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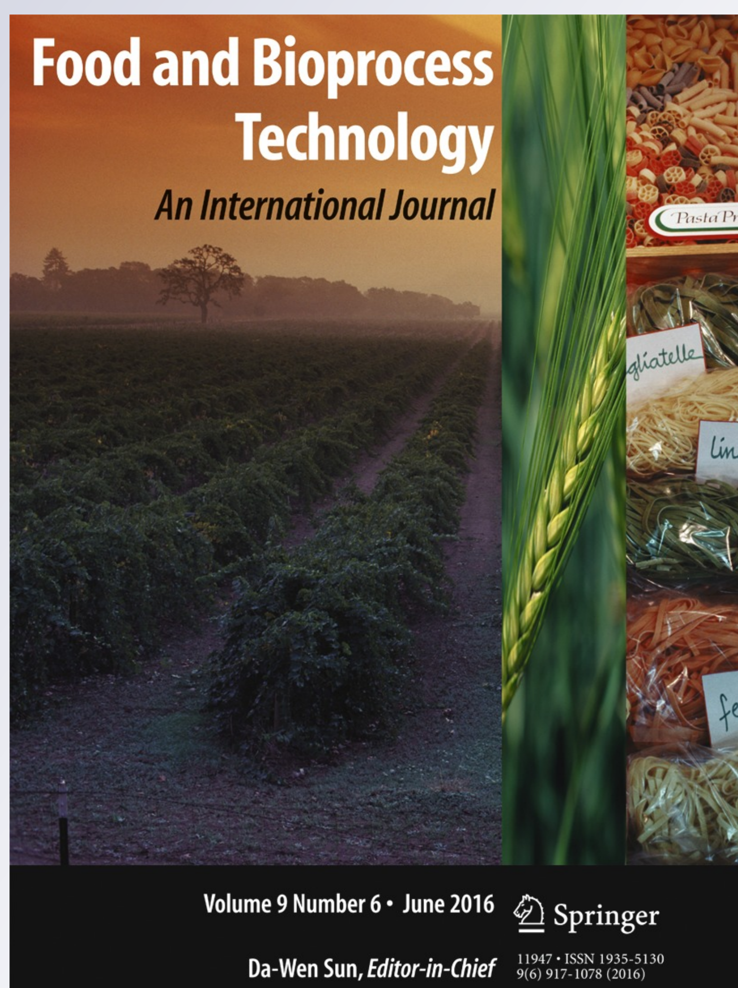

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ORIGINAL PAPER

An Approach to Standardize Methods for Fluence Determination in Bench-Scale Pulsed Light Experiments

Vicente M. Gómez-López[1] · James R. Bolton[2]

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Abstract Pulsed light (PL) is a fast non-thermal technology for decontamination based on the application of pulses of high-intensity polychromatic light including UV-C light. Continuous-wave (CW) ultraviolet (UV) light technology is based on the application of monochromatic or polychromatic low-intensity light for long times. Appropriate UV dosimetry is fundamental in order to intercompare results and for scaling up. There are standard methods for bench-top CW UV treatments but not for tests involving PL dosimetry. The present article introduces the fundamentals of photochemistry and photophysics, adapts a protocol for CW UV dosimetry to PL tests, and critically revises current ways of reporting results of PL tests.

Keywords Photon fluence · Germicidally weighted photon irradiance · UV light · Dosimetry · Disinfection

Introduction

Pulsed light (PL) is a non-thermal technology for decontamination of surfaces and translucent liquids based on the application of short pulses of high-intensity polychromatic light (Gómez-López et al. 2007), in which the ultraviolet-C (UV-C) component of the spectrum results in microbial inactivation. Its scope of application has been widened in recent years to enzyme inactivation (Janve et al. 2014), abatement of allergenicity (Shriver and Yang 2011), protein modification (Fernández et al. 2012), and mycotoxin destruction (Moreau et al. 2011). PL has been also shown to be able to increase skin anthocyanin concentrations and total phenolic content of harvested figs (Rodov et al. 2012); this effect seems to be the consequence of the stimulation of fruit physiology caused by PL used as an abiotic stressor and might be useful in the production of so-called functional foods, as suggested by Shama (2007). Another interesting use of this technology is the enrichment of vitamin D_2 concentration in white mushrooms arising from the photochemical conversion of its precursor ergosterol, naturally present in mushrooms (Koyyalamudi et al. 2011); this application has been industrially implemented (Xenon, 2015). Beyond food applications, PL has been also tested for the degradation of environmental pollutants (Baranda et al. 2012). On the other hand, continuous-wave ultraviolet (CW UV) light technology is based on the application of a steady UV light, which can be nearly monochromatic (at 253.7 nm) if delivered by low-pressure mercury lamps or polychromatic if delivered by medium-pressure mercury lamps (Bolton and Linden 2003; Orlowska et al., 2013).

PL technology is relatively new; its continuous research publication record only starts in 1998 (MacGregor et al. 1998). In contrast, CW UV light technology has been extensively studied and applied to many fields, such as water disinfection, where it has been uninterruptedly used for more than 50 years (Bolton 2013), with thousands of installations over the world. For example, the Catskill-Delaware UV Water Treatment Facility, which serves New York City, uses 11,760 UV lamps and has a treatment capacity of 8.5 billion liters/day (Water-technology, 2015). As with any other light-based technology, PL follows the laws of photochemistry and photophysics. We have serious concerns about the experimental design of studies in the PL

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research field, the possibility of comparing interlaboratory data, and the applicability of the results to real-life situations because the fundamental laws governing PL action seem not to have been taken into account. PL technology can take advantage of the knowledge accumulated by the more developed CW UV light technology branch of photoprocessing, but they seem to run in parallel.

PL can be considered a technology mature enough to take a qualitative step forward, since its efficacy on numerous microorganisms and substrates has been demonstrated by many studies (Cheigh et al. 2013; Farrell et al. 2009; Gómez-López et al. 2005ab; Huffman et al. 2000, Jun et al. 2003; Nicorescu et al. 2014). The goal of this article is to promote discussion on the state of the art of PL test design providing information about the basic rules governing photochemistry and photophysics relevant to the PL application field, highlighting important terms well defined by harmonization institutions, trying to transfer to PL technology knowledge accumulated by CW UV light technology, and proposing a UV dose method that could be used for standardization. This article is based on two previous fundamental manuscripts (Bolton and Linden 2003; Bolton et al. 2015).

The Multi-targeted Nature of Pulsed Light Effects on Microorganisms

The mechanism of microbial inactivation by PL can be described by the action of photons absorbed by DNA (Bohrerova et al. 2008); therefore, it is ruled by the laws of photochemistry and photophysics, which must be the basis to characterize PL processes. Yet, this is just a first approach because of the complex nature of the effects of PL on microorganisms. There are three inactivation mechanisms described for PL microbial inactivation, which have been classified by Krishnamurthy et al. (2010) as photochemical, photothermal, and photophysical effects.

The *photochemical effect* is mainly referred to chemical changes in the DNA molecule in which thymine dimer formation is the major photoproduct.

The *photothermal effect* is the localized heating of bacteria by PL.

The *photophysical effect* refers to structural damage to bacteria arising from the disturbance caused by PL.

It is generally accepted that photochemical damage of DNA, similar to that caused by CW UV light, is the principal lethal effect of PL. However, results show that microbial inactivation follows a rather complicated multi-hit process that includes phenomena such as the formation of reactive oxygen species (Rowan et al. 2015). Furthermore, structural collapse of microorganisms has been reported by different authors, such as Krishnamurthy et al. (2010) and Ferrario et al. (2014), although there are no studies correlating the onset of its occurrence to experimental variables. Additionally, PL-treated microorganisms can enter into a viable but non-culturable state, for which the importance for human safety must be established (Feuilloley et al. 2006).

Some Units

The basic units of photochemistry have been defined by the International Union of Pure and Applied Chemistry (IUPAC) (Braslavsky 2007). Extractions from this Glossary are indicated in quotes. It is worth noting that the notation of a superscript "0" indicates "incident" and of a subscript "p" indicates "photon."

Fluence (F_o) is "the total radiant energy traversing a small transparent imaginary spherical target containing the point under consideration, divided by the cross section of this target". The unit according to the International System of Units is joule per square meter, although joule per square centimeter is the most commonly used in PL technology. $F_{p,o}$ has the same meaning although referring to *photon fluence* (einstein/cm^2), that is, to the total number of moles of photons traversing that spherical target. $F_{p,o,\pi}$ is the $F_{p,o}$ corresponding to a single light pulse.

Radiant exposure (H) (J/m^2) is the radiant energy "of all wavelengths incident from all upward directions on a small element of surface containing the point under consideration divided by the area of the element."

Fluence rate (E_o) is the rate of fluence, and *irradiance* (E) the rate of radiant exposure, expressed in watt per square meter.

Spectral irradiance (E_λ) is the irradiance "at wavelength $\boldsymbol{\lambda}$ per unit wavelength interval" [W/(m^2 nm)]. $E_{p,\lambda}$ is the *spectral photon irradiance*; it is the same as E_λ although in terms of number of photons (einstein /s m^2 nm). When used in the form $E^0_{p,\lambda}$, it refers to *incident spectral photon irradiance* and it is used here with units: einstein /s cm^2 nm.

Overall average germicidally weighted photon irradiance ($\bar{E}_{p,o}$) [einstein/(s cm^2)] is the photon irradiance, the germicidal effect of which has been weighted by the light spectrum and the action spectrum of the target microorganism.

If the fluence rate is constant in time, the fluence is classically determined according to Eq. 1:

$$F_0 = E_0 t \tag{1}$$

where t is the exposure time in seconds.

Basics of Photochemistry and Photophysics

First Law of Photochemistry

The first law of photochemistry, also known as the Grotthus-Draper law, states that light must be absorbed by a compound in order for a photochemical reaction to take place (Rohatgi-Mukherjee 1986).

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Second Law of Photochemistry

The second law of photochemistry, also known as the Stark-Einstein law, states that the extent of any photochemical process must be proportional to the total number of photons absorbed (Rohatgi-Mukherjee 1986). It is worth noting that this law is rate-independent, which means that the rate of photons impinging on the target does not influence the extent of the photochemical reaction. As a consequence, the same photochemical effect can be achieved by a high fluence rate and short exposure time (such as in PL technology) or low fluence rate and long exposure time (typical of CW UV technology), which is known as the reciprocity law or Bunsen-Roscoe principle. Even though some violations to this law have been described since more than a century ago (Schwarzschild, 1900), it is the default way of interpreting photochemical processes.

The Inverse Square Law

The inverse square law states that the fluence varies in inverse proportion to the square of the distance (Ryer 1997; Gaertner 2012). This law is based on the geometry of the illumination. Considering that light propagates forming a cone with vertex in a point source, and considering two planes that cut the cone at different distances from the point source and perpendicularly to the light direction, the area of the closer plane will be smaller than the area of the distant plane; therefore, the amount of photons per unit area that crosses the closer plane (the photon fluence at that plane) will be higher than that crossing the distant plane. This law is not valid at distances near the light source because the lamps used in PL devices cannot be considered a point source. In such cases, the light source-target distance from the various points on the lamp to any target area element will change for all points on the source (Gaertner 2012). A rule of thumb indicates that the inverse square law is applicable at light source-target distances at least five times the largest dimension of the light source, when the light source can be considered a point source (Ryer 1997).

The Need for Proper Dosimetry

Since photobiological processes are driven by the incident photon flux, the treatment of any polychromatic light source (e.g., a medium-pressure UV lamp or a pulsed UV lamp) must be based on the incident photon irradiance [einstein/(m^2 s)]. Comparison of results produced in different studies, including contrasting efficacy between CW UV and PL technologies and scaling up to industrial applications, is only possible under standardized conditions. Taking the case of microbial inactivation in water as a common and simple model, we believe that a PL test must be designed, and results reported in terms of the photon fluence based on the determination of the overall average germicidally weighted photon irradiance per pulse in the water. Once the goal photon fluence is known, scaling up can be calculated to build a system design that accomplishes it.

Bolton and Linden (2003) described and explained a protocol that has become standard for CW UV tests with both monochromatic and polychromatic continuous light, which was designed to estimate fluence. More recently, Bolton et al. (2015) have updated this protocol proposing photon fluence as the parameter that best characterizes a UV-driven photochemical or photobiological process. We believe that this procedure can be adapted to PL tests. The protocol requires a UV lamp, a collimated beam apparatus (Fig. 1), a spectroradiometer, a spectrophotometer, a radiometer, a magnetic stirrer for sample homogenization, and the action spectrum of the target microorganism; the function of each of them is detailed below.

A collimator is a device that can be placed between the light source and the sample to produce almost parallel rays. Blatchley (1997) described a collimator consisting of a 20-cm-long tube with three vertically aligned plates 5.7 cm in diameter, which should be complemented by a mask over the sample cell to avoid reflection from the walls of the Petri dish or beaker (Bolton et al. 2015). In contrast to collimators for CW UV light sources, a shutter is not needed for PL tests because light generation can be easily switched off and the lamp does not need pre-heating. The irradiance and fluence rate are virtually the same in a collimated beam and are used interchangeably in this article. A collimator decreases the efficacy of PL because it absorbs a large part of the emitted light and consequently prolongs the exposure time required to reach the desired fluence. In contrast, PL systems are generally designed by commercial manufacturers with reflective materials in order to make the best use of the emitted light. A collimator consequently imposes the penalty of discarding most of the lamp emission with consequently longer treatment times, which is worth paying for in order to perform more accurate dosimetry. A difficulty for using collimators in PL tests is that they are performed in manufacturer-made systems with limited dimensions as opposed to CW UV light systems that can easily be self-made to include a collimator.

Protocol for Collimated Bean Measurements with a Polychromatic Light Source

Bolton et al. (2015) recommended that collimated beam measurements with a polychromatic light source should use the spectral photon irradiance. This is because photochemical and photobiological reactions are driven by the absorbed photon flux and not the energy flux.

The equation for calculating the overall average germicidally weighted photon irradiance [[$\bar{E}_p$ (water),

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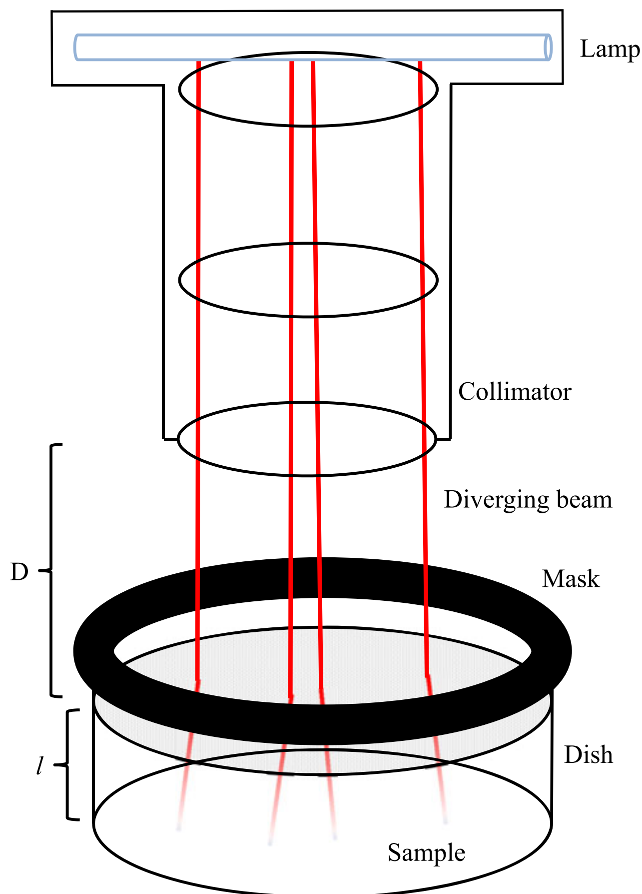

Fig. 1 Diagram of a quasi-collimated reactor showing a slightly diverging beam. *D* distance from the sample surface to the collimator exit, *l* sample depth

einstein/s cm^2] based on Bolton and Linden (2003) and Bolton et al. (2015), can be written as follows:

$$\overline{E}_{\mathrm{p}}\,(\mathrm{water}) = \mathrm{PF\ x\ DF\ x} \int_{\lambda_1}^{\lambda_2} \left[E^0_{\mathrm{p},\lambda}\ \mathrm{x\ RF}(\lambda)\mathrm{x\ WF}\ (\lambda)\ \mathrm{x\ GF}\ (\lambda) \right] \mathrm{d}\lambda \quad (2)$$

where PF is the Petri factor, DF is the divergence factor, RF(λ) is the reflection factor at wavelength λ, WF(λ) is the water factor at wavelength λ, and GF(λ) the germicidal factor at wavelength λ. Their determination is explained by Bolton and Linden (2003) and Bolton et al. (2015). The wavelength range in Eq. 2 is usually 200–300 nm; however, it must be case-dependent because longer wavelengths may be necessary to be considered. For example, Bohrerova et al. (2008) observed that 6 and 2 % of *Escherichia coli* inactivation by PL arose from wavelengths longer than 295 and 400 nm, respectively; these percentages reached 28 and 12 for phage T7.

PF is a correction factor for the non-homogeneity of illumination on the whole sample surface; it can be determined by using a radiometer according to Bolton and Linden (2003). PL systems do not illuminate surfaces homogeneously. For example, Farrell et al. (2009) reported using a PL system that had a 30 % variation in irradiance between the center and the edge of an 8.5-cm-diameter sample holder. Hsu and Moraru (2011) found that the fluence rate along the footprint of a PL system, which is very commonly used by many research laboratories, follows a Gaussian distribution, with a decrease of more than 50 % when moving only 1 cm away perpendicularly from the center of the UV distribution, and interestingly, with the peak fluence located 1 cm perpendicularly aside from the lamp. That work also reported a Gaussian distribution of emittance along the lamp axis, which shows that even using sample holders smaller than the lamp length does not guarantee a uniform exposure.

DF is a correction factor for light divergence within the liquid sample, since the beams are not perfectly collimated. It is estimated by:

$$DF = \frac{D}{D + l} \quad (3)$$

where D is the distance from the sample surface to the light source and l is the sample depth as represented in Fig. 1.

$E^0_{\mathrm{p},\lambda}$ is the spectral photon irradiance at wavelength λ. These values can be obtained from the readings of a properly calibrated spectroradiometer, which outputs the spectral irradiance (E_λ) over a wide spectral range. E_λ takes into account the variation of the lamp emission over the lamp emission spectrum. Note that there are significant differences in the spectral irradiance of different light sources, as can be appreciated in Fig. 2. Even though these figures cannot be compared in terms of absolute spectral irradiance because their units are not the same, it is obvious from them that the germicidal UV region (200–300 nm) is just a small portion of the emission spectrum. Therefore, a simple radiometric reading overestimates the irradiance because it also measures non-germicidal light and lacks a uniform spectral responsivity. Furthermore, the same system can produce different emission spectra depending on lamp operation conditions, such as voltage and aging; the latter is important to be aware of, since manufacturer fluence specifications may not hold up during the time span of usage.

$E^0_{\mathrm{p},\lambda}$ is given by $E_\lambda/U(\lambda)$, where $U(\lambda)$ is the photon energy per einstein at wavelength λ. This is calculated according to the Planck equation:

$$E^0_{\mathrm{p},\lambda}(\lambda) = \frac{E_\lambda}{h\,c\,N_A/\lambda} \quad (4)$$

where h is the Planck constant ($6.6260690 \times 10^{-34}$ J s), c is the speed of light in vacuum (2.99792458×10^8 m/s), and N_A is the Avogadro constant (6.0221418×10^{23} 1/mol).

RF(λ) is a correction factor for light reflection, since light reflected by the surface does not contribute to the photochemical process. It is 0.975 for an air-water interface at 253.7 nm, but varies slightly with wavelength. Instruction for its

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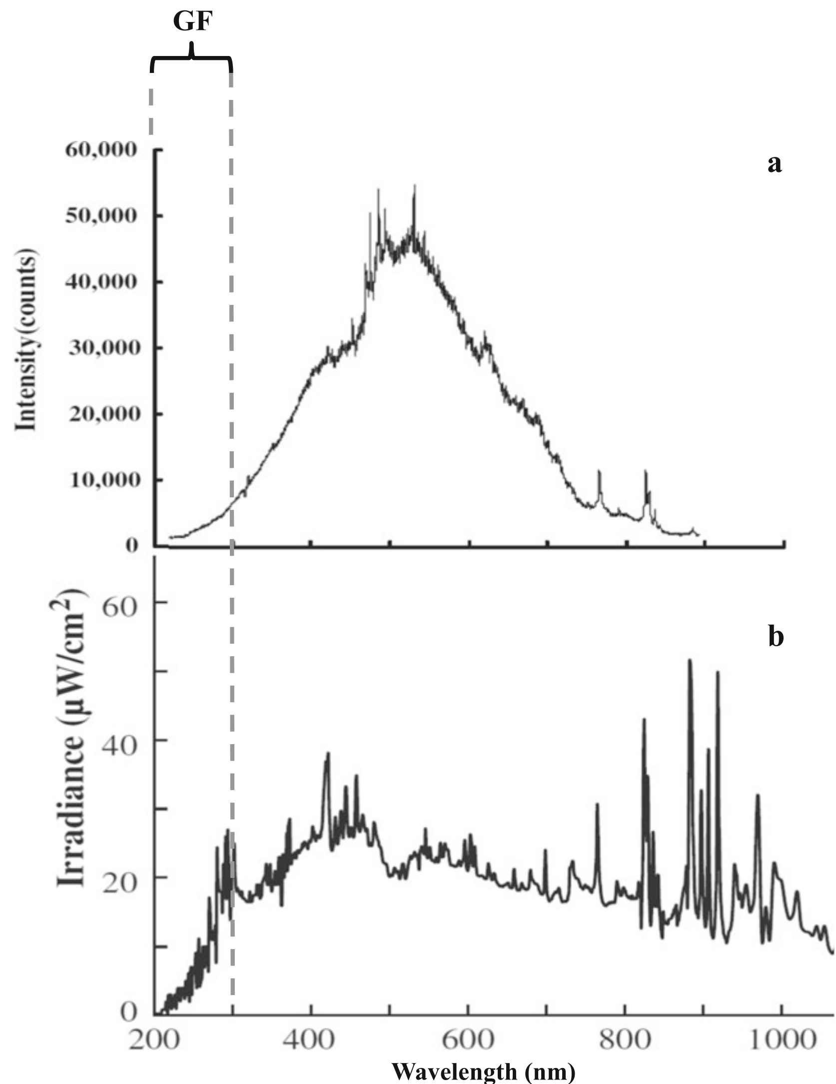

Fig. 2 Emission spectra of two different xenon pulsed light lamps. **a** From Lee et al. (2008) with permission of Springer. **b** From Cheigh et al. (2013) with permission of Elsevier

calculation can be found at Bolton et al. (2015) and references quoted therein. RF(λ) for other interfaces can be measured by spectroradiometry following the method described by Blatchley (1997).

WF(λ) corrects for the light absorption through the liquid sample at different wavelengths and can be determined by a common spectrophotometer. It is given by the following equation:

$$WF(\lambda) = \frac{1-10^{-a(\lambda)l}}{a(\lambda)\, l \ln(10)} \quad (5)$$

where $a(\lambda)$ is the absorption coefficient (1/cm) of the aqueous solution at wavelength λ and l is the vertical path length (cm) of the liquid as represented in Fig. 1.

GF is the germicidal factor. It corrects for the differences of target microorganism sensitivity to different wavelengths. Microorganisms have specific action spectra (inactivation response vs. wavelength). To calculate GF, accurate action spectra are required; however, unfortunately, good action spectra are available for only a few bacteria and viruses. A non-normalized action spectrum for the PL inactivation of *E. coli* has been reported by Wang et al. (2005), together with the method and equipment used to obtain it. If a monochromator is not available, a set of band-pass filters can also be used (Chen et al. 2009). A common surrogate is the absorbance spectrum of DNA (normalized to 1.000 at 253.7 nm), which is not useful if significant inactivation occurs at wavelengths >300 nm. Fig. 3 shows a photon fluence-based action spectrum of a bacterium generated using a tunable laser (Beck et al. 2015). It can be observed there the large differences in the response of *Bacillus pumilus* as a function of wavelength. Furthermore, observing simultaneously Figs. 2 and 3, it can be easily appreciated that the different spectral irradiances at each wavelength must be matched with the respective microbial response to those wavelengths in order to allow for accurate

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characterizations; it can also be appreciated that large part of the lamp emission plays no role in the lethality of the process.

Some researchers pack their samples into UV transparent films as a strategy to avoid post-processing contamination. When this is the case, Eq. 2 becomes Eq. 6 by including FF (λ), the film factor, which must be included to account for the wave-dependent light transmission through a film of specified thickness.

$$\overline{E}_p(\text{water}) = PF \times DF \times \int_{\lambda_1}^{\lambda_2} \left[E^0_{p,\lambda} \times RF(\lambda) \times WF(\lambda) \times GF(\lambda) \times FF(\lambda)\right] d\lambda \quad (6)$$

Either using Eq. 2 or 6, depending on the case, fluence is given by:

$$F_{p,o}(\text{water}) = \overline{E}_{p,o}(\text{water})\, t \quad (7)$$

While Eq. 7 can be applied to all photochemical processes, pulsed lamps are off most of the time during the PL application duty cycle; for example, many tests have reported using a treatment regime of 360 μs at 3 Hz (Ignat et al. 2014), which means a duty cycle of only 0.1 %. This discontinuous nature of PL makes other versions of Eq. 7 easier to use, such as Eq. 8:

$$F_{p,o}(\text{water}) = \overline{E}_{p,o}(\text{water})\, \tau\, n \quad (8)$$

where τ is the pulse width (s) and n the number of pulses. τ data is provided by PL system manufacturers, who determine it as full width at half maximum. Alternatively, if the photon fluence per pulse ($F_{p,o,\pi}$) is known, Eq. 9 or 10 can be used:

$$F_{p,o} = F_{p,o,\pi}\, n \quad (9)$$

$$F_{p,o} = F_{p,o,\pi}\, f\, t \quad (10)$$

where f is the pulse frequency (Hz).

Only a small part of the research results found in the PL scientific literature report as function of fluence, and within these, almost all report just F_o; only Orlowska et al. (2013) used a collimated beam for testing PL on foods. Therefore, the relevant wavelength-dependent target sensitivity [accounted for by the term GF(λ)] was ignored, as well as the important differences in the spectral irradiance in terms of photons as indicated by the term $E^0_{p,\lambda}$. Moreover, E_o is usually determined using radiometers or calorimeters without taking into consideration that only part of the light detected by the sensor has any photochemical action and the response of these detectors is not flat. Moreover, the correction terms PF, DF, RF(λ), and WF(λ) are also absent. We believe that it is impossible to obtain a quantitative assessment of inactivation unless the fluence rate is weighted by the germicidal action spectrum of the target microorganism, and unless this fundamental principle is recognized, there cannot be any valid scientific work produced in the PL field.

Some Factors Affecting Fluence

Besides fluence, other parameters are used to describe the effects of PL and/or introduced as experimental variables, for example, treatment time, number of pulses, lamp discharge voltage, and distance. While they can be useful in very specific cases, we believe, as stated before, that the variable that best describes a photochemical/ photobiological process is the photon fluence based on the determination of the overall average germicidally weighted irradiance per pulse in the water in tests run with quasi-collimated beams.

Time, Number of Pulses and Light Source-Target Distance

Reporting results in time units gives an idea of the time necessary to achieve a certain goal, such as a target microbial reduction. This way of reporting is advantageous for this technology because of the remarkable fastness of the effects of many PL systems, but it is not enough for making tests reproducible because of differences in pulse fluence, number of pulses, pulse repetition rate, and spectral irradiance, among others. For example, a PL system that produces fluence rates at the target level in the order of microjoules per square centimeter, such as that used by Luksiene et al. (2007), will take 1000 times longer time than a PL system that produces fluence rates of joules per square centimeter, such as that used by Nicorescu et al. (2014) under comparable test conditions. Moreover, the treatment time is also the function of the pulse repetition rate, which can be very different among tests, ranging from 0.5 Hz (Ignat et al. 2014) to 15 Hz (Choi et al. 2010).

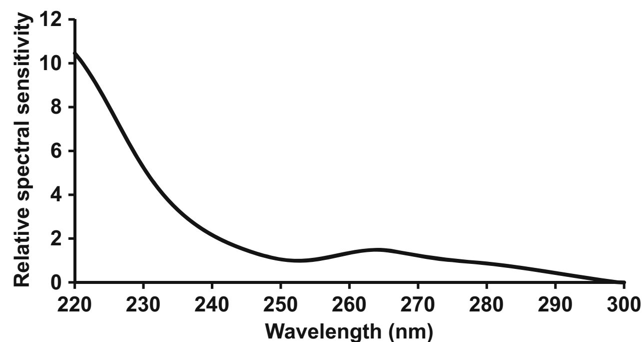

Fig. 3 Action spectrum of *Bacillus pumilus*. Replotted from Beck et al. (2015). With permission from Elsevier

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Similarly, reporting results in terms of the number of pulses gives little information because a desired fluence can be achieved by applying a low number of pulses of high energy and vice versa according to the Bunsen-Roscoe principle. Another approach that gives little information is testing different light source-target distances because it is known that a PL treatment is more effective at shorter distances, as predicted by the inverse square law.

Lamp Discharge Voltage

Increasing the lamp discharge voltage produces faster effects because this increases the fluence rate. In addition, there is a change in the emission spectrum of the lamp that increases the proportional output of lower wavelengths (Schaefer et al. 2007) because of a higher blackbody temperature of the plasma. Experiments testing different lamp discharge voltages conclude that the higher the lamp discharge voltage, the faster the inactivation (Choi et al. 2009, 2010; Artíguez et al. 2011). Furthermore, the higher UV output at high voltages makes it impossible to compare radiometric/calorimetric fluences because of the simultaneous change in lamp emittance and spectrum (Hancock et al. 2004; Schaefer et al. 2007).

Reactor Configuration

The reactor configuration will determine how the UV reaches microorganisms and consequently its efficacy (Gómez-López et al. 2005a), which depends on the overall average germicidally weighted fluence, food matrix (Gómez-López et al. 2005b), and hydrodynamics (Sauer and Moraru 2009; Artíguez et al. 2011; Hsu and Moraru 2011). The difference between a collimated system (Orlowska et al. 2013) and an industrial reactor such as a thin-film device (Chaine et al. 2012) is notorious (Grapperhaus et al. 2007). It must be stated that the industrial adaptation of laboratory results for CW UV light technology is a three-step process. First, the overall average germicidally weighted fluence required to inactivate the target microorganism must be determined in a collimated system, in which treatment conditions can be more easily controlled. Second, a reactor should be designed according to specific goals of fluence and product characteristics. Last, the reactor is validated (for example by biodosimetry); that is, the fluence is measured in the reactor in order to know if it is capable to deliver the goal fluence.

Three-Dimensional Bodies

The overall average germicidally weighted irradiance calculations on solid foods require fewer measurements and calculations because the water-related terms DF and WF of Eq. 2 or 6 can be excluded. Such measurements are, however, complicated when treating three-dimensional targets, such as small fruits, fruit pieces, or eggs because of the differences in illumination through the target as function of its size and geometry, with higher fluence at the top of the target and uneven fluence distribution through its curvature. Increasing lamp-target distance will minimize these effects, but this strategy is limited by the treatment chamber size. Since these kinds of tests are usually performed by exposing multiple targets (a specific number of pieces simultaneously), an additional strategy is randomization of the surfaces being exposed using a representative number of pieces (Lagunas-Solar and Gómez-López 2006) and a good characterization of piece size, geometry, and distribution through the footprint, although it must also be considered that, in this kind of experimental setup, the portion of light reflected by piece surfaces becomes a secondary source of UV light.

Treating Chemical Substances or Multiple Targets

Eqs. 2 and 6 are also valid for chemical substances replacing the germicidal factor for an equivalent factor related to the degradation response of the substance to PL as function of wavelength. A priority-based decision must be made when there are multiple targets. For example, if the inactivation of a foodborne pathogen in a fruit juice is tested, and simultaneously the effect of PL on quality parameters of interest that can have different stabilities as function of wavelength, such as color or ascorbic acid, a decision could be taken to use the action spectrum of the pathogen because inactivation is the major concern. When multiple microorganisms are targeted, the DNA absorption spectrum can be used as surrogate. In those cases, it could be better to report the photon fluence rate but not weighted by the GF. Knowing the action spectra of the different target microorganisms or chemicals, the germicidal photon fluence can be weighted for each one and, additionally, the results can even predict the responses of other microorganisms or chemicals under the same experimental conditions.

Conclusions

A protocol for dosimetry in bench-top PL tests is proposed, based on those elaborated for CW UV light technology and the laws of photochemistry and photophysics, and using water as reference matrix. While its implementation would improve the possibilities of intercomparison and scaling up, one must not neglect the complex nature of microbial inactivation by pulsed light, which is not purely a photochemical process.

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