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Multivariate optimization of fecal bioindicator inactivation by coupling UV-A and UV-C LEDs

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ABSTRACT

The development of new technologies for water recycling is a priority for arid and semi-arid countries such as those of the Mediterranean basin. The aim of this study was to test the efficiency of UV-A and UV-C light emitting diodes (UV-LEDs) on bacteria inactivation. We used *Escherichia coli* and *Enterococcus faecalis*, bioindicators of fecal pollution typically found in urban wastewaters. An experimental design was performed to discriminate weight of factors influencing bacteria inactivation yields and reactivation phenomena. Four parameters were tested on simple bacterial cultures: pH, bacterial density, exposure time and wavelength. It appears that the exposure time and wavelength used have a significant effect on the response. The 280/365 nm or 280/405 nm coupled wavelengths, have the most important bactericidal effect, and we also note the absence of bacterial reactivation after 60 s of exposure to UV.

1. Introduction

Continuous population growth and economic development increase the water demand and encourage governments to look for alternative water sources, such as reuse and recycling of wastewaters, especially for irrigation purposes [5, 23, 24]. Moreover, implementation of more stringent regulations about quality of wastewaters discharged into the aquatic environment (as the European Directive “concerning the management of bathing water quality”, 2006) implies design of more sophisticated or refining treatments. Consequently, developing new technologies for recycling wastewaters or treating raw waters is a priority for many countries, especially in relation to microbiological criteria.

Use of ultraviolet (UV) light has become an alternative to chlorine and other chemical disinfectants and has proven to be an efficient technique to eliminate many potentially pathogenic microorganisms, even in case of turbid waters [12, 13, 15, 18]. Indeed, UV radiations are known to cause damage on DNA, preventing replication, transcription and thus, indirectly, translation.

However, many microorganisms can repair DNA damages caused by UV radiation via enzymatic reactions. Two different mechanisms are distinguished depending on light availability: photoreactivation and dark-repair [19, 20, 25]. Photoreactivation is a natural process

in which bacterial cells can partially recover from ultraviolet damage when visible and UV wavelengths of light reverse DNA damage by monomerizing cyclobutane pyrimidine dimers. In this case, repair is due to an enzyme called photolyase. In dark repair the damage is reversed by the action of a number of different enzymes. All of these enzymes must initially be activated by an energy source, which may be visible light (300–500 nm) or nutrients that exist within the cell. [16] suggests that the enzymatic repair mechanism requires at least two enzyme systems: an exonuclease systems (i.e. to disrupt the thymine–thymine linkage), and a polymerase system to reinsert the thymine bases on the adenosine sites of the complementary strand of DNA.

The majority of UV disinfection systems use low or medium-pressure mercury lamps, first ones emitting predominantly monochromatic UV radiation at a wavelength of 254 nm and second ones emitting polychromatic UV radiation over the wavelength range from 200 to 400 nm. However, mercury vapor lamps have many disadvantages: large size, low resistance to shock and the energy required to operate. Furthermore, these lamps have a short lifespan of approximately 4000–10,000 h and contain mercury, a major environmental contaminant. On the other hand, the use of LEDs to produce UV radiations has many advantages. LEDs are very compact, shock-resistant, do not require much energy to operate and their lifetime exceeds 100,000 h [6]. In addition, LEDs do not contain toxic substances or pollutants: the materials typically used are gallium aluminum nitride (AlGaIn) and aluminum nitride (AlN), which are not toxic [14]. Furthermore,

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LEDs use electricity more efficiently, producing little heat. Last but not least, LEDs present the advantage upon lamps to be compact light sources and to produce UV at a one single wavelength, allowing thus to design UV disinfection systems with optimized numbers and wavelengths LED as a function of microorganisms to be inactivated.

Few studies have been conducted so far on water disinfection by UV-LED technology: [17] have tested the efficiency of commercially available UV-LEDs emitting at 365 nm and at 405 nm, [9] have tested high-UV LED emitting at 365 nm and [26] have shown that UV-LED irradiation was efficient to reduce *Escherichia coli* concentration using 269 nm. Until now, these studies have not been conducted with the objective to optimize choice of wavelengths, numbers of LEDs or even time of irradiation.

This preliminary study aims to develop a UV-LED-based protocol under in vitro conditions, by testing the effect of different factors on UV disinfection efficiency. We combine different parameters: bacterial density (10^5 or 10^7 cfu/mL), pH (6 and 8), UV exposure time (10, 20, 30, 60, 120 and 180 s), and wavelengths (254, 365, 280, 405 nm). Wavelengths 254 and 280 nm (UV-C) are potentially the most efficient to eliminate bacteria since they are close to the DNA maximum absorption rate and are responsible for the formation of cyclobutane pyrimidine dimers [9]. Wavelengths 365 and 405 nm are included in the UV-A range which is known to induce the formation of active substances having lethal effects [22], i.e. leading to the formation of oxidative DNA damage [9]. All these wavelengths were tested independently and also coupled (UV-C/UV-A) as follows: 254/365 nm; 280/405 nm and 280/365 nm. The study was performed according to an experimental design. The effectiveness of UV treatment was tested on *E. coli* and *Enterococcus faecalis*, fecal indicator bacteria commonly found in wastewater effluents. The bacterial reduction must reach at least 7 log to be considered as an efficient result in wastewater treatment [27]. To highlight potential different responses within the same species and between species, three strains of *E. coli* (American Type Culture Collection (ATCC) 11303 and ATCC 15597 [4] and Collection of Institut Pasteur (CIP) 6224) and two strains of *E. faecalis* (ATCC 19433 and ATCC 33186) were used. Moreover, specific attention was focused on the reactivation ability of selected microorganisms.

2. Materials and methods

2.1. Bacterial strains and growth conditions

To test the inter-specific variability, three strains of *E. coli* (ATCC 11303, ATCC 15597 (LGC Standards) and CIP 6224) and two strains of *E. faecalis* (ATCC 19433 and ATCC 33186) were cultivated for 3 h (exponential growth phase) on a nutrient broth medium. Cultures were centrifuged for 15 min at 10,000 rpm (Sorvall®, Evolution RC) and pellets were resuspended in either pH6 phosphate buffer or in pH8 tris(hydroxymethyl)aminomethane buffer. Their optical density (600 nm) was adjusted to 0.100 for *E. coli* and 0.050 for *E. faecalis* (10^7 cfu/mL) using the appropriate buffer for dilution. Buffered solutions containing 10^5 cfu/mL were obtained from dilutions of 10^7 cfu/mL solutions.

2.2. Experimental setup

Four LEDs (manufactured by Seoul Optodevice Co., Ltd., Korea) emitting at 255, 280, 365 or 405 nm respectively were used throughout experiments. These LEDs could be used together or separately and the electronic circuit was connected to a power supply (TTI EL302R Power Supply).

These four LEDs were used with their maximal amperage, as presented in Table 1, along with the other physical properties of these LEDs. Distance between the LEDs and the bacterial cultures was set at 1 cm (according to standard ÖNORM M5873-1). A scheme of the

Table 1

Optical and physical properties of UV-LED used during inactivation experiments (data provided by LED suppliers).

Peak wavelength (nm)	Packaging	Optical power (mW)	Viewing angle 2θ 1/2 (degree)	Maximum current intensity (mA)
255	TO-39	0.125	120	115
280	TO-39	0.55	120	77
365	P8	350	110	22
405	P8	210	130	46

experimental device is shown in Fig. 1. Bacterial cultures (10 mL) were placed in Petri dishes (55 mm diameter) and exposed to each wavelength or wavelengths coupling and for each exposure time. Cultures were homogenized using a magnetic stirrer during exposure.

2.3. Enumeration of bacteria

After irradiation by the UV-LED system, 1 mL of each bacterial suspension was diluted as necessary with a factor of ten and plated on Tryptone Soy Agar (TSA) medium. Cultures were incubated for 24 h at 37 °C and bacteria were then counted. Irradiated bacterial solutions were kept for 20 h at room temperature and in normal room lighting, and then diluted as necessary and plated on TSA medium to assess bacteria reactivation.

2.4. Experimental design and data analysis

As previously mentioned, different parameters are potentially influential on UV disinfection efficiency. On microorganisms, the various factors studied with the tested values were respectively 10^5 and 10^7 cfu/mL for the density of microorganisms; 6 and 8 for pH; 60, 120 and 180 s as exposure time; and 254, 280, 365, 405, 254/365, 280/365 and 280/405 nm as tested wavelengths.

In a first step, a screening study has been performed in order to select factors that are probably active and to get a preliminary idea as to their effects. Instead of a traditional “one factor at a time” approach, the experiments were carried out using an experimental design [3] for setting up experiments in such a manner that the required information was obtained as efficiently and precisely as possible.

A screening study allows the direct comparison of two or more values and the postulated mathematical model is simply additive. The reduced reference state model used in a screening design for 2 variables with 2 levels, 1 variable with 3 levels and 1 variable with 7 levels is the following:

$$\text{Disappearance\%} = a_0 + a_1X_{1A} + b_1X_{2A} + g_1X_{3A} + g_2X_{3B} + d_1X_{4A} + d_2X_{4B} + d_3X_{4C} + d_4X_{4D} + d_5X_{4E} + b_6X_{4F}$$

with $X_i = 0$ or 1 (presence-absence variables) in function of the level present for the four factors.

The coefficients $\alpha_1, \dots, \beta_1, \gamma_1, \gamma_2, \dots$ define the differential effect on the disappearance percentage of replacing a level (considering as reference state) by another level.

To optimize coefficients estimation of the model, an optimal design of experiments [1, 7] has been chosen: this asymmetrical design presents 14 experiments described in Table 2.

3. Results and discussion

The aim of this study was to develop the most effective UV LED protocol as a purification system and also to highlight different degrees in sensitivity to UV within a species and between species. Here we examined the effect of different UV wavelengths emitted by LEDs on three different strains of *E. coli* and two strains of *E. faecalis*. The ATCC 11303, ATCC 15597 (used in other experiments to test the effects of

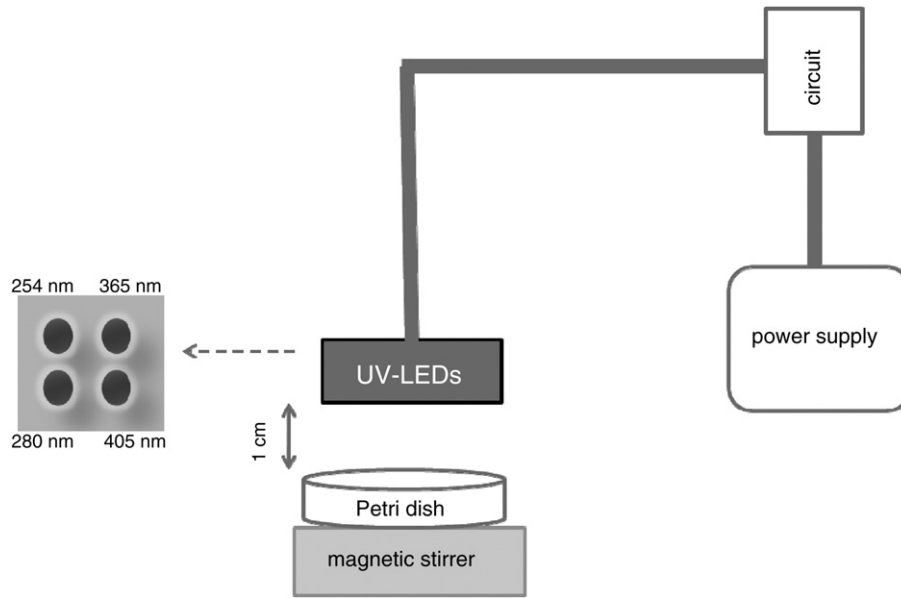


Fig. 1. Scheme of the experimental device used for LED-UV irradiation.

UV on enteric microorganisms [4, 11]) and CIP 6224 strains had been used for testing *E. coli* infraspecific response. For *E. faecalis*, we used ATCC 19433 and ATCC 33186 strains which are not known for their resistance or non-resistance to UV. To determine the best protocol we tested several factors: pH, bacterial density (10^5 or 10^7 cfu/mL), wavelength and exposure time. Two pH values were tested: pH 8, the pH of urban effluents, and pH 6 known to promote growth of microorganisms.

Preliminary experiments were performed to test the reproducibility of the results obtained after irradiation. The CIP 6224 strain (2.10^7 cfu/mL) was irradiated for 60 s at 254 nm and the percentage of bacteria disappearance was calculated. The test was repeated three times, each test resulting in three measures of the same dilution to avoid any error due to measurement. Since the experimental variance for bacterial reduction (expressed in percentages) was $\sigma^2 = 9.5926$ – with 2 degrees of freedom – for an average of 80.11% of bacterial reduction, quantitative statistical tests on the significance of the coefficients are possible on the experiments consecutively conducted. Considering short-term UV effect, four different factors were tested using an optimal screening design and the percentage of disappearance of each strain was calculated (Table 2).

From the experimental results reported in Table 2, we were able to calculate the estimations of the model coefficients a_i , b_i , ..., by least squares regression, representing the variation of the disappearance

percentage in comparison with the last level arbitrarily considered as reference.

For the three *E. coli* strains, the regression coefficients of the respective model were as follows:

$$\begin{aligned} \text{Disappearance \% (CIP6224)} = & 116.7 - 5.3X_{1A} - 12.5X_{2A} - 0.3X_{3A} \\ & - 15.7X_{3B} - 16.5X_{4A} - 0.9X_{4B} - 21.4X_{4C} \\ & - 16.4X_{4D} - 7.8X_{4E} - 7.7X_{4F} \end{aligned}$$

$$\begin{aligned} \text{Disappearance\% (ATCC11303)} = & 94.4 + 1.3X_{1A} + 3.5X_{2A} - 1.6X_{3A} \\ & + 6.3X_{3B} \\ & + 3.4X_{4A} - 1.3X_{4B} - 5.8X_{4C} - 5.9X_{4D} \\ & + 3.7X_{4E} + 3.9X_{4F} \end{aligned}$$

$$\begin{aligned} \text{Disappearance\% (ATCC15597)} = & 84.3 - 2.9X_{1A} + 13.9X_{2A} - 10.9X_{3A} \\ & + 11.9X_{3B} - 10.1X_{4A} - 3.8X_{4B} - 19.3X_{4C} \\ & - 60.3X_{4D} - 44.3X_{4E} + 2.6X_{4F} \end{aligned}$$

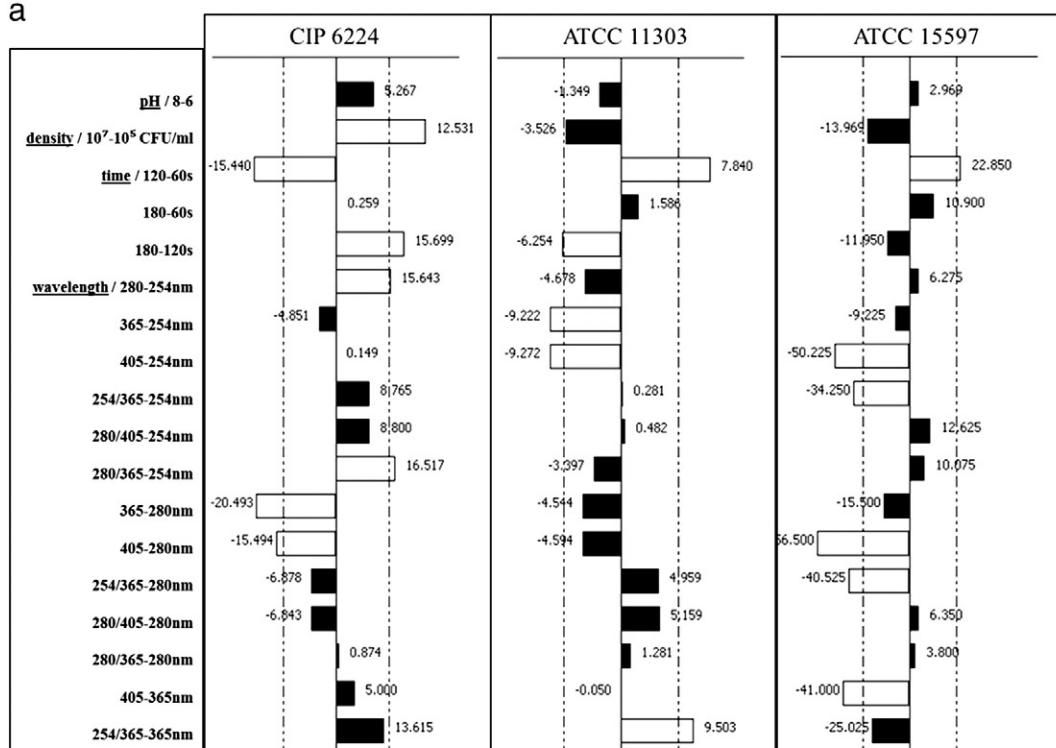
The same calculations were performed for both strains of *E. faecalis*. From these models, effect plots could be drafted to visualize and compare the behavior of each level of the studied factors. Fig. 2a shows the differences in statistical weight between the levels of each factor for *E. coli* strains and Fig. 2b for *E. faecalis* strains. The dotted lines represent the significance level calculated from the experimental variance

Table 2

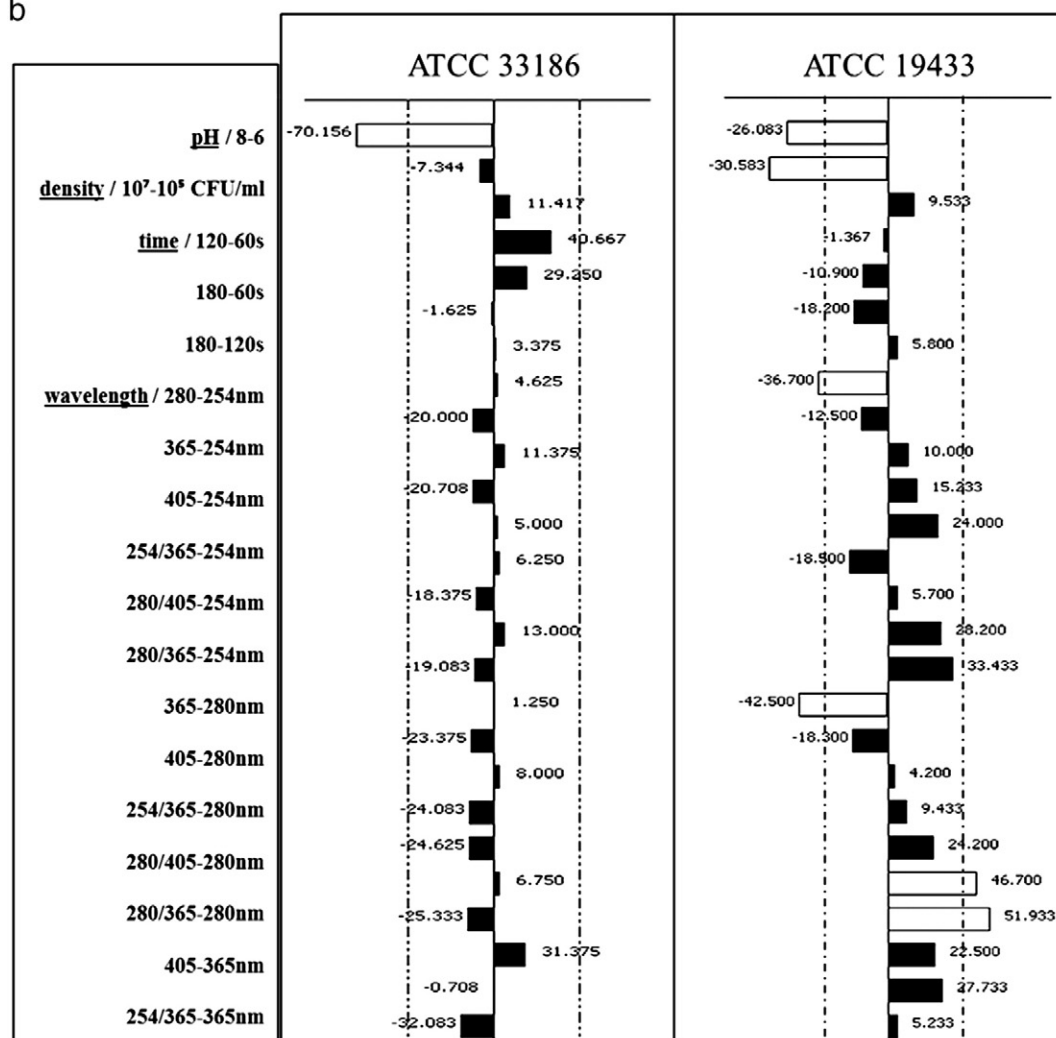
Design of experiments and experimental results (in percentage of disappearance) for the three *E. coli* strains and the two *E. faecalis* strains.

λ (nm)	Exposure time (s)	pH	Density (cfu/ml)	E. coli			E. faecalis	
				CIP 6224	ATCC 11303	ATCC 15597	ATCC 19433	ATCC 33186
254	60	6	10	83.00	100.00	70.50	100	100
254	180	8	10	99.40	98.84	78.00	22.5	40
280	60	8	10	98.00	95.75	78.00	25	7.5
280	120	6	10	99.99	99.99	95.00	72	100
365	60	8	10	97.00	86.65	55.00	45	17.5
365	120	6	10	60.00	100.00	87.00	100	100
405	60	6	10	97.00	87.13	10.00	42.5	100
405	120	8	10	70.00	99.43	50.00	17.5	20
254/365	60	8	10	99.93	99.98	50.00	65	0
254/365	180	6	10	100.00	99.43	30.00	32.5	100
280/405	60	6	10	100.00	99.86	74.50	42.5	70.8
280/405	180	8	10	100.00	99.95	99.25	100	92
280/365	120	8	10	99.99	99.89	91.50	62.5	10
280/365	180	6	10	100.00	100.00	100.00	100	100

a



b



($\sigma^2 = 9.5926$). These figures do not allow us to determine the best conditions of irradiation but only the key factors influencing bacterial mortality. These results show a first difference between the two species tested: only the *E. faecalis* reduction is affected by pH. This can be explained by the different sensitivities to pH for each bacterium [17]. The reduction of the ATCC 33186 strain seems to depend only on the pH of the medium. Bacterial density affects the bacterial reduction of one strain for each species: *E. coli* CIP 6224 and *E. faecalis* ATCC 19433. The effect of UV on bacterial strains within the same species greatly depends on turbidity: [26] had already shown that the turbidity increased UV resistance of *E. coli* strain K12. The irradiation time does not appear to affect the reduction of *E. faecalis* while it has a significant effect on the reduction of the three strains of *E. coli*. [17] have also shown that the irradiation time (15 or 30 min) had an influence on the survival of *E. coli* DH5 α , the survival of this strain decreased with time of UV exposure. The wavelength used is a key factor that significantly affects the reduction of all strains tested, except *E. faecalis* ATCC 33186.

Bacterial density and pH have fairly random effects on bacterial reduction and are not, in practice, easily adjustable in a wastewater treatment plant. For this reason, the optimization tests were based on only two factors: exposure time and wavelength.

Concerning long-term UV effect, we tested the enzymatic potential of bacteria to repair DNA. Percentages of bacteria disappearance 20 h after UV irradiation were calculated for all the conditions of the experiment. The effect of exposure time can have opposite effects. Indeed, longer exposure to UV can increase the mortality rate but may also increase bacterial photoreactivation. This phenomenon where the light causing lethal effects cause simultaneous photoreactivation is called “concomitant photoreactivation” [21]. Here, for UV exposure times of 60, 120 and 180 s, the three *E. coli* strains and the two *E. faecalis* strains tested are not able to repair DNA damage (data not shown). Moreover, the number of surviving bacteria decreases 20 h after irradiation, up to almost 100% reduction (5 to 7 log) for all strains and for all experiments. Results are almost the same for all the experiments of the screening design. Thus, 20 h after UV exposure, none of the four factors tested is discriminating. However, 7 log inactivation is obtained for the three strains of *E. coli* and for the two strains of *E. faecalis* 20 h after irradiation whatever the conditions tested.

A detailed quantitative study of the influence of the two key factors — exposure time and wavelength — was carried out. In this second study, additional interaction terms, assuming that the effect of a factor may depend on the level of other factors, are added to the model and the synergistic model is then:

$$\begin{aligned} \text{Disappearance \%} = & b_0 + b_{1A}X_{1A} + b_{1B}X_{1B} + b_{2A}X_{2A} + b_{2B}X_{2B} + b_{2C}X_{2C} \\ & + b_{2D}X_{2D} + b_{2E}X_{2E} + b_{2F}X_{2F} + b_{1A2A}X_{1A}X_{2A} \\ & + b_{1A2B}X_{1A}X_{2B} + b_{1A2C}X_{1A}X_{2C} + b_{1A2D}X_{1A}X_{2D} \\ & + b_{1A2E}X_{1A}X_{2E} + b_{1A2F}X_{1A}X_{2F} + b_{1B2A}X_{1B}X_{2A} \\ & + b_{1B2B}X_{1B}X_{2B} + b_{1B2C}X_{1B}X_{2C} + b_{1B2D}X_{1B}X_{2D} \\ & + b_{1B2E}X_{1B}X_{2E} + b_{1B2F}X_{1B}X_{2F} \end{aligned}$$

with $X_i = 0$ or 1 in function of the level of the factors.

For this second step, a complete factorial design 3^{17^1} with 21 experiments was chosen and as the first design shows that 60 s of UV exposure is sufficient to i) inhibit reactivation and ii) obtain a high percentage of disappearance for the three strains of *E. coli* and for the two strains of *E. faecalis* (Table 1) we studied shorter exposure times: 10, 20 and 30 s. The pH was set at 8, pH potentially found in the wastewater and bacterial density at 10^7 cfu/mL, maximum value of microorganisms in the effluent.

Table 3

Results of factorial design for *E. coli* and *E. faecalis* strains in log of reduction (maximum are in bold face).

Exposure time (s)	Wavelength (nm)	<i>E. coli</i>			<i>E. faecalis</i>	
		CIP 6224	ATCC 1130.03	ATCC 15597	ATCC 19433	ATCC 33186
10	254	0.5	2.9	0.0	4.8	0.0
20	254	0.8	3.3	0.0	4.8	0.0
30	254	1.7	3.5	0.0	7.0	0.0
10	280	0.4	3.8	0.0	4.5	0.0
20	280	0.6	7.0	0.0	7.0	0.0
30	280	0.8	7.0	0.3	7.0	0.2
10	365	0.0	2.4	0.0	3.0	0.0
20	365	0.3	2.7	0.0	3.6	0.0
30	365	0.5	2.7	0.3	3.8	0.0
10	405	0.5	2.9	0.0	3.0	0.0
20	405	0.6	3.0	0.0	3.3	0.0
30	405	0.7	3.3	0.0	4.0	0.0
10	254/365	0.5	3.9	0.0	3.0	0.0
20	254/365	1.7	4.5	0.1	3.6	0.0
30	254/365	2.3	7.0	0.3	3.7	0.0
10	280/405	0.7	1.5	0.0	3.5	0.0
20	280/405	1.6	4.3	0.2	3.8	0.0
30	280/405	2.0	7.0	0.2	7.0	0.0
10	280/365	0.8	4.3	0.0	2.7	0.0
20	280/365	1.0	7.0	0.0	4.0	0.0
30	280/365	1.5	7.0	0.4	4.5	0.0

After 10, 20 and 30 s of UV radiation the ATCC 15597 strain is still the most resistant strain of *E. coli* to UV, with a maximum of 0.4 log decrease after a 30-s irradiation using combined 280/365 nm wavelengths (Table 2). These results are comparable with those of the *E. faecalis* ATCC 33186 strain, which is also very insensitive to UV for short irradiation times with a maximum of 0.2 log reduction after 30 s of irradiation at 280 nm. The *E. coli* CIP 6224 strain is moderately sensitive to UV, with a maximum of 2.3 log decrease after a 30-s irradiation with combined 254/365 nm wavelengths while the ATCC 11303 strain is very sensitive to UV, with a 7 log decrease after a 20-s irradiation with 280 nm and 280/365 nm wavelengths. The *E. faecalis* ATCC 19433 strain is also UV sensitive with a maximum of 7 log inactivation under four conditions. Regarding the *E. coli* ATCC 15597 strain and the *E. faecalis* ATCC 19433 strain, for UV exposure times of 10, 20 and 30 s, most of the conditions tested have no direct effect on this strain (Table 3). No statistical treatments were possible under these conditions. Fig. 3 shows that the longer the irradiation time is (30 s), the more efficient the treatment is. For these short exposure times, the effect of the coupling wavelengths is only visible for two bacterial strains: *E. coli* ATCC 11303 and *E. coli* CIP 6224. Fig. 3 shows the interactions between the exposure times and the wavelengths used for these two strains. This graph indeed shows that for strain CIP 6224, coupled wavelengths 255/365 nm, 280/405 nm and 280/365 nm are more effective than wavelengths tested alone and, for strain ATCC 11303, coupled wavelengths 254/365 nm, 280/405 nm and 280/365 nm are as efficient as 280 nm. Thus, for these two strains the coupling 280/365 nm, which has a total optical power of 350.55 mW, is more effective than 365 nm used alone, even though this wavelength has an optical power of 350 mW. Such a small difference in optical power does not explain the difference in bacterial reduction achieved in this experiment. Furthermore, for these two strains, a wavelength of 280 nm is more effective than 365 nm, while the optical power of LEDs emitting at 280 nm is only 0.55 mW against 350 mW for the LED emitting at 365 nm.

Fig. 2. Differences in statistical weight of each level of the four factors on disappearance of *E. coli* (a) and *E. faecalis* (b) strains. The dotted lines represent the significance level; significant differences between statistical weights of levels are shown in white.

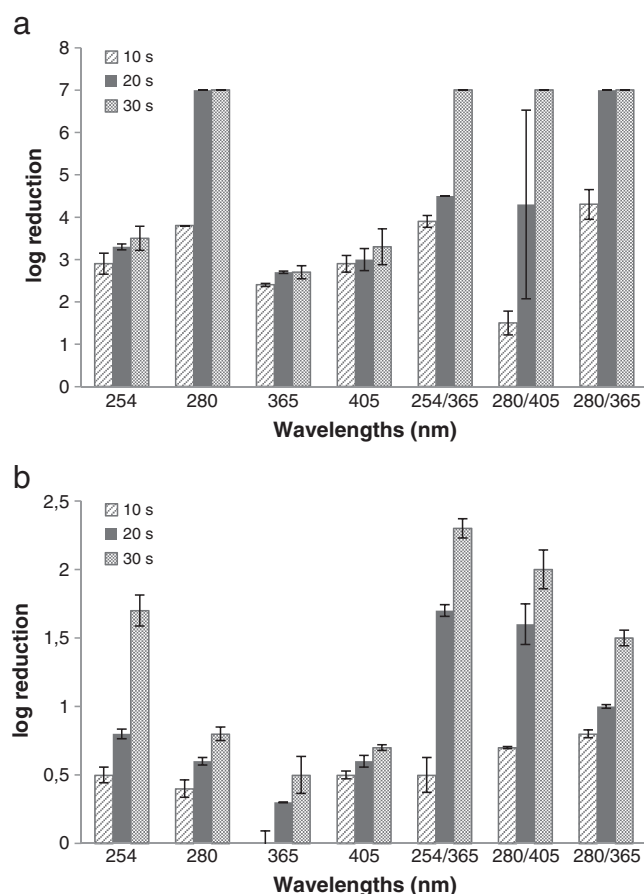


Fig. 3. Interaction diagram exposure time/wavelength for *E. coli* ATCC 11303 (a) and CIP 6224 (b).

The inactivation of *E. faecalis* ATCC 19433 is significantly higher (Wilcoxon test, $p < 0.05$) immediately after UV irradiation than 20 h later (data not shown). This means that this strain of *E. faecalis* is able to repair its DNA after UV irradiation of 10 to 30 s whereas for 60 s exposure, DNA repair is no more possible as mentioned above. The inactivation of *E. coli* ATCC 11303 is similar immediately or 20 h after irradiation (Wilcoxon test, $p = 0.768$). *E. faecalis* ATCC 33186, *E. coli* CIP 6224 and *E. coli* ATCC 15597 exhibit the same responses to UV treatment but with different intensities. For these three strains, the bacterial inactivation after 20 h is significantly higher than that measured immediately after irradiation (Wilcoxon test, $p < 0.05$). Regarding *E. coli* CIP 6224 and *E. faecalis* ATCC 33186, a 7 log inactivation is obtained 20 h after irradiation and only up to 1 log for *E. coli* ATCC 15597. Thirty seconds of irradiation were not sufficient to obtain interesting results for this strain since the decrease in bacteria must reach 7 log to be considered as an efficient result in wastewater treatment [27]. For this particularly UV-resistant strain, 60 s at 280 nm were needed to achieve a 0.8 log decrease (78% disappearance rate) as previously described (Table 1). The increase in bacterial inactivation 20 h after irradiation is probably due to the inactivation process of each range of UV rays. UV-C (200–280 nm) act immediately on bacteria DNA, inducing the formation of pyrimidine dimers which inhibit bacterial cell division whereas UV-A (320–400 nm) are not absorbed by DNA but inactivate microorganisms by damaging proteins, producing hydroxyl radicals, which destroy bacterial membranes [4]. This process needs more time than the formation of pyrimidine dimers and may consequently be responsible for the greater disappearance rate of bacteria 20 h after irradiation.

These results show a wide diversity of short-term response to UV irradiation within the same species and between two bacterial types. Very few studies have so far investigated the variability of infra-

specific UV resistance. Only studies comparing UV sensitivity of different species have been conducted, mainly comparing non spore-producing with spore-producing bacteria, which are more resistant to UV [8, 10, 17].

Coupled wavelengths and 280 nm appear to be optimal according to the results of certain strains of the log decrease just after irradiation. The wavelength typically used for water disinfection by mercury vapor lamps is 254 nm, which here is less effective than 280 nm. Recent studies on water disinfection by UV-LEDs also showed that wavelengths near 280 nm such as 275 nm were more efficient [2, 28]; the authors explain that this is probably due to the fact that, these wavelengths do not react on DNA but on proteins (avoiding DNA repair). However, the log decrease varies depending on the strain tested. Furthermore, under our experimental conditions, we show that DNA repair did not frequently occur for the strains tested.

4. Conclusion

This study compared the efficiency of four different UV-LED wavelengths toward inactivation of three strains of *E. coli* (ATCC 11303, ATCC 15597 and CIP 6224) and two strains of *E. faecalis* (ATCC 19433 and ATCC 33186), and by varying several factors: bacterial density, pH, UV exposure time, and use of single wavelength or coupled wavelengths. A multivariate approach has been carried out to point out most influential factors affecting yields of inactivation.

The results obtained here show that coupled wavelengths, 280/365, 280/405 nm and 255/365 nm, have a maximum effect on microorganisms with a limited impact on chemical by-product formation. In terms of cost, the most expensive LED emits at 255 nm, thus coupled wavelengths 280/365 nm and 280/405 nm should be considered. One hypothesis which may support the stronger efficiency of coupled wavelengths is the cumulative effect of the power emitted by both LEDs rather than a specific effect linked to the selected wavelengths. However differences in the power emitted do not justify such a discrepancy in the results. For instance, coupling 280/405 nm (210.55 mW) is more efficient than using 365 nm alone (350 mW). Regarding exposure time, 60 s are thus enough to eliminate the tested bacterial strains.

The first results obtained here indicate that UV treatment using LED is particularly effective. These preliminary experiments show a different sensitivity within a species and between two species to UV irradiation. The effect of wavelengths (with and without coupling), which seems to strongly affect microbial response should finally be investigated on an effluent. This complex medium will integrate microbial and chemical diversities in order to test the effectiveness of such a purification process.

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