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A contribution to the UV dose concept for bacteria disinfection in well mixed photoreactors

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Abstract

A modified dose concept for microorganism inactivation employing almost monochromatic, low wavelength radiation has been studied using disinfection data obtained with *Escherichia coli* bacteria and germicidal UV lamps. This first contribution has been applied to experiments performed in a well mixed reactor and describes the dose exploring two new concepts: (i) the use of the spatial distribution of the radiation absorption rates by the bacteria and (ii) the consideration that not necessarily the inactivation rate is of first order with respect to the radiation energy absorption rate. The proposed description agrees very well with the obtained experimental data for almost transparent water and for a medium having a concentrated culture with significant radiation absorption.

Keywords: Disinfection; Bacteria; UV radiation; Dose

1. Introduction

Disinfection of water employing UVC radiation (with emission wavelength between 200 and 280 nm) has become a convenient and beneficial alternative to the use of chemical processes. One of the critical concepts involved in its application is the UV dose. The idea evolved from the classical proposal in chemical disinfection based on the product of the reactant concentration (initial or average) with the reaction time or with the quotient of the reactor volume divided by the flowrate in continuous operations. The direct extension of this definition resulted in the proposal of:

$$\text{Dose} = I^* \times t_R \qquad \text{in batch systems} \tag{1}$$

$$\text{Dose} = I^* \times \frac{V_R}{Q} = I^* \times \tau \qquad \text{in continuous flow systems} \tag{2}$$

where I^* has been called radiation (or light) intensity expressed in W cm^{-2}, t_R is the reaction time and τ is the mean residence time (see for example, [1–4]). The value of I^* has been calculated with very different interpretations such as, considering for example two limiting cases: the lamp output emittance or the incoming irradiance at the reactor wall. In other cases some form of fluence rate inside the reactor has been used.

In more general terms, resorting to radiation field theory, it is possible to start from the definition of the specific intensity (also called radiance) for monochromatic radiation (λ) and for a particular direction $\underline{\Omega}$ [5,6]:

$$I_{\underline{\Omega},\lambda}(\underline{x}, t) = \lim_{\mathrm{d}A\,\mathrm{d}\Omega\,\mathrm{d}t\,\mathrm{d}\lambda \to 0} \left(\frac{\mathrm{d}E_\lambda}{\mathrm{d}A \cos\theta\, \mathrm{d}\Omega\, \mathrm{d}t\, \mathrm{d}\lambda} \right) \tag{3}$$

the units of $I_{\underline{\Omega},\lambda}$ being W cm^{-2} sr^{-1}. From this property one can derive the incident radiation at any point in the three-dimensional space that is equal to [5]:

$$G_\lambda(\underline{x}, t) = \int_\Omega I_{\lambda,\underline{\Omega}}(\underline{x}, t)\, \mathrm{d}\Omega \tag{4}$$

in units of W cm^{-2}. The incident radiation is the result of the integration of the incoming specific intensities from all directions (solid angle Ω) at any point inside the reaction space. Eq. (4) indicates that G could be a function of wavelength, position and time. For polychromatic light:

$$G(\underline{x}, t) = G_{\Sigma\lambda}(\underline{x}, t) = \int_{\lambda_1}^{\lambda_2} \mathrm{d}\lambda \int_\Omega I_{\lambda,\underline{\Omega}}(\underline{x}, t)\, \mathrm{d}\Omega \tag{5}$$

The time dependence of G may be originated in the unsteady state condition of some of the properties involved in the calcu-

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Nomenclature

A	area (cm^2)
$C_{Ec,i}$	*Escherichia coli* concentration of species with state of damage i (CFU cm^{-3})
C_m	medium concentration (g cm^{-3})
C_{mi}	microorganism concentration (CFU cm^{-3})
CFU	colony forming units
e^a	local volumetric rate of energy absorption (LVREA) (W cm^{-3})
E	radiation energy (W s)
G	incident radiation (W cm^{-2})
I	specific intensity (W cm^{-2} sr^{-1})
I^*	usually called light intensity (W cm^{-2})
k	inactivation kinetic constant (s^{-1} (cm^3 W^{-1})^m)
k_G	growth constant (CFU g^{-1} s^{-1})
k_{prot}	protection constant (s^{-1} g^{-1} W^{-m} $(cm)^{3(m+1)}$)
L_R	reactor length (cm)
m	reaction order with respect to e^a
n	threshold limit of damage
Q	flowrate (cm^3 s^{-1})
$R_{Ec,i}$	reaction rate corresponding to the bacteria a state of damage i (CFU cm^{-3} s^{-1})
t	time (s)
t_R	reaction time
V	volume (cm^3)
x	cartesian coordinate along the reactor length (cm)
$\underline{x}$	position vector (cm)

Greek letters

$\alpha_{Ec,i}$	*E. coli* specific Napierian absorption coefficient (cm^2 CFU^{-1})
α_m	medium specific Napierian absorption coefficient (cm^2 g^{-1})
α_{mi}	microorganism specific Napierian absorption coefficient (cm^2 g^{-1})
θ	angular coordinate (rad)
κ	Napierian absorption coefficient (cm^{-1})
λ	wavelength (nm)
τ	residence time (s)
Ω	solid angle (sr)

Subscripts

Ec	relative to *Escherichia coli*
i	relative to the damaging state i
R	relative to reactor
T	relative to total
W	relative to reactor wall
0	denotes initial value
λ	relative to wavelength

Special symbols

$\langle\rangle$	means reactor volume averaged value
$\overline{\langle\rangle}$	means time average of the reactor volume average

lation of the specific intensity such as the concentration of the radiation absorbing species. The spatial dependence of G may be due to the geometrical characteristics of the reactor and/or the attenuation of radiation produced by absorption and/or scattering in the participating medium. Thus, in practical situations the incident radiation at each point of the employed reactor must be known and cannot be considered constant as it is sometimes assumed when Eqs. such as (1) or (2) are applied. Understanding fully this limitation, an improvement in the definition of the UV dose considered that there is a spatial distribution of incident radiations in the reaction space and that the average value of G over the reactor volume could be used [7–10]:

$$\text{Dose} = \langle G(t)\rangle_{V_R} \times \tau \tag{6}$$

with:

$$\langle G(t)\rangle_{V_R} = \frac{1}{V_R}\int_{V_R} G(\underline{x}, t)\,\mathrm{d}V \tag{7}$$

The radiation distribution $[G(\underline{x}, t)]$ was calculated with different degrees of approximations from the simple application of the so called Lambert–Beer equation in planar reactors [7] to the linear source with spherical emission model [11]—also called the point source summation model—in continuous annular photoreactors (see for example, [12,13]). Even more realistic emission models have also been published elsewhere [14] and applied to very complex reactions.

The next significant improvement was the result of recognizing that in practical applications flow-through reactors under turbulent flow regime should be the preferred choice. In these cases, in real reactors, no two particle trajectories are the same and, consequently, there is not a unique exposure time to radiation for all the microorganisms present or, which is the same, there is a whole distribution of delivered doses in the reactor [3,12,13,15–19]. This observation led to the use of a detailed knowledge of the reactor's hydraulic profiles using computer fluid dynamics combined with UV light distribution models. Even fractal concepts as described by Lin and Blatchley III [19] have been used.

From all these contributions, it seems evident that, in order to pave the way for an a priori design of the reactor, the correct characterization of this critical parameter in disinfection processes employing continuous, turbulent flow systems, should require to have precise information of two interrelated phenomena: (i) the spatial (sometimes three-dimensional) distribution of the incident radiation and (ii) the spatial three-dimensional distribution of the fluid velocity under turbulent flow operation; i.e. the knowledge of the incident radiation "history" that the microorganism has been receiving during the exposure time. With this information and an intrinsic inactivation reaction model inserted in the appropriate colony forming units conservation equation, in principle, the reactor could be designed a priori. Implicit in all these increasing improvements to characterize such an important variable in UV disinfection, there are two aspects that have not been explored: (i) at each point inside the reactor, inactivation is the result of radiation absorption by the microorganism which is a property different than the incident radiation because it is

also proportional to its specific absorptivity and concentration:

$$e^a_\lambda(\underline{x},t) = \underbrace{\alpha_{\mathrm{mi},\lambda} C_{\mathrm{mi}}(\underline{x},t)}_{\text{Absorption coefficient}} \quad \underbrace{G_\lambda(\underline{x},t)}_{\text{Incident radiation}} \tag{8}$$

and (ii) the reaction order of the inactivation rate with respect to the absorbed radiation is not necessarily one. In Eq. (8) $e^a_\lambda(\underline{x},t)$ is the monochromatic radiation absorption rate per unit volume of fluid (or the local volumetric rate of energy absorption) in units of W cm^{-3} and a clear function of position and time, $\alpha_{\mathrm{mi},\lambda}$ is the Napierian absorptivity of the microorganisms in terms of cm^2 CFU^{-1} and C_{mi} is the CFU concentration. In a recent work [20,21] these two features have been taken into account in a modification of the Series-Event model proposed by Severin [22]. Based on the ideas of previous reports, as an additional contribution to the development of the dose concept, this work was performed in a specially designed well-stirred batch system and an analysis of those results in terms of a modified definition of the dose is presented in what follows.

2. Experimental set-up

The employed reactor has been described in details elsewhere [20,21]. The reactor is a Pyrex tube of circular cross section having two parallel, flat windows made of Suprasil quartz (volume equal to 74.5 cm^3). Each window is irradiated by an emission system made of a tubular germicidal lamp (90% plus emission at 253.7 nm) placed at the focal axis of a parabolic reflector. With the proper dimensions and geometric layout this system produces a very good approximation to a one-dimensional radiation field [23] facilitating the description of the radiation distribution inside the reactor. Using UVC lamps of different output power and neutral density filters, four different levels of irradiation rates were obtained. The reactor was placed inside a recirculating system that includes a pump (employed flowrate equal to 35 cm^3 s^{-1}) and a well stirred storage tank (liquid volume equal to 1000 cm^3) with provisions for sampling and temperature control. Good mixing in the reactor was achieved, by means of an intense recirculation of the liquid. This reactor set up was build for laboratory research and under no circumstances must be regarded as a proposal for industrial applications.

3. Experimental procedure

Escherichia coli strain ATCC 8739 was used throughout this work. The culture was grown in two different types of broth: (i) a complex medium (nutrient broth) having as main component beef extract and (ii) a synthetic medium of well-known composition having as main component glucose. Most of the initial *Escherichia coli* CFU concentration ranged from 10^4 to 10^7 CFU cm^{-3} depending upon the dilution of the culture, but some runs were also made with values above $C^0_{\mathrm{Ec}} = 10^8$ CFU cm^{-3}. Runs were duplicated for every operating condition and each sample was subjected to triplicate measurements of the following variables: absorbance at 253.7 nm and CFU counting using specific Petrifilm$^{\mathrm{TM}}$ plates (3M Microbiology Products) for *Escherichia coli*. The lower detection limit of the method was 15 CFU cm^{-3}. The radiation absorption characteristics of the reacting medium were significantly changed because about 50% of the runs were made with concentrated culture while the others were carried out with a large dilution (1/1000). More details can be found in the previously quoted references.

4. The resulting, experimentally validated model

The inactivation reaction model for diluted and concentrated media is described by the following set of equations [21]:

$$\begin{aligned}
&\text{for } i = 0 \rightarrow && R_{\mathrm{Ec},i}(x,t) = -(k - k_{\mathrm{prot}}C_{\mathrm{m}})C_{\mathrm{Ec},i}[e^{\mathrm{a}}_{\mathrm{Ec},i}(x,t)]^m + k_{\mathrm{G}}C_{\mathrm{m}} \\
&\text{for } i = 1,\ldots,n-1 \rightarrow && R_{\mathrm{Ec},i}(x,t) = (k - k_{\mathrm{prot}}C_{\mathrm{m}})C_{\mathrm{Ec},i-1}[e^{\mathrm{a}}_{\mathrm{Ec},i-1}(x,t)]^m - (k - k_{\mathrm{prot}}C_{\mathrm{m}})C_{\mathrm{Ec},i}[e^{\mathrm{a}}_{\mathrm{Ec},i}(x,t)]^m + k_{\mathrm{G}}C_{\mathrm{m}} \\
&\text{for } i = n \rightarrow && R_{\mathrm{Ec},i}(x,t) = (k - k_{\mathrm{prot}}C_{\mathrm{m}})C_{\mathrm{Ec},i-1}[e^{\mathrm{a}}_{\mathrm{Ec},i-1}(x,t)]^m
\end{aligned} \tag{9}$$

With the following values for the model parameters in a 95% confidence interval:

$n = 2; \qquad m = 0.205 \pm 0.015;$

$k = 9.03 \pm 0.36\,(\mathrm{cm}^3\,\mathrm{W}^{-1})^m\,\mathrm{s}^{-1};$

$k_{\mathrm{G}} = 1.50 \times 10^2 \pm 14.90\,\mathrm{CFU\,g^{-1}\,s^{-1}};$

$k_{\mathrm{prot}} = 5.56 \times 10^3 \pm 1.86 \times 10^2\,(\mathrm{cm}^3\,\mathrm{W}^{-1})^m\,\mathrm{cm}^3\,\mathrm{g}^{-1}\,\mathrm{s}^{-1}$

The model represented very well the experimental results as can be seen in Figs. 1 and 2.

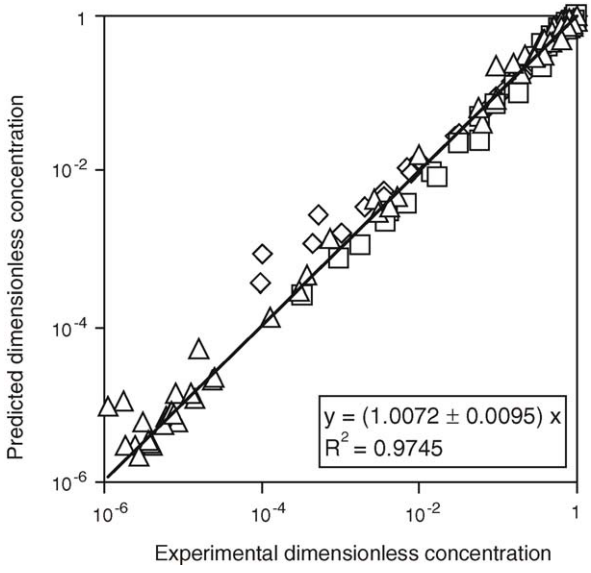

Fig. 1. Bacteria inactivation. Compendium of all experimental data comparing model predictions with experiments. (□) $C_{\mathrm{m}} = 4 \times 10^{-6}$ g cm^{-3} (nutrient broth); (◇) $C_{\mathrm{m}} = 5 \times 10^{-6}$ g cm^{-3} (synthetic broth); (△) $C_{\mathrm{m}} = 1 \times 10^{-3}$ g cm^{-3} (nutrient broth). Runs were made with Heraeus NNI40 and Philips TUV15 lamps (with and without filters) and different initial CFU concentrations.

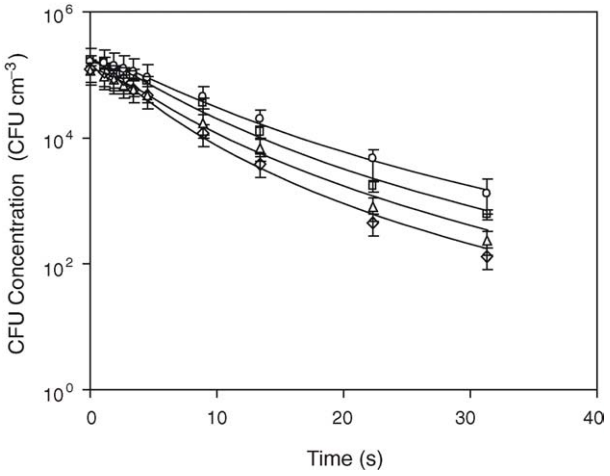

Fig. 2. Bacteria inactivation. Comparison of model predictions and experimental data. Solid lines: model predictions. (◯) Philips lamp with filter; (□) Heraeus lamp with filter; (△) Philips lamp; (◇) Heraeus lamp.

5. The radiation absorption rate

In Eq. (9) the value of $e^{a}_{Ec,i}$ was calculated according to:

$$G(x,t) = G_W \{\exp[-(\kappa_T(t)x)] + \exp[-(\kappa_T(t)x)(L_R - x)]\} \tag{10}$$

$$\kappa_T(t) = \sum_{i=0}^{n-1} \kappa_{Ec,i}(t) + \kappa_m = \sum_{i=0}^{n-1} \alpha_{Ec,i} C_{Ec,i}(t) + \alpha_m C_m \tag{11}$$

$$e^{a}_{Ec,i} = \kappa_{Ec,i}(t) G(x,t) \tag{12}$$

The values of $\alpha_{Ec,i}$ and α_m were obtained from spectrophotometric measurements at $\lambda = 253.7$ nm [20] and the one corresponding to G_W, the boundary condition for the one-dimensional radiative transfer equation (RTE), was extracted from actinometer measurements employing potassium ferrioxalate [20,21,24].

Note that a different type of reactor will require to use the appropriate and very likely different form of the RTE that, in the most general case, is a three-dimensional equation.

From Eqs. (10)–(12) it is clear that the absorbed radiation is a function of position and time. Experimental measurements represent average values of the reaction rates and in spite of the fact that, under well mixing conditions, concentrations inside the reactor have a unique value, depending on the characteristics of the reacting medium, the incident radiation may be a strong function of position. Moreover, if perfectly mixing conditions prevail, the bacteria during its short mean residence time per pass inside the reactor (ca. 2 s) may be exposed to different irradiation rates. Both problems (the second one as a reasonable approximation) can be solved employing the average value (over the reactor volume) of the reaction rate. Recalling that concentrations are uniform, we need to calculate the average value of the radiation component of the reaction rate model:

$$\left\langle [e^{a}_{Ec,i}(x,t)]^m \right\rangle_{V_R} = \frac{1}{V_R} \int_{V_R} [e^{a}_{Ec,i}(x,t)]^m \, dV \tag{13}$$

Since we are using a one-dimensional radiation model, if the reactor cross section is constant:

$$\left\langle [e^{a}_{Ec}(x,t)]^m \right\rangle_{L_R} = \frac{1}{L_R} \int_{L_R} [e^{a}_{Ec}(x,t)]^m \, dx \tag{14}$$

6. Application to a modified dose concept

Considering that the observed threshold limit in the disinfection model is equal to 2, after the first few seconds corresponding to a very short initial time lag (when the concentration of bacteria remains constant) the mass balance for the recirculating reactor [25], can be approximated by a system with $n = 1$.

$$\left.\frac{dC_{Ec}}{dt}\right|_{Tk} = -\frac{V_R}{V_T} \langle R_{Ec}(x,t) \rangle_{L_R} = -\frac{V_R}{V_T} (k - k_{prot} C_m) C_{Ec}(t) \left\langle [e^{a}_{Ec}(x,t)]^m \right\rangle_{L_R} \tag{15}$$

With the initial condition that at $t = 0$, $C_{Ec} = C^0_{Ec}$. This equation will be applied in the time interval when 99.9% of inactivation is obtained (three logs). During this time, as compared with the fast inactivation rates, $R_G \cong 0$ (the initial inactivation rate is more than eight orders of magnitude larger). With these approximations the parameters of the model were recalculated to give within a 95% confidence interval:

$$m = 0.205 \pm 0.015; \qquad k = 5.66 \pm 0.45 \, (\mathrm{cm^3\,W^{-1}})^m \, \mathrm{s^{-1}};$$
$$k_{prot} = 4.41 \times 10^3 \pm 2.16 \times 10^2 \, (\mathrm{cm^3\,W^{-1}})^m \, \mathrm{cm^3\,g^{-1}\,s^{-1}}$$

It is clear that imposing the approximation that $n = 1$ the errors in the estimated parameters are slightly larger.

Let us assume that the obtained kinetic parameters correspond to an intrinsic kinetic model and apply the results to the description of a simple batch reactor without recirculation.

The mass balance for this batch reactor with $V_R = V_T$ [25] takes the following form:

$$\left.\frac{dC_{Ec}}{dt}\right|_{Tk} = -\langle R_{Ec}(x,t) \rangle_{L_R} = -(k - k_{prot} C_m) C_{Ec}(t) \left\langle [e^{a}_{Ec}(x,t)]^m \right\rangle_{L_R} \tag{16}$$

With the initial condition that at $t = 0$, $C_{Ec} = C^0_{Ec}$. Eq. (16) can be put in an integrated form, rendering:

$$\ln \frac{C_{Ec}(t)}{C^0_{Ec}} = -(k - k_{prot} C_m) \int_{t^0}^{t_R} \left\langle [e^{a}_{Ec}(x,t)]^m \right\rangle_{L_R} dt \tag{17}$$

and

$$\int_{t^0}^{t_R} \left\langle [e^{a}_{Ec}(x,t)]^m \right\rangle_{L_R} dt = -\frac{\ln \left(C_{Ec}(t_R)/C^0_{Ec} \right)}{k - k_{prot} C_m} \tag{18}$$

In the proposed model the absorbed energy by the microorganism depends on the concentration of the surviving bacteria that is a function of time. Defining a second average along the reaction time interval, we have:

$$\frac{1}{\Delta t_R} \int_{t^0}^{t_R} \left\langle [e^{a}_{Ec}(x,t)]^m \right\rangle_{L_R} dt = \overline{\left\langle [e^{a}_{Ec}(x,t)]^m \right\rangle_{L_R}} \tag{19}$$

Table 1
Modified dose definition results (diluted medium)

Inactivation (%)	15 W Lamp with filter ((W cm^{-3})^m s)	15 W Lamp ((W cm^{-3})^m s)	40 W Lamp with filter ((W cm^{-3})^m s)	40 W Lamp ((W cm^{-3})^m s)
90	0.436	0.438	0.445	0.440
99	0.857	0.872	0.867	0.862
99.9	1.259	1.275	1.267	1.298

Table 2
Modified dose definition results (concentrated medium)

Inactivation (%)	15 W Lamp ((W cm^{-3})^m s)	40 W Lamp ((W cm^{-3})^m s)
90	1.863	1.873
99	3.724	3.728
99.9	5.588	5.594

In Eq. (19), $\Delta t_R = t_R - t^0$. Therefore, for the modified dose we can obtain:

$$\underbrace{\overline{\left\langle [e^a_{Ec}(x,t)]^m \right\rangle_{L_R}} \times \Delta t_R}_{\text{Modified dose}} = -\frac{\ln\left(C_{Ec}(t_R)/C^0_{Ec}\right)}{k - k_{prot} C_m} \quad (20)$$

For a fixed value of $C_{Ec}(t_R)/C^0_{Ec}$ it is clear that, with this definition, the modified dose should be a constant. In Tables 1 and 2 we present the results of calculating the modified dose for different operating conditions employing the left hand side of Eq. (20). Fixing the value of $C_{Ec}(t_R)$ we can obtain for each different irradiating condition the corresponding value of Δt_R. The time and reactor volume averaged of the local volumetric rate of energy absorption has been numerically calculated with $m = 0.205$. The value of the modified dose changes very dramatically according to the optical thickness of the reacting medium. Qualitatively this is not an unexpected result. What is somewhat a surprise is the very significant effect produced by the medium concentration because the absorbance of the liquid employed in our experiments with concentrated culture was not extremely high [20,21]. Employing this approach, it is possible to conclude that in well mixed reactors, this modified definition produce very good and consistent results.

This contribution is the first step of this research concerning the proposed approach for a different view of the dose concept. The next step is to work with a flow reactor with a well defined velocity field (fully developed laminar flow operation) and finally extend the study to a continuous reactor with turbulent flow.

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