PHOTOCATALYTIC INACTIVATION OF BACTERIA IN A FIXED-BED REACTOR: MECHANISTIC INSIGHTS BY EPIFLUORESCENCE MICROSCOPY

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ABSTRACT

The photocatalytic inactivation of *Escherichia coli* bacteria with titanium dioxide immobilised in a fixed-bed reactor has been studied and compared with the results obtained using aqueous titania suspensions. In both cases, the photocatalytic inactivation has been successfully achieved, reaching a 6-log decrease in the concentration of bacteria. The slurry system shows a higher inactivation rate at the beginning of the reaction that decreases progressively, whereas the fixed-bed reactor increases the inactivation rate as the irradiation time increases, leading both catalytic systems to comparable irradiation times for the total inactivation of bacteria, i.e. concentration below the detection limit. This opposite trend seems to be related to differences in the bacteria-TiO_2 interaction, being also observed that the fixed-bed catalytic system is less affected by the competition for the oxidant species of byproducts released after the bacterial cell lysis. Epifluorescence microscopy pictures taken after a dual DAPI/PI staining membrane permeability assay provide mechanistic insights into this different behaviour, showing for the fixed-bed experiments an increase in the damaged bacteria from the very beginning of the reaction, whereas for the slurry system they were not observed until longer irradiation exposition is achieved. These results suggest as hypothesis that although in absolute terms the number of hydroxyl radical attacks could be even lower in the immobilised systems, the damages are more concentrated on the area in which the bacteria-TiO_2 interaction takes place. Although the fixed-bed reactor requires comparable irradiation time for total inactivation of bacteria than that corresponding to the slurry system, it shows minor deactivation and allows the continuous operation without the need of particles removal, making this immobilised system a good alternative to successfully scale-up the photocatalytic disinfection technology.

KEYWORDS: photocatalysis, disinfection, *E.coli*, fixed-bed reactor, slurry reactor, epifluorescence microscopy.

1. INTRODUCTION

Chlorination processes have been traditionally used for disinfection of both drinking water and tertiary treatment of wastewater effluents, involving the expenses related to the dosing of chemical compounds but even more important, the formation of carcinogenic and mutagenic chloro-organic disinfection byproducts [1]. As a consequence, new technologies have to be developed to overcome the drawbacks of the present disinfection processes.

Heterogeneous photocatalysis using TiO_2 as catalyst has been long studied due to the advantages over other advanced oxidation processes (AOPs), such as operation under ambient temperature and pressure, no requirements of pH adjustment or additional oxidant chemicals apart from air, and also the possibility of using solar light as radiation source. Since the early work of Matsunaga et al. [2], titania-based photocatalytic processes have been reported by many research groups [3,4] as alternative technology to inactivate microorganisms successfully.

Most of the reports about photocatalytic disinfection of water sources use TiO_2 slurries [5,6], reaching a high efficiency to inactivate bacteria. Some efforts have been also focused on using immobilised TiO_2 systems [7-12], usually showing to be less active and require more irradiation time compared with TiO_2 slurries due to several problems such as mass transfer limitations and lower titania surface area. Moreover, the immobilised catalysts must keep the stability, avoiding the possible leaching of TiO_2 particles to the solution [13]. However, from an engineering viewpoint, the real application of this technology has to avoid the separation step for a feasible continuous water treatment.

Different reactor configurations of the catalytic material inside the reactor can lead to changes in the bacteria-catalyst interaction and consequently, not only yielding different absolute activities in the photocatalytic inactivation of bacteria at long irradiation time [14], but also variations in the mechanism of bacterial damages generation that could explain the differences in the shape of the inactivation profiles reported in the literature for immobilised and slurry TiO_2 [7,9]. As certain amount of damages is necessary to cause the irreversible inactivation of the bacteria [15], plots of viable bacteria calculated by counting colony forming units (CFU) on agar plates (commonly used to follow bacterial inactivation experiments) could mask significant changes in the damaging mechanism, which makes

interesting the possibility to discriminate the concentration of bacteria with intermediate levels of damage.

This work deals with the photocatalytic inactivation of *Escherichia coli* with TiO_2 in slurry and in a fixed-bed reactor, focusing the study on the novel application of epifluorescence microscopy techniques after a dual DAPI/PI staining membrane permeability assay to provide mechanistic insights into the possible differences between both photocatalytic systems concerning bacteria-TiO_2 interaction.

2. EXPERIMENTAL

2.1. Photoreactor and catalysts.

Photocatalytic experiments of bacteria inactivation were carried out using two different catalytic systems: (i) a slurry reactor, using 0.1 g L^{-1} of Degussa P25 TiO_2 in suspension, and (ii) a fixed-bed reactor with Degussa P25 TiO_2 immobilized onto 6x6 mm glass Raschig rings. The illumination source was a Philips TL 6W black light lamp placed in the axis of the annular photoreactor. The UV-A incident photon flow, determined by ferrioxalate actinometry, was 2.8×10^{-6} Einstein s^{-1} with a maximum emission peak centred at 365 nm. More details of the experimental setup, the immobilisation procedure and the optimisation of the reactor system can be found elsewhere [16].

2.2. Photocatalytic experiments.

Escherichia coli K12 provided by the Colección Española de Cultivos Tipo (CECT 4624, corresponding to ATCC 23631) was used as model microorganism. *E. coli* is frequently used as a model organism in microbiology studies and it is a common faecal contamination indicator to evaluate the microbiological quality of water. K12 is an *E. coli* strain well-adapted to the laboratory environment, and, unlike wild type strains, has lost its ability to thrive in the intestine, making safer the experimental work. Fresh liquid cultures with a stationary concentration around 10^9 colony forming units (CFU) mL^{-1} were prepared by inoculation in a Luria–Bertani nutrient medium (Miller's LB Broth, Scharlab) and incubation at 37 ºC for 24 h under constant stirring on a rotary shaker, being diluted to the initial

concentration of bacteria required for the experiments. Two different kinds of water have been used: (i) deionised water; and (ii) synthetic municipal wastewater [17] diluted to a total organic carbon value of 15 mg L^{-1} to simulate the effluents of a wastewater treatment plant. The analysis of the samples along the reaction was carried out following the concentration of viable bacteria through a standard serial dilution procedure and agar plating, using eight independent measurements of each sample to obtain statistically significant data. Additionally, key experiments were repeated three times to test the reproducibility of the disinfection results. More details of the procedure followed to prepare the cultures, the initial reaction suspension and the bacterial quantification can be found elsewhere [16].

2.3. DAPI/PI staining permeability assay.

The physiological bacterial state was examined by a permeability assay using two nucleic acid stains, 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) and propidium iodide, (PI) (Sigma-Aldrich). DAPI is used as specific DNA fluorochrome to stain all DNA bacteria cells (viable and non viable) since it is able to cross uncompromised cell membranes and bind DNA. In contrast, PI is an indicator of the membrane integrity since it can not cross the cell membrane unless it has been damaged or compromised. If the cell membrane is damaged, PI enters the cell and binds to the nucleic acids [18-22]. Therefore, the combination of both stains assess the physiological state of the bacteria by establishing a relationship between staining and membrane integrity. DAPI stock solution was made up to a concentration of 5 mg/5 mL, in sterile deionised water and 0.5 mL of 2.5 % glutaraldehyde. PI stock solution was made up to a concentration of 25 mg/25 mL in sterile deionised water. Both of them were stored at 3-5 ºC in the dark.

A volume of 80 µL of DAPI and PI (1 mg mL^{-1}) was added to 4 mL of the suspension sample during the photocatalytic experiment, incubated in the dark at room temperature for 10 min and filtered onto 0.22 µm black polycarbonate filters (Millipore). The filters were placed on glass slides, covered with a cover slip and visualised under immersion oil with a 100x objective on a Leica DMI 4000B microscope fitted with a fluorescence attachment ebq-100mc-L and coupled with a Canon Power Shot S80 digital camera. For each sample, three different areas from the inner part of the filter (to avoid edge effects) were randomly chosen, taking two images in each of them using a filter with excitation at 340-380 nm and

suppression at 425 nm, and a filter with excitation at 515-560 nm and suppression at 590 nm to visualise DAPI-stained cells and PI-stained cells, respectively. Counting of the cells has been carried out with the help of image processing software (ImageJ 1.42, National Institutes of Health, http://www.nih.gov), averaging the three independent bacteria counting measurements to obtained statistically significant data.

3. RESULTS

3.1. Photocatalytic disinfection experiments.

Figure 1 shows the comparison between the photocatalytic inactivation of aqueous suspensions of increasing concentration of *E. coli* in deionised water using a slurry reactor with TiO_2 in suspension and the immobilised fixed-bed reactor, both catalytic systems using the optimal TiO_2 loading determined previously [16]. TiO_2 suspensions lead to higher inactivation activities for short reaction time, decreasing the rates for longer irradiation time, probably due to the competition for the hydroxyl radical of the compounds released after the bacterial cell lysis. In contrast, the inactivation curves obtained with fixed-bed reactor show a longer initial delay, but for longer irradiation time, the bacterial inactivation is accelerated, reaching a 6-log decay in *E.coli* concentration after an irradiation time comparable to that of the TiO_2 slurry. This somewhat unexpected high activity of the immobilised system might be due to a relatively high active surface given titania supported on the Raschig rings placed in the whole photoreactor volume, in comparison with other immobilised systems such as wall reactors [7, 16].

FIGURE 1

As the initial concentration of *E. coli* increases, more irradiation time is required to reach the total inactivation below the bacterial detection limit, in agreement with other authors [23,24,25]. These results were expected, as a higher concentration of bacteria obviously requires a higher number of •OH radical attacks to be inactivated. However, it is observed that an increase in the initial concentration of bacteria does not lead to a proportional increase in the irradiation time required to reach a complete inactivation. Therefore, there is not a linear correlation between the amount of inactivated bacteria and the amount of hydroxyl

radicals generated, which can be assumed to be proportional to irradiation time. In fact, plots of inactivation profiles in relative terms with respect to the initial concentration of bacteria (insets of figure 1) clearly overlap, suggesting that phenomena involved in the photocatalytic inactivation of bacteria are essentially first-order processes regarding the concentration of bacteria. This means that each step of the serial-event inactivation mechanism in which bacteria with different degrees of damage are successively attacked by ·OH radicals until being totally inactivated follows a exponential decay kinetics. However, this does not mean that inactivation curves should be fitted by pure log-linear equations, as the concentration of viable bacteria determined by counting the CFU grown on agar plates includes viable bacteria with different level of damages, and consequently the curves do not represent a single elemental first-order reaction step, but a sequence of them, as discussed previously [15]. Therefore, these results suggest that the inactivation rate is more dependent on the bacteria concentration than on the •OH radical generation rate for the reactor configuration here studied, suggesting than bacteria-TiO_2 interaction could be the limiting step of the process.

Water composition has been shown to play an important role on the photocatalytic activity for bacterial inactivation [6,8,9,16]. In addition to the possible competition for the hydroxyl radicals of the organic and inorganic species, it could affect the bacteria-TiO_2 interaction [14,26,27]. Figure 2 shows the comparison of the activity of the slurry and fixed-bed reactor on the photocatalytic inactivation of *E. coli* in a simulated effluent of a wastewater treatment plant (WTP). In this case the irradiation time required to reach the complete inactivation for the fixed-bed reactor was far shorter than for the slurry TiO_2.

FIGURE 2

When using TiO_2 in suspension, a longer initial delay is observed in comparison with inactivation in deionised water, probably related to the competition of ions and organic matter for the •OH radicals, or maybe changes on the bacteria-catalyst interaction. Moreover, the presence of ions and organic matter in WTP effluents might lead to a higher resistance of *E. coli* for the inactivation compared with the results in deionised water, where the osmotic stress weakens the bacterial cell wall. [28]. In contrast, only a slight effect is observed when the bacterial inactivation with the fixed-bed reactor is compared between deionised water and the simulated effluent of WTP, where the initial delay observed is scarcely longer and the irradiation time to achieve the total inactivation below the detection limit is quite similar. As

the competition of the chemical substances present in the water for the reactive species and the osmotic effects on the viability of *E.coli* cells should be essentially the same in slurry and fixed-bed reactor, these results point out the relevance of the bacteria-catalyst interaction as responsible for the decrease in activity, since the bacteria-TiO_2 contact is the main different between both types of reactors. In addition to that, it must be noticed that no loss of the catalytic activity of the fixed-bed reactor (deactivation) is observed even after ten cycles of reuse (data not shown)..

3.2. DAPI/PI staining membrane permeability assays.

These assays were designed to look for mechanistic insights related to the differences of the bacteria-catalyst interaction, and consequently the photocatalytic activity, of TiO_2 in suspension and the fixed-bed reactor. As it was stated before, one of the limitations of using CFU on agar plates as method to follow bacterial inactivation experiments is that this technique only provides information about the viability for growing of the bacteria, and does not give any information regarding the physiological state or the average level of damage of the total amount of viable bacteria.

Membrane permeability can be used as a measurement of the photocatalytic damage over the bacteria, considering that the most commonly accepted photocatalytic inactivation mechanism is based on the attack of •OH radicals and other reactive oxygen species (ROS) to the bacteria cell wall, where the bacteria-catalyst contact takes place [29,30]. Cumulative damages lead to the cell membrane disorder, increasing its permeability as demonstrated by the leakage of potassium ions [31,32], and finally the cell lysis and death.

Figure 3 shows the progress of an experiment of *E. coli* inactivation in a simulated WTP effluent with the fixed bed reactor followed by epifluorescence microscopy after dual DAPI/PI staining. Under UV excitation (figure 3 left), DAPI-stained cells appear blue fluorescent, whereas DAPI/PI-stained cells appear red fluorescent. On the other hand, under green excitation (figure 3 right), DAPI-stained cells show no fluorescence, whereas DAPI/PI-stained cells appear brightly red fluorescent. Considering that DAPI is able to cross undamaged cell membranes whereas PI is only able to cross damaged membranes, the combination of both stains allows the assessing of the membrane integrity along the reaction.

Blue cells showed in Figure 3 represent the undamaged or not badly damaged bacteria, whereas red cells correspond to bacteria with seriously damaged membrane.

FIGURE 3

It can be noticed that as the irradiation time increases, the number of blue cells decreases whereas the number of red cells rises. The same trend was observed for an experiment carried out with the slurry reactor (data not shown). It means that the number of undamaged bacteria decreases along the reaction whereas the number of damaged bacteria increases. These results seem to agree with the most accepted photocatalytic inactivation mechanism according to which the cell wall is the initial point of attack by the •OH radicals, causing damages on the cell wall that lead to an increase in the permeability of the cell membrane up to finally cause the irreversible inactivation.

Quantitative analysis of the epifluorescence microscopy pictures have been carried out with the help of image processing software. The cells have been divided into blue cells (undamaged) and red cells (damaged). The value (and its error) of the percentage of each group of cells has been calculated by averaging the results of three images taken in different regions of the whole microscope observation area.

Figure 4 shows the evolution of the percentage of blue and red cells along the experiments of photocatalytic inactivation of *E.coli* in simulated WTP effluents for the slurry and the fixed-bed reactor.

FIGURE 4

The comparison between these membrane permeability assays and the percentage of viable for growing CFU determined by agar plating (also shown in figure 4) suggest that red cells (damaged bacteria) do not exactly correspond to inactivated bacteria (CFU). In fact, in the fixed-bed reactor experiment the increase in the amount of red cells (and the decrease increases in blue cells) is observed from the very beginning of the reaction, whereas a delay appears before the amount of CFU starts to decrease. Consequently, it can be said that membrane-damaged-bacteria profiles do not exactly correspond to non-viable-for-growing-

bacteria curves, being both types of methods to follow photocatalytic reactions of bacterial inactivation complementary from a mechanistic viewpoint.

Figure 4 also shows that significant differences can be found when comparing both slurry and fixed-bed catalytic systems. With the immobilised catalyst, a decrease in the undamaged bacteria (blue cells) together with a parallel increase in the damaged bacteria (red cells) is observed from the beginning of the irradiation, despite the decrease in the number of CFU shows certain delay. In contrast, for the slurry reactor there is a delay before the decrease in the damaged bacteria takes place, remaining the amount of undamaged bacteria (blue cells) essentially constant and only starting to decrease together with the amount in CFU counted on plates. Moreover, the results obtained with TiO_2 in suspensions show that the decrease in the CFU grown in agar plates is faster than that observed in the conversion of the undamaged blue cells to damaged red cells, suggesting the existence of an inactivation mechanism not exclusively related to membrane damaging that is not present when using the fixed-bed. A possible explanation is that small titania particles enter the cytoplasm, producing additional photocatalytic damages inside the cell, as suggested in the literature [5].

4. DISCUSSION

The results presented in the previous section point out to significant differences in the mechanism of bacteria inactivation with the fixed-bed photocatalytic reactor in comparison with the use of TiO_2 in suspension. These differences are revealed in:

i) a different shape of the inactivation curves, leading the fixed-bed reactor to a slower decrease in the concentration of CFU at the beginning of the reaction whereas for long irradiation times the process accelerates, in comparison with the initially faster but later slower inactivation achieved with TiO_2 in suspension;
ii) a different deactivation by the presence of organic and inorganic species in water, showing the slurry a much lower activity in the treatment of simulated wastewater treatment plant effluents, whereas the activity achieved with the fixed-bed reactor is essentially the same as with pure water;
iii) different profiles of undamaged/damaged bacteria obtained through a membrane permeability assays, leading the fixed-bed reactor to an increase in the damaged bacteria from the very beginning of the reaction, whereas the slurry shows a delay in

the inactivation process and the damaged bacteria started to be detected once the CFU grown on plates begin to diminish.

As heterogeneous photocatalytic process, bacteria inactivation is the result of several processes, simplified as follow [33]:

i) Photon absorption by the TiO_2 semiconductor and hydroxyl radical generation. Radiation transport takes place simultaneously with the remaining steps, related to the bacteria balance.
ii) Bacteria transport from the suspension bulk to the TiO_2 surface or vice versa.
iii) Adsorption of TiO_2 onto the bacteria surface (and possible incorporation of small titania particles in suspension to the bacteria cytoplasm). In fact this process should be considered as bacteria-TiO_2 interaction, as it is not a true adsorption phenomenon because the size of bacteria is much bigger than that of the TiO_2 particles in suspension.
iv) Attack of the hydroxyl radicals to the bacteria through the bacteria-TiO_2 interface (or additionally to the cytoplasmic components in the case of TiO_2 suspensions).
v) Desorption of bacteria from the TiO_2 surface.
vi) Transport of the bacteria from the TiO_2 surface to the bulk of the suspension or vice versa.

It can be reasonably assumed that bacteria transport steps (ii and vi) through the suspension should take place in similar way in both photocatalytic reactors. Concerning the attacks (step iv), the intrinsic kinetic of the process should not be different, although the reaction rate would obviously depend on the hydroxyl radical generation rate. This step is more dependent on the radiation absorption step (i), which could be very different in each reactor. However, differences in the photon absorption rate would lead to a different inactivation rate, but should not lead to significant differences in the shape of the bacterial inactivation profiles. Consequently, the experimental results point out to differences in the mechanism of bacteria-TiO_2 interaction (steps iii and v) as responsible for the different observed behavior of each catalytic system. This hypothesis is in agreement with the significant influence of the bacteria-catalyst contact reported by other authors [26,27].

Figure 5 shows schematically how the use of a slurry or immobilised TiO_2 can affect the bacteria-catalyst interaction. Whereas particles of TiO_2 in suspension can cover most of the

external wall of the bacteria and the smallest can even access the cytoplasm, in immobilised TiO_2 systems the contact bacteria-TiO_2 takes place in a reduced fraction of the microorganism external surface, excluding in principle the possibility of inactivation from titania particles inside the bacteria. Consequently, the attacks to the membrane should be concentrated on a specific area of the cell wall whereas the damages produced by TiO_2 in suspension would be distributed all over the cell wall.

FIGURE 5

Even with a lower radiation absorption and hydroxyl radical generation rates, the immobilised system could be more effective producing damages that increase the membrane permeability, as it is suggested by the DAPI/PI staining membrane permeability assays, in which the fixed-bed reactor lead to damaged cells from the very beginning of the reaction. In contrast, the slurry TiO_2 requires longer irradiation time to achieve an increase in the damaged cells, probably because damages are distributed over the whole membrane and therefore the increase in the membrane permeability is not so effective.

As it was shown before [14], the bacterial inactivation processes are quite different from the photocatalytic oxidation of chemical compounds. The adsorption of molecules on the TiO_2 surface should not be so different in both types of catalytic systems, being in this case much more favored for the TiO_2 in suspension due to its higher contact surface area. Moreover, the oxidation of a molecule is the result of a chemical reaction with the hydroxyl radicals that modify the molecular structure of the compound. Consequently the efficiency of the photocatalytic oxidation of chemicals is much more dependent on the hydroxyl generation rate and the specific surface area of the catalyst, being a more direct relation between the number of absorbed photons and the number of oxidized molecules. In contrast, bacterial inactivation is produced when the cell wall and membrane are damaged enough to increase the permeability of the membrane, no acting longer as a barrier between the cell and the surroundings. To achieve this goal, a concentrated attack on a small region on the external bacteria surface could be much more efficient.

Additionally, it has to be considered the auto-recovery mechanisms of the microorganisms capable of repairing damages on the cell structures, including the membrane, which can act when bacteria are in the dark, as it is the case for the parts of the experimental setup outside

the photoreactor (reservoir and pipes). The efficiency of the repairing mechanisms can be even higher for small damages (even although they are distributed over the whole membrane) than for large damage on a specific area of the cell.

Moreover, the suppression of osmotic stress when real aqueous effluent is treated instead of deionised water has a significant impact on the efficiency of the process when titania particles are suspended in the aqueous solution, because bacteria could be more resistant when a low level of damages is homogeneously distributed. In contrast, a high level of damage on a small area of the membrane seems to make bacteria much more prone to the inactivation, in such a way that the presence of organic and inorganic species (and the consequently reduction of the osmotic stress) does not reduce the efficiency of the process.

5. CONCLUSIONS

DAPI/PI staining assays confirmed that bacterial inactivation is based on cumulative damages on the bacteria membrane that increase its permeability. Moreover, this technique has provided mechanistic insights into the higher efficiency achieved by using a fixed-bed photocatalytic reactor in comparison with TiO_2 in suspension. Differences in bacteria-TiO_2 interaction between both catalytic systems are quite significant, what could lead to damages homogeneously distributed over the whole external surface of bacteria when using slurries, whereas immobilised systems would produce damages more concentrated on a small area of the membrane. This last strategy could be more efficient, probably requiring a lower amount of hydroxyl radicals to achieve the irreversible inactivation of bacteria as a result of a more effective increase of the permeability, a more difficult action of the auto-recovery mechanism of the cell for repairing damages, and a higher resistance to deactivation by the presence of organic and inorganic species in the water.

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FIGURE CAPTIONS

Figure 1. Photocatalytic inactivation of *E. coli* suspensions with different initial concentration in deionised water using TiO_2 in suspension (top) and the fixed-bed reactor (bottom). Solid lines correspond to the fitting with the kinetic model detailed previously [9]. Error bars calculated from eight independent bacteria counting.

Figure 2. Photocatalytic inactivation of *E. coli* suspensions in a simulated wastewater treatment plant effluent using TiO_2 in suspension and the fixed-bed reactor. Solid lines correspond to the fitting with the kinetic model detailed previously [9]. Error bars calculated from eight independent bacteria counting.

Figure 3. Epifluorescence microscopy pictures under UV excitation (left) and green excitation (right) of *E.coli* after dual staining with 1 mg mL^{-1} DAPI and PI. Samples taken during a photocatalytic inactivation experiment in a simulated wastewater treatment plant effluent in the fixed-bed reactor after 0 (top), 60 (middle) and 120 (bottom) min of irradiation.

Figure 4. Comparison of results of the DAPI/PI membrane permeability assay and the concentration of viable bacteria determined by agar plating on the photocatalytic inactivation of *E.coli* in a simulated wastewater treatment plant effluent with TiO_2 in suspension (top) and the fixed-bed reactor (bottom). Error bars calculated from three independent measurements.

Figure 5. Schematic representation of the differences in the bacteria-TiO_2 interaction and membrane distribution of the photocatalytic attacks for slurry and fixed-bed systems.

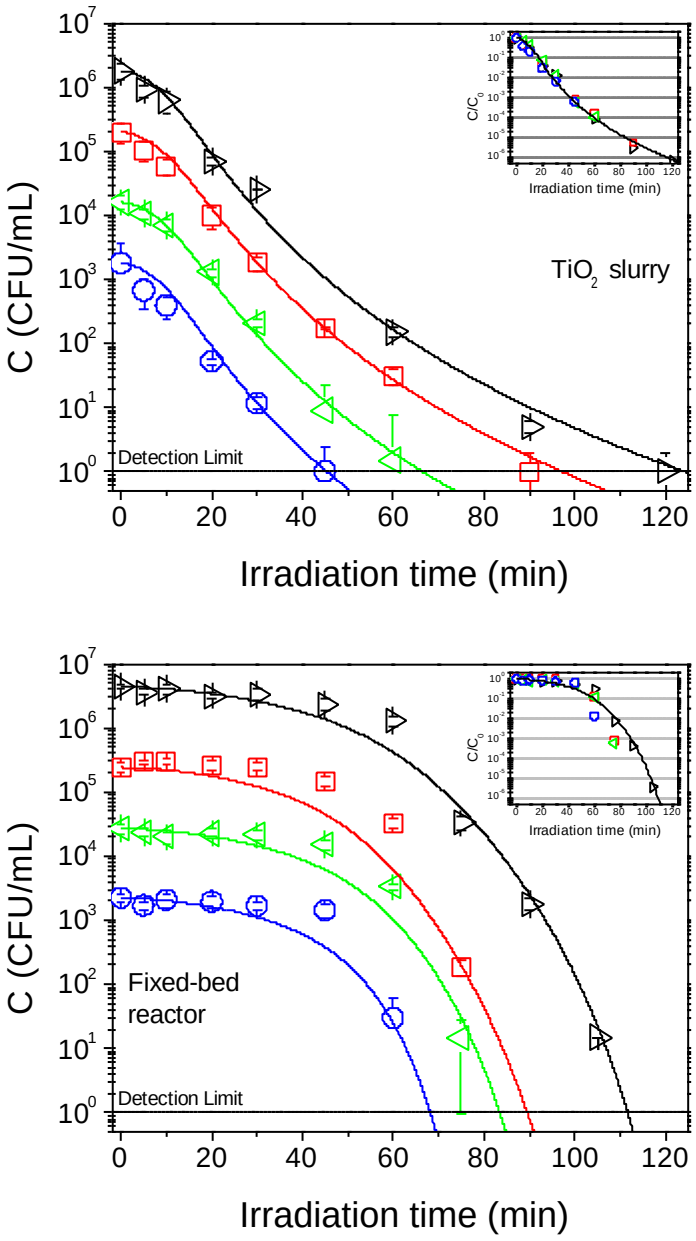

Figure 1. Photocatalytic inactivation of *E. coli* suspensions with different initial concentration in deionised water using TiO_2 in suspension (top) and the fixed-bed reactor (bottom). Solid lines correspond to the fitting with the kinetic model detailed previously [9]. Error bars calculated from eight independent bacteria counting.

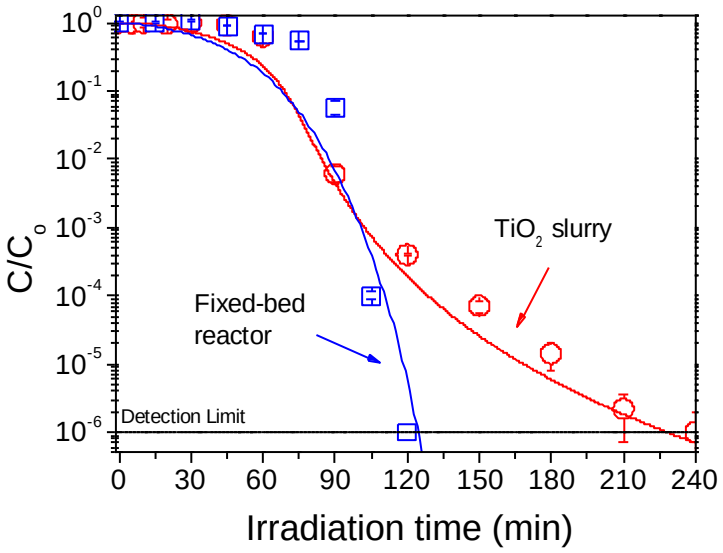

Figure 2. Photocatalytic inactivation of *E. coli* suspensions in a simulated wastewater treatment plant effluent using TiO_2 in suspension and the fixed-bed reactor. Solid lines correspond to the fitting with the kinetic model detailed previously [9]. Error bars calculated from eight independent bacteria counting.

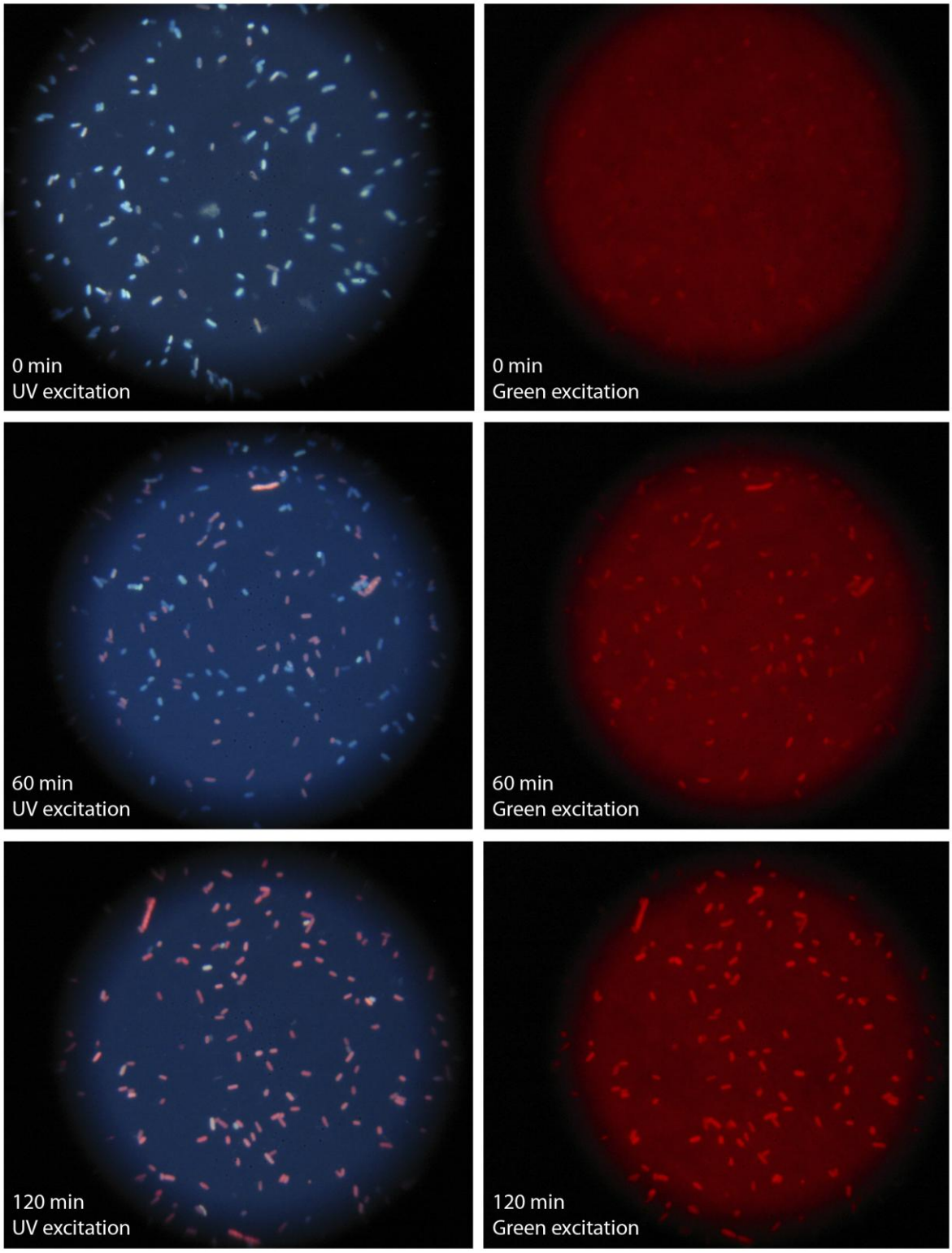

Figure 3. Epiflueresce microscopy pictures with UV excitation (left) and green excitation (right) of *E.coli* after dual staining with 1 mg mL $^{-1}$ DAPI and PI. Samples taken during a photocatalytic inactivation experiment in a simulated wastewater treatment plant effluent in the fixed-bed reactor after 0 (top), 60 (middle) and 120 (bottom) min of irradiation.

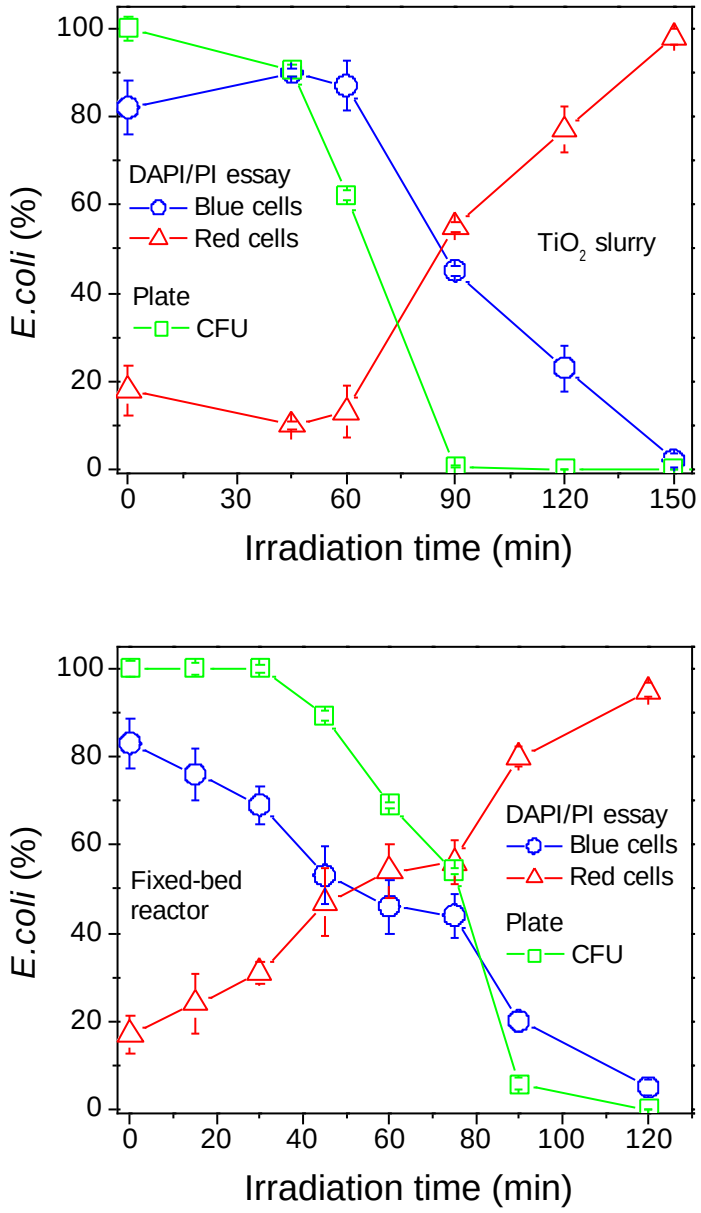

Figure 4. Comparison of results of the DAPI/PI membrane permeability assay and the concentration of viable bacteria determined by agar plating on the photocatalytic inactivation of *E.coli* in a simulated wastewater treatment plant effluent with TiO_2 in suspension (top) and the fixed-bed reactor (bottom). Error bars calculated from three independent measurements.

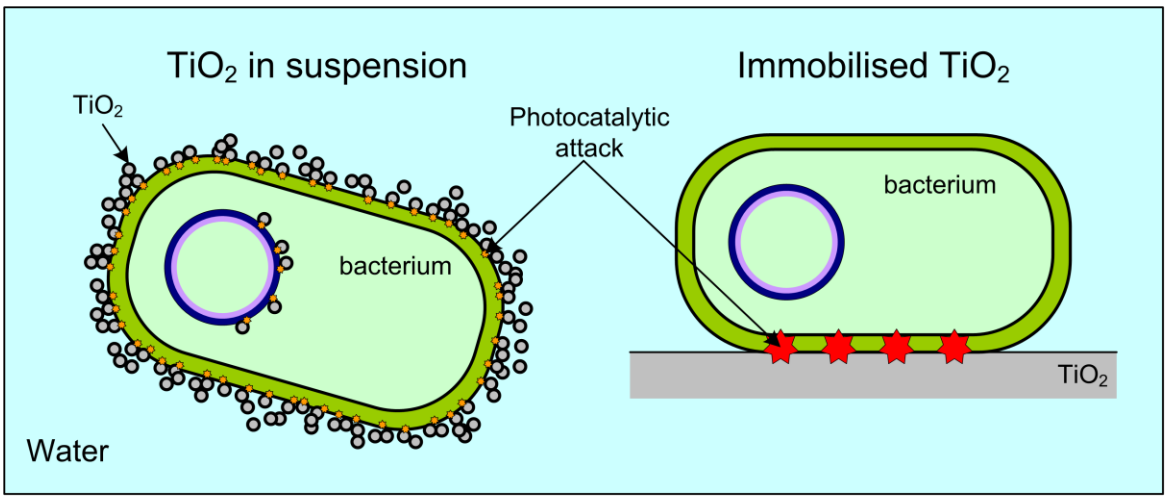

Figure 5. Schematic representation of the differences in the bacteria-TiO_2 interaction and membrane distribution of the photocatalytic attacks for slurry and fixed-bed systems.