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Modelling of reactivation after UV disinfection: Effect of UV-C dose on subsequent photoreactivation and dark repair

E. Nebot Sanz[a,*], I. Salcedo Dávila[a], J.A. Andrade Balao[b], J.M. Quiroga Alonso[a]

[a]Department of Chemical Engineering, Food Technologies and Environmental Technologies, Faculty of Marine and Environmental Sciences, University of Cádiz, Cadiz, Spain
[b]Aguas de Jerez, Empresa Municipal, Spain

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ABSTRACT

The increased use of UV radiation as a wastewater treatment technology has stimulated studies of the repair potential of microorganisms following treatment. In this study, samples of unfiltered secondary effluent were irradiated with seven levels of UV-C doses (50–200 mW s/cm^2) from six low-pressure lamps in an open-channel UV disinfection system. Following irradiation, samples were incubated at 20 °C under photoreactivating light or in darkness. Samples were analysed for 240 min following incubation.

The logistic model is proposed to explain the relation between photoreactivation and the UV-C dose received by the microorganisms. That model accurately fitted the data obtained in photoreactivation experiments, permitting interpretation of the estimated kinetic parameters: S_m and k_2. In the experiments carried out in darkness, a slight reactivation is observed (<0.1%), followed by a decay period in which survival decreases. In order to model this last period, a modification was made to the logistic model by including a term of mortality that assumes a zero-order kinetic. The parameters S_m and k_2, in both photoreactivation and darkness, show an exponential dependence on the UV-C inactivating dose. It is possible to predict their values, and hence the reactivation curve, from the equations proposed in this work.

1. Introduction

Ultraviolet (UV) light is increasingly being considered as a promising alternative to chlorine for the disinfection of wastewater (Lindenauer and Darby, 1994). Reclaimed wastewater reuse for agricultural purposes and golf course irrigation is now expanding, and the advantages of applying UV disinfection to enable wastewater to be reused are widely recognized (Kashimada et al., 1996).

The inactivation of microorganisms by far-UV light (UV-C: 200–280 nm) is effected through the formation of lesions in the genomic DNA of the organisms (Friedberg et al., 1995; Harm, 1980). The major lesion induced by germicidal UV-C light (254 nm) is the formation of pyrimidine dimers (Harm, 1980; Rothman and Setlox, 1979; Tyrrell, 1973). The presence of lesions induced by UV-C would inhibit the normal replication of DNA and therefore result in inactivation of the microorganisms (Oguma et al., 2001). However, many organisms are known to possess the ability to repair their DNA damage by light-dependent (photoreactivation) as well as light-independent (dark repair) mechanisms (Friedberg et al., 1995; Harm, 1980). This phenomenon therefore represents a potential disadvantage for UV-C disinfection methods in water reclamation (Weinbauer et al., 1997; Oguma et al., 2001; Liltved and Landfald, 1996).

Photoreactivation is the phenomenon by which inactivated microorganisms recover activity through the repair of pyrimidine

*Corresponding author. Tel.: +34 956 016198.
E-mail address: enrique.nebot@uca.es (E. Nebot Sanz).
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Nomenclature

k_1	reactivation first-order reaction rate constant, min^{-1}
k_2	reactivation second-order reaction rate constant, min^{-1}
M	mortality zero-order reaction rate constant, min^{-1}
N_o	concentration of microorganisms before disinfection, FCU/100 mL
N_d	concentration of microorganisms after disinfection (before reactivation), FCU/100 mL
N_m	maximum concentration of microorganisms, FCU/100 mL
Nr	concentration of microorganisms at time t after the beginning of the reactivation phase, FCU/100 mL
S_o	survival immediately after UV disinfection $(N_d/N_o) \times 100$, (%)
S	survival ratio at time t, (%)
S_m	maximum survival ratio $(N_m/N_o) \times 100$, (%)

dimers in the DNA under near-UV (UV-A) and visible light (310–480 nm) with the enzyme photolyase and without excising the distorted region (Walker, 1984; Oguma et al., 2001; Liltved and Landfald, 1996). The dark repair mechanism, as its name suggests, can repair the damaged DNA without light. This mechanism is a multi-enzyme repair process involving the excision of dimers.

Many studies have demonstrated the possibility for photoreactivation or dark repair of UV-C damaged microorganisms, enabling regrowth of the microbial population under certain conditions, thus reducing the efficacy of UV-C inactivation (Chan and Killick, 1995; Lindenauer and Darby, 1994; Whitby and Palmateer, 1993; Eker et al., 1991; Harris et al., 1987; Levine and Thiel, 1987; Machida et al., 1986).

Systematic quantitative study of photoreactivation, the more important of the two mechanisms, has suggested a two-step reaction scheme (Harm, 1980):

Step 1: Formation of a complex between a photoreactivation enzyme (PRE) and the dimer to be repaired. This step does not require light, but is dependent on temperature, pH and ionic strength (Lindenauer and Darby, 1994).

Step 2: Release of PRE and repaired DNA. The restoration of the dimer to its original monomerized form is absolutely dependent upon light energy intensity. The reaction occurs in less than a millisecond; consequently the limiting step of the whole reactivation process is the formation of the PRE–dimer complex. An extended period of exposure to photoreactivating light would enable the release of PRE that would then be available to form new complexes (Step 1).

The effect of temperature on the reactivation phase is still little studied. Chan et al. (1995) investigated the effect of salinity and temperature on the reactivation of *Escherichia coli* in a marine environment. They found that the effect of salinity is greater than temperature, although an Arrhenius tendency was confirmed for the rate constants calculated.

The factors that influence the rate and extent of reactivation are beginning to emerge through scientific research, but not enough is known at present to make quantitative predictions in most cases. It was showed (Kalisvaart, 2004) that medium-pressure UV lamps produce a broad, "polychromatic" spectrum of UV wavelengths that inflict irreparable damage not only on cellular DNA, but on other molecules, such as enzymes, as well. However low-pressure lamps emit a single wavelength peak which only affects DNA. Therefore, the microorganism reactivation is more difficult with medium-pressure lamps. Several studies have noted that if reactivation is observed in a microbial species, the extent of reactivation is often inversely related to the applied UV-C dose (Baron and Bourbigot, 1996; Lindenauer and Darby, 1994). The repair was generally found to be higher at low doses (Hu et al., 2005). In an interesting recent review (Hijnen et al., 2006), it is explained that reactivation entails a lower inactivation kinetic, and it means that higher UV-C fluence are required to obtain the same level of inactivation. Quantitative data showed a 2.8–4.6 higher UV fluence requirement for 1–3 log inactivation of *Legionella pneumophila* (Knudson, 1985).

Few papers focus on modelling the reactivation processes: most simply describe qualitative studies of the process (Harris et al., 1987; Lindenauer and Darby, 1994; Hassen et al., 2000; Oguma et al., 2004). Other authors have tried to model jointly the inactivation and reactivation phases (Tosa and Hirata, 1999; Beggs, 2002), by comparing experiments with and without reactivation. Kashimada et al. in 1996 proposed a model to predict independently the reactivation phase, but the experiments carried out were insufficient to be generalized. Therefore, it is pertinent to investigate new kinetic models that permit reactivation processes to be predicted, and provide a better understanding of the factors affecting this interesting phenomenon.

Thus, in this study, photoreactivation and dark repair of three bacterial indicators, total coliforms (TE), faecal coliforms (FC) and faecal streptococci, were investigated in order to develop a kinetic model which allows prediction of their reactivation after UV disinfection depending on the UV-C dose applied. For this purpose, the relationship between the repair and the UV-C irradiation dose was particularly examined. The extent and rate of reactivation were also studied.

2. Materials and methods

2.1. UV-C irradiation

UV-C irradiation treatment was performed with a 5.0 m^3/h horizontal-lamp open-channel UV disinfection system (Trojan Technologies, Spain, S.L.) and six low-pressure high-intensity mercury UV lamps (Philips 30W UV-C at 254 nm). The UV channel received the water from the unfiltered secondary effluent of the Municipal Wastewater Treatment Plant of Jerez de la Frontera (Spain).

Bacteria were exposed to seven levels of UV-C fluence (50, 75, 100, 125, 150, 175 and 200 mW s/cm^2). The UV-C fluence (mW s/cm^2) applied was calculated as a product of the average UV fluence rate in the reactor (mW/cm^2) and the irradiation time (s). The average UV fluence rate was calculated by the Point Source Summation (PSS) method (Ho et al., 1998; Braunstein et al., 1996; USEPA, 1986, 1992; Qualls et al., 1989). The exposure time was calculated from the channel volume and the influent flow rate, after first ensuring that the plug flow condition existed in the channel.

2.2. Repair conditions

After UV-C irradiation, the water sample was divided and transferred into two 500 mL glass Erlenmeyer flasks (95% transparent for 360 nm light). One of the two Erlenmeyer flasks was thermostated in a controlled-environment incubator (FOC 225E, Refrigerated Incubator, VELP Scientifica), which was equipped with one fluorescent lamp (3.7 W, PHILIPS TLD), at six different temperatures: 5, 10, 15, 20, 25 and 30 °C (photoreactivation). The range of the lamp wavelengths was 310–420 nm with a broad peak at 360 nm. Irradiation periods were in the range of 30–240 min (minimum and maximum values). The UV-A fluence rate of the fluorescent lamp was 0.1 mW/cm^2 at 360 nm at the sample surface, estimated by the PSS method and the distance between the samples and the lamp. The other Erlenmeyer flask was covered immediately with aluminium foil and incubated simultaneously at the same temperature for 240 min (dark repair). Concentration of bacteria was measured every 30 min taking samples from each Erlenmeyer flask with a pipette.

2.3. Enumeration of microorganisms

Three bacterial indicators of microbiological contamination have been analysed: TC, FC and *Streptococcus faecalis* (SF).

All the microorganisms were analysed according to Standard Methods for the Examination of Water and Wastewater (APHA et al., 1992) by using the membrane-filter technique. TC were cultured on MF-Endo agar and incubated at 35 °C for 24 h. FC were determined on m-FC agar and 24 h incubation at 44.5 °C. Finally, *S. faecalis* were grown on KF agar at 37 °C for 48 h. After the incubation period, bacterial colonies were counted and the results calculated as colony forming units per 100 mL of sample (CFU/100 mL). For each microorganism and each experimental condition used, tests were repeated at least three times and mean values were obtained from these repeated experiments.

Experiments were repeated three times independently for each bacterium and experimental condition used. Standard deviations of triplicates are not presented on the graphs, in the interests of clarity. When standard deviation was disproportionate (CV > 20%), data were rejected.

The rates of reactivation were assessed by determining microorganism survival from microbial numbers before disinfection and after reactivation phenomenon.

2.4. Modelling the reactivation kinetics

Reactivation is frequently expressed as a function of the survival ratio in respect of the initial microorganism concentration existing before the inactivating treatment.

Therefore, the survival values were calculated using the following equation:

$$S = \frac{N_r}{N_o} \cdot 100, \tag{1}$$

where S is the survival ratio at time t, N_o is the concentration of microorganisms before disinfection and N_r is the concentration at time t after the beginning of reactivation.

A typical inactivation–reactivation curve as a function of time is shown in Fig. 1. In that figure it is possible to differentiate the various phases of the process: exponential UV inactivation, reactivation process which includes an induction period, growth phase, stabilization phase and decay period. As stated, the reactivation can occur by two mechanisms according to the exposure of the samples to light or in darkness.

2.5. Photoreactivation kinetic

Kashimada et al. (1996) proposed an asymptotic model, assuming the photoreactivation phenomenon follows a saturation-type first-order reaction, as:

$$\frac{dS}{dt} = k_1 \cdot (S_m - S), \tag{2}$$

where S_m is the maximum survival ratio and k_1 the first-order reactivation rate constant.

In the model, the term (S_m–S) acts as driving force for the reactivation. As the survival ratio, S, is reaching its maximum value (S_m), the process decelerates showing an asymptotic tendency.

Reactivation curves have been obtained by means of the experiments described below. In Fig. 2a the typical asymptotic-sigmoidal shape of these curves can be observed. In the induction period, the curve suggests imperceptible reactivation; then a rapid exponential growth can be seen, and finally a stabilization period is reached when growth ceases. After the application of the Kashimada et al. (1996) model, we observed that it did not fit the data correctly, mainly at the beginning of the curve, when an induction period is observed (Fig. 2b). Therefore we decided to modify the model, but without increasing the number of parameters. The new model is represented by the following equation:

$$\frac{dS}{dt} = k_2(S_m - S) \cdot S, \tag{3}$$

where k_2 is the new growth second-order reactivation rate constant. This relationship is simply a combination of the second-order equation and the driving force concept employed by Kashimada et al. (1996). The equation is really not new, because it coincides, in its mathematical form, with the logistic equation proposed by Verhulst in 1838 for interpreting biological population growth. Nevertheless, the originality of our work lies in its innovative application to microorganism reactivation prediction. The model has the advantage that both kinetic parameters: S_m and k_2, have a clear physical significance. On the one hand, S_m is the maximum limit of the microorganisms' survival by reactivation, and on the other hand, k_2 represents the rate at which that value is reached. It can be seen in Fig. 2b that this proposed model correctly fits the experimental data.

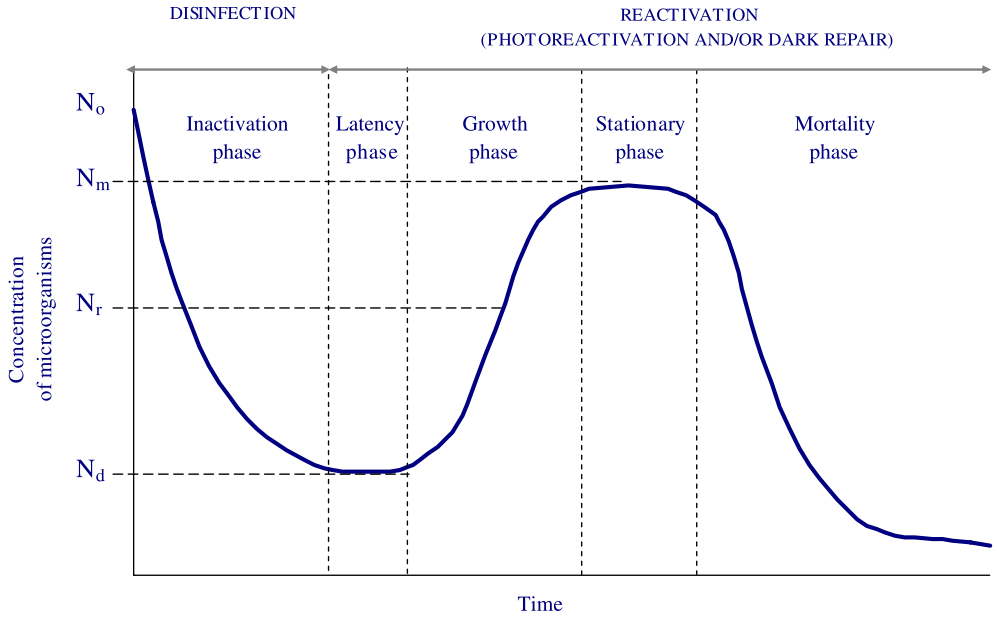

Fig. 1 – A typical inactivation–reactivation curve as a function of time, where N_o is the concentration of microorganisms before disinfection, N_d, after disinfection but before reactivation and N_r at time t after the beginning of the reactivation phase. N_m is the maximum concentration of microorganisms reached by reactivation.

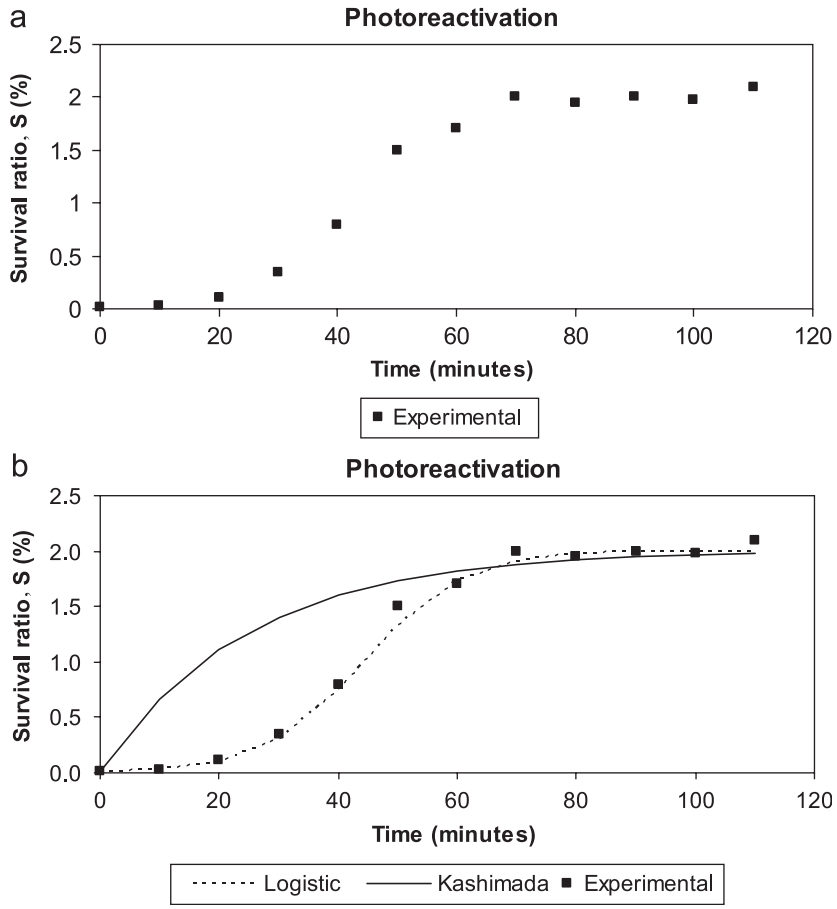

Fig. 2 – (a) Typical photoreactivation curve. (b) Curves from the model proposed by Kashimada et al. (1996), and the logistic model.

By the integration of Eq. (3), the following is obtained:

$$\ln\frac{S[S_o - S_m]}{S_o[S - S_m]} = k_2 \cdot S_m \cdot t, \quad (4)$$

where S_o is the survival immediately after UV disinfection (N_d/N_o).

From Eq. (4), it is possible to express the variable S as a function of the kinetic parameters k_2, S_m, S_o and time (Eq. (5)) and, as a result, we can easily obtain the two parameters, S_m and k_2 by non-linear regression.

$$S = \frac{S_m}{1 + [S_m/S_o - 1] \cdot e^{-k_2 \cdot S_m \cdot t}}. \quad (5)$$

This Eq. (5) allows the photoreactivation curve over time to be simulated (Fig. 3).

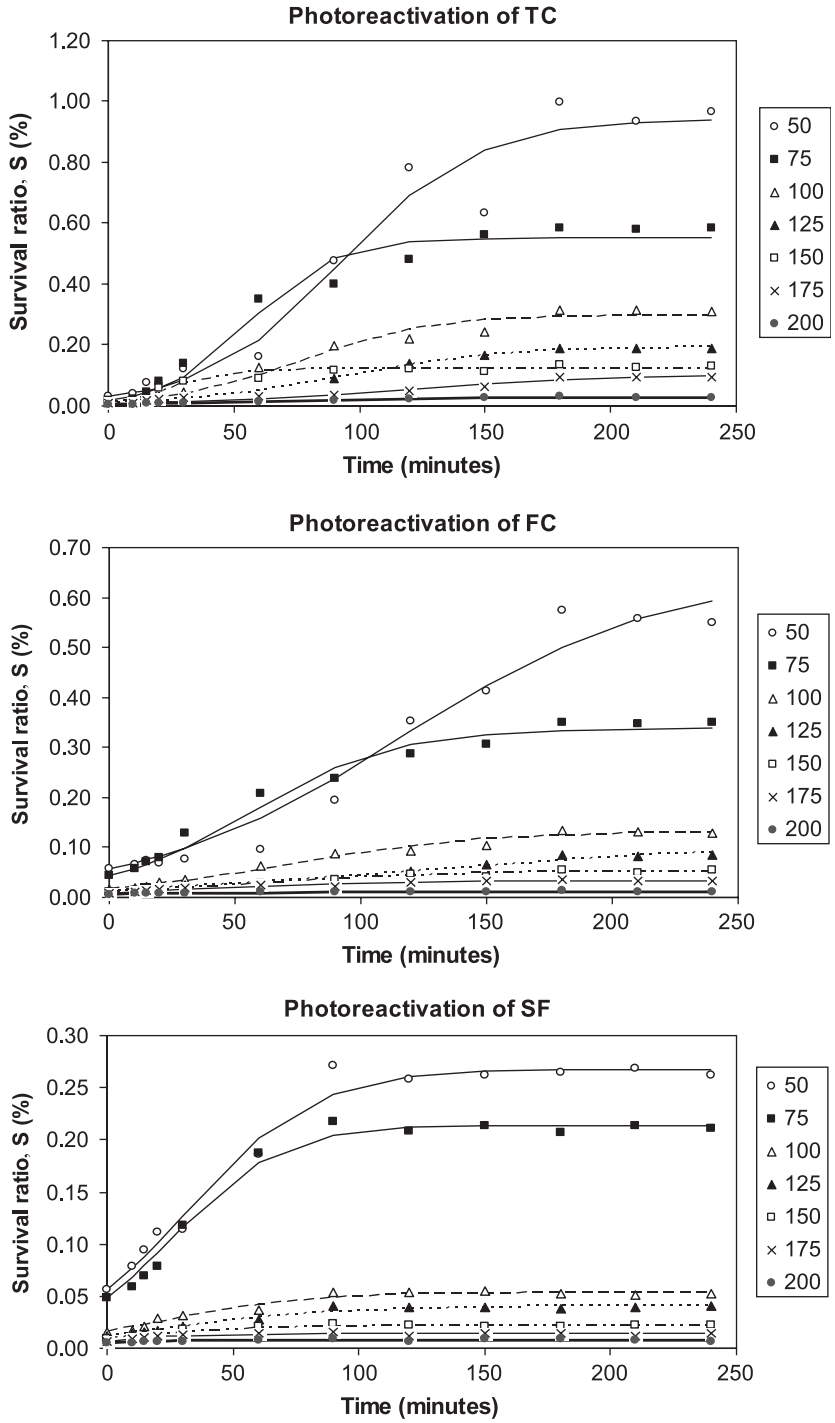

Fig. 3 – Survival ratio versus time of exposure to photoreactivating light for different inactivating UV-C doses applied (50–200 mW s/cm^2). Experimental data and prediction of the logistic model.

2.6. *Dark repair kinetic*

After carrying out the dark repair experiments, it was observed that the curve of microorganisms' survival versus time showed, after a low and brief reactivation period, a decay phase, not detected in photoreactivation experiments. We concluded that in darkness, the reactivation did occur but to a less extent than in illumination conditions, and then the survival commenced a decreasing tendency (Fig. 4). Therefore the model proposed (Eq. (5)) did not fit these data, and it was

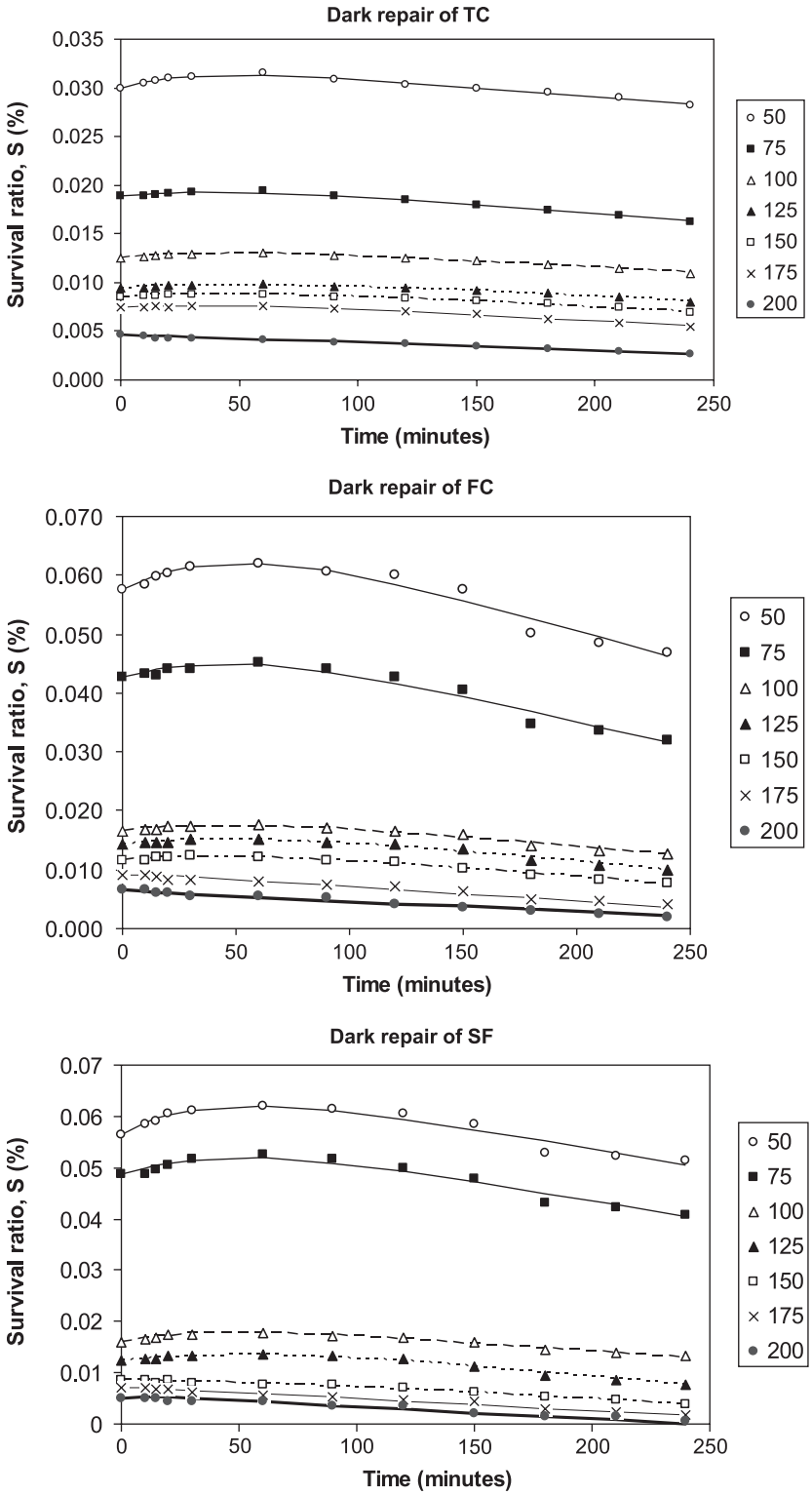

Fig. 4 – Survival ratio versus time of exposure to darkness for different inactivating UV-C doses applied (50–200 mW s/cm^2). Experimental data and prediction of the logistic model.

necessary to include a term corresponding to the decay process. Observing the experimental curves, we decided to test a zero-order kinetic for this decay process, in the following equation:

$$\frac{dS}{dt} = -M, \tag{6}$$

where M, mortality, is a zero-order decay rate constant.

Eq. (7) is obtained by integrating Eq. (6), and it indicates that the decay term is linear over time

$$\Delta S_d = -M \cdot t. \tag{7}$$

By combining Eqs. (5) and (7), the modified version of the integrated model is obtained, which is also valid for dark repair:

$$S = \frac{S_m}{1 + \left[S_m/S_o - 1\right] \cdot e^{-k_2 \cdot S_m \cdot t}} - M \cdot t. \tag{8}$$

3. Results

3.1. Photoreactivation

As stated, prior to photoreactivation, samples were exposed to seven levels of UV-C fluence (50, 75, 100, 125, 150, 175 and 200 mW s/cm^2). Fig. 3 represents the survival ratio versus time for TC, FC and SF, and the asymptotic shape of the curves can be seen, including an induction period, an exponential growth and finally a stabilization phase. The model described in Eq. (5) was applied to experimental data using non-linear regression. Table 1 gives the values of the estimated kinetic parameters: S_m and k_2, together with the r^2 statistics and the optimization function (observed−Predicted)^2. The good fit of the model to the experimental data can be observed.

This study indicated a ranking of maximum photoreactivation limits as follows: TC > FC > faecal streptococci. Even though the maximum survival ratio does not exceed 1%, this apparently low percentage, for a N_o of 10^6 FCU/mL, would produce a reactivation of 10^4 colonies, which could cause serious health and environmental problems. On the other hand, if the value of k for the different microorganisms is compared (Table 1), it can be seen that TC and FC have values similar to each other but lower than SF. This means that SF reaches the maximum survival ratio sooner than TC and FC (see Fig. 3), although this maximum is lower than those reached by TC and FC. Therefore, k_2 depends on particular biochemistry repair mechanisms that vary between the different microorganisms.

In Table 2 and Fig. 5, the consistent behaviour of S_m and k_2 with the UV-C dose can be seen. On the one hand, S_m (the asymptotic limit of the microorganism survival due to photoreactivation) shows a negative exponential tendency with the UV-C dose. This finding indicates that high UV-C doses produce severe alteration on bacteria, making their reactivation non-viable, while low doses permit the photoreactivation process to take place, and to a greater extent when microorganisms had received a lower dose. On the other hand, k—the kinetics rate constant—shows an inverse tendency: it increases jointly with UV-C. This finding means

Table 1 – Kinetic parameters of the logistic model applied to photoreactivation experiments

UV-C doses (mW s/cm^2)	TC				FC				SF			
	S_m (% survival)	k_2 (% min)$^{-1}$	(obs-pred)^2 (% survival)^2	r^2	S_m (% survival)	k_2 (% min)$^{-1}$	(obs-pred)^2 (% survival)^2	r^2	S_m (% survival)	k_2 (% min)$^{-1}$	(obs-pred)^2 (% survival)^2	r^2
50	0.945	0.039	6.71E-02	0.962	0.644	0.031	1.39E-02	0.976	0.268	0.151	1.36E-03	0.984
75	0.551	0.107	1.87E-02	0.972	0.339	0.102	3.43E-03	0.982	0.214	0.220	7.27E-04	0.990
100	0.298	0.134	4.52E-03	0.975	0.131	0.204	4.36E-04	0.982	0.053	0.686	9.55E-05	0.964
125	0.196	0.162	2.88E-04	0.997	0.105	0.146	2.93E-04	0.969	0.040	0.791	3.27E-05	0.979
150	0.120	0.859	1.70E-03	0.937	0.054	0.435	1.03E-04	0.968	0.022	2.174	2.55E-05	0.939
175	0.103	0.215	6.64E-04	0.961	0.033	0.858	5.89E-05	0.944	0.014	4.955	1.05E-05	0.856
200	0.029	0.830	2.76E-05	0.977	0.012	1.301	3.49E-06	0.932	0.008	7.703	2.73E-06	0.807

Table 2 – Kinetic parameters of the logistic model applied to dark repair experiments

Doses	TC					FC					SF				
	S_m (%)	k_2 (% min)$^{-1}$	M (%/min)	(obs-pred)^2 (%)^2	r^2	S_m (%)	k_2 (% min)$^{-1}$	M (%/min)	(obs-pred)^2 (%)^2	r^2	S_m (%)	k_2 (% min)$^{-1}$	M (%/min)	(obs-pred)^2 (%)^2	r^2
50	0.033	1.0150	1.89E-05	1.41E-07	0.986	0.073	0.341	1.10E-04	1.23E-05	0.960	0.076	0.460	1.09E-04	1.16E-05	0.930
75	0.021	1.0616	1.95E-05	7.26E-08	0.994	0.053	0.464	8.81E-05	8.48E-06	0.965	0.061	0.458	8.92E-05	8.17E-06	0.953
100	0.013	1.3106	7.45E-06	1.18E-06	0.942	0.021	1.136	3.33E-05	2.64E-06	0.946	0.019	0.870	2.29E-05	1.44E-06	0.955
125	0.010	1.6567	7.26E-06	4.97E-07	0.929	0.019	1.192	3.69E-05	1.07E-05	0.821	0.015	1.440	2.61E-05	4.61E-06	0.898
150	0.010	2.0439	1.25E-05	3.54E-08	0.992	0.015	1.565	3.06E-05	2.17E-06	0.924	0.013	1.146	4.43E-05	4.35E-05	0.902
175	0.009	2.9999	1.26E-05	7.80E-08	0.988	0.010	2.265	2.43E-05	7.38E-07	0.979	0.009	1.545	3.40E-05	1.43E-05	0.946
200	0.004	4.3728	7.30E-06	9.41E-08	0.982	0.006	4.737	1.70E-05	3.88E-07	0.987	0.005	1.224	1.81E-05	1.06E-06	0.960

that the reactivation phenomenon is more rapid with high doses, although a lower survival percentage is reached.

3.2. *Dark repair*

Fig. 4 shows that, in conditions of darkness, the reactivation occurs to a considerably lower degree than in the case of photoreactivation. After the maximum survival was reached, a decay process is observed, with survival diminishing according to a linear trend over time. For this reason, a modification has been made to the model (Eq. (9)). In Table 2 the results of applying the model are listed, including the values of the three kinetic parameters estimated: S_m, k_2 and M (mortality rate constant). The last parameter, M, is calculated experimentally from the slope of the linear end of the survival curve (between the 120 min point and the final point at 240 min). The good fit of the model to the experimental data from dark repair experiments can also be seen. The parameters S_m and k_2 have an exponential relationship with UV-C dose, similar to that found in photoreactivation experiments, whereas M seem to be independent of dose and microorganism, having an average value of between 1 and 4×10^{-5} (% survival/min).

In Fig. 5, it is possible to compare the photoreactivation and dark repair processes through the kinetic parameters obtained by the application of the model. It can be seen that S_m is one order of magnitude higher in the case of photoreactivation, unlike k_2 which is higher in dark experiments where reactivations finish earlier. This last finding is not very significant, since it only means that in dark reactivation, the maximum survival (very small) was reached sooner than in the case of photoreactivation. Table 3 lists the coefficients of the fitted exponential that are useful for predicting the S_m and k_2 parameters in function of type of microorganism, of light or dark conditions, and UV-C dose administrated.

4. Conclusions

(a) *Proposed model for reactivation process*

The kinetic model proposed by Kashimada et al. (1996)—a first-order saturation type—does not adequately fit the data obtained in reactivation experiments conducted in conditions of both light and darkness, because it does not take into account the existence of an induction period.

A modification of that model is proposed, which consists in changing from first- to second-order reaction, while keeping the concept of saturation model suggested by Kashimada et al. (1996). That new model, which actually coincides with the classic logistic equation, incorporates two kinetic parameters: S_m (maximum survival ratio) and k_2 (reactivation rate constant). In order to interpret correctly the reactivation that occurs in conditions of darkness, a new term for decay—based on the parameter M, the mortality rate constant—is added to the logistic equation. The model accurately fits the data obtained in photoreactivation experiments, and permits the interpretation of the estimated kinetic parameters: S_m, k_2 and M (only for dark repair) and their relationship with various environmental conditions: microbial type, light and UV-dose exposure.

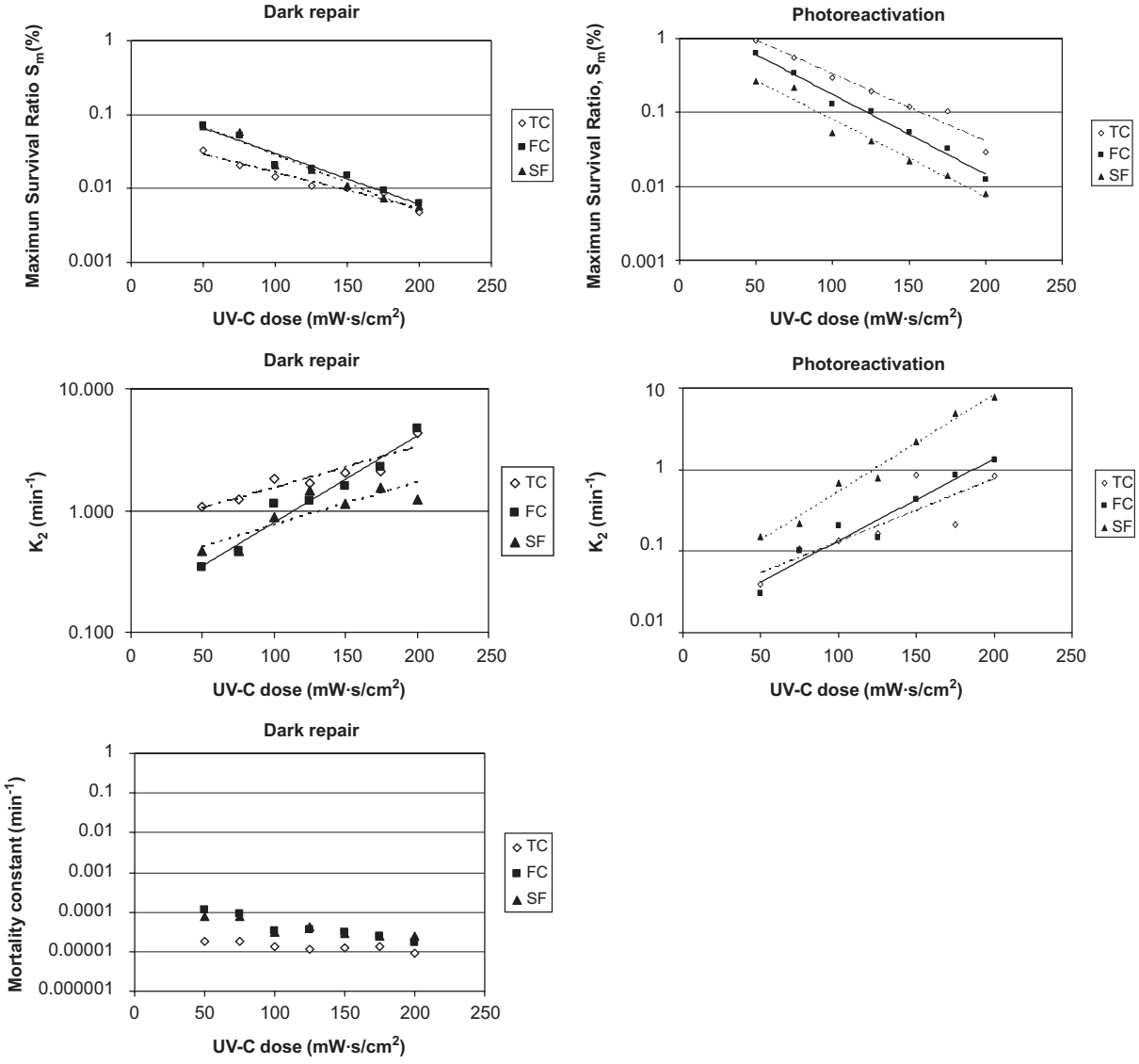

Fig. 5 – Kinetic parameters of the logistic model: S_m, k_2 and M, for the three groups of microorganism assayed: total coliforms (TC), faecal coliforms (FC) and *Streptococcus faecalis* (SF). Comparison between dark repair and photoreactivation.

Table 3 – Exponential dependence of the reactivation parameters, S_m, k_2 and M, on the UV-C disinfection dose

Reactivation conditions	Microorganism	$k_2 = b \cdot e^{a \cdot \text{UV-C dose}}$			$S_m = b \cdot e^{a \cdot \text{UV-C dose}}$		
		b	a	r^2	b	a	r^2
Photoreactivation	TC	0.022	0.018	0.743	2.695	−0.021	0.964
	FC	0.013	0.023	0.938	2.112	−0.025	0.985
	SF	0.034	0.027	0.982	0.892	−0.024	0.968
Dark repair	TC	0.704	0.008	0.843	0.050	−0.011	0.947
	FC	0.157	0.016	0.955	0.146	−0.016	0.958
	SF	0.413	0.007	0.725	0.153	−0.017	0.946

b) *Effect of microbial indicator type*

In respect of the type of microorganism exposed to photoreactivating light, the maximum survival ratio, S_m, follows the sequence: TC > FC > SF, whereas, for the pseudo second-order kinetics constant, k_2, the sequence is: SF > TC = FC. Dark repair is less important than photoreactivation, with the differences between the microbial indicator types therefore being less relevant. The maximum survival

ratio, S_m, does not exceed 1% but, despite this apparently low percentage, the surviving bacteria could still cause serious health and environmental problems, and an increment of UV fluence would be required to ensure adequate inactivation (Hijnen et al., 2006).

(c) *Effect of light/darkness conditions*

In the experiments carried out in darkness, it can be seen that S_m is one order of magnitude lower than in the case of photoreactivation and that, to the contrary, k_2 is greater in darkness experiments. No clear distinction is found between the different types of microorganisms in terms of survival. The new kinetic parameter *M*, seem to be independent of dose and microorganism, having an average value of between 1 and 4×10^{-5} (%survival/min). Those values are really small, since during the 240 min of reactivation a maximum reduction of survival of approximately 0.01% will be produced. We suppose that this mortality rate is due to the residual effect of radiation on the bacterial DNA, since the biochemical mechanism of actuation needs some time to be manifested completely. It is possible that mortality does not occur in photoreactivation experiments, since in this situation the repair of damaged DNA is more effective.

(d) *Effect of the UV-C dose administered*

The parameters S_m and k_2, in both photoreactivation and conditions of darkness, show an exponential dependence on the UV-C inactivating dose, and it is possible to predict their values, and hence the reactivation curve, from the equations proposed in this work.

S_m decreases with the UV-C dose, indicating that the extent of reactivation is limited by elevated UV-C doses. However, k_2 increases with the UV-C dose. This fact is due to k_2 not being a pure reaction rate constant: it is a model parameter that is adjusted to predict the experimental data. Its physical meaning is related to the time required to reach the maximum survival ratio and then the stabilization phase: high values of k_2 signify short induction and growth phases.

M seems to be independent of UV-C dose, having a value of between 1 and 4×10^{-5} (%survival/min).

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