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Ultraviolet germicidal efficacy as a function of pulsed radiation parameters studied by spore film dosimetry

Stefan Bauer[a,*], Hans Holtschmidt[b], Günter Ott[a]

[a] Unit Physical Agents, Federal Institute for Occupational Safety and Health (BAuA), Dortmund, Germany
[b] Laboratory for Biosensory Systems, BioSense, Bornheim, Germany

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ABSTRACT

Disinfection by pulsed ultraviolet (UV) radiation is a commonly used method, e.g. in industry or medicine and can be carried out either with lasers or broadband UV radiation sources. Detrimental effects to biological materials depending on parameters such as pulse duration τ or pulse repetition frequency f_p are well-understood for pulsed coherent UV radiation, however, relatively little is known for its incoherent variant. Therefore, within this work, it is the first time that disinfection rates of pulsed and continuous (cw) incoherent UV radiation studied by means of spore film dosimetry are presented, compared with each other, and in a second step further investigated regarding two pulse parameters. After analyzing the dynamic range of the *Bacillus subtilis* spore films with variable cw radiant exposures $H = 5–100\ \mathrm{Jm^{-2}}$ a validation of the Bunsen-Roscoe law revealed its restricted applicability and a 28% enhanced detrimental effect of pulsed compared to cw incoherent UV radiation. A radiant exposure $H = 50\ \mathrm{Jm^{-2}}$ and an irradiance $E = 0.5\ \mathrm{Wm^{-2}}$ were found to be suitable parameters for an analysis of the disinfection rate as a function of $\tau = 0.5–10$ ms and $f_p = 25–500$ Hz unveiling that shorter pulses and lower frequencies inactivate more spores. Finally, the number of applied pulses as well as the experiment time were considered with regard to spore film disinfection.

1. Introduction

The detrimental effects of UV radiation to germs and bacteria [1] or to living organisms in general [2] are well-understood and utilized, e.g. for water disinfection [3] or for food decontamination [4,5] to reduce the spreading of pathogens. This antiseptic action is also applied in medicine for virus inactivation of blood products [6–8] with the advantage that the biological properties of the sample will remain unchanged in contrast to a chemical treatment. Upon advancing technological development, pulsed UV disinfection becomes more and more popular due to reduced exposure times [9] and operational costs. Additionally, Ben Said and Otaki [10] found a four- to sixfold enhanced disinfection efficiency of pulsed compared to cw UV irradiation.

At a first glance, this result might be astonishing as the fundamental law in photobiology states that an equal radiant exposure always leads to the same photobiological or photochemical reaction irrespective of the choice of exposure duration or irradiance. However, it is obvious that this law discovered 1859 by Bunsen and Roscoe [11] can only be valid within certain limits at least for most biological systems [12]. For example, an ultra-short high-energy laser pulse can evaporate cells whereas the same cw radiant exposure applied on a much longer timescale can even stimulate DNA synthesis [13].

Early experiments verifying the Bunsen-Roscoe law were based upon fractionated irradiation where a constant radiant exposure was applied either as a single dose or in several smaller fractions. It was found that "…UVA-induced cell death is increased by about 100% upon fractionated exposure…" [14]. This result, though, depends on the recovery time (seconds to several days) between the individual fractionated doses and thus a contrary outcome was also found that indicated an enhanced capacity of pulsed UV radiation for DNA repair [15].

Another kind of dose experiment can be performed by gradually increasing the number of constant doses each separated by a certain recovery time. Several publications can be found dealing with such exposure scenarios with regard to disinfection of fungi and bacteria [16], apoptosis of human cells [17], or tumor growth in animals [18–20] all concluding that detrimental UV radiation effects enhance upon increasing radiant exposure.

From a present point of view with the growing number of pulsed UV radiation sources in mind the question arises what happens to the disinfection rate when the recovery time decreases in such a way that a

* Corresponding author.
E-mail address: bauer.stefan@baua.bund.de (S. Bauer).
URL: http://www.baua.de (S. Bauer).

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low-frequency pulsed exposure scenario prevails? How do pulse duration τ and pulse repetition frequency f_p influence UV germicidal effects? A recent study treats these questions focusing on narrowband UV-LEDs [21], though, for incoherent broadband UV radiation corresponding knowledge does not exist. However, most conventionally used germicidal lamps emit this kind of radiation so that studying the effect of variable pulse parameters on the disinfection rate can optimize the germicidal process.

To shed a different light on the topic there is no scientific basis for the protection of workers being exposed to pulsed incoherent UV radiation. At the moment the "International Commission on Non-Ionizing Radiation Protection (ICNIRP)" suggests to assess pulsed incoherent like pulsed laser radiation [22] without considering their different physical characteristics. Of course, the main focus of radiation protection is on human skin and eye but preliminary investigations regarding other biological materials can lead to a better understanding of the detrimental effects of pulsed incoherent UV radiation in general.

Within the present work, the disinfection rate for a *Bacillus subtilis* spore film was examined depending on pulse duration and pulse repetition frequency for the UV radiation of a broadband xenon lamp. First, the dynamic range of the spore film dosimeters was investigated. With a suitable constant radiant exposure found, cw and pulsed irradiations were performed to determine the validity range of the Bunsen-Roscoe law. In a second step, based upon the former results, τ and f_p were varied to study their effect on the disinfection rate.

2. UV Irradiation Setup

2.1. Beam Control

The beam of a LOT-Quantum Design 550 W ozone-free xenon short arc lamp (not shown) enters from the right side of Fig. 1 onto an aperture system used to reduce the amount of stray light. A manually operated beam blocker serves as an irradiation start and stop trigger. The optical bandpass filter (UG5, Schott) suppresses most of the visible spectrum and parts of the infrared radiation from the xenon lamp (Fig. 2). Afterward, the beam is focused by a UV optimized lens into an integrating sphere with a diameter of $D = 15$ cm that emits perfectly homogenized radiation ($D = 25$ mm of the exit port). The irradiation setup was equipped with an additional aperture ($D = 9$ mm) located very close to a Polytec Ithaco (model 383B) chopper disc to ensure a rectangular pulse shape (Fig. 3). Finally, the beam passes through one of the N apertures of the chopper disc and strikes a modified VioSpor dosimeter from BioSense (Section 2.4).

All experiments were performed in a 22 °C air-conditioned dark room. A cover (not shown) was utilized to guarantee that no remaining scattered light but only UV radiation from the integrating sphere

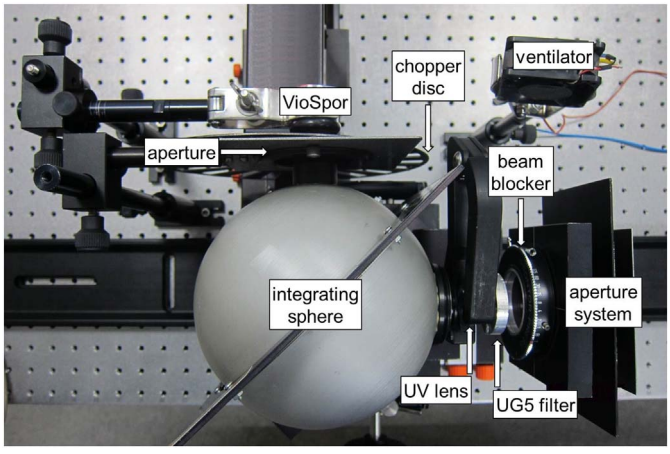

Fig. 1. Experimental setup for pulsed and cw irradiation of the VioSpor dosimeters.

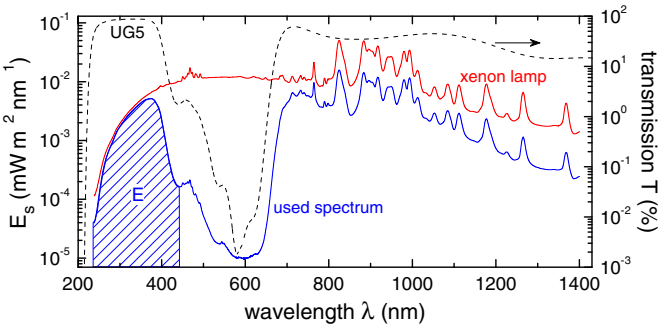

Fig. 2. (left scale) Spectral irradiance $E_s(\lambda)$ emitted from the xenon short arc lamp in the wavelength region from 200 up to 1400 nm (red solid line). Due to the spectral transmission of the applied UG5 bandpass filter (dashed line, right ordinate as indicated by the arrow) most of the visible radiation is filtered from the lamp emission spectrum (blue solid line). All spectra were measured at the exact position of the VioSpor dosimeters in Fig. 1. The hatched area ranging from 237 to 442 nm visualizes the (integral) UV irradiance E that was used for the calculation of the radiant exposure $H = E \cdot t$. Notice that the bandpass filter reduces IR radiation from the xenon lamp by a factor of approximately two third, too. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

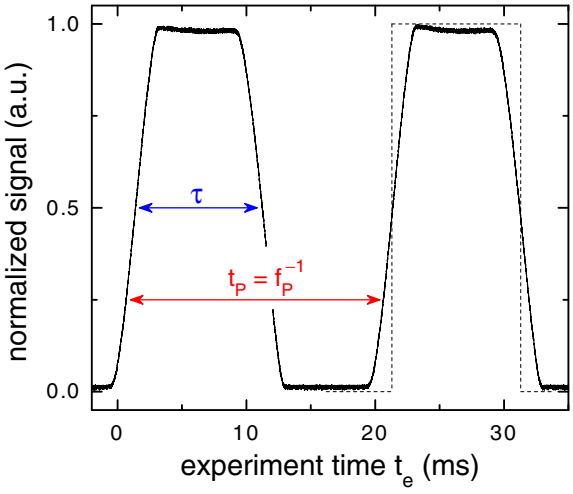

Fig. 3. Exemplary presentation of the rectangular pulses used within this work. The signal from a UV photoreceiver (normalized subsequently) was recorded as a function of experiment time t_e by means of a 200 MHz oscilloscope. Pulse duration τ (10 ms) as well as period duration $t_p = f_p^{-1}$ (20 ms) with pulse repetition frequency f_p (50 Hz) are visualized by horizontal double arrows. The dashed line represents an ideal rectangular pulse.

reached the spore film dosimeter. During the first irradiation attempts several UG5 filters heated up too much and got a crack, thus, a ventilator had to be added to the setup to cool the bandpass filter. This experimental design was used for pulsed as well as for cw irradiation of the VioSpor dosimeters, however, for the latter experiments the chopper disc did not rotate but was fixed.

2.2. Spectral Irradiance

The spectral irradiance $E_s(\lambda)$ emitted by the xenon lamp is presented in Fig. 2. The two decade $E_s(\lambda)$ loss for $\lambda < 400$ nm is characteristic for xenon short arc lamps as well as the comparatively flat spectral irradiance for visible wavelengths and the high emission lines in the IR region. Most of the visible radiation was suppressed by means of a UG5 bandpass filter whose spectral transmission $T(\lambda)$ is also depicted in Fig. 2 but with regard to the right ordinate. Notice the almost five decades decrease in $T(\lambda)$ between ~ 400 to ~ 600 nm. The final $E_s(\lambda)$ for irradiating the VioSpor dosimeters, i.e. the used spectrum in Fig. 2, had a high amount of UV spectral irradiance, nearly no visible radiation