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Abstract

Salmonellosis is one of the most common causes of foodborne bacterial human disease worldwide and the emergence of multidrug resistant (MDR) strains of *Salmonella enterica* serovar Typhimurium (*S. typhimurium)* was associated to the incidence of invasive salmonellosis. The objective of the present work was to investigate the effects of the TiO_2 photocatalysis process in terms of both bacteria inactivation and the emergence of mutants, on *S. typhimurium* TA102 water suspensions. The TiO_2 photocatalysis was compared with a conventional disinfection process *i.e*. UV-C radiation. In spite of the faster bacterial inactivation obtained in UV-C disinfection experiments (45, 15 and 10 min for total inactivation for initial cell density 10^9, 10^8 and 10^7 CFU mL^{-1}, respectively), photocatalytic disinfection (60, 30 and 15 min) was more energy efficient because of a lower energy requirement (2 - 20 mWs cm^{-2}) compared to the UV-C disinfection process (5 - 30 mWs cm^{-2}). During the photocatalytic experiments, the mutation frequency increased up to 1648 fold compared to background level for a 10^8 CFU mL^{-1} initial bacterial density, and mutants were inactivated after 1 - 10 min treatment, depending on initial bacterial cell density. In UV-C disinfection experiments, the mutation frequency increased up to 2181 fold for a 10^8 CFU mL^{-1} initial bacterial cell density, and UV-C doses in the range 0.5 - 4.8 mWs cm^{-2} were necessary to decrease mutation frequency. In conclusion, both disinfection processes were effective in the inactivation of *S. typhimurium* cells and mutants release into the environment can be avoided if cells are effectively inactivated.

Keywords: advanced oxidation processes, Ames test, mutagenicity, *Salmonella typhimurium*, water disinfection.

1. Introduction

Salmonellosis is one of the most common causes of foodborne bacterial human disease worldwide and a significant increase in the number of *Salmonella* infections has been observed in many countries over the past decade (Rahmani et al. 2013). Specifically, *Salmonella* spp. results in approximately 93.8 million cases of gastroenteritis annually worldwide, leading to 155,000 deaths each year (Majowicz et al. 2010). The genus *Salmonella* comprises two species *S. bongori* and *S. enterica,* the latter one being in turn divided in six sub-species that cover more than 2550 serovars according to their O and H antigens. Among them, *S. enterica* serovar Typhimurium, is a primary enteric pathogen mainly associated to human and other warm-blooded vertebrates (Popoff 2001, Fàbrega and Vila 2013). In the last years, a close association was reported between the emergence of multidrug resistant (MDR) strains of *Salmonella* and the incidence of invasive salmonellosis (Gordon et al. 2008). The increased frequency of MDR *Salmonella* strains in human infections and the ensuing consequences it has on a health perspective (Oubrim et al. 2012; Rahman et al. 2014), brings to light the possible role of fecally contaminated waters in the spread of MDR *Salmonella* (Levantesi et al. 2012).

Food and water still play the main roles in the transmission of *Salmonella*. *Salmonellae* are excreted through animal and human feces into the environment and are frequently found in aqueous matrices. In particular, they typically occur in large numbers in raw sewage (Oubrim et al. 2012) and can still persist in wastewater effluents (Masarikova et al. 2016) even after a disinfection process (Wéry et al. 2008). A study indicated that 80% of wastewater samples used for irrigation was positive for *Salmonella* (Nutt et al. 2003). Moreover, it has been detected in various types of natural waters such as rivers, lakes, coastal waters, as well as contaminated ground water (Polo et al. 1999; Martinez-Urtaza et al. 2004; Haley et al. 2009; Levantesi et al. 2010).

Different studies have investigated the inactivation of *Salmonella* strains by disinfection processes in water and wastewater. In particular, Koivunen and Heinonen-Tanski (2005) evaluated the effect of different disinfection processes on *Salmonella* strains in synthetic wastewater where the treatment with peracetic acid and UV radiation has been shown to be faster than UV radiation alone and chlorine processes for the inactivation of *Salmonella enteritidis*. In some instance, *Salmonella* has been found to be more resistant to disinfection processes than others bacterial indicators (Berney et al. 2006; Rincon and Pulgarin 2007, Sciacca et al. 2011; Li et al. 2012). On the other hand, conventional disinfection by either chlorination or UV radiation may not be effective enough in controlling the spread of pathogens including ~~resistant microorganisms and~~

~~particularly~~ antibiotic resistance microorganisms (Rizzo et al. 2013), therefore alternative disinfection methods should be investigated. Advanced oxidation processes (AOPs) (e.g., Fenton, photo- Fenton, TiO_2 photocatalysis, UV/O_3, UV/H_2O_2 etc.) have been found effective in the removal of a wide range of contaminants (Rizzo 2011). Among AOPs, TiO_2 photocatalysis is a promising water disinfection option, because of its capacity to inactivate a wide range of pathogens in water (Dunlop et al. 2011; Polo-López et al. 2014; Rizzo et al. 2014a); however, to the best of our knowledge, the effectiveness of TiO_2 photocatalysis on the inactivation of selected *Salmonella* strains and/or its possible effect in terms of mutagenicity has not been investigated so far. Indeed, if the inactivation of microbial indicators during treatment/disinfection processes is well documented, specific process-induced mutagenicity is generally occulted, apart from demonstrating the production of genotoxic by-products. Yet, in *Salmonella* as for other organisms, gene mutations can confer resistance to antimicrobial by altering the corresponding protein target or by altering the substrate specificity of existing antimicrobial degrading enzymes (Michael et al. 2006). Thus, beyond bacterial inactivation, considering process-induced mutagenicity might prove to be of prime importance for a better control of antibiotic resistance pathogens in the released effluents. In fact, antibiotic resistances can be acquired either through gene transfer or by mutation of the target gene. If the effects of various treatment strategies on the persistence of resistance genes/bacteria start now to be relatively well documented, the effects of such treatments on the emergence (mutation) and dissemination (transfer) of antibiotic resistance genes remains scarce nay inexistent, and needs further consideration (Sharma et al 2016).

The objective of the present work was to investigate the effects of the TiO_2 photocatalysis process in terms of inactivation and mutagenicity on a model microorganism. *Salmonella* was chosen as target/model organism (its genus belongs to the *Enterobacteriaceae*, the same family as *Escherichia*, which includes the species *E. coli*) because of (i) its spread in aqueous matrices (particularly in natural water and wastewater), (ii) the related concern for human health (it can cause illnesses such as typhoid fever and paratyphoid fever) and (iii) its role in antibiotic resistance spread. Moreover, the effect of TiO_2 photocatalysis on the inactivation and mutagenicity of *Salmonella* was compared with a conventional disinfection process *i.e.* UV-C disinfection.

2. Material and methods

2.1 Bacterial strain and media

Salmonella enterica serovar Typhimurium strain TA102 was used as tester strain for general mutagenicity testing according to Maron and Ames (1983). Strain TA102 has been extensively used and reported under its former species name "*Salmonella typhimurium"* and will be reported as such in this manuscript to avoid unnecessary confusion. Strain TA102 exhibits a requirement for histidine and therefore cannot grow on minimal medium unless the *his428* mutation, carried on its plasmid pAQ1, mutates back to a prototrophic form. *S. typhimurium* TA102 was routinely cultured at 37°C on Vogel-Bonner minimal medium supplemented with D-Glucose, D-Biotin and L-Histidine (Maron and Ames 1983). For isolation of His+ revertants, the histidine supplement was omitted from the medium. Strain *S. typhimurium* TA102 was selected among a variety of other *Salmonella* tester strains because of its noticeable sensitivity to numerous mutagenic agents, including UV, that are otherwise poorly detected by alternative tester strains (Maron and Ames 1983).

TA102 bacteria were unfrozen and revived by streaking on Vogel-Bonner agar plate supplemented with Glucose and Histidine and incubated at 37 °C for 48 h. A single colony from the plate was inoculated into 10 mL sterile lysogeny broth (LB, Sigma-Aldrich, USA) and incubated at 30°C for 18 h under agitation (160 rpm) in a rotating shaker to obtain a stationary phase culture. Cells were washed twice by alternating centrifugations at 5000 rpm for 2 min and pellets re-suspensions in 10 mM $MgSO_4$. The final volumes were adjusted to obtain cell concentrations at either 10^9, 10^8 or 10^7 CFU mL^{-1}; these suspensions were prepared independently and then used in independent disinfection experiments.

2.2 TiO_2 photocatalysis tests

Photocatalytic tests were carried out in a 2.2 L cylindrical glass reactor (13.0 cm in diameter) filled in with the 400 mL of sample. The thickness of liquid was constant and equal to the liquid depth (3 cm). The reactor was placed in a water bath to control the temperature at 30°C during the experiments. The aqueous suspension in the reactor was stirred continuously. A wide spectrum 250 W lamp (Procomat, Italy) with a peak of light intensity in the UV-A range of 0.012 mW cm^{-2} at 350 nm was used as light source. The lamp was placed horizontally, 40 cm above the surface of the water. Degussa P25 TiO_2 was used as slurry as received from the manufacturer to perform heterogeneous photocatalytic experiments. They were carried out at concentration of 100 mg L^{-1} according to previously set conditions (Rizzo et al. 2014b). Finally, control tests with TiO_2 under dark conditions and UV irradiation alone were carried out in order to distinguish their respective contribution

119 to *S. typhimurium* TA102 inactivation and stress-induced mutagenicity compared to the photocatalytic
120 process.
121

122 ### *2.3 UV-C disinfection tests*

123 UV-C experiments were carried out in 40 mL glass Petri dishes (diameter: 90 mm) filled in with 25 mL of
124 sample. Petri dishes were gently stirred throughout the exposure time under a collimating apparatus (Simonet
125 and Gantzer, 2006) equipped with a low-pressure mercury vapor lamp (10-W Slimline germicidal lamp,
126 ozone-free Ster-L-Ray G12T6L; Atlantic UV Corporation) emitting monochromatic (253.7-nm) UV light.
127 The irradiance at the center of the beam at the water surface was about 0.008 mW cm^{-2}, and was delivered by
128 a UV tube positioned horizontally, 20 cm above the surface of the water. Different UV-C doses were achieved
129 by varying the exposure time
130

131 ### *2.4 Bacterial count and mutation frenquencies*

132 Bacterial cells exposed to UV-C and TiO_2 photocatalysis treatment were recovered, serially diluted in 10 mM
133 $MgSO_4$, and spread in triplicate on minimal glucose agar with and without histidine. Plates were incubated at
134 37°C for 48-72h. Each sample was analyzed in triplicate. Mutation frequencies were obtained as ratios
135 between the number of revertants (counts on plates without histidine) and the total bacterial cell counts (on
136 medium with histidine).
137

138 ### *2.5 Statistical analysis*

139 The correlations between the bacterial mortality (log (N/N_0)) and the duration of the treatments, or the doses
140 received, were assessed using the Pearson's test. Then, regression data analyses were performed on log-
141 transformed data: $\log(N/N_0)$ for the total cell counts and $\log(F/F_0)$ for the frequencies of mutation. The effects
142 of both the treatment and the initial bacterial cell load were analyzed using a Fisher F-test and a Student T-
143 test. The "Fisher F-test" was used to test data for homogeneity of variances. Thereafter, once homogeneity
144 was verified, a "Student T-test" was further used to compare the trends (slopes) of linear regressions. A
145 statistical difference revealed by a Student T-test, combined to the comparison of regression trendlines (slopes)

allowed determining whether a given treatment or condition had stronger effects than another one. Statistical correlations and statistical comparisons are presented in supplementary material.

3. Results and discussion

3.1 TiO_2 photocatalytic tests

3.1.1 Control tests

Photocatalytic tests were carried out by using a wide spectrum lamp with the main emission in UV-A wavelength range (400-315 nm) and TiO_2 as photocatalyst. Therefore, control tests were carried out using UV-A radiation and TiO_2 as stand-alone processes in order to evaluate the effective contribution of photocatalytic process (UV/TiO_2) on both the inactivation of strain TA102 and the induction of His+ revertant mutants.

TiO_2 control experiments were carried out in the dark and did not result in any significant change neither in the initial bacterial density and nor the mutants count (2h treatment; data not shown). UV-A control tests were carried out up over time to 15, 30 and 60 min irradiation time with initial cell suspension density of 10^7, 10^8 and 10^9 CFU mL^{-1}, respectively, according to the respective minimum treatment time necessary to achieve total *S. typhimurium* TA102 inactivation by the corresponding UV/TiO_2 process. As shown in Figure 1 and Supplementary section 3, the UV-A control tests resulted in a moderate (but statistically significant) cell inactivation and weak changes in mutation frequency over the course of the UV-A exposure time

Figure 1

The bacteria inactivation clearly appears exposure time dependent, it did not seem to be significantly influenced by initial cell load as for a given time of UV-A exposure a similar cell count reduction is observed (ca. 1 log reduction for a 15 min exposure). On a statistical point of view, the decreasing trend in viable cell counts correlates with the exposure time but the absence of homogenous variances does not allow drawing any conclusion regarding a possible effects of the initial cell load (Supplementary sections 2 and 3). The evolution of the His+ mutant counts appear to decrease at a slower pace compared to the total viable count, with 0.5 log units decrease after 15 min to 2.2 log units decrease after 60 min treatment time (Figure 1a). This

may indicate that an increase of the mutation frequency, therefore producing more His+ mutants, could partially compensate the inactivation of the His+ subpopulation. This seems to be particularly true for the 10^9 CFU mL^{-1} initial cell density suspension, where a 231 fold increase of the mutation frequency was reached after 60 min of UV-A exposure. Nevertheless, when comparing the different evolution of the mutation frequencies (log F/F_0) as a function of the treatment dose (exposure time), no statistical difference was observed between the different initial cell loads (Supplementary Fig. S2, Table S3).

The inactivation of *S. typhimurium* TA102 can be explained through the damage caused by UV-A radiation following its absorption by cellular components called intracellular chromophores. Light absorption through chromophores contributes to the generation of reactive oxygen species (ROS). According to McGuigan et al. (2012), the UV-A wavelengths bordering on visible light are not sufficiently energetic to alter DNA directly. Nevertheless, UV-A play an important role in promoting the formation of intracellular ROS (singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radical), which can in turn damage DNA.

3.1.2 S. typhimurium TA102 inactivation by TiO_2 photocatalysis

The results of *S. typhimurium* TA102 inactivation by TiO_2 photocatalysis are plotted as function of irradiation time in Figure 2.

Figure 2

The initial bacterial density strongly affected the inactivation rate by TiO_2 photocatalysis (Fig. 2; Supplementary section 4). The complete bacterial inactivation was achieved after 60, 30 and 15 min of irradiation for initial bacterial densities of 10^9, 10^8 and 10^7 CFU mL^{-1}, respectively. Compared to control test with UV-A radiation alone (Fig.1), TiO_2 photocatalytic process was found to be undoubtedly more effective, which was further demonstrated by a Student comparison T-tests on both series of data (Supplementary section 6). Cho et al. (2004) evaluated the correlation between hydroxyl radicals ($^{\bullet}OH$) and *E. coli* inactivation rates under UV/TiO_2 treatment. The concentration of $^{\bullet}OH$ during photocatalytic treatment was quantified by measuring p-chlorobenzoic acid (a probe compound) degradation rate. The results demonstrated an excellent linear correlation between $^{\bullet}OH$ and the rate of *E. coli* inactivation, which indicates that the $^{\bullet}OH$ radical is the primary oxidant species responsible for inactivating *E. coli* in the UV/TiO_2 process. The inactivation of *S. typhimurium* TA102 by photocatalysis with UV-A lamp in combination with TiO_2 at concentration of 0.5 g L^{-}

[1] was studied by Long et al. (2014). Three different initial cell loads were investigated: 10^7, 10^6 and 10^5 CFU mL^{-1}, and a complete inactivation was reached after 180, 70 and 60 min respectively. Slower inactivation rates, as might be expected, were obtained with only the UV-A lamp. A similar study, with the inactivation of *Salmonella enteretidis* (initial bacterial density of 10^8 CFU mL^{-1}) by UV-A lamp and TiO_2 at concentration of 1.0 g L^{-1} was carried out by Robertson et al. (2005). A decrease of 5 log units was reached after 120 min of treatment, however, quite surprisingly, little difference was observed in experiments without catalyst and only with UV-A lamp. In our previous experiments, the effect of TiO_2/sunlight experiment on antibiotic resistant *Escherichia coli* strains was investigated by using the same experimental set up (2.2 L cylindrical glass reactor and 250 W lamp) and a total inactivation was achieved after 60 min, with 100 mg L^{-1} of TiO_2 and an initial bacterial load of 10^6 CFU mL^{-1} (Rizzo et al. 2014b). Wang et al. (2014) also reported that the rate of bacterial inactivation, or real antimicrobial activity by TiO_2, depends on the initial bacterial concentration. In the present study, we also found that the inactivation rate of strain TA102 was exposure time-dependent and statistically stronger as the initial cell load decrease (Supplementary section 4). Possibly, when the initial bacteria population is too high, there is a bigger buffering capacity for the hydroxyl radicals, which is not only due to the large amount of cells but also from the mineralization products in the reaction process. In 2011, Foster et al. proposed a killing mechanism where hydroxyl radicals progressively damage the cell surface structures leading to the release of intracellular material/molecules that can in turn be undergo further degradation up to a complete mineralization. From this it can then be assumed that the released intracellular components here seen as intermediate products of photocatalysis, have a protective effect on live bacteria as they compete with live cells for interacting with hydroxyl radicals, and thus contribute to the loss of antibacterial activity. ~~In our previous experiments, the effect of TiO_2/sunlight experiment on antibiotic resistant *Escherichia coli* strains was investigated by using the same experimental set up (2.2 L cylindrical glass reactor and 250 W lamp) and a total inactivation was achieved after 60 min, with 100 mg L^{-1} of TiO_2 and an initial bacterial load of 10^6 CFU mL^{-1} (Rizzo et al. 2014b).~~

In spite of the debate over which process lead to death of an organism exposed to photocatalytic action, most of the experimental evidences show that the destruction of the cell membrane is an important issue for inactivation (Dalrymple et al. 2010). The mechanism for bacterial destruction by TiO_2 photocatalysis has been proposed to occur via attack by $^{\bullet}OH$ generated on the photocatalyst surface and the mode of microbial destruction suggest that initial target for photocatalytic attack is the bacterial cell wall. Gram-negative cell wall includes a thin peptidoglycan layer, and an external membrane made of lipopolysaccharide (LPS) and

234 phospholipid. In gram-negative bacteria, peptidoglycan layer makes up only about 10% of the cell wall and it
235 is likely that it may be susceptible to radical attack (Lu et al. 2003). LPS layer and the phospholipid bilayer
236 are made up of fatty acids, which may be also susceptible to radicals attack. The possibility of radicals to travel
237 only very short distances and the presence of an intact membrane reduce the probability of the radical reaching
238 intracellular components such as DNA, but once the radicals are generated in close proximity to the target
239 molecules, they will be able to inflict injury directly (Dalrymple et al. 2010). On the other hand, two $^{\bullet}OH$
240 radicals may recombine to form hydrogen peroxide, which may inactivate bacterial cells on a longer distance
241 between the source of radicals and the target (Foster et al. 2011).
242
243 *3.1.3* S. typhimurium *mutagenicity during TiO_2 photocatalysis tests*

244 The mutation frequencies were also evaluated following the TiO_2 photocatalytic tests, and displayed a 42,
245 1648, 32 fold increase compared to background level for a 10^9, 10^8 and 10^7 CFU mL^{-1} initial bacterial density,
246 respectively (Fig. 2). The evolution of the mutation frequencies as a function of the treatment exposure time
247 displayed biphasic aspects with an initial increase followed by a slow-down or a decrease as the exposure time
248 increases. It is worth noting that the increased mutation frequencies are not associated to dramatic increases
249 of the mutants counts; considering the fact that the emerging mutants are also subjected to an increase loss of
250 cell viability, the apparent stability of the mutants counts probably result from an increased mutation frequency
251 counterbalanced by an increased cell killing. If the levels of mutation reached were clearly different depending
252 on the initial cell load (Fig. 2), statistical analyses demonstrated that the evolution of the mutation frequency
253 (as a function of the exposure time) were similar for the 10^8 and 10^7 CFU mL^{-1} initial cell load (Supplementary
254 section 4). This tends to show (i) that the treatment is in fact equally effective for these two initial cell loads
255 in terms of dose-dependent effects, but (ii) the 10^7 CFU mL^{-1} suspension stops accumulating mutations earlier
256 than the 10^8 CFU mL^{-1} probably because of a stronger killing effect.
257 The effect of different oxidants/disinfectants (namely chlorine, chlorine dioxide and ozone) on genotoxicity
258 has been studied by different authors (Monarca et al. 2000; Mišík et al. 2011; Magdeburg et al. 2014), but no
259 information is available in scientific literature (to authors' knowledge) on the effect of photocatalytic processes
260 on the induction of mutants in *Salmonella* strains after treatment. Basically, *Salmonella* disinfection-induced
261 mutagenicity has been mainly related to the formation of disinfection by-products. For example, Magdeburg
262 et al. (2014) studied the effect of ozone, as tertiary treatment, in a pilot scale wastewater treatment. The Ames
263 assay using *Salmonella typhimurium* strain YG7108 revealed an ozone-dose dependent mutagenicity increase

after wastewater ozonation, indicating the formation of alkylating mutagenic oxidation by-products. The impact of ozone treatment on genotoxic and acute toxic effects of tertiary treated municipal wastewater was investigated by Mišík et al. (2011). After ozone treatment, they observed a decrease of the mutagenic activity of the samples and the bactericidal effects were reduced by ozonation. The influence of disinfectants such as chlorine dioxide, ozone, peracetic acid and, UV radiation, on the formation of mutagenic compounds has been evaluated by Monarca et al. (2000). They evaluated the mutagenicity using the Ames test and all disinfectant treatments induced the formation of mutagenic compounds, particularly chemical treatment with ClO_2 or ozone, in contrast with physical treatment with UV-C lamp.

Despite the lack of work available in scientific literature concerning the effect of TiO_2 photocatalytic process on the formation of mutants in *Salmonella* strains, the possible role of hydroxyl radicals has been investigated (Kanno et al. 2012). De Kok et al. (1992) used electron spin resonance (ESR) spectroscopy to detect and identify oxygen species generated by fecapentaenes (potent genotoxins found in human feces) and investigated the influence of scavenging reactive oxygen species (dimethyl sulfoxide (DMSO), t-butyl alcohol, t-butyl hydroperoxide (TBOOH), 2,2,6,6-tetramethylpiperidine and 5,5-dimethyl-lpyrroline-A'-oxide) on the mutagenicity of *Salmonella* strains including TA102. All hydroxyl radical scavengers significantly reduced the number of revertants (except for DMSO in combination with TBOOH), thus showing that hydroxyl radical could mediate mutagenicity of the *Salmonella* strains although the precise mechanisms did not appear clearly identified. In another study, hydrogen peroxide-induced mutagenicity in *Salmonella typhimurium* TA102 was assumed to be caused by hydroxyl radicals generated by iron ions closely associated with DNA, since iron chelators or hydroxyl radical scavengers (ascorbic acid or DMSO) exhibited effective anti-mutagenic effects (Grey and Adlercreutz, 2003).

3.2 UV-C disinfection tests

3.2.1 S. typhimurium TA102 inactivation by UV-C disinfection tests

UV-C irradiation tests were carried out in order to compare the results from TiO_2 photocatalytic process with a more conventional disinfection process. Figure 3 shows the evolution of cell counts and mutation frequencies as a function of UV energy. Control tests carried out in the dark (sample stored at room temperature for 4 hours) did not show any change in bacterial cultivability (data not shown).

293 Figure 3

294

295 Whatever the initial cell load, a progressive loss of cultivability of strain TA102 was observed as the UV-dose
296 was increased, which was confirmed by a Pearson correlation test (Supplementary section 2). For the highest
297 initial cell load (10^9 CFU mL^{-1}), the total cell count dropped down by 9 log units and total inactivation was
298 reached after about 45 min of UV exposure (Fig.3a), which correspond to a 30 mWs cm^{-2} (45 min irradiation
299 time) UV-C dose. As the initial bacterial density was decreased to 10^8 (Fig. 3b) and 10^7 (Fig. 3c) CFU mL^{-1},
300 the total bacteria inactivation was achieved in 15 and 10 min, respectively while the corresponding UV-C
301 doses were relatively close in both experiments (approximately 5-10 mWs cm^{-2}). Statistically, the cell count
302 abatements (log (N/N_0)) appeared similar in terms of dose-dependent decrease for the two lower initial cell
303 loads (Supplementary section 5) although longer treatment are necessary to achieve a full inactivation of the
304 higher initial cell load. The cell count abatement obtained for the 10^9 CFU mL^{-1} suspension appeared to
305 decrease at a significant lower pace (Supplementary Fig. S5) compare the two other suspensions, which
306 probably reflect the shield effect exerted by bacteria when the cell density is too high. However, a direct
307 dependence of initial concentration of microorganism and UV dose necessary to reach a full cell inactivation
308 was expected. Its estimation was based on the averaged incident radiation energy over the reactor volume that
309 is a dynamic function of the absorption coefficient, which itself depends dynamically on the concentration of
310 active cells (Silva et al. 2013). UV-C disinfection relies on the sensitivity of the microorganism to UV
311 radiation. This is unique to each microorganism and is determined by its ability to absorb at 200–280 nm
312 (germicidal wavelength range), so inactivating their active cells through UV-induced damages such as the
313 formation of pyrimidine dimers in their DNA. UV-induced damages disrupt the DNA structure, so that, if a
314 critical number of dimers is formed, the DNA cannot replicate (Hignen et al. 2006).

315 At a first glimpse, the cell complete inactivation was faster in the UV-C disinfection experiments compared
316 to photocatalytic disinfection experiments for all initial bacterial densities investigated (Fig.2). However,
317 considering the energy engaged in both treatments, the photocatalytic process was more efficient than the UV-
318 C process because a lower energy was required to achieve a total *S. typhimurium* inactivation (in the range 2
319 - 20 mWs cm^{-2}).

320

3.2.2 *S. typhimurium mutagenicity during UV-C disinfection tests*

The mutation frequencies, increased in a UV-C dose dependent manner to reach a maximum corresponding to a 21, 2181 and 26 fold increase compared to background level, for 10^9, 10^8 and 10^7 CFU mL^{-1} initial bacterial density, respectively (Fig. 3). UV-C doses higher than 7.2, 2.4 and 0.5 mWs cm^{-2} respectively, resulted in absence of detectable mutants, where the deleterious effect of the UV-C treatment ultimately dominates over the emergence of mutants (Fig.3). As for the photocatalytic treatment, the evolution of the mutation frequencies displayed a dose-dependent biphasic aspects with an initial increase followed by a slow-down/decrease for higher UV-C doses. Here again, statistical analysis demonstrated that the initial increase of the $\log(F/F_0)$ ratio appeared to evolve at a lower pace for the 10^9 CFU mL^{-1} cell suspension than the 10^8 and the 10^7 CFU mL^{-1} ones (Supplementary section 5), which mean that different initial cell load are not equally sensitive as far as cell are numerous enough to exert a shield effect. UV radiation produces many types of photochemical alterations (e.g., in DNA, RNA, protein, membranes) but DNA remains the major target for the deleterious effects of UV radiation all together because of its large size, its efficient UV absorption, its low number of copies per cells, and finally because it is the support of genetic information (Grossweiner et al. 2005). A large number of different types of damage are produced in DNA by UV irradiation however, mutagenic lesions principally result from the formation of pyrimidine dimers and pyrimidine adducts (Sinha and Häder 2002). UV-induced mutagenesis is a highly complex process involving different photoproducts and repair mechanisms that deal with them. Tate et al. (2006) characterized the damage of UV-C radiation in *Salmonella typhimurim* strain TA98. The damage was found primarily localized in the nucleic acids and it results from direct absorption of photons by the target molecules. According to the authors, the damage produced by UV-C was predominately in the form of pyrimidine dimers and 6-4 photoproducts resulting from the direct photon absorption by DNA. UV-induced mutations in *Salmonella* are relevant not only regarding lethality, but also because it may favor the appearance of some antibiotic resistances (Michael et al. 2006). With this respect, it is worth noting that in the case of UV-C disinfection tests carried out with 10^7 CFU mL^{-1}, a one log increased titer of His+ mutants was observed together with the increasing mutation frequency. Possibly in this setup, from 0 to 0.2 mWs cm^{-2}, the appearance of mutants is not compensated by cell death as the mutation frequency increases. This was also observed but to a lesser extent with the other treatment.

4. Conclusions

TiO_2 photocatalysis was compared with UV-A (control) and UV-C radiations both in terms of cell inactivation and appearance of mutants in *Salmonella typhimurium* TA102 dispersed in water. In the investigated conditions, the inactivation rate appeared faster in the UV-C radiation experiments (10 min for total inactivation with an initial density of 10^7 CFU mL^{-1}) than the photocatalytic experiments (15 min). But photocatalytic process was more energy efficient because a lower energy was required (2 - 20 mWs cm^{-2}, depending on initial bacterial density) compared to UV-C disinfection process (5 - 30 mWs cm^{-2}). In most experiments, the appearance of mutants seemed to be counterbalanced by cell death, which results in an increasing mutation frequency with no increase of the His+ mutant titers. In some case though, a significant increase of the His+ mutant titers was observed before full inactivation. Possibly, in particular conditions where disinfection is incomplete, the selected process may pose a risk of releasing more mutants than untreated cell suspensions, with possible consequences regarding mutation-dependent acquisition of antibiotic resistances. Statistically, both the bacterial inactivation and the evolution of the mutation frequencies appeared directly dependent of initial concentration of microorganism and treatment dose, whatever the treatment, UV-C or photocalysis respectively. This necessarily implies to carefully consider the cell density if the generation of mutants is to be prevented in disinfection setup.

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379 **References**

380 Berney HU, Weilenmann A, Simonetti T (2006) Efficacy of solar disinfection of *Escherichia coli,*
381 *Shigella flexneri, Salmonella* Typhimurium and *Vibrio cholerae*. J Appl Microbiol 101(4):828-836
382 Cho M, Chung H, Choi W, Yoon J, (2004) Linear correlation between inactivation of *E. coli* and OH
383 radical concentration in TiO2 photocatalytic disinfection. Water Res 38:1069–1077
384 Dalrymple OK, Stefanakos E, Trotz MA, Goswami DY (2010) A review of the mechanisms and
385 modeling of photocatalytic disinfection. Appl Catalysis B: Environ 98:27–38
386 de Kok TM, van Maanen JM, Lankelma J, ten Hoor F, Kleinjans JC (1992) Electron spin resonance
387 spectroscopy of oxygen radicals generated by synthetic fecapentaene-12 and reduction of fecapentaene
388 mutagenicity to *Salmonella typhimurium* by hydroxyl radical scavenging. Carcinogenesis 13:1249–
389 1255
390 Dunlop PSM, Ciavola M, Rizzo L, Byrne JA (2011) Inactivation and injury assessment of *Escherichia*
391 *coli* during solar and photocatalytic disinfection in LDPE bags. Chemosphere 85:1160-1166
392 Fàbrega A, Vila J (2013) *Salmonella enterica* serovar Typhimurium skills to succeed in the host:
393 virulence and regulation. Clin Microbiol Rev 26(2):308-341
394 Foster HA, Ditta IB, Varghese S, Steele A (2011) Photocatalytic disinfection using titanium dioxide:
395 spectrum and mechanism of antimicrobial activity. Appl Microbiol Biotechnol. 90(6):1847-1868
396 Grossweiner LI, Jones LR, Grossweiner JB, Rogers BHG (2005) Photochemical Damage to Biological
397 Systems. In *The Science of Phototherapy: an Introduction* (Ed Jones), Springer the Netherlands.
398 Gordon MA, Graham SM, Walsh AL, Wilson LK, Phiri A, Molyneux EM, et al. (2008) Epidemics of
399 invasive *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium
400 infection associated with multidrug resistance among adults and children in Malawi. Clin Infect Dis
401 46:963-969
402 Grey CE, Adlercreutz P (2003) Ability of antioxidants to prevent oxidative mutations in *Salmonella*
403 typhimurium TA102. Mutat Res 527:27–36
404 Haley BJ and Cole D (2009) Distribution, diversity, and seasonality of waterborne salmonellae in a
405 rural watershed. Appl Environ Microb 75(5):1248-1255
406 Hignen WAM and Medema GJ (2006) Inactivation credit of UV radiation for viruses, bacteria and
407 protozoan (oo)cysts in water: A review. Water Res 40(1):3-22

Kanno T, Nakamura K, Ikai H, Kikuchi K, Sasaki K, Niwano Y (2012) Literature review of the role of hydroxyl radicals in chemicallyinduced mutagenicity and carcinogenicity for the risk assessment of a disinfection system utilizing photolysis of hydrogen peroxide. J Clin Biochem Nutr 51:9–14

Koivunen J and Heinonen-Tanski H (2005) Inactivation of enteric microorganisms with chemical disinfectants, UV radiation and combined chemical/UV treatments. Water Res 39(8):1519-1526

Levantesi C, Bonadonna L, Briancesco R, Grohmann E, Toze S, Tandoi V (2012) *Salmonella* in surface and drinking water: Occurrence and water-mediated transmission. Food Res Internat 45(2): 587-602

Levantesi C, La Mantia R, Masciopinto C, Uta Böckelmann C et al. (2010) Quantification of pathogenic microorganisms and microbial indicators in three wastewater reclamation and managed aquifer recharge facilities in Europe. Sci Total Environ 408(21):4923-4230

Li H, Bhaskara A, Megalis C, Tortorello ML (2012) Transcriptomic analysis of *Salmonella* desiccation resistance. Foodborne Pathog Dis 9:1143–1151

Long M, Wang J, Zhuang H, Zhang Y, Wu H, and Zhang J (2014) Performance and mechanism of standard nano-TiO2 (P-25) in photocatalytic disinfection of foodborne microorganisms—*Salmonella* typhimurium and *Listeria monocytogenes*. Food Control 39(1):68–74

Lu Z-X, Zhou L, Zhang Z-L, Shi W-L, Xie Z-X, Xie H-Y, Pang D-W, Shen P (2003) Cell Damage Induced by Photocatalysis of TiO2 Thin Films. Langmuir 19:8765-8768

Magdeburg A, Stalter D, Schlsener M, Ternes T, Oehlmann J (2014) Evaluating the efficiency of advanced wastewater treatment: target analysis of organic contaminants and (geno-)toxicity assessment tell a different story. Water Res 50(0):35-47

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM (2010) The global burden of nontyphoidal *Salmonella gastroenteritis*. Clin Infect Dis 50:882–889

Maron DM, Ames BN (1983) Revised methods for *Salmonella* mutagenicity test. Mutat Res 101: 173-215

Martinez-Urtaza J, Saco M, de Novoa J, Perez-Pineiro P, Peiteado J, Lozano-Leon A, Garcia-Martin O (2004) Influence of environmental factors and human activity on the presence of *Salmonella* serovars in a marine environment. Appl Environ Microbiol 70:2089–2097

Masarikova M, Manga I, Cizek A, Dolejska M, Oravcova V, Myskova P, Karpiskova R, Literak I (2016) *Salmonella enterica* resistant to antimicrobials in wastewater effluents and black-headed gulls in the Czech Republic, 2012. Sci Total Environ 542:102–107

McGuigan KG, Conroy RM, Mosler H-J, du Preez M, Ubomba-Jaswa E, Fernandez-Ibanez P (2012) Solar water disinfection (SODIS): A review from bench-top to roof-top. J Hazard Mater 235– 236:29–46

Michael GB, Butaye P, Cloeckaert A, Schwarz S (2006) Genes and mutations conferring antimicrobial resistance in *Salmonella*: an update. Microbes Infect 8(7):1898-1914

Mišík M, Knasmueller S, Ferk F, Cichna-Markl M, Grummt T, Schaar H, Kreuzinger N (2011) Impact of ozonation on the genotoxic activity of tertiary treated municipalwastewatr. Water Res 45(12):3681-3691

Monarca S, Feretti D, Collivignarelli C, Guzzella G, Zerbini I, Bertanza G, Pedrazzani R (2000) The influence of different disinfectants on mutagenicity and toxicity of urban wastewater. Water Res 34(17):4261-4269

Nutt JD, Li X, Woodward CL, Zabala-Diaz IB and Ricke SC (2003) Growth kinetics response of a *Salmonella* Typhimurium poultry marker strain to fresh produce extracts. Bioresource Technol 89:313-316

Oubrim N, Ennaji MM, Badri S, Cohen N (2012) Removal of Antibiotic-Resistant *Salmonella* in Sewage Water from Wastewater Treatment Plants in Settat and Soualem, Morocco. Eur J Sci Res 68(4):565-573

Polo F, Figueras MJ, Inza I, Sala J, Fleisher JM, Guarro J (1999) Prevalence of *Salmonella* serotypes in environmental waters and their relationships with indicator organisms. A Van Leeuw 75:285–292

Polo-López MI, Castro-Alférez M, Oller I, Fernández-Ibáñez P (2014) Assessment of solar photo-Fenton, photocatalysis, and H2O2 for removal of phytopathogen fungi spores in synthetic and real effluents of urban wastewater. Chemi Eng J 257:122-130

Popoff M Y (2001) Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Center for Reference and Research on Salmonella (8th ed). Institut Pasteur Paris, France.

Rahmani M, Peighambari S M, Svendsen C A, Cavaco L M, Agersø Y, Hendriksen RS (2013) Molecular clonality and antimicrobial resistance in *Salmonella enterica* serovars Enteritidis and Infantis from broilers in three Northern regions of Iran. BMC Vet Res 9:66

Rahmani BA, Wasfy MO, Maksoud MA, Hanna N, Dueger E, House B (2014) Multi-drug resistance and reduced susceptibility to ciprofloxacin among *Salmonella* enterica serovar Typhi isolates from the Middle East and Central Asia. New Microbes and New Infect 2(4):88-92

468 Rincon AG, Pulgarin C (2007) Fe3+ and TiO2 solar-light-assisted inactivation of *E. coli* at field scale
469 – implications in solar disinfection at low temperature of large quantities of water. Catal Today 122:
470 128–136.
471 Rizzo L (2011) Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater
472 treatment. Water Res 45:4311-4330
473 Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D (2013)
474 Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the
475 environment: A review. Sci Total Environ 447:345-360
476 Rizzo L, Ferro G, Manaia CM (2014a) Wastewater disinfection by solar heterogeneous photocatalysis:
477 Effect on tetracycline resistant/sensitive enterococcus strains. Global Nest Journal 16: 455-462
478 Rizzo L, Della Sala A, Fiorentino A, Li Puma G (2014b) Disinfection of urban wastewater by solar
479 driven and UV lamp - TiO2 photocatalysis: Effect on a multi drug resistant *Escherichia coli* strain.
480 Water Res 53:145-152
481 Robertson GP (2005) Functional and therapeutic significance of Akt deregulation in malignant
482 melanoma. Cancer Metast Rev 24:273–285
483 Sciacca J, Rengifo-Herrera J, Wethe, Pulgarin C (2011) Solar disinfection of wild *Salmonella* sp. in
484 natural water with a 18 L CPC photoreactor: Detrimental effect of non-sterile storage of treated water.
485 Sol Energy 85:1399–1408
486 Sharma VK, Johnson N, Cizmas L, McDonald TJ, Kim H (2016). A review of the influence of treatment
487 strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere 150:702-714
488 Silva RC, Cardoso WM, Teixeira RSC, Albuquerque ÁH, Horn RV, Cavalcanti CM (2013) *Salmonella*
489 *Gallinarum* virulence in experimentally infected Japanese quails (Coturnix japonica). Braz J of Poult
490 Science 15(1):39-45
491 Sinha RP, Häder DP (2002) UV-induced DNA damage and repair: a review. Photochem Photobiol Sci
492 1(4):225-236
493 Simonet J, Gantzer C (2006) Inactivation of poliovirus 1 and F-specific RNA phages and degradation
494 of their genomes by UV irradiation at 254 nanometers. Appl Environ Microbiol 72(12):7671-7677
495 Tate P, Stannera A, Shieldsa K, Smithc S, Larcom L (2006) Blackberry extracts inhibit UV-induced
496 mutagenesis in *Salmonella typhimurium* TA100. Nutrition Research 26:100–104

497 Wang J, Zhuan H, Hinton A Jr., Bowker B, Zhang J (2014) Photocatalytic disinfection of spoilage
498 bacteria *Pseudomonas fluorescens* and *Macrococcus caseolyticus* by nano-TiO_2. LWT - Food Science
499 and Technology 59(2):1009–1017
500 Wéry N, Lhoutellier C, Ducray F, Delgenès JP & Godon JJ (2008) Behaviour of Pathogenic and
501 Indicator Bacteria During Urban Wastewater Treatment and Sludge Composting, as Revealed By
502 Quantitative PCR. Water Res 42:53-62
503

Figure captions

Fig. 1 Control tests on TiO_2/UV experiments: effect of UV-A radiation on the inactivation and the formation of mutants for three different initial cell loads of strain TA102: 10^9 (a), 10^8 (b) and 10^7 (c) $CFU.mL^{-1}$. Error bars represent standard deviation from three independent experiments

Fig. 2 Effect of TiO_2/UV process on the inactivation and the formation of mutants for three different initial cell loads of strain TA102: 10^9 (a), 10^8 (b) and 10^7 (c) $CFU.mL^{-1}$. Error bars represent standard deviation from three independent experiments

Fig. 3 Effect of UV-C process on the inactivation and the formation of mutants for three different initial cell loads of strain TA102: 10^9 (a), 10^8 (b) and 10^7 (c) $CFU.mL^{-1}$. Error bars represent standard deviation from three independent experiments

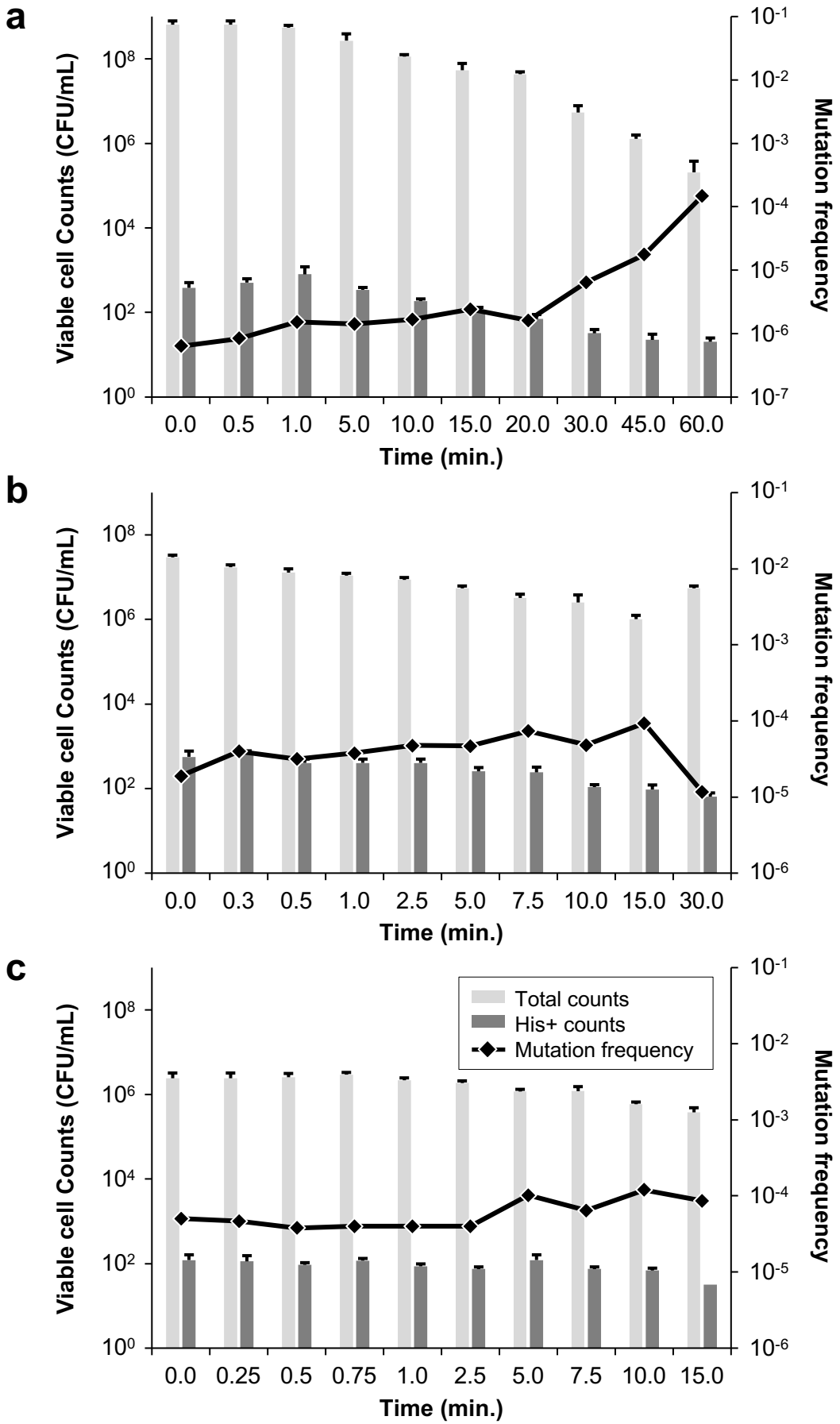

a
Viable cell Counts (CFU/mL)
Mutation frequency
Time (min.)
0.0 0.5 1.0 5.0 10.0 15.0 20.0 30.0 45.0 60.0
b
Viable cell Counts (CFU/mL)
Mutation frequency
Time (min.)
0.0 0.3 0.5 1.0 2.5 5.0 7.5 10.0 15.0 30.0
c
Total counts
His+ counts
Mutation frequency
Viable cell Counts (CFU/mL)
Mutation frequency
Time (min.)
0.0 0.25 0.5 0.75 1.0 2.5 5.0 7.5 10.0 15.0

Figure 1

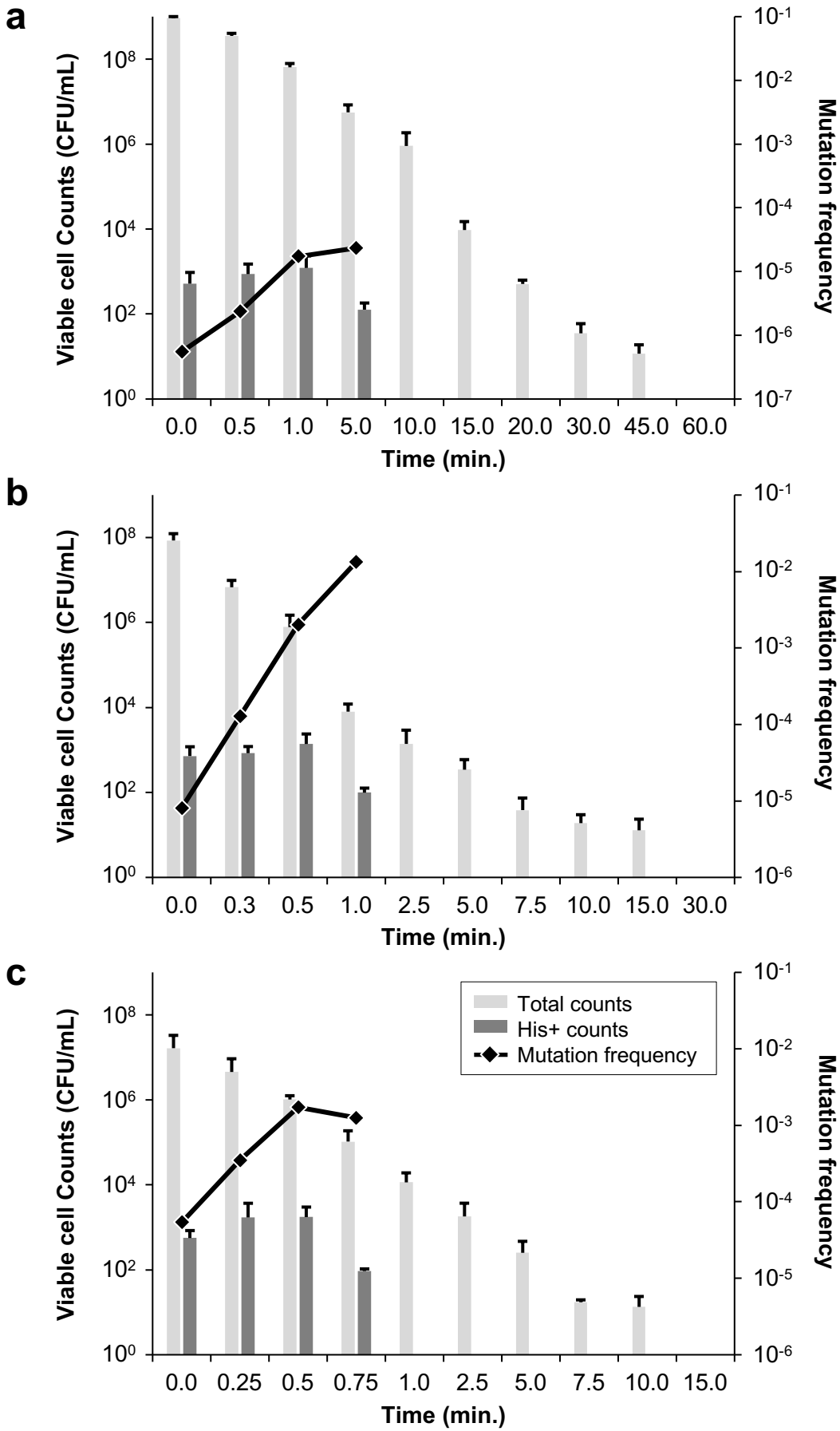

a
b
c
Viable cell Counts (CFU/mL)
Mutation frequency
Time (min.)
Total counts
His+ counts
Mutation frequency
0.0 0.5 1.0 5.0 10.0 15.0 20.0 30.0 45.0 60.0
0.0 0.3 0.5 1.0 2.5 5.0 7.5 10.0 15.0 30.0
0.0 0.25 0.5 0.75 1.0 2.5 5.0 7.5 10.0 15.0

Figure 2

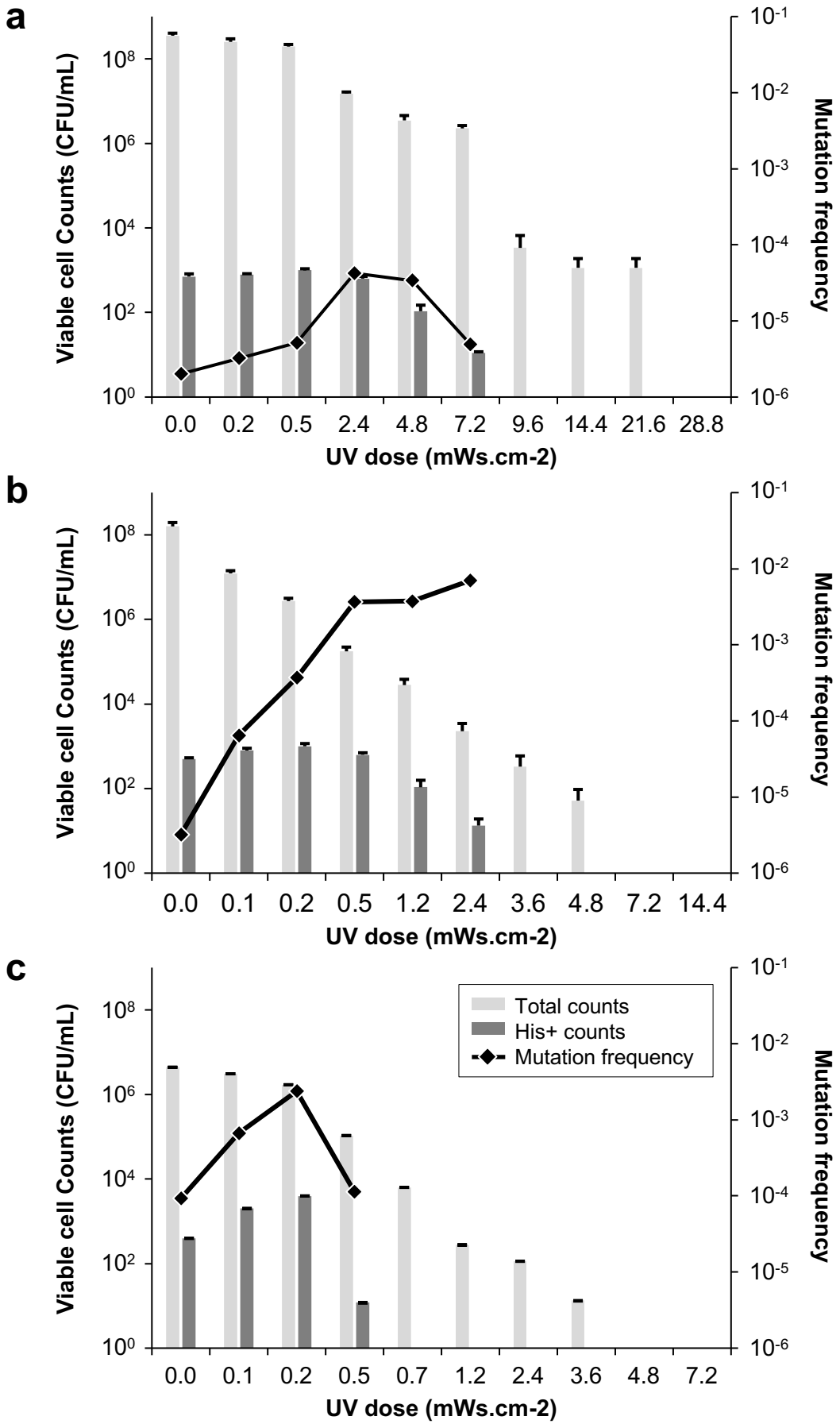

a
b
c
Viable cell Counts (CFU/mL)
Mutation frequency
UV dose (mWs.cm-2)
0.0 0.2 0.5 2.4 4.8 7.2 9.6 14.4 21.6 28.8
0.0 0.1 0.2 0.5 1.2 2.4 3.6 4.8 7.2 14.4
0.0 0.1 0.2 0.5 0.7 1.2 2.4 3.6 4.8 7.2
Total counts
His+ counts
Mutation frequency

Figure 3

Supplementary material

Comparing TiO_2 photocatalysis and UV-C radiation for inactivation and mutants formation of *Salmonella typhimurium* TA102

Antonino Fiorentino, Luigi Rizzo, Hélène Guilloteau, Xavier Bellanger, Christophe Merlin.

Table of content:

1. Statistical analysis procedures

The correlations between the bacterial mortality (log (N/N_0)) and the duration of the treatments, or the doses received, were assessed using the Pearson's test. Then, regression data analyses were performed on log-transformed data: log(N/N_0) for the total cell counts and Log(F/F_0) for the frequencies of mutation. The effects of both the treatment and the initial bacterial cell load were analyzed using a Fisher F-test and a Student T-test. The Fisher F-test was used to test data for homogeneity of variances. Thereafter, once homogeneity was verified, a Student T-test was further used to compare the trends (slopes) of linear regressions. A statistical difference revealed by a StudentT-test, combined to the comparison of regression trend-lines (slopes) allowed determining whether a given treatment or condition had stronger effects than another one.

2. Dose-dependent effects of the treatments on bacterial viability

The dose-dependent effect of each treatment (UV-C, UV-A, and UV-A + TiO_2) on the bacterial viability (total cell count) was evaluated using a Pearson correlation test. For all conditions, the cell count abatements appeared statistically correlated to the treatment dose whatever the initial cell load (Table S1). Data were further analyzed to evaluate the statistical significance of the initial cell load and to compare treatments where possible.

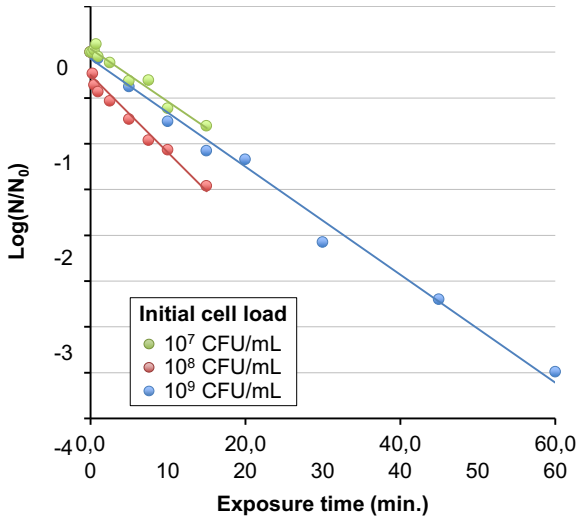
Table S1: Correlation between viable cell counts (log(CFU/mL)) and the treatment dose (UV-C)/treatment duration (UV-A ± TiO_2) for the three different initial cell loads. The table provides "Pearson correlation coefficient" (PCC) and p-values (between parentheses).

Initial cell loads / treatment	10^9 CFU/mL	10^8 CFU/mL	10^7 CFU/mL
UV-C	-0.957 (1.5×10^{-5})	-0.840 (2.0×10^{-3})	-0.876 (8.9×10^{-4})
UV-A	-0.933 (7.9×10^{-5})	-0.750 (1.2×10^{-2})	-0.900 (3.8×10^{-4})
UV-A + TiO_2	-0.988 (1.0×10^{-7})	-0.976 (1.3×10^{-6})	-0.981 (5.2×10^{-7})

3. Statistical analysis of the UV-A treatment efficiency

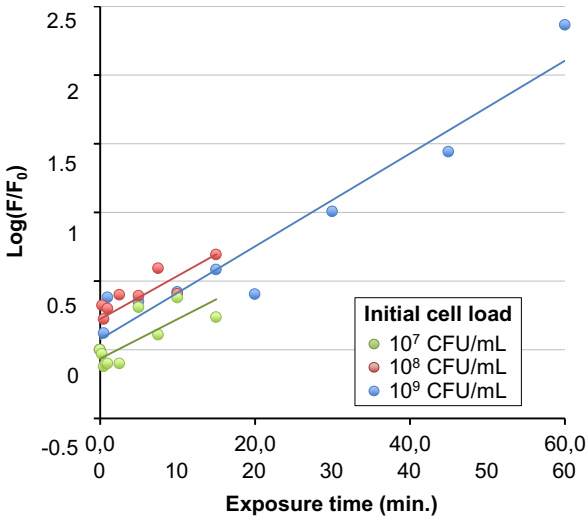
3.1. Regression analyses and trendline equations summaries

Fig. S1: Total viable cell counts abatements



Initial cell load	10^9 CFU/mL	10^8 CFU/mL	10^7 CFU/mL
Slope	-0.059	-0.085	-0.058
r^2	0.991	0.942	0.964

Fig. S2: Evolution of mutation frequencies



Initial cell load	10^9 CFU/mL	10^8 CFU/mL	10^7 CFU/mL
Slope	0.034	0.031	0.029
r^2	0.933	0.674	0.609

3.2. Statistical comparison of the treatment efficiency as a function of the cell load

At a first glimpse, the cell count abatement profiles (Fig. S1) and the evolution of the frequencies profiles (Fig. S2) for the UV-A treatment appeared relatively close to each other whatever the initial cell loads. This similarity was further confirmed by Student T-tests, whenever the comparison was authorized by Fisher F-tests (Tables S2 and S3).

Table S2: Comparison of total cell counts abatements for the UV-A treatment.

Initial cell load	10^9 CFU/mL	10^8 CFU/mL	10^7 CFU/mL
10^9 CFU/mL		≈	ND
10^8 CFU/mL			ND
10^7 CFU/mL			

Table S3: Comparison of the evolutions of mutation frequencies for the UV-A treatment.

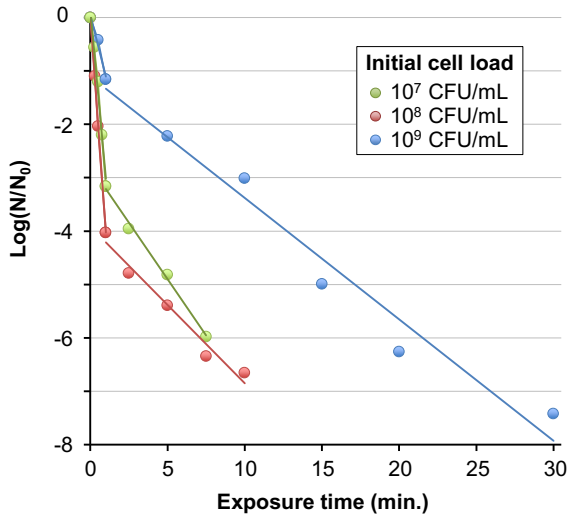
Initial cell load	10^9 CFU/mL	10^8 CFU/mL	10^7 CFU/mL
10^9 CFU/mL		≈	≈
10^8 CFU/mL			≈
10^7 CFU/mL			

NA : Not applicable (not enough data in the regression curve (2 points) to authorize a statistical comparison); **ND :** Not determined (variances not homogenous according to a Fisher F-test); **≠ :** the trends (slopes) are significantly different ($p>0.05$); **≈ :** the trends (slopes) are statistically similar ($p\leq0.05$).

4. Statistical analysis of the UV-A + TiO_2 treatment efficiency

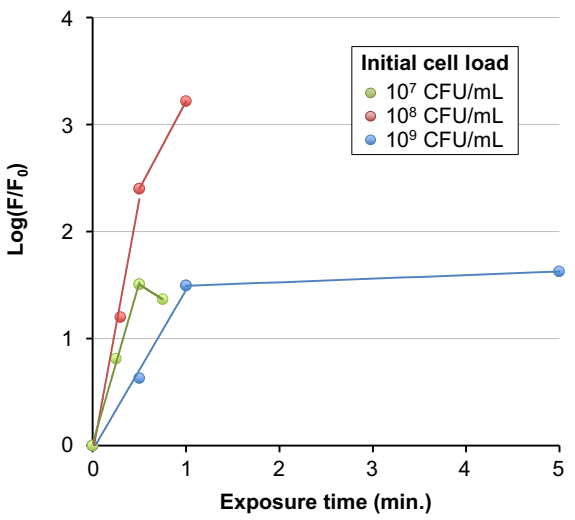
4.1. Regression analyses and trendline equations summaries

Fig. S3: Total viable cell counts abatements



Initial cell load	10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
Curves	part 1	part 2	part 1	part 2	part 1	part 2
Slope	-1.160	-0.227	-4.062	-0.293	-3.182	-0.422
r^2	0.976	0.966	0.999	0.970	0.984	0.995

Fig. S4: Evolution of the mutation frequencies



Initial cell load	10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
Curves	part 1	part 2	part 1	part 2	part 1	part 2
Slope	1.494	0.033	4.737	1.634	3.011	-0.553
r^2	0.992	1.000	0.987	1.000	0.998	1.000

4.2. Statistical comparison of the treatment efficiency as a function of the cell load

Both, the cell count abatement profiles (Fig. S3) and the evolution of the mutation frequencies profiles (Fig. S4) obtained for the UV-A+TiO_2 treatment tend to display a biphasic aspect with a trend linked to the initial cell loads. The biphasic aspects of the curves could be confirmed statistically by comparing the different parts of a same curve. When statistical comparisons were authorized by the Fisher F-test, the Student T-test showed that the treatment-dependent cell killing is significantly more reduced as the initial cell load increases (Fig. S3 and Table S2). Similarly, the analysis of the first part of the mutation frequency evolution curves, show that for an initial cell load of 10^9 CFU/mL, the time-dependent increase of the mutation frequency is significantly lower than for the two other cell concentrations (Fig. S4 and Table S3).

Table S2: Comparison of total cell counts abatements for the UV-A+TiO_2 treatment.

Initial cell load / curve part		10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
		part 1	part 2	part 1	part 2	part 1	part 2
10^9 CFU/mL	part 1		ND	≠		≠	
	part 2			ND	≈		ND
10^8 CFU/mL	part 1				≠	≠	
	part 2						≠
10^7 CFU/mL	part 1						≠
	part 2						

Table S3: Comparison of the evolutions of mutation frequencies for the UV-A+TiO_2 treatment.

Initial cell load / curve part		10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
		part 1	part 2	part 1	part 2	part 1	part 2
10^9 CFU/mL	part 1		NA	≠		≠	
	part 2				NA		NA
10^8 CFU/mL	part 1				NA	≈	
	part 2						NA
10^7 CFU/mL	part 1						NA
	part 2						

NA : Not applicable (not enough data in the regression curve (2 points) to authorize a statistical comparison); **ND :** Not determined (variances not homogenous according to a Fisher F-test); **≠ :** the trends (slopes) are significantly different ($p>0.05$); **≈ :** the trends (slopes) are statistically similar ($p\leq0.05$).

5. Statistical analysis of the UV-C treatment efficiency

5.1. Regression analyses and trendline equations summaries

Fig. S5: Total viable cell counts abatements

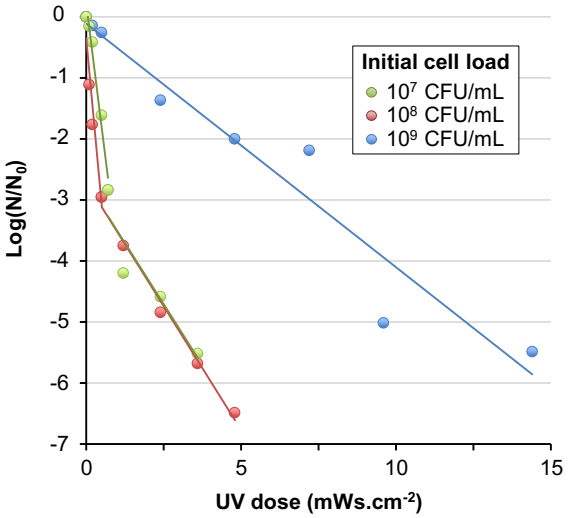
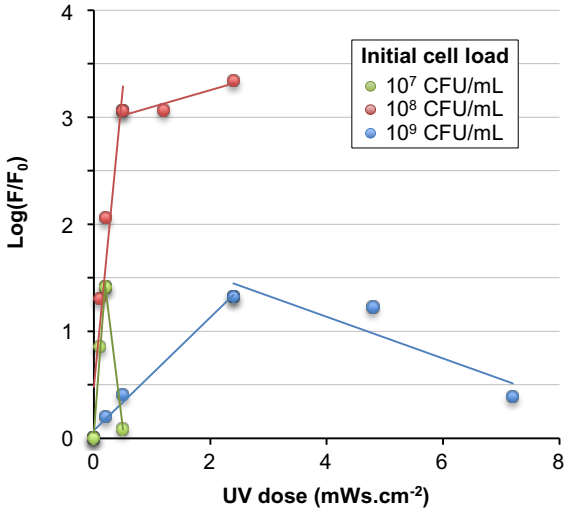


Fig. S6: Evolution of the mutation frequencies



Initial cell load	10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
Curves	part 1	part 2	part 1	part 2	part 1	part 2
Slope	-0.399	-	-5.539	-0.811	-4.080	-0.800
r^2	0.937	-	0.937	0.990	0.971	0.872

Initial cell load	10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
Curves	part 1	part 2	part 1	part 2	part 1	part 2
Slope	0.527	-0.195	5.618	0.157	7.048	-4.419
r^2	0.989	0.828	0.887	0.888	0.985	1.000

5.2. Statistical comparison of the treatment efficiency as a function of the cell load

With the UV treatment, the biphasic aspect of the cell count abatement curves where solely observed for the initial cell loads of 10^7 and 10^8 CFU/mL (Fig. S5). When statistical comparison was authorized by the Fisher F-test, Student T-tests showed that for these two initial cell loads (i) the abatement curves are statistically similar and (ii) that the treatment is relatively more efficient than for an initial cell load of 10^9 CFU/mL (Fig. S5 and Table S4). Concerning the mutation frequencies profiles, the lack

of analysable datapoints (valid Fisher tests and/or enough datapoints on the trend line) authorized comparisons in a limited number cases, mainly the first part of the curves for the two extreme initial cell loads. In such cases the Student T-test demonstrated that mutation frequencies increased faster as the initial cell load is smaller (Fig. S6 and Table S5).

Table S4: Comparison of total cell counts abatements for the UV-C treatment.

Initial cell load / curve part		10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
		part 1		part 1	part 2	part 1	part 2
10^9 CFU/mL	part 1			≠	ND	ND	≈
10^8 CFU/mL	part 1				≠	≈	NA
	part 2					NA	≈
10^7 CFU/mL	part 1						≠
	part 2						

Table S5: Comparison of the evolutions of mutation frequencies for the UV-C treatment.

Initial cell load / curve part		10^9 CFU/mL		10^8 CFU/mL		10^7 CFU/mL	
		part 1	part 2	part 1	part 2	part 1	part 2
10^9 CFU/mL	part 1		≠	ND		≠	
	part 2				≈		NA
10^8 CFU/mL	part 1				≠	ND	
	part 2						NA
10^7 CFU/mL	part 1						NA
	part 2						

NA : Not applicable (not enough data in the regression curve (2 points) to authorize a statistical comparison); **ND :** Not determined (variances not homogenous according to a Fisher F-test); **≠ :** the trends (slopes) are significantly different ($p>0.05$); **≈ :** the trends (slopes) are statistically similar ($p\leq0.05$).

6. Statistical comparison of the UV-A and UV-A + TiO2 treatments

UV-A and UV-A/TiO_2 treatments, which were carried out in the same conditions, were compared using Student T-tests. Whenever it was authorized by Fisher F-tests, the comparisons of the cell count abatements showed that (i) UV-A and UV-A+TiO2 treatments are significantly different for a given initial cell load (Table S8), and (ii) with a stronger effect of the photocatalytic treatment according to the trends (slopes) of the linear regressions (Fig. S1, Fig. S3). The same tendencies were observed for the evolution of the mutation frequencies although the statistical comparison were authorized (Fisher F-test) for the two higher initial cell loads only (Table S7, Fig. S2, Fig. S4).

Table S6: Comparison of total cell counts abatements for the UV-A and UV-A + TiO_2 treatments.

Initial cell load and treatment	10^9 CFU/mL UV-A + TiO_2		10^8 CFU/mL UV-A + TiO_2		10^7 CFU/mL UV-A + TiO_2	
	part 1	part 2	part 1	part 2	part 1	part 2
10^9 CFU/mL UV-A	≠	ND				
10^8 CFU/mL UV-A			≠	≠		
10^7 CFU/mL UV-A					ND	≠

Table S7: Comparison of the evolutions of mutation frequencies for the UV-A and UV-A + TiO_2 treatments.

Initial cell load and treatment	10^9 CFU/mL UV-A + TiO_2	10^8 CFU/mL UV-A + TiO_2	10^7 CFU/mL UV-A + TiO_2
	part 1	part 1	part 1
10^9 CFU/mL UV-A	≠		
10^8 CFU/mL UV-A		≠	
10^7 CFU/mL UV-A			ND

NA : Not applicable (not enough data in the regression curve (2 points) to authorize a statistical comparison); **ND :** Not determined (variances not homogenous according to a Fisher F-test); **≠ :** the trends (slopes) are significantly different ($p>0.05$); **≈ :** the trends (slopes) are statistically similar ($p\leq0.05$).