely and effectively through experiments. At present, the primary ultraviolet source of ultraviolet disinfection systems is low-pressure or medium-pressure mercury lamps, but they still have many problems[16].

1 UV LED Devices and The Problems Encountered in Their Development

LED is a semiconductor light-emitting device. Its core is a particular p-n junction. The p-type and n-type layers inject holes and electrons into the active area in the middle. The electron holes recombine in the active layer and emit light. The UV LEDs with a wavelength of 200 ~ 320 nm can be manufactured by combining semiconductor materials in an appropriate ratio[17]. The most common ones are gallium nitride, aluminum gallium nitride, and aluminum nitride, etc.[18]. Standard deep-ultraviolet LEDs are flip-chip structures because the flip-chip structure UVC LED can improve the external quantum efficiency. The substrate position is set to lead out light, and the lower end of the electrode is set with a circuit to ensure stable heat dissipation of the LED.

Whether ultraviolet rays can kill microorganisms in water, the radiation dose is a crucial factor. Because different kinds of

microorganisms have different sensitivity to ultraviolet rays, the inactivation rate constant K is usually used to evaluate the sensitivity of microorganisms to ultraviolet rays. The higher the K value, the more sensitive the microorganisms are to ultraviolet light and can be inactivated with lower radiation flux. Fig. 2 shows the inactivation constants of common challenging microorganisms[19].

It can be seen from the figure that the K value of MS2 and Bacillus subtilis spores is low, so they are less sensitive to ultraviolet light. Escherichia coli and T7 phage show low resistance to ultraviolet light. If the radiation dose is too high, there will be a safety hazard, and if the radiation dose is low, it will not be able to kill microorganisms. In ultraviolet sterilization equipment, the power of a single UVC LED chip is low, and it is challenging to meet the requirements for microbial inactivation. Thus, high-power UV LEDs must be obtained by integrating multiple chips.

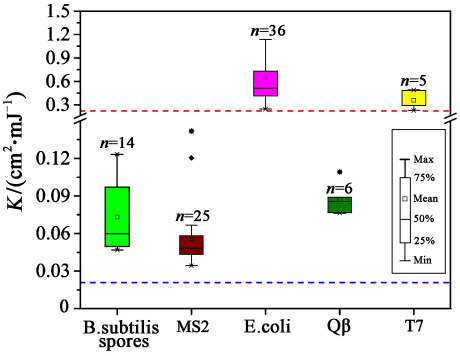

Fig. 2　Inactivation constants for common challenge microorganisms[19]

External quantum efficiency (EQE) refers to the ratio of photons emitting from a device to the number of carriers injected into the device, which is one of the most effective parameters for quantifying LED performance. Although the UVC LED chip has a device structure similar to that of the traditional blue LED, the external quantum efficiency of the two is quite different. The EQE of blue LEDs is relatively high and can reach more than 50%[20], but UV LEDs based on AlGaN materials are still in the exploratory stage, and their EQE is low. Fig. 3 is the current EQE distribution diagram of UV LEDs with different wavelengths[9].

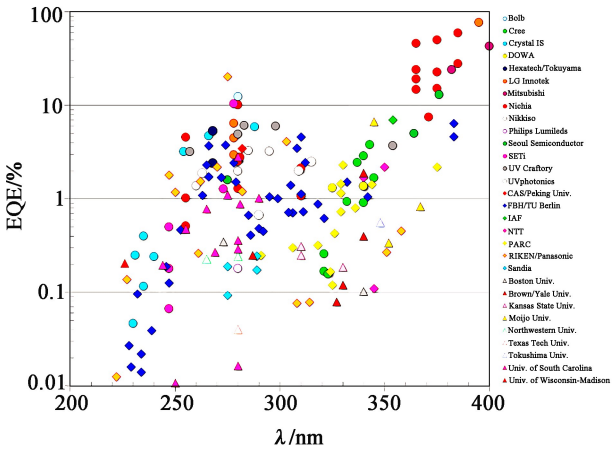

Fig. 3　EQE distribution of UV LED at different wavelengths[9]

2　The Inactivation Mechanism of Ultraviolet Light-Emitting Diodes

The traditional chemical water disinfection method mainly destroys the structure of cells, uses chemical substances to penetrate the bacterial body so that the bacterial protein is coagulated and denatured, interferes with the activity of bacterial enzymes, and inhibits the metabolism and growth of bacteria. It can also damage the bacterial membrane structure, thereby playing a role in disinfection and sterilization. Ultraviolet rays are electromagnetic waves, the shorter the wavelength in electromagnetic waves, the higher the energy and the stronger the penetrating power. Ultraviolet rays have shorter wavelengths and higher energy, which can act on the nuclei of microorganisms. A broad-spectrum and rapid bactericidal effect can be achieved by destroying DNA and RNA to prevent reproduction[21]. Different wavelengths of ultraviolet rays have different types of DNA damage. Specifically, UVA radiation generates some reactive oxygen species (ROS) through photo-oxidation reactions. These reactive oxygen species induce oxidative damage to DNA, proteins, and cell membranes and cause their growth delay[22], but UVA is not for all microorganisms which have an inactivation

effect. Among the four nucleotides of DNA, thymine has a unique photochemical reaction. If two *T* thymine bases are adjacent to each other, after UVC irradiation, the absorption of ultraviolet photons by one thymine will cause chemical bonds between two thymine molecules. These are the cyclobutane pyrimidine dimers (CPDs). Cytosine can also form dimers, but the amount is much lower than thymine. For viruses that only contain RNA, a similar reaction occurs between two uracil bases. The formation of dimers destroys the structure of DNA. DNA cannot be replicated after a certain number of dimers are formed, and microorganisms will slowly die[23]. Fig. 4 shows a schematic diagram of the formation of thymine dimers in DNA and the photochemical dimerization process of two thymine bases[24].

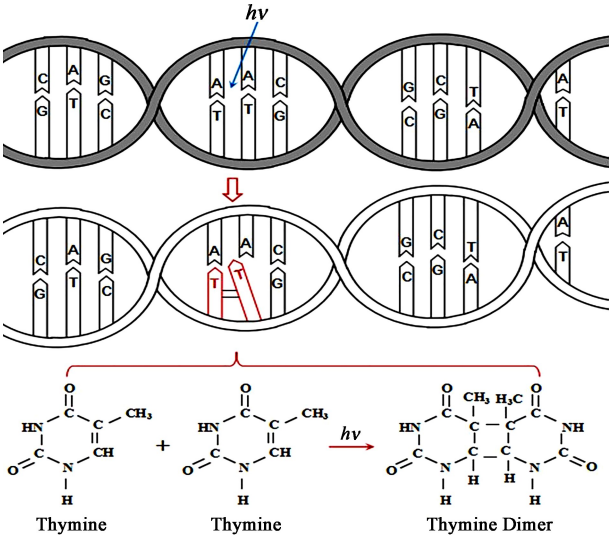

Fig. 4 The formation of thymine dimers caused by disruption to double-stranded DNA by UV radiation. A: adenine, G: guanine, T: thymine; C: cytosine.

3 Application Type of Deep Ultraviolet Light-Emitting Diode in Drinking Water Treatment

3.1 Application of UV LED in Different UV Reactors

Factors such as the structure of the UV reactor, the arrangement of the light source, and the hydraulic conditions will affect the dose of UV radiation received by the microorganisms in water[25]. In the process of ultraviolet inactivation of microorganisms, the radiation dose is significant. When the ultraviolet radiation dose reaches the corresponding amount, the organisms will be inactivated. However, when the quantity is insufficient, some viruses and bacteria that have been inactivated by ultraviolet radiation can repair their damaged structures through the assistance of light[26]. Therefore, it is essential to match the design of the UV reactor with the irradiation mode of the UV light source. According to the combination of UV light source and UV reactor, all UV disinfection systems can be divided into three types: immersive, external, and distributed[27]. The immersion type means that the ultraviolet light source is ultimately placed inside the ultraviolet reactor. The external type means that the ultraviolet light source is placed outside the reactor. Distributed is that the light source is placed outside the reactor, but some optical means (such as adding a TIR lens behind the UV LED to increase its collimation) make the light shine inside the reactor.

The radiation shape of the ultraviolet lamp is mainly cylindrical. It is difficult to change its radiation model through some optical means, which has a significant limitation on the structure of the ultraviolet reactor, and most ultraviolet lamps can only be placed inside. Therefore, the construction of the entire reactor is more complicated and the design flexibility is poor, thus it will cause specific interference to the fluid flow in the reactor. The radiation profile of UV LED is mainly concentrated in one direction. Although the radiated light is relatively divergent and uneven, some optical lenses can change their radiation profile. For example, the collimation of light lines can be achieved by using mirrors or Fresnel lenses. In addition, the volume of UV LEDs is small. These characteristics make it possible to install inside or outside the reactor. There is more flexibility in the matching process with the UV

reactor, which is more conducive to design a high-efficiency UV disinfection system[1,28]. In a flow-type UV reactor, microorganisms move with the water flow, and the light intensity distribution in the reactor is also very uneven. Moreover, the path on which each organism travels through the reactor is different, which can easily cause the light intensity to be weaker where the flow velocity is fast, and the light intensity to be more vigorous where the flow velocity is slow. This causes the microorganisms to receive different UV doses, which leads to very uneven sterilization. Therefore, it is essential to construct a more uniform light field inside the UV reactor to become a constant. It is more convenient to study the radiation flux received by microorganisms. It is also more conducive to the uniformity of sterilization. Due to the large volume of traditional ultraviolet lamps, and generally can only be installed inside the reactor. The radiated light field is relatively fixed, and it isn't easy to form a uniform light lot. However, due to its small size, the radiation profile and other characteristics of UV LED are easy to change. It is easier to form a relatively uniform light field in matching with the reactor[29]. Therefore, UV LED has a broader application prospect in fitting with the reactor. Tab. 1 shows a summary of the classification of reactors and the arrangement of light sources. It can also be seen from the table that UV LEDs have more matching methods with the reactor, and a UV lamp can only be placed inside the reactor.

Tab. 1　Summary of The Classification of Reactors and The Arrangement of Light Sources

UV source	Reactor type	Arrangement of the light source	Reactor shape	Reference
UV Lamp	immersive	perpendicular and parallel to the reactor axis	cylinder	[30]
UV Lamp	immersive	perpendicular to the reactor axis	cylinder	[25]
UV Lamp	immersive	perpendicular and parallel to the reactor axis	cuboid	[31]
UV Lamp	immersive	parallel to the reactor axis	U-shape (Internal baffle)	[32]
UV Lamp	immersive	parallel to the reactor axis	U-shape L-shape	[33]
UV LED	external	On the outer wall of the reactor	cuboid	[34]
UV LED	external	In the middle of the helical structure	spiral-channel configuration	[35]
UV LED	immersive	The light source module is placed in the center of the reactor	U-shape	[36]
UV LED	immersive	The light source module is placed in the center of the reactor	L-shape	[37]
UV LED	external	The outer wall of the reactor	cylinder	[38]

3.2　Advanced Oxidation Processes (AOPs) Based on UV LED

This method refers to the interaction of ultraviolet rays and some catalysts, including H_2O_2, TiO_2, O_3, to produce some reactive radicals to kill harmful microorganisms in water[39-40]. Fig. 5 shows the schematic diagram of AOPs microbial inactivation of microorganisms and degradation of organic pollutants[41].

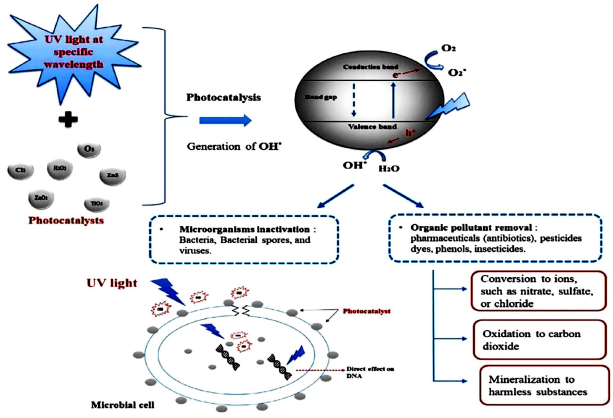

Fig. 5　Schematic diagram of AOPs microbial inactivation of microorganisms and degradation of organic pollutants[41]

The catalyst is H_2O_2. As an example, H_2O_2 will decompose to generate a large number of hydroxyl radicals after absorbing the specific wavelength ($\lambda < 280$ nm) of ultraviolet light emitted by UV LED. Hydroxy radicals can degrade organic pollutants in water quickly and are non-selective to sterilize. Hydroxyl efficiently reacts with the 2-deoxyribose moieties by hydrogen abstraction that leads to the DNA strand breaks. The concentration of hydrogen peroxide will also

affect the effect of sterilization and disinfection. Under constant UV intensity irradiation, more hydrogen peroxide will decompose to generate more hydroxyl radicals, accelerate the decomposition rate, and rapidly decompose organic pollutants in the water. However, more than H_2O_2, hydroxyl radicals are consumed to form hydroperoxyl radicals with lower oxidation capability, slowing down the degradation rate. In addition, the pH value of the water will also affect the rate of degradation because hydrogen peroxide will dissociate to hydroperoxyl anion in the alkaline medium. The dissociated hydroperoxy anion will consume the hydroxyl radicals, which will also slow down the degradation rate of pollutants. Experimental studies have also proved that combining the pulsed ultraviolet light source and hydrogen peroxide is more effective in inactivating bacillus subtilis. Thus, the advanced oxidation process is an option for drinking water disinfection and degradation of pollutants. Several published works have described the reaction and kinetics of the ultraviolet-based advanced oxidation method to degrade organic pollutants in water[42-43].

3.3 Combined Wavelength Irradiation

Studies have shown that UV bactericidal effect depends heavily on wavelength and the spectral sensitivity of microorganisms is not necessarily consistent with the DNA absorption spectrum[44]. Fig. 6 shows the inactivation spectra of several common microorganisms and the absorption spectra of DNA and protein[19]. It should be noted that all inactivation constant and absorbance values are normalized to their corresponding values at 254 nm (i.e., relative sensitivity).

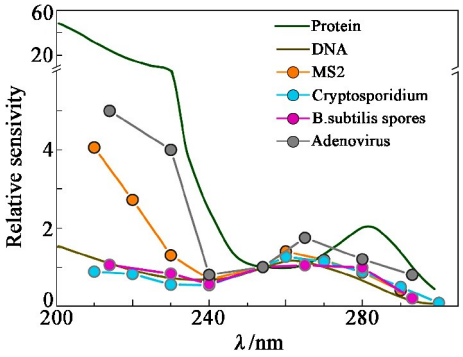

Fig. 6 Action spectra for MS2, B. subtilis spores, cryptosporidium, adenovirus, and the absorbance spectra for DNA and protein[19]

It can be seen from the figure that the action spectrum of several microorganisms has a peak near 260 nm, and the absorption spectrum of DNA reaches a maximum near 260 nm. Therefore, it is generally believed that the ultraviolet wavelength around 260 nm has the best sterilization effect. However, the discussion about the optimal inactivation wavelength has never stopped, mainly because the types of the studied microorganisms are too few, and the samples are not comprehensive enough. Therefore, in addition to the radiation dose, the sterilization efficiency also depends on the wavelength. According to international regulations, the disinfection of drinking water should reach the standard of a healthy human drinking[45]. Tab. 2 shows the UV dose or exposure time required for some microorganisms in the water to achieve at least 4 log inactivation at different wavelengths.

Tab. 2 The Dose and Exposure Time under Inactivation that Meets The International Requirements

λ/nm	Microorganism	Disinfection medium	U.V. dose/ (mJ/cm^2)	Log inactivation	Reference
265	E. coli	Water	10.8	4	[38]
365	E. coli	Water	315 000	5.7	[14]
280	E. coli	Water	Exposure Time: 30 s	7	[46]
265/285	E. coli	Water	Exposure Time: 40 s	4.6	[47]
254/365	E. coli	Water	Exposure Time: 30 s	7	[46]
280/365	E. coli	Water	Exposure Time: 30 s	7	[46]
280/405	E. coli	Water	Exposure Time: 30 s	7	[46]
269	B. subtilis	Water	40	5.9	[48]
282	B. subtilis	Water	60	7.2	[48]
265	B. subtilis	Water	28	4	[28]
280	B. subtilis	Water	48	4	[28]
265	Qβ	Water	39	4	[28]
280	Qβ	Water	75	4	[28]
266	Qβ	Water	9	7	[49]
266	MS2	Water	9	7	[49]
275	T7	Water	20	4.7	[1]
266	ΦX174	Water	1	7	[49]
375	Streptococcus mutans	Water	Exposure Time: 15 min	4	[50]

The inactivation effect of single-wavelength ultraviolet light on microorganisms in water has been studied in detail. Aoyagi et al.[51] showed that the UV light-emitting diode at 255 nm had a better inactivation effect on phage ΦX174, Qβ, and MS2. Bowker et al.[1] studied the inactivation of MS2 T7 and E. coli by UV light-emitting diodes at 255 and 275 nm. The results showed that the inactivation effect of the UV light-emitting diode at 255 nm was better than that at 275 nm under single-wavelength irradiation. For T7 and E. coli, the UV dose-response at 255 nm was slightly higher than at 275 nm, indicating that the inactivation effect at 275 nm was better.

Different wavelengths can cause damage to various key cell components (proteins and nucleic acids)[52-54]. Therefore, the combination of different ultraviolet wavelengths is considered more effective than only one wavelength of ultraviolet radiation. However, the inactivation effect of different UV wavelength combinations on microorganisms is less studied. Chevremont et al.[46] studied the inactivation effect of UVA LED and UVC LED on thermophilic bacteria. The results showed that the inactivation effect of UVA LED and UVC LED was better than that of UVA LED alone. Kai Song et al.[47] proved that the combination of UVC and UVB wave irradiation could cause higher log inactivation.

In comparison, Oguma et al.[37] used 265, 280 and 310 nm UV LEDs for E. coli inactivation, but no synergy was observed. On the contrary, these researchers found that the inactivation efficiency of the combined wavelength was lower than that of the individual wavelength. Wan et al.[48] compared the performance of low-pressure UV and UV light-emitting diodes at 265, 280 and 265/280 nm combined emission. Then they tested their inactivation efficiency for three waterborne fungal species in an intermittent water disinfection system. The results showed that the inactivation effect of a UV light-emitting diode on fungal spores was better than that of 254 nm low-pressure UV. Still, there was no statistical difference in the inactivation effect of a UV light-emitting diode on fungal spores. The germicidal efficacy of wavelength combination in UVC and UVB range was also studied. It was found that no combination of wavelengths was better than a single wavelength[55-56]. These studies show that combining the selected wavelength may be a promising method to improve the disinfection efficiency of ultraviolet light-emitting diodes. However, it is difficult to identify and distinguish which wavelength or combination of specific wavelengths has a better bactericidal effect in the ultraviolet wavelength range. In addition, the researchers' different experimental conditions may cause different results, so more in-depth researches are needed.

4 Conclusion

At present, the primary ultraviolet light source of the ultraviolet disinfection system is a low- and medium-pressure mercury lamp[57]. Although this is widely used in the water treatment system, they still present lots of problems. It is mainly the fragile ultraviolet lamp, containing toxic mercury, short life, harmful to the environment and so on[58-59]. Compared with the mercury lamp, UV LED has the advantages of environmental protection, rapid start-up, and can emit various wavelengths. These advantages attract much interest in researching the application of UV LEDs in water treatment. It is believed that shortly, with the rapid development of semiconductor technology, the technical bottlenecks encountered in the research and development of UV LEDs, such as low efficiency of external quantum, easy aging of packaging materials, and heat dissipation problems, will be solved.

In short, UV LED has a broad application prospect in water treatment, but there are still some problems to be considered and solved: (Ⅰ) At present, the luminous efficiency of deep

ultraviolet LEDs is still very low, and its EQE is generally lower than 10%. Therefore, the output power of a single UV LED chip is difficult to meet the needs of microbial inactivation, and multiple chips are required to be integrated. But at the same time, it will cause the integrated module heat to be too high, which will affect the life of the chip. On the other hand, the manufacturing cost of UV LED is high, and it is difficult to be used in large-scale commercialization. These problems must be solved from the chip structure and epitaxial growth. Thus we need to design a set of stable and heat-resistant packaging structure to ensure the stable operation of the chip; (Ⅱ) In the UV LED water treatment system, it is difficult to ensure that ultraviolet light is uniformly irradiated in the whole water flow. Therefore, it is necessary to over-irradiate the areas under-irradiated by the UV LED, while the over-irradiated areas waste resources. We can try to change the radiation model of the UV LED by installing an optical lens to make it irradiate more uniformly; (Ⅲ) There is no uniform standard for the amount of inactivation of each microorganism in water by UV LEDs. Many researchers have different inactivation doses for the same microorganism, which may be due to other experimental conditions. Moreover, the research on the types of microorganisms is not perfect, which will bring some problems to the application of UV LED in water treatment; (Ⅳ) We should accurately study the optimal inactivation wavelength and inactivation dose of each microorganism in the water, and complete the kinetic data of UVC LED irradiation disinfection and sterilization. Ultraviolet rays can cause special damage to the human body. Therefore, the radiation dose for disinfection and sterilization should be strictly controlled; (Ⅴ) The inactivation mechanisms of each microorganism in water by UV LED should be studied in more detail, and more experimental data of UV LED needs to be explored.

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About the Authors:

YANG Chengshuang (1996 —), Male, born in Dengzhou City, Henan Province, master degree candidate, his main research interests are optoelectronic materials and semiconductor lighting;

XIE Jianing (1971 —), Female, born in Chaozhou City, Guangdong Province, PhD, Professor, mainly engaged in the research of optical information processing, semiconductor devices and other directions.