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Inactivation efficacy and reactivation of fecal bacteria with a flow-through LED ultraviolet reactor: Intraspecific response prevails over interspecific differences

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ABSTRACT

Treatment with ultraviolet (UV) light is a common option for inactivating waterborne organisms. The mercury vapor lamps conventionally used as a source of UV-C light for water disinfection are eventually replaced by light emitter diodes (LEDs) in the middle term due to their higher efficiency and lack of hazardous materials. Nonetheless, biological mechanisms for repairing UV damage caused by the UV treatment are some of its significant undesirable features. The objective of this study is to evaluate and compare the UV-resistance and the reactivation degree in different strains of *E. coli* and *E. faecalis* treated with a flow-through reactor equipped with LEDs with an emission range between 265 and 285 nm. The treated organisms were subjected to various illumination regimes after the UV irradiation. The results obtained indicated that intraspecific differences between the strains of *E. coli* were greater than the interspecific differences with respect to *E. faecalis* in terms of UV-resistance and repairing potential. The UV doses necessary to achieve four log-reductions ranged from 10.2 to 16.3 mJ cm^{-2} for *E. coli* and from 11.1 to 11.4 for mJ cm^{-2} for *E. faecalis*. Dark repair was not observed within 24 h after the UV irradiation whereas the degree of photorepair depended on both the bacteria strain and the applied UV dose. The exposure of the irradiated organisms to an illuminated environment entailed and increasing between the 18 % and the 160 % of the UV dose required to achieve four log-reductions.

1. Introduction

Irradiation with UV light is used for water disinfection in a variety of application fields such as water purification and wastewater reclamation as well as other specific purposes like the treatment of aquaculture effluents and ballast water [1–3]. The UV light in the UV-C (200–280 nm) range interacts with the cell DNA and primarily causes the formation of cyclobutane-pyrimidine dimers that interfere with the DNA replication and consequently prevent the cells from reproducing [4]. Additionally, the UV-C light induces oxidative damage on other cell structures such as membranes and proteins [5,6]. The advantages of the UV treatment with respect to other disinfection technologies include the absence of chemicals that facilitates the treatment application, avoids the need for storing potentially hazardous materials, and limits the generation of disinfection by-products [7,8]. On the other hand, using UV irradiation for water disinfection has some undesirable features including the lack of a residual disinfection effect and the potential of DNA reparation by the treated organisms [9].

The UV DNA-damage repairing mechanisms involve photoreactivation and dark repair. The former is an ancestral repair system that is extensively present in organisms in which the photolyase enzyme is activated by the energy of photons with wavelengths from 330 to 480 nm and specifically binds to cyclobutane–pyrimidine dimers and reverses the damage [10]. On the other hand, the latter is a process that is independent from the light that replaces the damaged DNA with new, undamaged nucleotides [11]. Between both of the processes,

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photoreactivation is the major one in terms of the amount of damage that can be repaired [12]. The existence of these repairing mechanisms may compromise the quality of the UV-treated water due to a dependence of the treatment efficacy on the post-treatment illumination conditions.

The conventional sources of UV light based on mercury vapor are destinated to be replaced by the recently developed UV-C light emitter diodes (LEDs) in the middle term [13]. The current primary disadvantage of UV-C LEDs is their low power conversion into UV light, although this occurrence is being addressed in order to increase their feasibility for the water disinfection treatment [14]. On the other hand, using LEDs for water disinfection has numerous advantages such as the absence of mercury in their construction, higher energy efficiency, longer lifetime, no need for a warming-up period, and greater flexibility in the wavelength of emissions and the design of reactors [15,16].

Recent studies revealed that treatment with varying wavelengths within the UV-C spectrum induces different responses in the treated organisms in regard to their UV-resistance and their UV damage repair potential [17,18]. In addition, the survival response to the UV treatment is determined by a set of factors that includes the use of different indicator organisms, UV devices, and manipulation procedures. Although the most studied microorganism in water disinfection is *Escherichia coli* because it is the reference microorganism in biology, the selected strain for evaluating the treatment can be a crucial parameter [19]. The organism *Enterococcus faecalis* is also a bacterial indicator in some specific fields such as the treatment of industrial effluents and ballast water [20]. In general, the UV-resistance of *E. faecalis* is greater than *E. coli* when using traditional mercury UV-C lamps [21]. This poses a fundamental question regarding the relevance of adopting *E. coli* as a surrogate for water-borne pathogenic bacteria for UV disinfection [22]. Similarly, the type of UV device used for treatment determines the survival responses in the tested organisms. Generally, UV inactivation of microorganisms is believed to adhere to the Bunsen-Roscoe photochemical principle. It states that the photochemical inactivation depends only on the total energy dose following a time-dose reciprocity [23,24]. However, there may be discrepancies in time-dose reciprocity [25]. In this context, the type of UV reactor that is used may have a certain influence on the treatment outcome; in fact, the same organisms subjected to the same experimental procedures demonstrated different inactivation kinetics after their irradiation with either a collimated beam reactor or a flow-through reactor [26]. Finally, factors such as the growth phase and the pretreatment conditions of the target organisms also influence the survival response by the treated organisms [27–29]. This variability in the survival response hinders the comparison of the results from different studies and is thus a concern for the integration of their outcomes in the overall scenario.

The objective in this study is to quantify the UV-resistance and the reactivation degree in different strains of *E. coli* and *E. faecalis*. The target organisms were subjected to different UV doses using a flow-through reactor equipped with LEDs with emission between 265 and 285 nm. The concentration of viable organisms was measured immediately after the UV irradiation and following an incubation period of 24 h under either an illuminated or dark environment. The inactivation curves were fitted into inactivation models to determine the kinetics parameters which allowed the quantitative comparison of the UV-resistance and damage repair potential between the different organisms and post-treatment conditions that were tested.

2. Material and methods

2.1. Target organisms and microbiological procedures

Four strains of *E. coli* (ATCC 11775, ATCC 11229, ATCC 8739 and ATCC 23716 K12 wildtype) and two strains of *E. faecalis* (ATCC 19443 and ATCC 27285) were used and compared in this study. They were acquired from the Spanish Type Culture Collection (CECT) in a lyophilized format. The organisms were reactivated in recommended culture medium, i.e. Tryptic Soy Broth (Scharlab, Barcelona, Spain) for *E. coli* and Brain Heart Infusion Broth (Scharlab) for *E. faecalis* and incubated for 24 h at 37 °C. Aliquots of 1 mL were subsequently subcultured into fresh medium and again incubated for 24 h. The resulting cultures were placed into Eppendorf vials and centrifugated at 3000 rpm for 10 min; the supernatant was discarded, and 1 mL of glycerol-water 50:50 was added to every vial. Pellets were stirred, resuspended, and subsequently stored in a freezer at −20 °C.

For preparing the experimental inoculum, one vial with the bacterial suspension was taken from the freezer and reactivated following the same described protocol with a minimum of one and a maximum of three sub-culturing steps. Upon centrifugation, the pellet was resuspended in 50 mL of distilled water with an added 1.5 mL per liter of phosphate buffer at pH 7.20. The bacterial inoculum was combined with 20 L of buffered distilled water in the plastic deposit attached to the experimental rig (see Section 2.2 for experimental setup). The organisms acclimated for 40 min after which the bacterial concentration was between $1.3 \cdot 10^6$ and $2.6 \cdot 10^6$ CFU mL^{-1} except for *E. coli* ATCC 11229 with a mean concentration of $3.4 \cdot 10^5$ CFU mL^{-1}.

After the treatment, samples were subjected to membrane filtration [30], agar plating, and incubation procedures in order to determine the bacterial concentration. Depending on the UV dose that was applied, volumes between 10^{-5} and 100 mL were filtered through cellulose acetate membrane with a pore size of 0.45 μm (Pall Corporation, NY, USA). Volumes below 1 mL were achieved by ten-fold dilutions of the original sample. Membranes were plated in Petri plates with specific chromogenic agar, i.e. Microinstant® Colinstant Chromogenic Agar (Scharlab) for *E. coli* and Slanetz and Bartley Agar (Scharlab) with a TTC indicator for *E. faecalis*. These were incubated at 37 °C for 24 h for the *E. coli* and for 48 h in the case of *E. faecalis*. Colony forming units were counted after the incubation and divided between the filtered volume for determining the bacterial concentration.

2.2. UV reactor description and experimental procedure

UV treatment was applied by means of a flow-through reactor (Photolab LED275–0.4c, APRIA Systems S.L., Cantabria Spain) mounted onto a laboratory experimental rig, which consisted of a 20 L plastic deposit, a peristaltic pump, and the reactor. The reactor was equipped with 40 LEDs with 10.5 mW, and emission was between 265 and 275 nm (UVC) with a maximum of 275 nm. LEDs were distributed in four rows along a square-section structure that was placed longitudinally at the center of the reactor. They were separated from the target water by a quartz sleeve, forming an annular configuration with an irradiated water volume of 0.36 L. The UV dose that was applied to the samples was calculated as the product of the mean intensity (I_m) and the exposure time. According to previous biodosimetry and actinometry experiments, the mean intensity (I_m) provided by the reactor with 100 % power was 0.86 mW cm^{-2} for target water with 275 nm transmittance of 95 % [31]. The optimum range of flow rates was from 55 L h^{-1} (exposure time of 23.6 s and UV dose of 20.3 mJ cm^{-2}) to 230 L h^{-1} (exposure time of 5.7 s and UV dose of 4.9 mJ cm^{-2}). To achieve UV doses below this value, the power of the LEDs was reduced to a minimum of 20 %.

The UV treatment was applied in an experimental series of samples collected at various flow rates and thus subjected to different UV doses. Three experimental series were performed for each bacterial strain. The samples in the same experimental series came from the same 20 L of inoculated water. Prior to the experiments, the experimental rig was cleaned with water to which hypochlorite was added, and it was subsequently rinsed with tap water. For the treatment application, the UV lamp was switched on, and the target water was pumped at a flow rate below 55 L h^{-1}. When the hydraulic system was full of water and the outlet flow was stable, the flow rate was increased to a minimum of 55 L h^{-1}, and its exact value was measured with a cylinder and a timer. Once measured the flow rate, a minimum volume of 2 L of treated water was

wasted to ensure that the taken sample was not mixed with previous samples or rinse water. At this moment, the sample was taken at the reactor outlet. Higher UV doses were first applied in order to avoid contamination of the tubing following the reactor which could affect the subsequent samples. After taking the sample, the flow rate was increased, and the procedure was repeated. When all of the treated samples were taken, the UV lamp was turned off and the control was taken at the same flow rate as the last treated sample. The 20 L of target water allowed taking five treated samples and the control. Three samples of 250 mL were taken in borosilicate flasks for each applied UV dose; each flask was subjected to a different analysis. One of the flasks was immediately subjected to membrane filtration and incubation for determining the bacterial concentration. The other two flasks, one of them covered with aluminum foil (dark conditions), were incubated for 24 h in a climate room at 20 °C under white light 36 μEinstein m^{-2} s^{-1} for 24 h. After the incubation, samples were subjected to membrane filtration and incubation procedures in order to determine the bacterial concentration.

2.3. Data treatment

The survival in every sample was obtained as the quotient between the bacterial concentration in that sample and the mean concentration of the control on the day of the experiment (day 0) from the same experimental series. Survival data from the three experimental series from one determined strain and one determined post-treatment condition (i.e. 0 days, 1 day under light, and 1 day in dark) were merged to obtain the inactivation curve represented as Log (*S*) with respect to the UV dose that was applied. Inactivation curves were then fitted to inactivation kinetic models using the GInaFiT tool for MS Excel [32]; additionally, *p*-values for the different model parameters were determined using the SigmaPlot (v.11.0) software. The inactivation kinetics parameters were utilized for calculating the UV doses necessary to achieve "n" log-reductions of the initial concentration (D_n). Values of D_n were employed as a comparative parameter for the treatment efficacy among the different bacterial strains and post-treatment conditions.

The percentage of photoreactivation (*P*) was calculated for each bacterial strain that was tested according to Eq. (1) in which N_{pr} is the bacteria concentration in samples incubated for 24 h under illumination, *N* is the bacteria concentration in samples measured immediately after the UV irradiation, and N_0 is the initial bacterial concentration [33].

$$P = 100 \cdot \frac{N_{pr} - N}{N_0 - N} \quad (1)$$

For determining the existence of significant differences between bacterial strains and post-treatment conditions, the data corresponding to the inactivation curves were subjected to a two-way ANCOVA analysis (Statgraphics Centurion XVI, ver. 16.1.03) using Log (*S*) as the dependent variable, UV dose as the covariate, and the bacterial strain and post-treatment conditions as qualitative factors. Similarly, the percentage of photoreactivation was subjected to the same analysis using *P* as the dependent variable, the UV dose as a covariate, and the bacterial strain as a qualitative factor.

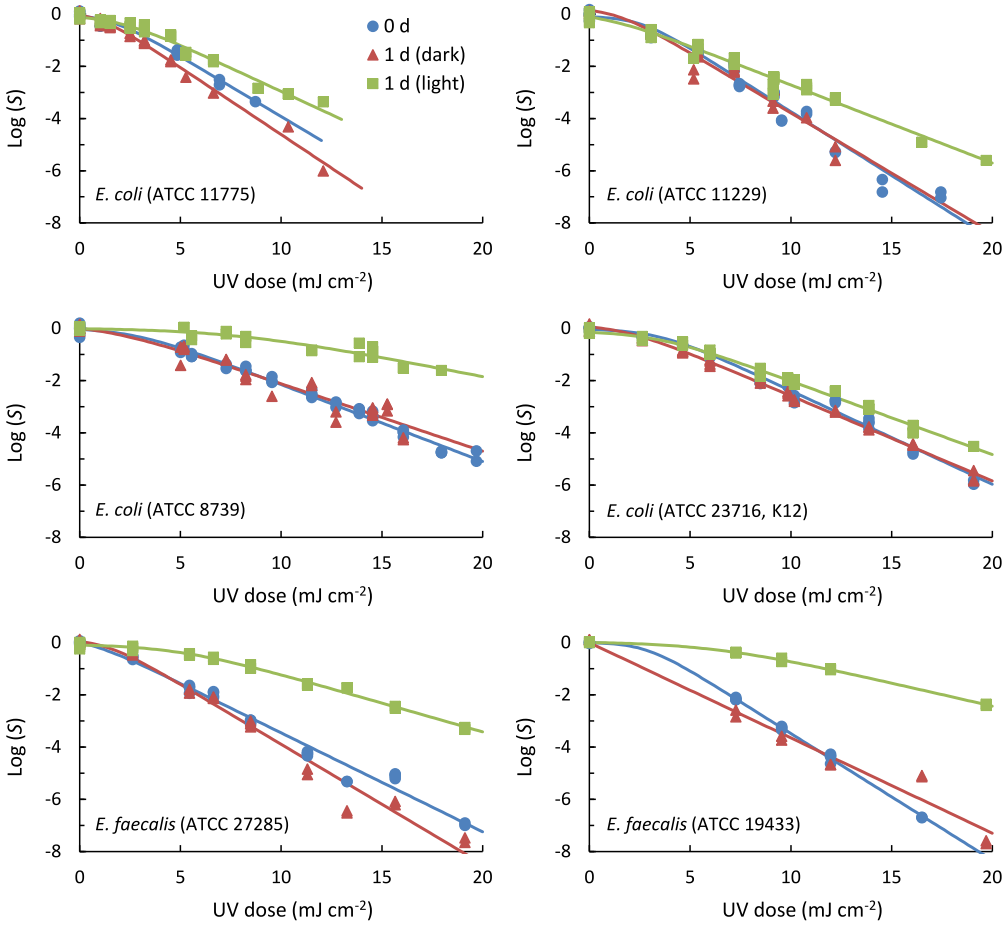

Fig. 1. Inactivation curves for the treatment of the bacterial strains that were tested and subjected to different post-treatment conditions. Symbols represent the experimental Log (*S*) data, and lines represent the log-linear + shoulder model fitted.

3. Results

3.1. Features and comparison of the inactivation curves

The inactivation curves obtained in this study depicted a shoulder region with a low UV dose, and a tail was absent in the experimental range of UV doses (Fig. 1); therefore, the inactivation curves accorded with a log-linear + shoulder model (Eq. (2) in which *S*: survival at a given UV dose; S_0: survival in absence of UV irradiation; *SL*: shoulder length; *k*: inactivation rate; *D*: UV dose).

$$S = S_0 \cdot \frac{e^{-k \cdot D} \cdot e^{k \cdot SL}}{1 + e^{-k \cdot D} \cdot (e^{k \cdot SL} - 1)} \quad (2)$$

The inactivation achieved with 24 h of incubation in darkness was similar to that achieved without incubation, indicating that the dark repair is practically negligible. On the contrary, the inactivation achieved after 24 h incubation in light conditions was lower than that without incubation in all of the cases which reveals the importance of the photorepair in these bacterial strains. The inactivation curves also revealed differences between the UV resistance and the effect by illuminated post-treatment conditions in the different strains of *E. coli* according to the level of inactivation achieved for the various UV doses that were applied. Inactivation curves data were analyzed using a two-way ANCOVA with one interaction term using Log (*S*) as the dependent variable, UV dose as covariate, and bacterial strain and dark post-treatment condition as factors with one interaction term for both factors. The analysis indicated that the UV dose, strain, and post-treatment condition as well as the interaction between the strain and post-treatment condition were significant in the determination of Log (*S*) ($p < 0.001$). The same analysis divided the tested strains into four homogenous groups (Table 1). Groups a and b contained the strains of *E. coli* ATCC 11775 and ATCC 11229 as well as the two strains of *E. faecalis*. The two other strains of *E. coli*, ATCC 8739 and ATCC 23716 were each distributed in the two homogenous groups of c and d. The analysis divided the post-treatment conditions into two groups. Group a' contained the absence of incubation and incubation for 24 h in darkness whereas group b' contained the incubation for 24 h under the light. These results confirm the existence of more intraspecific than interspecific differences in terms of their UV resistance and photoreactivation features as well as the negligibility of the dark repair and the significance of the photoreactivation.

3.2. Analysis of the inactivation kinetics parameters

The inactivation curves were fitted according to the log-linear + shoulder model. The inactivation kinetics parameters that were obtained allowed quantitatively evaluating the UV treatment efficacy and the importance of the reactivation processes. The Log (S_0) indicates the survival of the organisms in absence of UV irradiation; for incubated samples, the Log (S_0) quantifies the impact of the incubation on the bacterial survival. The values of the Log (S_0) were not significant (*p*-value >0.05) in all but three of the cases (Fig. 2 - top). The three significant values of the Log (*S*) were obtained with 24 h incubations under light for the strains of *E. coli* ATCC 11775 (-0.12 ± 0.05; *p*-value = 0.034) and ATCC 23716 (-0.15 ± 0.06; p-value = 0.015) and the strain of *E. faecalis* ATCC 27285 (-0.09 ± 0.03; p-value = 0.004). According to these Log (S_0) values, the maximum significant mortality due to the incubation was 29 % of the organisms whereas the UV irradiation reached several log-reductions; this implies that the mortality due to the incubation was negligible in comparison with the inactivation due to the UV treatment. The parameter *SL* indicates the section of the curve in which the inactivation rate is below the maximum value. In general, the *SL* increases in the case of 24 h incubation under light, implying a reduction of the treatment efficacy. Finally, the value of *k* indicates the inactivation rate once the UV dose exceeds the shoulder phase. The 24 h

Table 1
Two-way ANCOVA for the inactivation curves using Log (*S*) as the dependent variable, UV dose as covariate, and bacterial strain and dark post-treatment conditions as qualitative factors. "n" indicates the number of observations in each case.

	n	Mean LS	Sigma LS	Groups
Strain ($p < 0.001$)				
E. coli (ATCC 11229)	90	−2.394	0.0651	a
E. coli (ATCC 11775)	72	−2.289	0.0783	ab
E. faecalis (ATCC 27285)	92	−2.183	0.0637	b
E. faecalis (ATCC 19433)	43	−2.054	0.0955	b
E. coli (ATCC 23716)	103	−1.719	0.0600	c
E. coli (ATCC 8739)	106	−1.058	0.0626	d
Dark post-treatment ($p < 0.001$)				
0 d	190	−2.310	0.0479	a'
1 d (dark)	155	−2.363	0.0518	a'
1 d (light)	161	−1.176	0.0510	b'

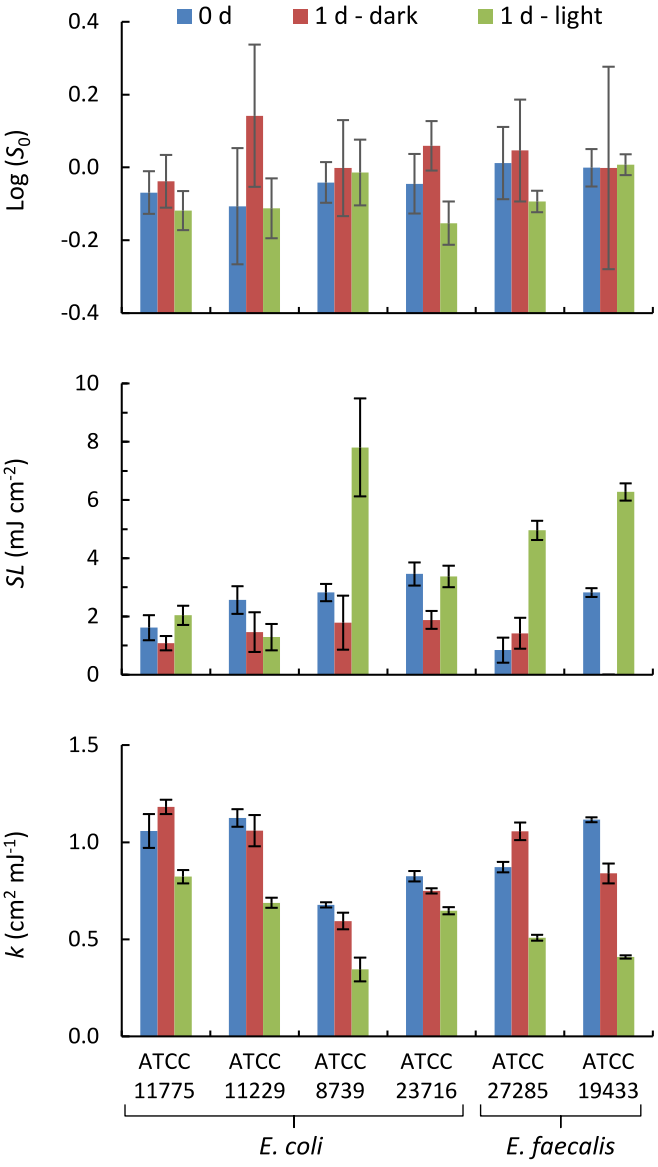

Fig. 2. Inactivation kinetics parameters obtained by fitting the inactivation curves with a log-linear + shoulder model. S_0: survival at UV dose equal to 0 mJ cm^{-2}. *SL*: shoulder length. *k*: inactivation rate at UV doses beyond the shoulder phase. Error bars represent the standard error calculated by GInaFit.

incubation in dark conditions did not cause relevant changes in k values with respect to the samples without incubations whereas the incubation under light decreased the k in all cases (Fig. 2 – bottom).

The UV resistance and reactivation features for each bacterial strain that was tested were examined according to the UV dose required to achieve four log-reductions from the initial concentration (D_4). This was calculated using the three inactivation kinetics parameters obtained in the log-linear + shoulder model fitting (Table 2). According to the D_4 values, the most UV-resistant strains were *E. coli* ATCC 8739 and *E. coli* ATCC 23716. The remainder of the *E. coli* and *E. faecalis* strains reported similar UV resistance. In general, with one exception, the D_4 values decreased slightly in the samples that were incubated for 24 h in the dark, although with minimal differences with regards to the samples that were not incubated. Conversely, the D_4 increased considerably if the samples were exposed to the light after the UV treatment due to photoreactivation. A D_4 increase was especially relevant in both *E. faecalis* strains and *E. coli* ATCC 8739 whereas the strain that was less affected by the photoreactivation was *E. coli* ATCC 23716.

3.3. Percentage of photoreactivation of the different bacterial strains

The percentage of photoreactivation (P) decreased according to the UV dose that was applied (Fig. 3). The maximum percentage of detected photoreactivation was 48 % and was reached by *E. faecalis* ATCC 27285 treated with 2.7 mJ cm $^{-2}$. The two-way ANCOVA analysis applied to the Log (P) data with the UV dose as the covariate and the bacterial strain as the qualitative factor divided the six bacterial strains into three groups (Table 3). Three species of *E. coli* (ATCC 11229, ATCC 11775, and ATCC 23716) were classified as similar in terms of dependence of percentage of reactivation with respect to the UV dose that was applied. Both strains of *E. faecalis* were classified as a different group with respect to the three mentioned *E. coli* strains. The remaining strain of *E. coli* (ATCC 8739) was more similar to *E. faecalis* than to the other *E. coli* strains. P decreased with the applied UV dose according to a log-linear relationship ($a \cdot e^{-b \cdot D}$). The UV dose required limiting the photoreactivation into the 1 % ranged from 6.5 up to 23.2 mJ cm^{-2}, obtained for *E. coli* ATCC 11229 and *E. coli* ATCC 8739, respectively. Once four log-reductions from the initial bacterial concentration were achieved, P varied between the 0.05 % obtained for *E. coli* ATCC 23716 and the 12.3 % determined for *E. faecalis* ATCC 19433.

4. Discussion

4.1. Inactivation kinetics

The inactivation curves obtained in this study depicted a shoulder region at a low UV dose, and a tail was absent in the experimental range of UV doses (Fig. 1). Although the inactivation curves are based on the classic model [34] in which the Log (S) decreases linearly with the UV dose that is applied, the appearance of variants such as "shouldering" and "tailing" are frequent in disinfection procedures [35]. The causes of both phenomena are a matter of debate [21]. Shouldering in the inactivation curves (Fig. 1) corresponds to a lower inactivation rate with low UV doses that is attributed to multitarget kinetics and the need of cumulative damage to inactivate the cell [36,37]. Studies [31] found that the inactivation curves for *E. coli* ATCC 8739 were similar for a treatment applied with a collimated beam reactor with lower intensity and longer exposure times and a flow-through reactor that applies higher intensity in shorter exposure times at a wavelength of 275 nm. However, the inactivation curve for *E. faecalis* showed a longer shoulder when treated with the collimated beam reactor. This fact may be relevant in the design and optimization of UV reactors. On the other hand, the causes of tailing are stated as the presence of a resistant subpopulation, hydraulic issues in treatment with recirculation and shielding by suspended solids and flocs as well as the achievement of inactivation levels that are close to the detection limit [21,38–42]. In previous studies using *E. coli* ATCC 11229 and *E. faecalis* ATCC 27285 in buffered distilled water and irradiation with conventional low-pressure mercury lamps, the occurrence of shouldering and tailing depended on the UV device that was used. Inactivation curves obtained using a collimated beam reactor lacked a shoulder but depicted tailing whereas the curves obtained using a flow-through reactor showed a shoulder, and tailing was not observed in the experimental range of UV doses up to 30 mJ cm^{-2} [26,43]. Shoulders and tails have also been observed using UV-LED treatment on *E. coli* [17,18]. The relationship between UV intensity and exposure time also defined the inactivating efficacy by the

Table 2
UV doses required to achieve 4 log-reductions (D_4) for the different bacterial strains and post-treatment conditions. Percentages indicate the increasing of the UV dose that was required to achieve and maintain 4 log-reductions after an incubation of 24 h under light or dark conditions after the UV irradiation taking samples without incubation as a control.

Organism	D_4 (mJ cm^{-2})		
	0 d	1 d - dark	1 d - light
E. coli (ATCC 11775)	10.2 ± 1.3	8.8 ± 0.6 (−13 %)	12.9 ± 1.0 (27 %)
E. coli (ATCC 11229)	10.5 ± 1.2	10.5 ± 1.9 (−1 %)	14.3 ± 1.2 (36 %)
E. coli (ATCC 8739)	16.3 ± 0.8	17.3 ± 2.7 (6 %)	34.4 ± 8.2 (111 %)
E. coli (ATCC 23716, K12)	14.5 ± 1.0	14.3 ± 0.7 (−1 %)	17.0 ± 1.0 (18 %)
E. faecalis (ATCC 27285)	11.4 ± 1.0	10.2 ± 1.2 (−10 %)	22.6 ± 1.0 (98 %)
E. faecalis (ATCC 19433)	11.1 ± 0.4	11.0 ± 1.5 (−1 %)	28.8 ± 0.9 (160 %)

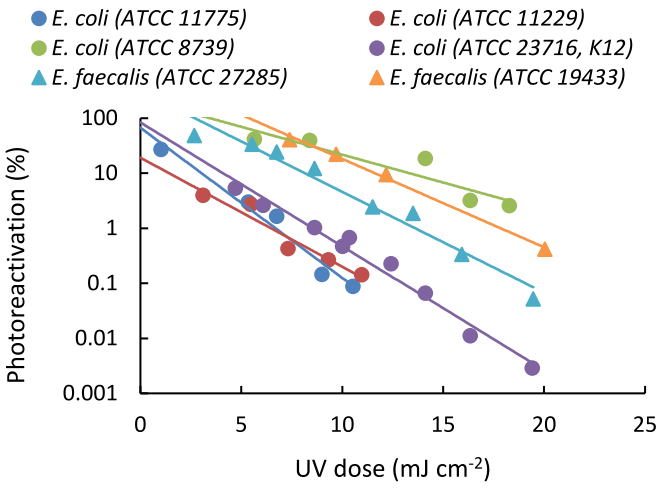

Fig. 3. Relationship between the percentage of photoreactivation and the UV dose for the different tested bacterial strains.

Table 3
Parameters corresponding to the log-linear relationship between the percentage of photoreactivation (P) and the applied UV dose ($P = a \cdot e^{-b \cdot D}$), values of P calculated at the UV dose required to achieve four log-reductions, and homogenous groups reported in the two-way ANCOVA analysis.

Strain	a	b (cm^2 mJ^{-1})	R^2	P (D_4) (%)	Group
E. coli (ATCC 11229)	19.2	0.456	0.935	0.16	a
E. coli (ATCC 11775)	67.3	0.628	0.970	0.11	a
E. coli (ATCC 23716, K12)	83.8	0.518	0.979	0.05	a
E. faecalis (ATCC 27285)	311.2	0.422	0.966	2.50	b
E. faecalis (ATCC 19433)	731.5	0.369	0.995	12.30	bc
E. coli (ATCC 8739)	223.5	0.233	0.856	5.04	c

treatment for one determined dose [23]. In this context, the cause of the shouldering and absence of tailing within the experimental range of UV doses can be attributed to the features of the UV device used for the treatment, such as hydraulics and UV intensity distribution.

4.2. UV resistance in the different bacterial strains

The results that were obtained revealed significant differences in the UV-resistance between the different strains of *E. coli*; in some of them, their resistance was more similar to *E. faecalis* than to the other strains of the same species. The UV-resistance has traditionally been attributed to morphological and physiological bacterial features. In the case of Gram-positive bacteria, the conventional thought is that the structure of the outer membrane behaves as a shield against the UV radiation and provides higher UV-resistance with respect to the Gram-negative bacteria [19,44,45]. The differences in UV-resistance between different strains of the same species can be due to the presence or absence of RecA protein [46], the total amount of genetic material with respect to the other strains [47], and the amount of enzymes produced to protect them from oxidative stress [48]. Additionally, the UV resistance is dependent on culturing and experimental conditions such as the growth phase of the irradiated organisms [49], the pH of the acid medium [50], and the UV device that is used [5,19,23]. These facts imply the necessity of comparing similar strains under identical experimental conditions to suitably assess the UV treatment efficacy. The six bacterial strains tested in this study were subjected to the same experimental procedure; therefore, the differences between them are attributed to features of the proper strain.

The Type *E. coli* strain ATCC 11775 and the strain ATCC 11229 that are commonly used as indicators in disinfection studies were both the most sensitive strains. On the contrary, the strain of *E. coli* ATCC 23716, also known as K12 wildtype, had relatively greater UV-resistance, and the strain of *E. coli* ATCC 8739 demonstrated the greatest resistance to the UV treatment with respect to the rest of the *E. coli* strains. The UV-resistance in *E. coli* ATCC 23716 is well known and attributed to the presence and expression of the RecA gen and the lower amount of genetic material [47,51,52]. On the other hand, the strain *E. coli* ATCC 8739 is endorsed in different studies as a surrogate or indicator organism for evaluating the UV treatment on bacteria due to its high resistance to the UV light at 254 nm [53–55]. The results obtained in this study regarding UV-resistance and photorepair potential confirm the suitability of *E. coli* ATCC 8739 for providing conservative estimations of the inactivation with LEDs emitting at 275 nm.

Both strains of *E. faecalis* showed relatively high sensitivity to the irradiation at 275 nm. The values of the D_4 obtained in this study using UV-C LEDs for irradiation were 10.2 mJ cm^{-2} for *E. coli* ATCC 11229 and 11.4 mJ cm^{-2} for *E. faecalis* ATCC 27285. However, previous experimentation using similar strains, procedures, and conditions but utilizing a conventional LP-Hg UV-C lamp reported D_4 values of 17.9 mJ cm^{-2} for *E. coli* ATCC 11229 and 31.2 mJ cm^{-2} for *E. faecalis* ATCC 27285 [26]. The comparison between both studies revealed a reduction of the D_4 values and thus greater inactivating efficacy with similar UV doses using UV-C LEDs with respect to the LP-Hg UV-C lamp. Additionally, the UV-resistance of *E. coli* and *E. faecalis* was more similar between them when they were treated with LEDs emitting in the range 265–285 nm whereas the UV-resistance of *E. faecalis* is considerably higher if the treatment is applied with conventional mercury UV lamps [21,26]. This similarity in the UV-resistance by *E. coli* and *E. faecalis* treated with UV-LEDs supports the fact that irradiation at 275 nm involves damaging other cell structures in addition to the DNA damage. Recent studies revealed that irradiation at 265 and 285 nm do not significantly affect the cell membrane integrity [56]. However, aromatic amino acids such as Tryptophan and Tyrosine have a peak absorption at approximately 280 nm [57,58]; therefore, the irradiation in this wavelength range can excite and destroy the proteins [59]. In this context, the irradiation at different wavelengths effectuates different damaging mechanisms that may change the established outlook on the bacteria UV-resistance.

4.3. Implications of the repairing mechanisms in the UV treatment of waterborne bacteria

The results that were obtained showed that the treatment with LEDs emitting in the range between 265 and 285 nm did not prevent the bacteria from repairing part of the damage received if the bacteria were exposed to the light after the treatment. The percentage of photoreactivation was dependent on the UV dose that was applied with those between 6.5 and 23.2 mJ cm^{-2} repairing 1 % of the organisms in accordance with the value of approximately 11 mJ cm^{-2} obtained for natural *E. coli* irradiated with low-pressure mercury lamps [33]. However, it is stated in different studies that the irradiation with LEDs emitting at a wavelength closer to 285 nm reduces the photoreactivation percentage with respect to a wavelength that is more approximate to 254 nm [17,18,60]. In this study, the irradiated samples were exposed to the light in a culture chamber for maximizing the possibilities of photorepair. However, the photoreactivation rate depends on the environment in which the irradiated water is stored [61,62]; in the case of storing in sunlight, there is a compromise between the photoreactivation and the further inactivation due to the UV-A and UV-B radiation, respectively [63]. There was an absence of dark repair within the 24 h following the UV irradiation, which is similar to previous studies [18,60]. These facts substantiate the need of considering the destination of the water once it is treated which requires applying increased UV doses for maintaining the achievement of 4 log-reductions if the treated water is exposed to an illuminated environment upon the irradiation (Table 2). In this context, the bacterial strains contained in the treated water define the increase on the UV dose, requiring applying a UV dose 160 % higher in the most extreme case which corresponds to the strain *E. faecalis* ATCC 19433.

The evolution of the treated bacteria once it is released into the environment depends on a series of processes, i.e. reproduction, reactivation, and repair [64]. Reproduction is defined as the increase in bacterial population due to their ability to reproduce. This fact was not observed in the samples stored in darkness for 24 h. The reproduction of fecal bacteria in a nonhost environment depends on factors such as the availability of nutrients and the temperature, and this is not frequent in most environmental conditions [65]. Reactivation refers to the viable but non-culturable (VBNC) organisms that become fully viable; in this context, the absence of increasing the number of culturable cells in the dark-stored samples indicated a lack of reactivation within 24 h following the treatment. Repair refers to the processes of DNA damage reversal such as photoreactivation and dark repair. The results obtained in this study indicated that the dark repair was not relevant within the 24 h following the treatment whereas the photoreactivation was dependent on the bacterial strain and the UV dose that was applied. This should be considered if the treated water is exposed to an illuminated environment upon the UV irradiation. However, it is known that a delay of 3 h in the light exposure after the UV irradiation noticeably reduces the photoreactivation fractions [29]. Therefore, this dark delay can be introduced as part of the water treatment for preventing risks due to the photoreactivation.

The significant differences between the bacterial strains of *E. coli* regarding their UV-resistance and photoreactivation features imply that the selection of an appropriate indicator organism is critical for obtaining suitable and useful information in the research and trials of UV disinfection devices. In practical terms, the properties of the UV light source and the post-treatment condition of the target water are two important factors in the choice of the proper test organism. An appropriate indicator should have high UV-resistance to ensure that its inactivation entails the inactivation of weaker organisms; in parallel, the indicator organism should have high photoreactivation potential in the event that the treated water will be exposed to environmental light upon treatment. Whereas the organisms *E. faecalis* are a suitable indicator for

the conventional Hg lamps due to the higher resistance with respect to *E. coli* [21], it was observed that the differences in their UV-resistance are reduced by using LEDs emitting in the 265–285 nm range. In the context of LED-UV disinfection, the strain of *E. coli* ATCC 8739 is a suitable indicator since it requires higher UV doses for its inactivation (Table 2) in comparison to the rest of *E. coli* and *E. faecalis* strains.

5. Conclusions

In this study, four strains of *E. coli* and two strains of *E. faecalis* were subjected to UV irradiation using a flow-through reactor equipped with UV-C LEDs emitting between 265 and 285 nm. The objective was to compare their UV-resistance and determine their potential for UV damage repair.

The inactivation curves were fitted with log-linear + shoulder kinetics which implies a lower inactivation rate at low UV doses; tailing was absent in the range of UV doses up to 20 mJ cm^{-2}, reaching inactivation levels greater than six log-reductions without a loss of efficacy. The determination of the viable cell concentration upon the UV irradiation indicated the existence of significant differences in the UV resistance of the bacterial strains that were tested with intraspecific differences that prevail over those that are interspecific. According to the comparison of the data obtained in this study with previous studies using the same bacterial strains and experimental procedures, the UV treatment with LEDs emitting between 265 and 285 nm was more effective than the treatment with conventional mercury UV-C lamps for the same applied doses. In addition, the greater UV-resistance in *E. faecalis* with respect to *E. coli* that is traditionally observed in the treatment with the mercury UV-C lamps was reduced by the use of an LED UV source.

The obtained results indicated that the dark repair was negligible within 24 h following the UV irradiation whereas the photoreactivation was relevant and dependent on the bacterial strain that was tested and the UV dose that was applied. Regarding the photoreactivation potential, noticeable differences were observed between the tested strains with *E. coli* ATCC 23716 (K12) showing the lowest percentage of photoreactivation and *E. faecalis* ATCC 19433 showing the greatest. In practice, the photoreactivation implies the necessity of applying greater UV doses to maintain the inactivation level that was achieved with the irradiation in the event that the treated water will be exposed to an illuminated environment. In terms of the UV dose required to achieve four log-reductions, the strain *E. coli* ATCC 8739 was the most resistant strain when the treated water was exposed to either an illuminated or a dark environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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