中国激光

# 全固态 228 nm 远紫外脉冲激光的灭菌效果

赵志斌[1,2]，程成[2]，金映虹[2]，曾文博[2]，金秦隆[2]，李权[2]，李娇娇[2]，曲轶[2**]，薄报学[1*]

[1]长春理工大学高功率半导体激光国家重点实验室，吉林 长春 130022；

[2]海南师范大学激光技术与光电功能材料省重点实验室，热带岛屿生态学教育部重点实验室，海南 海口 571158

**摘要** 持续的新型冠状病毒肺炎疫情大流行下，世界各国亟需可在空气中灭活细菌病毒的新方法。典型的杀菌紫外线波长为 254 nm，但该辐照对人体细胞有害。有研究表明，200～230 nm 远紫外线能灭活病原体，且对人体细胞无害。目前通常采用准分子灯发射的远紫外线来灭活细菌病毒。激光能实现远距离传输，在远距灭菌消毒领域可弥补准分子灯光源的不足。本文采用自主研制的全固态 228 nm 远紫外脉冲激光作为光源，在 2 mJ/cm$^2$ 和 6 mJ/cm$^2$ 低剂量照射下对大肠杆菌和芽孢杆菌的灭活率均高达 100%。该实验结果表明，228 nm 远紫外脉冲激光具有较强的抗菌特性。

**关键词** 生物光学；全固态激光；200～230 nm 远紫外线；228 nm 脉冲激光；抗菌特性

**中图分类号** TN248.1 **文献标志码** A **DOI**：10.3788/CJL202249.1515001

新型冠状病毒（SARS-CoV-2）肺炎疫情大流行下，国内外均在探寻可在空气中灭活细菌病毒的方法。紫外线能有效灭活 SARS-CoV-2[1]，证明了 SARS-CoV-2 与多数细菌病毒一样对紫外线敏感。紫外线杀菌机理是通过辐射微生物并破坏其脱氧核糖核酸（DNA）或核糖核酸（RNA）[2]。传统的杀菌紫外线光源是汞蒸气灯，然而汞蒸气灯发射的峰值波长为 254 nm 的紫外线对人体细胞和组织有害，重则导致皮肤癌[3]或白内障[4]。近 10 年来的研究结果表明，200～230 nm 波段的紫外线可以灭活细菌、空气中的流感病毒和 SARS-CoV-2 病毒等病原体而不损害人体细胞[5-8]。国际上把 200～230 nm 波段深紫外光命名为“远紫外线”。2022 年北京冬季奥运会广泛使用远紫外线进行杀菌消毒，并称之为“光疫苗”。与杀菌用的典型 254 nm 紫外线相比，远紫外线对人体细胞无害的生物物理原理是蛋白质对该波段存在吸收峰[9]：远紫外线可以穿过比人体细胞小得多的微生物（细菌或病毒的典型直径为 1 μm 和 0.1 μm）[10]，而典型人体细胞的直径范围为 10～25 μm，远紫外线被人体细胞质中的蛋白质强烈吸收，并且在到达人体细胞核之前急剧减弱[6]。例如，对于人体皮肤，其最外层是角质层，由已死亡的无核角质细胞组成。角质层的主要作用是保护其皮下组织。大多数辐照的远紫外线被皮肤角质层胞质中的蛋白质吸收，无法穿透皮肤角质层到达下面的关键基底细胞或黑素细胞[11]。对于人眼睛，其对紫外线敏感的组织是晶状体。然而，晶状体位于角膜的后端，角膜厚度约为 500 μm[12]，因此，远紫外线通过角膜到达晶状体的透过率基本为 0[13]。

远紫外线具有抗菌性，而且对人体几乎无伤害，因此，在 SARS-CoV-2 肺炎疫情大流行下，开展该波段内新型光源及其抗菌特性研究具有非常重要的意义。在远紫外线波段范围内，目前通常采用准分子灯光源灭活细菌病毒[5-8,14]。基于激光器和准分子灯发射光的原理不同，两者的输出光特性有较大区别。相较于准分子灯发射光，激光具有方向性好、亮度高、单色性强等优点。激光能实现较远距离传输，在远距灭菌消毒领域可弥补准分子灯发射光的不足；激光能量较集中且较容易实现高性能脉冲运转光输出，使其与物质相互作用时可瞬间产生较高温度，这一特性有利于增强光热作用来灭活细菌病毒，提高灭活效率。本研究以大肠杆菌和芽孢杆菌为例，利用自主研制的发射峰值波长为 228 nm 的远紫外脉冲激光开展抗菌效果研究。

1）细菌样品制备。大肠杆菌广泛地存在自然界中，是人类公共卫生中重点防御的一类病原，且是肠杆菌群中抗药性最强的物种之一，常被用于紫外线杀毒和环境卫生的研究；芽孢杆菌容易引起食物中毒，由于其孢子具有耐热性和耐酸性，不能通过巴氏杀菌或正常的卫生程序消除。因此，本研究使用的菌种为大肠

**收稿日期**：2022-04-11；**修回日期**：2022-05-30；**录用日期**：2022-06-10

**基金项目**：海南省重点研发项目（ZDYF2020020）、海南省重大科技计划（ZDKJ2019005）、国家自然科学基金（61774024，61864002，61964007，11764012）、海南省院士创新平台科研专项（YSPTZX202034）

**通信作者**：*bbx@cust.edu.cn；**quyihainan@126.com

杆菌(*Escherichia coli*)和芽孢杆菌(*Bacillus cereus*)。大肠杆菌和芽孢杆菌分别是由海南师范大学热带岛屿生态学教育部重点实验室提供的大肠埃希氏菌[*Escherichia coli* CMCC(B) 44102]和海南师范大学环境微生物生态研究室提供的苏云金芽孢杆菌库斯塔克亚种 HD-1。大肠杆菌和芽孢杆菌在营养琼脂培养基中培养,放置在温度为 35 ℃的培养箱中,培养周期为 24 h。营养琼脂培养基由广东环凯微生物科技有限公司提供。紫外线辐照源为自主研制的全固态 228 nm 远紫外激光器[15-16],最高输出平均功率为 35 mW,脉冲宽度为 46 ns,重复频率为 10 kHz。该激光器是通过 LD 泵浦 $Nd^{3+}$ 激光晶体,利用调 $Q$ 技术和非线性光学频率转换技术实现激光输出。全固态激光器具有体积小、效率高、光束质量好、可靠性高、寿命长和便携等优点。

2) 228 nm 激光杀菌。整个实验操作过程在超净室中进行,使用高灭菌锅对实验工具进行灭菌,实验前对工作台进行 1 h 的紫外线照射,避免环境的细菌污染。比色皿在放入细菌悬浮液后进行封口,以避免悬浮液直接接触外界空气环境。整个实验过程细菌处于营养体中,避免由细菌自然死亡带来的实验误差。取 1 mL 一定浓度的大肠杆菌和芽孢杆菌悬浮液样品放入高透 UVC 波段的比色皿中,通过调节激光输出功率和比色皿的放置位置,得到辐照度为 0.1 mW/cm$^2$ 的 228 nm 激光,并使用该激光分别以不同的辐照时间对大肠杆菌和芽孢杆菌悬浮液样品进行照射。图 1(a)所示为 228 nm 激光垂直照射中的大肠杆菌悬浮液。将对照样品和辐照样品再培养 24 h 后,得到的无辐照和辐照后的细菌分布如图 1(b)、(c)所示。采用营养琼脂平板计数法测定对照组和辐照后的大肠杆菌细菌计数,为提高实验结果的准确度,在相同的辐照剂量下,对每个样品重复测量 3 次取平均值。

检测结果如表 1 所示。利用 228 nm 激光对大肠杆菌悬浮液照射 10 s(剂量为 1.0 mJ/cm$^2$),灭活率

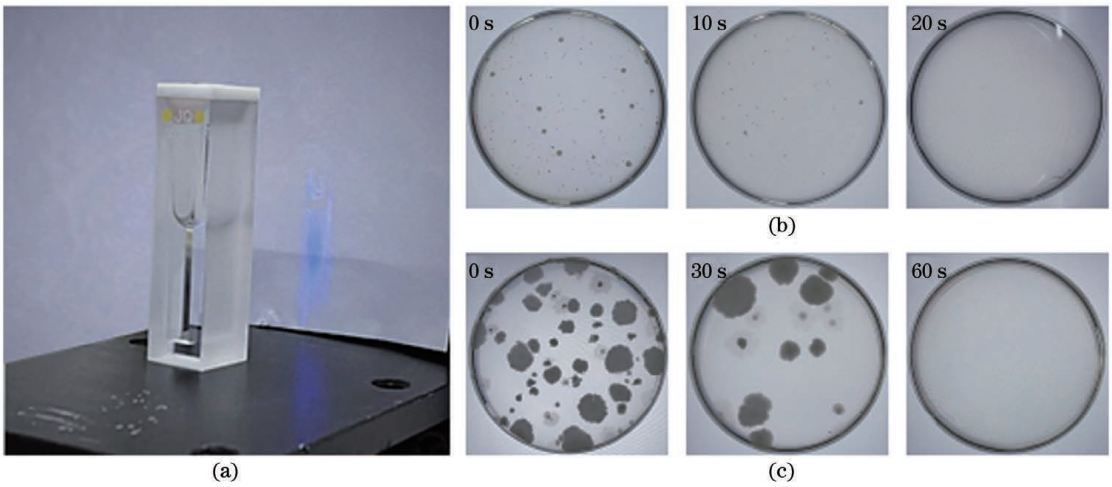


图 1　228 nm 激光杀菌实验。(a)照射中的大肠杆菌悬浮液;(b)辐照 0,10,20 s 后大肠杆菌分布;(c)辐照 0,30,60 s 后芽孢杆菌分布

Fig. 1　228 nm laser sterilization experiment. (a) *Escherichia coli* suspension in irradiation; (b) distribution of *Escherichia coli* under irradiation time of 0, 10, 20 s; (c) distribution of *Bacillus cereus* under irradiation time of 0, 30, 60 s

表 1　228 nm 激光灭活大肠杆菌和芽孢杆菌的效果

Table 1　228 nm laser inactivation effect of *Escherichia coli* and *Bacillus cereus*

| Test strains | Irradiation time /s | 228 nm laser dose /(mJ·cm$^{-2}$) | Average bacteria count /(CFU·mL$^{-1}$) | Inactivated rate /% |
|---|---|---|---|---|
| *Escherichia coli* | 0 | 0 | 382 | – |
| | 5 | 0.5 | 60 | 84.3 |
| | 10 | 1.0 | 36 | 90.7 |
| | 15 | 1.5 | 12 | 96.9 |
| | 20 | 2.0 | 0 | 100.0 |
| *Bacillus cereus* | 0 | 0 | 284 | – |
| | 15 | 1.5 | 114 | 50.9 |
| | 30 | 3.0 | 33 | 88.4 |
| | 45 | 4.5 | 4 | 98.6 |
| | 60 | 6.0 | 0 | 100.0 |

为 90.7%；照射 15 s(剂量为 1.5 mJ/cm$^2$)，灭活率为 96.9%；照射 20 s(剂量为 2.0 mJ/cm$^2$)，灭活率高达为 100.0%。利用 228 nm 激光对芽孢杆菌悬浮液照射 30 s(剂量为 3.0 mJ/cm$^2$)，灭活率为 88.4%；照射 45 s(剂量为 4.5 mJ/cm$^2$)，灭活率高达为 98.6%；照射 60 s(剂量为 6.0 mJ/cm$^2$)，灭活率高达为 100.0%。

本实验的研究结果表明，使用 2.0 mJ/cm$^2$ 和 6.0 mJ/cm$^2$ 剂量的远紫外 228 nm 脉冲激光照射可分别有效灭活大肠杆菌和芽孢杆菌，采用远紫外 222 nm 准分子灯发射光照射，则分别需要 40 mJ/cm$^2$ 和 60 mJ/cm$^2$ 剂量[5,17]。与准分子灯光源相比，本研究使用的远紫外脉冲激光光源展现更强的杀菌效果，其原因是准分子灯光源主要通过光化作用灭活细菌病毒，远紫外脉冲激光是通过光化和光热两种方式共同作用杀菌，使得灭活细菌病毒的效率更高。另外，从本实验和其他文献报道的结果可知，紫外线灭活芽孢杆菌所需辐照剂量比大肠杆菌大，其原因是采用紫外线灭活细菌病毒，所需辐照剂量主要和微生物的尺寸、细胞膜(壁)厚度和核酸结构(单链或双链)等因素有关，通常情况下，微生物尺寸越大、细胞膜越厚，所需辐照剂量越高；双链结构微生物修复能力比单链结构强，所需辐照剂量也较高；芽孢杆菌和大肠杆菌都是双链结构，但是芽孢杆菌的尺寸和细胞壁厚度约为(1.0～1.2 nm)×(3.0～5.0 nm)和 20～80 nm，大肠杆菌的仅为(0.5～0.8 nm)×(1.0～3.0 nm)和 11 nm，因此灭活芽孢杆菌所需紫外线辐照剂量比大肠杆菌高。

## 参　考　文　献


[1] Raeiszadeh M, Adeli B. A critical review on ultraviolet disinfection systems against COVID-19 outbreak: applicability, validation, and safety considerations[J]. ACS Photonics, 2020, 7(11): 2941-2951.

[2] Budowsky E I, Bresler S E, Friedman E A, et al. Principles of selective inactivation of viral genome[J]. Archives of Virology, 1981, 68(3): 239-247.

[3] Pfeifer G P, Besaratinia A. UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer [J]. Photochemical & Photobiological Sciences, 2012, 11(1): 90-97.

[4] Söderberg P G. Acute cataract in the rat after exposure to radiation in the 300 nm wavelength region. A study of the macro-, micro- and ultrastructure[J]. Acta Ophthalmologica, 1988, 66(2): 141-152.

[5] Sosnin E A, Stoffels E, Erofeev M V, et al. The effects of UV irradiation and gas plasma treatment on living mammalian cells and bacteria: a comparative approach[J]. IEEE Transactions on Plasma Science, 2004, 32(4): 1544-1550.

[6] Buonanno M, Stanislauskas M, Ponnaiya B, et al. 207-nm UV light-a promising tool for safe low-cost reduction of surgical site infections. II: *in-vivo* safety studies[J]. PLoS One, 2016, 11(6): e0138418.

[7] Welch D, Buonanno M, Grilj V, et al. Far-UVC light: a new tool to control the spread of airborne-mediated microbial diseases [J]. Scientific Reports, 2018, 8: 2752.

[8] Buonanno M, Welch D, Shuryak I, et al. Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses[J]. Scientific Reports, 2020, 10: 10285.

[9] Hessling M, Haag R, Sieber N, et al. The impact of far-UVC radiation (200–230 nm) on pathogens, cells, skin, and eyes: a collection and analysis of a hundred years of data[J]. GMS Hygiene and Infection Control, 2021, 16: Doc07.

[10] Metzler D E. Biochemistry: the chemical reactions of living cells [M]. 2nd ed. San Diego: Academic Press, 2001.

[11] Narita K, Asano K, Morimoto Y, et al. Disinfection and healing effects of 222-nm UVC light on methicillin-resistant *Staphylococcus* aureus infection in mouse wounds[J]. Journal of Photochemistry and Photobiology B, 2018, 178: 10-18.

[12] Doughty M J, Zaman M L. Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach[J]. Survey of Ophthalmology, 2000, 44(5): 367-408.

[13] Kolozsvári L, Nógrádi A, Hopp B, et al. UV absorbance of the human cornea in the 240- to 400-nm range[J]. Investigative Ophthalmology & Visual Science, 2002, 43(7): 2165-2168.

[14] Kitagawa H, Nomura T, Nazmul T, et al. Effectiveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination[J]. American journal of infection control, 2021, 49(3): 299-301.

[15] Zhao Z B, Chen H, Liu G J, et al. All-solid-state DUV light source by quadrupling of an acousto-optically *Q*-switched Nd∶$YVO_4$ laser[J]. IEEE Access, 2021, 9: 165989-165995.

[16] 赵志斌, 陈浩, 徐东昕, 等. LD 端面抽运全固态声光调 *Q* 228.5 nm 深紫外激光器[J]. 中国激光, 2022, 49(3): 0315001.
Zhao Z B, Chen H, Xu D X, et al. LD end-pumped all-solid-state acousto-optical *Q*-switched 228.5 nm deep ultraviolet laser [J]. Chinese Journal of Lasers, 2022, 49(3): 0315001.

[17] Matafonova G G, Batoev V B, Astakhova S A, et al. Efficiency of KrCl excilamp (222 nm) for inactivation of bacteria in suspension[J]. Letters in Applied Microbiology, 2008, 47(6): 508-513.

# Inactivation Effect of All-Solid-State 228 nm Far-UVC Pulsed Laser

Zhao Zhibin[1,2], Cheng Cheng[2], Jin Yinghong[2], Zeng Wenbo[2], Jin Qinlong[2], Li Quan[2], Li Jiaojiao[2], Qu Yi[2**], Bo Baoxue[1*]

[1] *State Key Laboratory of High Power Semiconductor Laser, Changchun University of Science and Technology, Changchun* 130022, *Jilin, China*;

[2] *Key Laboratory of Laser Technology and Optoelectronic Functional Materials of Hainan Province, Ministry of Education Key Laboratory for Ecology of Tropical Islands, Hainan Normal University, Haikou* 571158, *Hainan, China*

## Abstract

**Objective** The ongoing coronavirus pandemic has propelled the need for new approaches to disinfection, especially for airborne viruses. The 254 nm emission of low-pressure vacuum lamps is known for its antimicrobial effect; however, its radiation is harmful to human health, causing skin cancer and cataracts. Some studies have shown that short-wavelength ultraviolet (UV) light in the spectral region of 200–230 nm (far-UVC) can inactivate pathogens without harming human cells. Thus, it has great prospects for many applications. Sufficient studies have proved the antibacterial performance of far-UVC band range in an excimer lamp emitting a peak wavelength of 222 nm light. Furthermore, laser light sources can realize long-distance transmission and complement the deficiency of excimer lamps in remote sterilization and disinfection. This study investigates the antibacterial effect of a self-developed far-UVC laser with a peak wavelength of 228 nm and hopes to provide a new technical approach for the inactivation of the novel coronavirus and other microbial pathogens.

**Methods** Bacterial sample preparation: *Escherichia coli* (*E. coli*) widely exists naturally and is a pathogen of major focus in human public health defense. It is also one of the most drug-resistant species in the enterobacterium group. Therefore, it is often used in ultraviolet disinfection and environmental health research. *Bacillus cereus* (*B. cereus*), which is closely related to humans, causes food poisoning and cannot be eliminated by pasteurization or normal hygiene procedures due to the heat and acid resistance of its spores. Therefore, the strains used in this experiment are *E. coli* and *B. cereus*. *E. coli* and *B. cereus* are provided by the Ministry of Education Key Laboratory for Ecology of Tropical Islands, Hainan Normal University, and subsp. Kustaki HD-1, provided by the Environmental Microbial Ecology Laboratory of Hainan Normal University. Both strains are cultured in a nutrient agar medium and placed in an incubator at 35 ℃ for 1 day. Nutrient agar medium is provided by Guangdong Huankai Microbial Technology Co., LTD, China. UV irradiation source: the irradiation source is a self-developed all-solid-state 228 nm far-UVC laser, which provides UV irradiance of up to 35 mW/cm$^2$, and its spectral linewidth is less than 0.1 nm. The laser is realized by LD-pumped $Nd^{3+}$ laser crystal, *Q*-switched technology, and nonlinear optical frequency conversion technology. All-solid-state lasers have the advantages of small size, high efficiency, good beam quality, high reliability, long life, and portability. 228 nm far-UVC laser sterilization: we input a certain concentration of 1 mL bacterial suspension sample into a high permeability UVC cuvette. The 228 nm laser irradiance of 0.1 mW/cm$^2$ is obtained by adjusting the laser output power and the placement of the colorimeter. *E. coli* suspension samples are irradiated for 5, 10, 15, and 20 s [Fig. 1(b)], and *B. cereus* suspension samples are irradiated for 15, 30, 45, and 60 s [Fig. 1(c)] at 228 nm far-UV light of 0.1 mW/cm$^2$. The experiment is repeated three times for each sample at the same irradiation dose.

**Results and Discussions** Figs. 1 (b) and (c) show the distribution of bacteria before and after 228 nm laser irradiation. The concentration of bacterial suspension samples in the control and irradiated groups is determined using the nutrient agar plate counting method. The detection results are shown in Table 1. When the *E. coli* suspension is irradiated by 228 nm laser for 10, 15, and 20 s (1, 1.5, and 2 mJ/cm$^2$), the inactivation rates are 90.7%, 96.9%, and 100%, respectively. When the *B. cereus* suspension is irradiated by 228 nm laser for 30, 45, and 60 s (3, 4.5, and 6 mJ/cm$^2$), the inactivation rates are 88.4%, 98.6%, and 100%, respectively.

**Conclusions** This experimental study shows that the use of several mJ/cm$^2$ doses of far-UVC 228 nm pulsed laser irradiation can effectively inactivate *E. coli* and *B. cereus*, whereas the use of excimer lamps requires dozens of mJ/cm$^2$ doses. Compared with the excimer light source, the far-UVC pulsed laser light source shows a stronger sterilization effect. The next step is to conduct experimental research on the inactivation of the influenza virus using a far-UVC 228 nm pulsed laser.

**Key words** bio-optics; all-solid-state laser; 200–230 nm far-UVC; 228 nm pulsed laser; antibacterial properties