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Limits of the Bunsen-Roscoe Law in UV Disinfection: Identifying Low-Irradiance Thresholds for *Escherichia coli* Inactivation

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Abstract: Although experimental evidence shows that the response of biological systems to UV radiation could deviate from the Bunsen–Roscoe law, the precise operational threshold where this rule fails during air and surface UV disinfection has not been established for standard bacterial models. To define these practical boundaries, the susceptibility of *Escherichia coli* K12 exposed to 254 nm radiation was evaluated across a representative fluence-rate range (0.0025 to 2.056 Wm^{-2}). Inactivation kinetics for *E. coli* K12 remained unchanged from 0.0212 to 2.056 Wm^{-2}, demonstrating that the Bunsen–Roscoe law holds over most of the fluence-rate range occurring during air and surface disinfection. However, at 0.0106 Wm^{-2}, a fluence rate comparable to the lower values estimated during disinfection procedures, there was a reduction in lethality, and prolonged exposure resulted in higher bacterial survival at identical total fluences. Reciprocity law validity appears to be limited at the lower end of the range of fluence rates which typically occurs during disinfection procedures. Consequently, to assess the lethal effect of a treatment from exposure time and radiation intensity, it should be verified that the susceptibility of the target microorganism is the same at the maximum and minimum fluence rates that occur within the irradiation device.

Keywords: ultraviolet; disinfection; reciprocity law

1. Introduction

In UV disinfection treatment design, it is essential to estimate the required dose to inactivate the target microorganisms. This estimate is usually based on the lethal effect observed under laboratory conditions. Applying this criterion assumes that, according to the Bunsen-Roscoe law, the effect depends on the imparted dose, but not on the intensity of the incident radiation or the duration of exposure. Whether this assumption is correct is a relevant topic for UV disinfection, and many studies have endeavored to obtain experimental evidence supporting or refuting compliance with the Bunsen-Roscoe law (also called reciprocity law) during microbial inactivation by UV [1–10]. Results from some of these studies, in which *Escherichia coli* was the model organism, are compiled in Table 1. While the use of different bacterial strains, culture conditions and irradiation procedures hinder direct comparison, it is worth noting that deviations from reciprocity appear consistently linked to the lower limit of the tested fluence-rate range. Some studies [2,5] suggest the existence of a threshold fluence rate below which radiation loses germicidal efficacy, a critical factor in system design. Indeed, irradiance gradients from 0.12 to 0.87 Wm^{-2} have been measured in healthcare rooms disinfected with low-pressure mercury vapor lamps [11], while gradients from 0.002 to 2 Wm^{-2} are expected to occur near the ceiling in rooms equipped with upper-air ultraviolet C devices [12]. The lower boundaries of these operational ranges closely match the lowest intensities where bacterial inactivation remains independent of fluence rate (Table 1). Therefore, the objective of this study

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was to know the threshold fluence rate above which inactivation by 254 nm radiation of the model organism *E. coli* fulfils the Bunsen-Roscoe law under conditions mimicking, in terms of fluence rate, practical air and surface disinfection. Based on our results, we propose a practical recommendation for system validation, and discuss the potential mechanisms responsible for increased bacterial survival at low fluence rates.

Table 1. Some previous studies on the effect of fluence rate on *E. coli* inactivation by radiation of 254 or 265 nm.

Radiation Source	Fluence Rate Range (Wm^{-2})	Irradiation Medium	*E. coli* Strains	Observations	Reference
LP [a]	64.3–0.12	liquid [c]	NCTC wild-type	Survival was independent of the fluence rate	[1]
LP [a]	0.72–0.002	liquid [c]	B wild-type B_{s-1} C_{syn}-	Survival increased at low fluence rate	[2]
LP [a]	2–0.02	liquid [c]	ATCC 25922	Survival was independent of the fluence rate	[3]
LED [b]	1.12–0.012	liquid [c]	CN13	Survival was independent of the fluence rate	[4]
LED [b]	1–0.0002	solid [d]	ATCC 15597	Survival increased at low fluence rate	[9]

[a] Low-pressure mercury vapor lamps emitting mainly at 254 nm, [b] Light Emitting Diodes emitting at 265 nm, [c] Cells were irradiated while suspended in a liquid medium (water, saline solution or phosphate buffer) in the absence of nutrients, [d] Cells were irradiated while they were actively growing on the surface of rich medium agar plates.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

E. coli K12 was selected as model organism. Cultures were grown to stationary phase in rich medium (Nutrient Broth), and then bacterial cells were resuspended in phosphate-buffered saline solution to be irradiated in a medium that was transparent to the incoming radiation, and nutrient-free, since nutrient depletion is assumed to be the usual condition during ultraviolet treatments. The origin of the strain, as well as the culture conditions and the procedures followed to prepare bacterial suspensions for irradiation, have been described previously [13].

2.2. Irradiation Procedures

The radiation source was a Philips TUV 15 W/G15 T8 tube (Philips, Poland) placed inside a pseudo-collimated device [14] in which the collimating tube was positioned horizontally. The fluence rate could be adjusted between 2.056 and 0.0025 Wm^{-2} by changing the distance from the source to the samples from 0.47 to 7.49 m, thereby testing distances ranging from those typical in devices such as laminar flow cabinets to those expected in rooms equipped with ceiling devices or UV disinfection robots. Four-mL samples of bacterial suspension were placed in spectrophotometer quartz cuvettes (1 cm path length) closed with Teflon lids and sealed with Parafilm. A magnetic stirrer was modified by replacing the magnet with a cell holder in which 4 quartz cuvettes could be placed symmetrically around the rotation axis on a plane perpendicular to it. The stirrer was placed at the end of the collimating tube with the rotation axis at the center of the radiation beam. During irradiation, rotation ensured a homogeneous distribution of bacteria within the suspensions, and equivalent exposure in all four quartz cuvettes. In addition, the use of closed quartz cuvettes rather than Petri dishes prevented evaporation during long time exposures. The fluence imparted was determined by the exposure time. An aluminum plate interposed at the end of the collimating tube was used as a shutter to start and stop the exposures. Fluence rates for the different configurations of the irradiation device were measured by actinometry using a KI/KIO_3 solution [15]. As dark controls, samples of bacterial suspensions were placed in the irradiation device with the radiation source off during a time lapse equivalent to the longest exposure time used (8 h 54 min). Since the cell holder allowed irradiating only 4 quartz cuvettes simultaneously, several irradiations were performed with the system configured to irradiate at a specific fluence rate, in order to test each fluence value three times. In this way, each irradiation condition (a given fluence imparted at a given fluence rate) was tested in three independent assays.

2.3. Counting Procedures

After irradiation, the quartz cuvettes were removed from the holder and viable counts were obtained by plating serial dilutions of the suspensions on Nutrient Agar plates. These procedures were performed immediately after the end of irradiation. The protocol followed for this purpose has been described previously [13]. In order to

avoid photoreactivation, irradiation procedures and post irradiation manipulation of the samples were performed under the dim light of a Philips SON 70 W E lamp (Philips, Belgium). Plates were then incubated in the dark.

2.4. Data Analysis

Survival curves were analyzed using models derived from the target-theory for the action of radiation. The parameters of the equations:

$$\log (N/N_0) = \log (e^{-qHo}) \quad (1)$$

$$\log (N/N_0) = \log (1-(1-e^{-kHo})^n) \quad (2)$$

were fitted to the experimental data by least-square non-linear regression. In this expressions N is the number of viable cells after application of a fluence H_0, and N_0 is the initial number of viable cells. Equation (1) describes bacterial inactivation predicted by the single-hit single-target model, which assumes that the lethal effect relays on single events of energy deposition occurring on single targets. The parameter q is the reciprocal of the fluence required to obtain a survival fraction equal to $1/e$. ts Equation (2) describes bacterial inactivation according to the single-hit multiple-target model, which predicts that survival will be unaffected at low fluences, but it will exhibit an exponential decay at fluences above a threshold value. Limited effect of radiation at low fluences is frequently called "shoulder", and ascribed to the ability of the cells to tolerate or repair small amounts of damage. The parameter k is the reciprocal of the fluence required to reduce the survival fraction to $1/e$ when the imparted fluence exceeds the threshold value and the survival decays exponentially. In the target theory the parameter n is interpreted as the number of targets that should be inactivated in order to kill a cell, and determines the extent of the shoulder. The influence of n can be appraised by the quasi-threshold fluence, calculated as $\ln(n)/k$, which indicates the fluence for transition from the shoulder to the exponential decay. The parameter n is also named "extrapolation number" because the value of $\ln(n)$ is given by extrapolation of the portion of the curve exhibiting exponential decay to the axis corresponding to fluence = 0. In the analysis of survival curves, fitting to Equation (1) is interpreted as the absence of cumulative effects ($n = 1$), which results in curves with no changes in slope. On the other hand, fitting to Equation (2) suggests the occurrence of lethal effect dependent on damage accumulation ($n > 1$), which manifests as an increase in the slope once threshold fluence is exceeded. Results from each fluence rate were described using the model providing the best fit to the experimental results. Data from different irradiations performed at the same fluence rate, expressed as survival fraction at a given imparted fluence, were pooled and analyzed collectively. In this way, a single curve represents the response of the bacterial population exposed to a given fluence rate.

3. Results

Survival curves for *E. coli* exposed to UV at different fluence rates are shown in Figure 1. At 2.056 and 0.0212 Wm^{-2}, the curves exhibit a shoulder (up to approximately 20 Jm^{-2}) with a slight negative slope, followed by an exponential reduction in the survival fraction. The two curves overlap indicating that, within this interval, the lethal effect is independent of the fluence rate. Conversely, in the curve obtained at 0.0106 Wm^{-2}, the slight slope of the shoulder is maintained even at the highest fluences tested. At this lower fluence rate, when a fluence of 80 Jm^{-2} was imparted, the survival fraction was about 200 times higher than that estimated at higher fluence rates. Survival-curves obtained at 0.0055 and 0.0025 Wm^{-2} exhibited additional, albeit minor, increases in survival relative to the response at 0.0106 Wm^{-2}. From the results of regression analysis (Table 2) a change in the radiation response is apparent at some value in the range of fluence rates between 0.0212 and 0.0106 Wm^{-2}.

Table 2. Regression analysis of the data obtained at 0.0212 and 0.0106 W m^{-2}.

	0.0212 W m^{-2}		**0.0106 W m^{-2}**	
Parameter	**Model Equation (1)**	**Model Equation (2)**	**Model Equation (1)**	**Model Equation (2)**
χ^2	0.18742	0.06535	0.0072	0.00797
R^2	0.88616	0.96428	0.94778	0.94859
q	0.08842 (0.00618)		0.03157 (0.00105)	
k		0.1283 (0.01023)		0.03305 (0.03371)
n		10.11122 (5.77383)		1.10418 (0.26261)

Good fitting should have a minimal χ^2 value and an R^2 value as close as possible to 1. At 0.0212 W m^{-2} these conditions are given for the model described by Equation (2). On the other hand, inclusion of an additional parameter n did not improve fitting when data obtained at 0.0106 W m^{-2} were analyzed. In this case, since n is

near a value of 1, Equation (2) equals Equation (1). Curves obtained at fluence rates of 0.0212 W m^{-2} or higher reflect a radiation response different from that evidenced by curves obtained at fluence rates of 0.0106 W m^{-2} or lower. The change in the slope of the survival curve, attributed to the accumulation of damage in the model applied for data analysis, appeared to be absent at low fluence rates.

No significant change was observed in the viable count for the dark control. Taken together, the results indicate that the lethal effect of radiation was independent of the fluence rate within the range from 2.056 to 0.0212 Wm^{-2}, but decreased at fluence rates below this value. These results are consistent with previous observations reported by Harm [2] and Mohsin and Lanzarini-Lopes [9]. On the other hand, unlike what was observed by Shi et al. [10] when evaluating fluence rates between 5 and 100 Wm^{-2}, survival did not decrease with increasing fluence rate within the 0.0212 to 2.056 Wm^{-2} range.

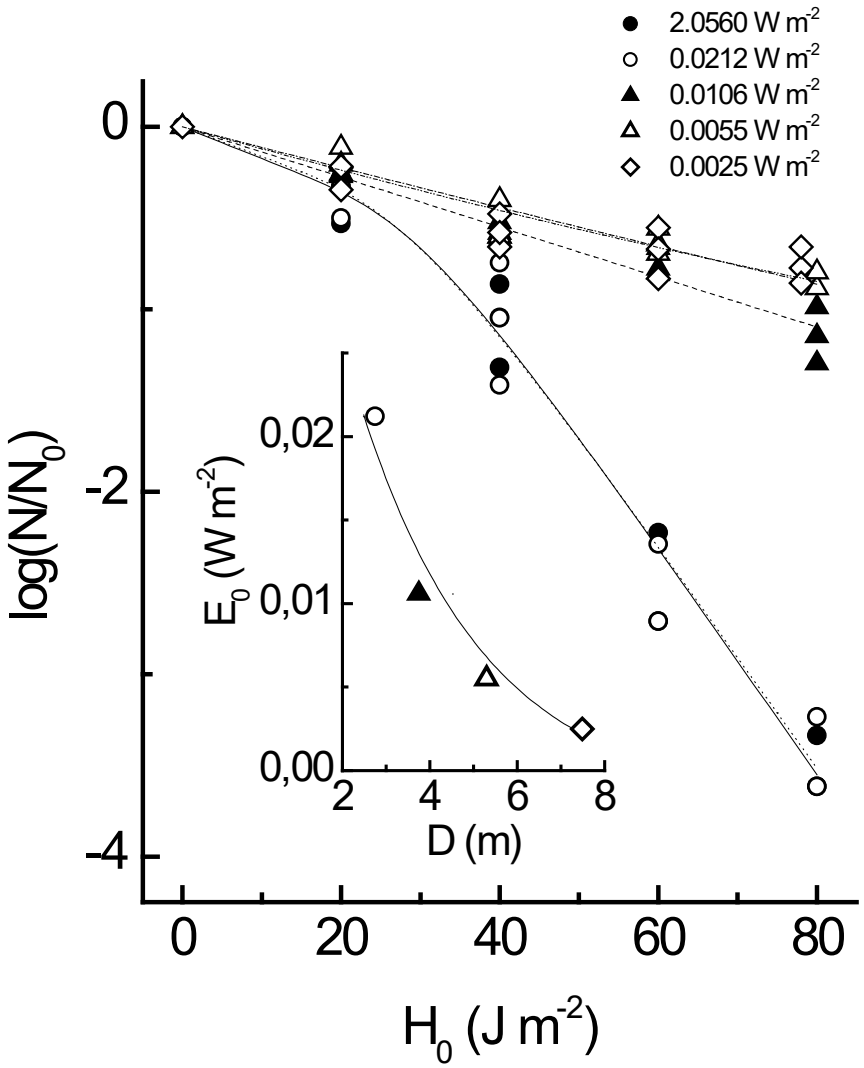

Figure 1. Survival curves for *E. coli* exposed to the radiation emitted by a low-pressure mercury vapor lamp at fluence rates of 2.056 W m^{-2} (closed circles), 0.0212 Wm^{-2} (open circles), 0.0106 Wm^{-2} (closed triangles), 0.0055 Wm^{-2} (open triangles) or 0.0025 Wm^{-2} (open diamonds). N: viable counts after an imparted fluence H_0, N_0: viable counts at the start of the irradiation. Each symbol represents a mean value obtained for a given fluence imparted at a given fluence rate in an independent assay. Curves corresponding to 2.056 and 0.0212 Wm^{-2} were obtained by fitting the Equation (2) to the set of results available for these conditions. Curves corresponding to 0.0106, 0.0055 and 0.0025 Wm^{-2} were obtained following the same procedure with Equation (1). Insert: change in the fluence rate E_0 as a function of distance to the radiation source D.

4. Discussion

Under the experimental conditions tested in the present study, the Bunsen-Roscoe law holds across most of the fluence-rate range expected during air and surface disinfection procedures [11,12], but at the lower limit of this range, the effectiveness of radiation drops significantly. In our experimental setup, survival exceeded the

expected levels for samples placed at 3.74 m from the radiation source, a distance comparable to that reached by radiation emitted by upper-air UV-C devices [12].

It is possible that in some studies [1,3,4] improvement in survival at low fluence rates was undetectable simply because the fluence rates assayed were above the threshold values for the specific experimental conditions tested. In time intervals such as those required to deliver lethal doses of radiation at low fluence rates, *E. coli* has the ability to repair radiation-induced damage, even when in a nutrient-free environment. This notion is relevant for the interpretation of the results obtained after prolonged irradiation. Harm [2] reported that the survival of wild type *E. coli* cells increased considerably when the fluence rate was reduced, but that increase was very limited to DNA repair-deficient mutants. Mohsin and Lanzarini-Lopes [9] observed that at low fluence rates the transcription of genes related to DNA repair was upregulated by UV exposure, even when measurable bacterial inactivation was absent. As an interpretation of their results, these authors proposed that at low fluence rates, the long exposure time required to deliver a lethal fluence allows active DNA repair processes to compete with damage accumulation, thereby increasing cell survival. A mechanism based on DNA repair and damage accumulation would also explain why the lethal effect on bacterial spores [3] and host-free viruses [2,3] remains independent of the fluence rate. In bacterial spores, DNA repair occurs during germination, whereas repair of viral DNA depends on the host cell machinery following infection. Consequently, for these non-vegetative models, and because DNA repair is temporally decoupled from UV exposure, the fluence rate cannot influence the interplay between damage accumulation and repair, explaining why reciprocity holds for these microorganisms. Nevertheless, the repair-versus-damage explanation is a hypothesis, not a validated mechanism. Qualitatively, it provides a reasonable explanation for our results. However, the reason why modifying the damage-repair balance should cause such an abrupt transition in radiation sensitivity within a narrow fluence-rate window, as shown in Figure 1, is a question that remains intriguing. This transition will be the subject of further studies. The contradiction between our results and those reported by Shi et al. [10] is only apparent. The fluences and fluence rates applied by these authors are considerably higher than those used in the present study. Phenomena like inactivation of repair systems, membrane damage or generation of reactive oxygen species are not expected to occur under the conditions assayed here. Different mechanisms would probably underline changes in UV susceptibility at low and high fluence rates.

5. Conclusions

The validity of the Bunsen–Roscoe law during UV disinfection is limited to a specific range of fluence rates. Consequently, to properly assess treatment efficacy based on exposure time and radiation intensity, it must be verified that the susceptibility of the target microorganism remains identical at the maximum and minimum fluence rates that occur within the irradiation device.

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The author declares no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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