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Article

Antibacterial Activity of TiO_2 Photocatalyst Alone or in Coatings on *E. coli*: The Influence of Methodological Aspects

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Abstract: In damp environments, indoor building materials are among the main proliferation substrates for microorganisms. Photocatalytic coatings, including nanoparticles of TiO_2, could be a way to prevent microbial proliferation or, at least, to significantly reduce the amount of microorganisms that grow on indoor building materials. Previous works involving TiO_2 have already shown the inactivation of bacteria by the photocatalysis process. This paper studies the inactivation of *Escherichia coli* bacteria by photocatalysis involving TiO_2 nanoparticles alone or in transparent coatings (varnishes) and investigates different parameters that significantly influence the antibacterial activity. The antibacterial activity of TiO_2 was evaluated through two types of experiments under UV irradiation: (I) in slurry with physiological water (stirred suspension); and (II) in a drop deposited on a glass plate. The results confirmed the difference in antibacterial activity between simple drop-deposited inoculum and inoculum spread under a plastic film, which increased the probability of contact between TiO_2 and bacteria (forced contact). In addition, the major effect of the nature of the suspension on the photocatalytic disinfection ability was highlighted. Experiments were also carried out at the surface of transparent coatings formulated using nanoparticles of TiO_2. The results showed significant antibacterial activities after 2 h and 4 h and suggested that improving the formulation would increase efficiency.

Keywords: antibacterial activity; TiO_2; photocatalysis; *E. coli*; transparent coating

1. Introduction

Indoor air pollution is a serious public health concern and a major cause of morbidity and mortality worldwide. In Europe, the total disease burden due to indoor air is about two million DALY (disability-adjusted life year) a year [1]. In 2006, the World Health Organization (Regional Office for Europe) started to draw up guidelines for indoor air quality [2] and addressed the three causes of indoor pollution that were most relevant for public health [3]:

- Biological indoor air pollutants (damp and mold) [4];
- Chemical indoor air pollutants (selected products) [5];
- Pollutants from indoor combustion of fuels (in progress).

The presence of microbial populations in damp indoor environments is one of the main causes of the degradation of indoor air quality and contributes to Sick Building Syndrome [6,7]. In Northern Europe and North America, the prevalence of mold contamination in buildings is estimated at between 20% and 40% [8]. Among the hundreds of microbial species that can be found in indoor environments [9–11], some are listed as potentially pathogenic species by the French High Council for Public Health and the France Environment Health Association [8,12,13]. Various studies have reported associations of mold growth with respiratory diseases in buildings, especially damp and water-damaged buildings [14]. Microorganisms may produce contaminants, *i.e.*, aerial particles, such as spores, allergens, toxins and other metabolites, that can be serious health hazards to occupants [15–23]. Frequent exposure to these contaminants can lead to various health troubles, including irritations and toxic effects, superficial and systemic infections, allergies and other respiratory or skin diseases [13,23–26]. Sick Building Syndrome has extensive economic and social impact [27–29]. A number of researchers have already pointed out that indoor building materials can become major sites of microbial growth when promoting conditions, such as high humidity and nutrient content, are present [30]. These conditions are easily satisfied in water-damaged buildings, damp buildings and badly-insulated buildings. Results from earlier studies have revealed that various microorganisms, including potentially pathogenic species, are detected on building materials [30].

A substantial amount of literature has been published on the effect of photocatalytic TiO_2 nanoparticles on microorganisms [31–34]. These studies show that the photocatalytic process in water is effective against a wide range of organisms, such as algae, viruses, fungi and bacteria. It should be noted that the different tests were carried out in aqueous slurry or with aqueous inoculum (sprayed or dropped), emphasizing the major role of water in the microorganism photo-killing process. In addition, TiO_2 nanoparticles can be used as (I) powder, usually dispersed in aqueous slurry or (II) film/coating applied to various substrates. Several works have highlighted very high bactericidal efficiency on different microorganisms: around 3 log after 30 min [35] and 6 log after 90 min [36] on *E. coli*, approximately 8 log after 90 min on mutans streptococci [37], *etc.* However, studies reporting such efficiencies used relatively strong light intensity, close to 10 W/m^2, and sometimes even beyond intensities in everyday use, up to 500 W/m^2, with photon wavelengths usually between 300 and 400 nm [38–40]. To our

knowledge, no study reports such inactivation values with weaker light intensity, closer to a passive photocatalytic device. The efficiency of photocatalytic disinfection is attributed to the oxidative damage mainly induced by reactive oxygen species (ROS), such as $O_2^{\bullet-}$, H_2O_2 and $HO^{\bullet}$. These reactive oxygen species are produced by redox reactions between adsorbed species (such as water and oxygen) and electrons and holes photo-generated by the illumination of TiO_2. On the basis of studies on *Escherichia coli*, OH radicals were assumed to be the major cause of the bactericidal effect [41,42], although direct oxidation by "holes" (h+) from the valence band on the TiO_2 surface is also highlighted in some works [43,44]. Regarding the process of degradation, the authors agree that the outer membrane, if present (Gram-negative bacteria), is the first barrier and, once it is damaged, the cytoplasmic membrane is attacked. The loss of cytoplasmic membrane integrity, which is involved in the process of cellular respiration, leads to the death of the cell.

This work is a preliminary study on transparent coatings formulated using TiO_2 nanoparticles to fight against microbial proliferation in indoor conditions. As such, the first step of our work was to explore the different parameters influencing the efficiency of TiO_2 nanoparticles when used alone for disinfection, *i.e.*, before being included in coatings. The aim of the paper was to emphasize the different factors determining disinfection efficiency and to show that the various performances reported in the literature should be correlated with experimental parameters. Passive devices in the form of semi-transparent photocatalytic coatings, easy to apply to the building material surfaces, are also considered.

Our previous investigations have already shown the efficiency of semi-transparent coatings on the abatement of NO_x and VOC in air under various environmental conditions (Relative Humidity—RH, concentration of polluting gas, *etc.*) [45,46]. Such coatings consisted of ultra-light varnishes formulated using nanoparticles of TiO_2, acrylic resin and silicates as the inorganic binder. The results obtained in air purification point out the interest of testing these transparent coatings for the photocatalytic disinfection of microorganisms. However, the coatings were found to be inefficient against green algae colonization in accelerated tests [47]. Regarding TiO_2 nanoparticles alone, very good antibacterial performance is sometimes reported for photocatalytic TiO_2, but may be related to very specific experimental conditions that are not representative of the natural conditions to be considered for passive devices. Three sets of experiments were carried out to highlight different factors determining the extent to which *Escherichia coli*, a Gram-negative bacterium, was inactivated by TiO_2 photocatalysis: (1) the activity of TiO_2 in the dark allowed the photocatalytic effect to be dissociated from the physical effect; (2) the deposited drop experiment was carried out to evaluate the influence of forced conditions between bacteria and particles; and (3) the stirring experiment, which was easier to carry out for the kinetics evaluation, enabled the effect of the suspension to be estimated.

We also highlight some of the issues to be faced in the formulation of such a product, for example the inclusion of nanoparticles within a binder matrix (acrylic resin here), which can act as a mask against UV absorption and/or can react with photogenerated radicals.

2. Materials and Methods

2.1. Cultivation of Bacteria

Escherichia coli CIP 53126 was obtained from Institut Pasteur Collection, Paris, France. The strain was preserved at −80 °C in Eugon medium supplemented with 10% glycerol. Before each experiment, bacterial cells were pre-cultured on a nutrient agar slant. They were then transferred to a trypticase soy agar and incubated at a temperature of 36 °C ± 1 °C for 16 to 24 h. In addition, one plastic loop of bacteria was transferred to a fresh trypticase soy agar and incubated at a temperature of 36 °C ± 1 °C for 16 to 20 h prior to the test. For testing, one plastic loop of bacteria was dispersed evenly in a small amount of 1/500 nutrient broth (NB) [48] or of sterile distilled water, depending on the test, and the bacterial cell content of the suspension for inoculation was adjusted to about 10^8 cells/mL with a spectrophotometer (640 nm). The cell suspension was then 10-fold steps diluted, and 1 mL of each dilution was incorporated in trypticase soy agar to determine the number of CFU/mL. The test suspensions were prepared by 10-fold dilutions.

2.2. Antibacterial Activity of TiO_2 in the Dark

TiO_2 nanoparticles (KRONOClean 7050) were suspended in 1/500 NB [48] at the concentration of 13.9 g/L. Eleven milliliters of the suspension were then deposited onto a sterile Petri dish, so that the total area of the inside part of the dish was covered. The Petri dishes were placed in a sterile flow hood for air drying until the water had totally evaporated. A film of TiO_2 was visible at the bottom. Then, 11 mL of the inoculum (between 8×10^4 and 2×10^5 cells/mL) were deposited on the TiO_2 film, and the Petri dishes were covered with a lid [48]. After a fixed time (0 and 24 h), the lid was removed, the bottoms of the Petri dishes were gently scraped with a plastic loop in order to remove any adhered cells and 1 mL of the suspension was collected and diluted in phosphate buffer. Control samples were studied in Petri dishes without TiO_2.

One-mL quantities of the appropriate dilutions were then dropped into distilled sterile water and filtered on cellulose ester filters ($\phi = 0.45$ μm) in order to separate bacterial cells from nanoparticles. The filters were then deposited on trypticase soy agar and incubated at a temperature of 36 °C ± 1 °C for 40 to 48 h. After incubation, the number of viable cells was estimated in CFU/mL.

2.3. Deposited-Drop Experiment

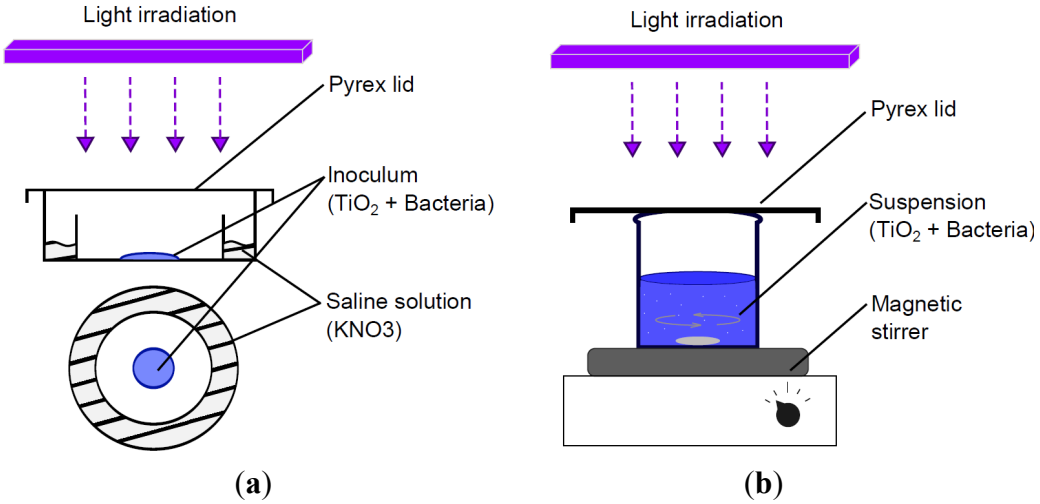
To avoid damage by UV irradiation alone [49], the maximum UV intensity was maintained at 2.5 W/m^2. Previous tests with higher UV intensity had shown total drying of the inoculum during the experiment and led to the inactivation of bacteria in control samples. The light intensity was measured on the samples using a UV-A radiometer (Gigahertz-Optik, GmbH Türkenfeld, Germany) in the 310–400 nm range.

Various configurations were studied: samples under UV irradiation (TiO_2-bearing samples and control specimen without TiO_2) and samples kept in the dark (TiO_2-bearing samples and control specimen without TiO_2). All tests were carried out in triplicate. The data shown are the average of triplicates, with the corresponding standard errors.

2.3.1. With TiO_2 Powder

The experiment was based on the standards JIS Z 2801 (Japanese Industrial Standard) and ISO 27447 [48,49]. TiO_2 nanoparticle powder (KRONOClean7050–anatase) was suspended in 9 mL of 1/500 NB [48], and 1 mL of the bacterial suspension (Section 2.1) was added. Final concentrations were 1 g/L for TiO_2 and between 8×10^4 and 2×10^5 CFU/mL for bacteria. The bacterial suspension (Section 2.1) without TiO_2 was used as a control. Then, 0.4 mL of the inoculum were instilled onto a Pyrex Petri dish designed so that an external ring could receive 2 mL of a supersaturated saline solution (KNO_3) to maintain 90% RH and was covered with a Pyrex lid (Figure 1). The Petri dishes were placed in a sterile flow hood and illuminated with an 8-W black-light bulb. After a few minutes, the TiO_2 nanoparticles were observed to have sedimented at the bottom of the drop.

Figure 1. Schematic illustration of the deposited-drop experiments with TiO_2 powder and TiO_2 semi-transparent coatings (**a**) and stirring experiment (**b**).



A Soybean Casein Lecithin Polysorbate 80 Medium, also known as SCDLP broth, was prepared in sterile distilled water as recommended in standard JIS Z 2801 [48], using casein peptone, soybean peptone, sodium chloride, disodium hydrogen phosphate, glucose, lecithin and Tween 80.

After different contact times (2 h, 4 h, 6 h), the suspension was washed out with the appropriate amount of SCDLP broth and with sterile glass beads (d = 4 mm). When necessary, the washed-out suspension was diluted X times in a phosphate buffer, so that it contained 30 to 300 cells per mL. For each sample, 1 mL of the appropriate dilution was dispensed into two sterilized Petri dishes with 15 to 20 mL of trypticase soy agar (TSA) and incubated at a temperature of 36 °C ± 1 °C for 40 to 48 h. After incubation, the number of viable cells was estimated in terms of CFU. The overall procedure was also systematically carried out immediately after inoculation (t = 0 h) to validate the test. The antibacterial activity was then calculated as the difference between the average logarithm of the number of viable bacteria on the control without TiO_2 and the average logarithm of the number of viable bacteria on the TiO_2 sample:

$$A = \log\left(N_{TiO_2}\right) - \log\left(N_{controrl}\right) = \log\left(\frac{N_{TiO_2}}{N_{controrl}}\right) \quad (1)$$

where, A: antibacterial activity; N_{TiO_2}: average number of CFU on TiO_2 sample at time t; $N_{control}$: average number of CFU on control sample at time *t*.

The test was then repeated with a transparent film (9–10 cm^2) gently placed on the inoculum before irradiation in order to increase the probability of contact between bacteria cells and TiO_2 nanoparticles (forced contact).

2.3.2. With TiO_2 Semi-Transparent Coating

The deposited-drop experiment was repeated with semi-transparent coating formulated using TiO_2 nanoparticles as an antibacterial product: TiO_2 powder (KronoClean 7050, KRONOS/Société Industrielle du Titane, Paris, France) and TiO_2 dispersion (Kronos type 7454, trial product, KRONOS/Société Industrielle du Titane, Paris, France). The coating formulation included water and acrylic based on the work of Martinez *et al.* [46], as shown in Table 1. Sterilized cover-glasses (26 × 76 mm^2) were covered with the coatings by instilling 1 mL, so that the total area of each glass was coated. The cover-glasses were then placed under a sterile flow hood for air drying. After drying, the semi-transparent coatings with TiO_2 powder (STC-SP (with silicates), STC-P) were gently sanded with fine sandpaper in order to prevent the possible inclusion of nanoparticles in the binder. The semi-transparent coatings with TiO_2 in aqueous suspension (STC-A) were pre-aged by irradiating them with UV light (2.5 W/m^2) for 80 h. The amount of TiO_2 was estimated at 2.5 mg/cm^2 for samples coated with TiO_2 powder (STC-SP, STC-P) and 0.63 mg/cm^2 for samples coated with TiO_2 aqueous suspension (STC-A). In order to evaluate the possible inclusion of nanoparticles in the binder of STC-A, samples were also prepared with water and TiO_2 aqueous suspension, without acrylic resin (STC-A2). For each test sample, corresponding controls were prepared in the same way with water and acrylic resin, but without TiO_2.

Table 1. Formulation of semi-transparent coatings. STC: semi-transparent coating.

STC-SP	STC-P	STC-A	STC-A2
Water	Water	Water	Water
Acrylic resin (7.5 wt%)	Acrylic resin (12 wt%)	Acrylic resin (2 wt%)	–
TiO_2 powder (KronoClean 7050)	TiO_2 powder (KronoClean 7050)	TiO_2 in aqueous suspension (Kronos trial product 7454)	TiO_2 in aqueous suspension (Kronos trial product 7454)
Silicates (12.5 wt%)	–	–	–

The inoculation suspension (of Section 2.1) was diluted to make the concentration of inoculum 8×10^4 to 2×10^5 CFU/mL. The coated cover-glasses were placed over the internal ring of the Pyrex Petri dishes shown in Figure 1. Relative humidity was maintained with 2 mL of the supersaturated saline solution (KNO_3) deposited in the external ring of each dish. Then, 0.4 mL of the inoculum were instilled on each coated cover-glass, and a transparent plastic film was applied, spreading the inoculum over a surface area of 10 cm^2. The Petri dishes were then covered with a Pyrex lid (Figure 1), placed in a sterile flow hood and illuminated with an 8-W black-light bulb.

After different contact times (2 h, 4 h, 6 h), the cover-glasses were recovered with sterile pliers and placed in plastic Petri dishes for wash-out. The wash-out of bacteria cells and the following procedures for CFU counting were repeated as in Section 2.3.1.

2.4. Stirring Experiment

For the stirring experiment; TiO_2 nanoparticles (KronoClean7050) were suspended in a sterile beaker in 27 mL of 1/500 NB or sterile distilled water; depending on the test; and 3 mL of the cell suspension were added to make the final test suspension. Final concentrations of the suspension were 1 g/L for TiO_2 and 7×10^4 to 1×10^5 CFU/mL for bacteria. A suspension of bacterial cells without TiO_2 was prepared as a control. The beakers (test sample and control) were placed in a sterile flow hood; covered with a Pyrex lid and illuminated with an 8-W black-light bulb at a light intensity of 5 W/m^2.

An aliquot of 1 mL was taken from each beaker every 30 min during 4 h and, when necessary, diluted in phosphate buffer before inclusion in trypticase soy agar as in the deposited-drop experiment. Controls were also carried out without TiO_2 and in the dark, with/without TiO_2. The data presented are the average of three experiments with the corresponding standard errors.

To assess the influence of the nature of the water during the experiment, two solutions were used: 1/500 nutrient broth and sterile distilled water. The two conductivities of the solutions were compared using a conductivity meter before the test. At room temperature (~21 °C), the conductivities were 4.437 mS/m for 1/500 NB and 1.1 mS/m for distilled water.

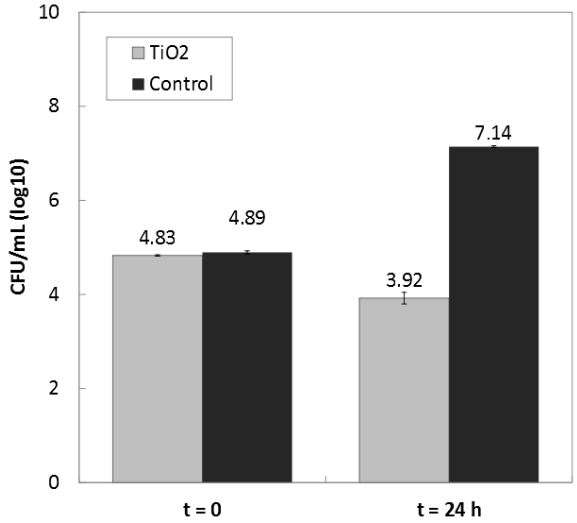
3. Results and Discussion

3.1. Effect of TiO_2 in the Dark

Figure 2 shows the bacterial concentration of *E. coli* cells after 0 and 24 h of contact with TiO_2 nanoparticles (11 mL inoculation of air-dried TiO_2 film in Petri dishes). From these data, an increase can be seen in the number of CFUs for control samples (+2.25 ± 0.06 log) and a decrease in the number of CFUs for TiO_2 samples (−0.91 ± 0.14 log). The corresponding antibacterial activity, calculated from Equation (1), is 3.22 ± 0.14 log. It is therefore likely that the activity of TiO_2 on *E. coli* in the dark is correlated with a growth inhibitory effect as a major pathway and a bactericidal effect as a minor pathway. These results highlight the physical impact on *E. coli* cells induced by contact with TiO_2 nanoparticles, without regard to the photocatalytic process. This also agrees with earlier observations by Liu *et al.* [50] and Gogniat *et al.* [38], which showed a loss of bacterial culturability after contact with TiO_2 nanoparticles in the dark. A study by de Niederhãusen and Bondi [51] on the self-cleaning of Ag-TiO_2-coated ceramic tiles also showed significant antibacterial activity for 24 h in the dark.

Interestingly, we detected no difference in the CFU counts between bacterial suspensions with TiO_2 (1 g/L and 10 g/L) and a control bacterial suspension (without TiO_2) after direct plating of 2 × 1 mL on TSA and 48 hours' incubation (data not shown). It seems possible that the physical damage sustained is not sufficient to kill bacterial cells when they are growing in a nutrient-rich culture medium. Such a "neutralizing" effect of culture media is current with antiseptic and disinfectant molecules, which highlights the impact of test conditions on efficiency evaluation.

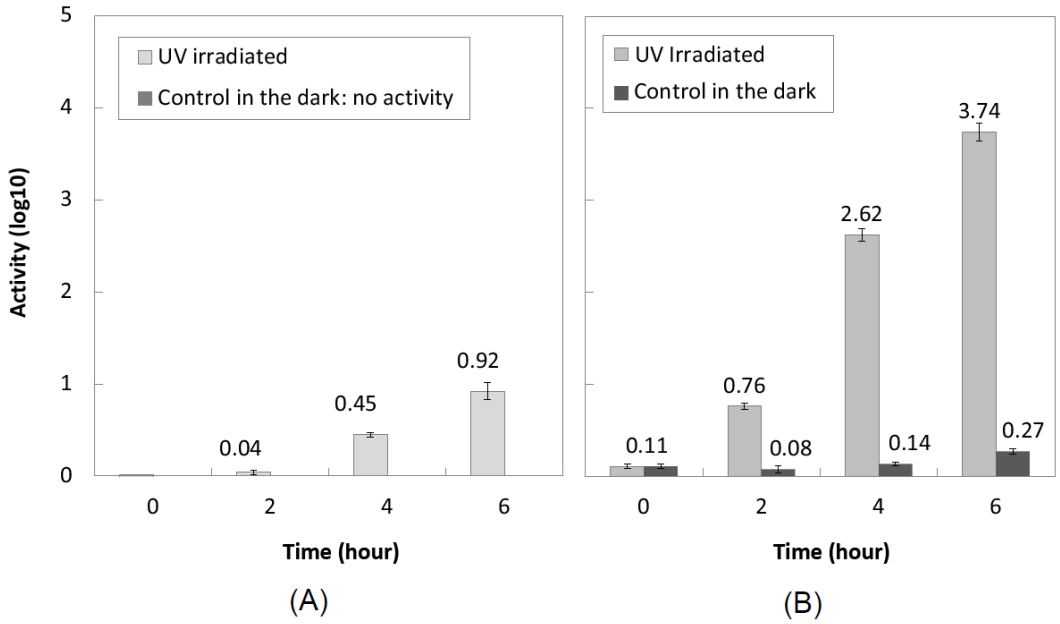
Figure 2. Bacterial concentration after 24 h with TiO_2 nanoparticles in the dark. Mean ± SE, $n = 3$.



3.2. Free Surface Drop Deposit vs. Forced Contact between Bacteria and Nanoparticles

The antibacterial activities of TiO_2 nanoparticles on *E. coli* during the deposited-drop experiment are presented in Figure 3. No activity was detected for samples kept in the dark for 6 h under normal testing conditions (A). The bacterial reduction reached 0.92 ± 0.09 log after 6 h of irradiation (A).

Figure 3. Antibacterial activity of TiO_2 as a support under UV irradiation (2.5 W/m ^{2}): (**A**) standard conditions; (**B**) after application of a transparent plastic film on the inoculum. Mean ± SE, $n = 3$.



Since the bactericidal effect induced by photocatalysis of TiO_2 nanoparticles depends on many factors, such as the amount [35,37,52–55] and the crystalline nature [39,55–57] of TiO_2, the irradiation time and intensity [35,52,55,58] and the inoculum concentration [42,53,59], it is reasonable to assume

that the dispersion of TiO_2 particles and bacteria (low probability of contact) and the very low light intensity used in our experiment (2.5 W/m^2 in order to avoid any UV-damage) could be major factors explaining the low activity observed after 6 h of irradiation. Various studies from the literature show intensities of over 10 W/m^2 and antibacterial activities on *E. coli* easily greater than 3 log after 90 min of irradiation [39,40,42,60].

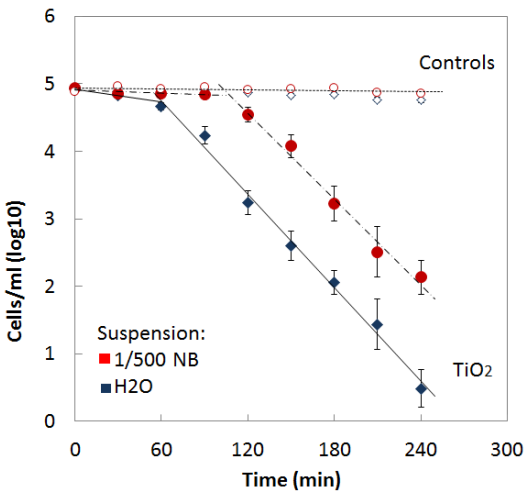
Following the application of a transparent film (9 cm^2) onto the inoculum, a significant increase in the antibacterial activity was observed. As shown in Figure 3B, the activity was 3.74 ± 0.1 log after 6 h under UV irradiation and 0.27 ± 0.03 log after 6 h in the dark.

The present findings seem to support the idea that reducing the distance between bacterial cells and TiO_2 nanoparticles enhances the photocatalytic disinfection process. This also agrees with earlier research highlighting the importance of the contact between bacterial cells and the surface of TiO_2 [39–41,43,44,50,61–63]. In addition to the oxidative stress induced by reactive oxygen species (ROS) on bacterial cells, contact with the TiO_2 surface leads to the direct oxidation of cells by photogenerated holes, which also reduces the recombination of charges inside the photocatalyst [38,43,44,64]. Moreover, it has been suggested that direct contact and adsorption on TiO_2 nanoparticles cause (I) a loss of membrane integrity [38,50,61] and possibly (II) a process of phagocytosis of the nanoparticles by the cells (the findings of Cai *et al.* [64] must be interpreted with caution in this paper, because they focused on HeLa cells and not bacterial cells.) [64], both leading to the reduction of the number of cultivable cells, if not to cell death. These results also agree with the findings of other studies that have highlighted the major role of surface radicals compared to free radicals in photocatalytic disinfection [40,41,62].

3.3. Influence of the Nature of the Solution for Suspension

The results obtained from the stirring experiment are presented in Figure 4. For TiO_2 samples, both inactivation curves consist of two steps: the first with a very low inactivation rate followed by the second with a higher inactivation rate. In addition, the second rate appears to be the same for both distilled water and 1/500 NB. It is likely that 1/500 NB acts as a retarding agent of the photocatalytic disinfection process.

Figure 4. Survival of *E. coli* cells *vs.* irradiation time at ~5 W/m^2 with the standard error of three experiments. Mean ± SE, n = 3.



The present finding is in full agreement with the inhibitory effect of various ions and organic compounds on photocatalytic disinfection, which is widely reported in the literature [34,37,56,65,66]. The presence of ions and organic compounds can reduce the efficiency in different ways:

- Competition between ions, compounds and bacteria for the adsorption on the TiO_2 surface [37,38,56,65,67];
- The ROS mobilized by ions and compounds cannot oxidize bacterial cells [65];
- Aggregates of organic compounds could create a barrier filtering UV.

According to Dunlop *et al.* [56] and Sunada *et al.* [42], the low rate of inactivation in the first step may be due to the preliminary attack of the outer membrane of cells by ROS.

During this first step, the damage sustained by the outer membrane may be insufficient to kill bacteria: they can recover from the damage and re-grow once they are plated in agar media [42,56]. After some time, degradation of the outer membrane enables reactive species to penetrate, which induces damage, leading to the death of the bacterial cells (second, higher rates on the curves of Figure 4). This hypothesis has also been considered by other researchers [58,68]. Mitoraj *et al.* [68] explained this "incubation period" as the time for the concentration of photogenerated ROS to increase to a level that is harmful to bacteria.

Another possible explanation for the first step with the low inactivation rate is proposed by Gogniat *et al.* [38]. In their works, they observed the two-stage curve only in a sodium phosphate solution and not in a NaCl-KCl solution. They hypothesized that the change of adsorption properties of TiO_2 when illuminated led to a photo-desorption of ions previously adsorbed on its surface. Thus, the time taken for the photo-desorption process explains the low inactivation rate observed during the first minutes of the experiment [38].

Interestingly, the third step observed in earlier studies [58,68–70] and consisting of strong attenuation of the bacterial inactivation was not observed here. One of the hypotheses suggested is that photocatalytic inactivation is built up by bacterial growth after a certain period of time [69]. It can be supposed that bacterial growth in pure water is slowed down or stopped. Further investigations in 1/500 NB after longer times could show similar attenuation of the inactivation rate.

Some authors have compared efficiencies between scattered and fixed TiO_2 [71–73]. Pablos *et al.* [72] observed a higher inactivation rate at the beginning of the reaction with fixed TiO_2. They suggested that damage was uniformly distributed over the whole cell wall in slurries, whereas it was more concentrated on small areas with fixed TiO_2, requiring smaller amounts of radicals to achieve inactivation. However, they observed similar times for total inactivation of bacteria (*E. coli*) for both implementations (fixed and scattered). On the other hand, Gumy *et al.* [40] found higher inactivation efficiency with suspended TiO_2 than with TiO_2 coated on a fibrous web and suggested that particles dispersed in slurry would provide more surfaces for the adsorption of bacteria. In addition, inactivation of bacteria has been observed in the presence of TiO_2 nanoparticles in the dark, suggesting that phenomena other than photocatalytic processes can explain inactivation [50,61]. Although the complete process is not perfectly understood yet, the overall literature points to the importance of the contact between bacteria and TiO_2 for improving disinfection efficiency, suggesting both chemical and physical influences.

In their work, Gomes *et al.* [71] also reported a higher inactivation rate in slurry than with TiO_2 supported on Ahlstrom paper. They suggested that such results could be explained by competitive

reactions of TiO_2 with the organic matter released by the paper during the experiment. Accordingly, the presence of ions and/or organic compounds in the slurry/inoculum considerably reduced the efficiency by reacting with ROS and being adsorbed on TiO_2 in place of bacterial cells [38,65]. These works also raised the problem of TiO_2 coatings in which the organic matter from the binder can monopolize photogenerated radicals and, thus, lead to a decrease in disinfection efficiency.

3.4. Semi-Transparent Coating

Figure 5 presents the experimental data on the antibacterial activity of semi-transparent coatings formulated with silicate and TiO_2 powder (STC-SP). Surprisingly, antibacterial activity was also observed on samples kept in the dark. A quick estimation of the pH of the inoculum with indicator paper showed a pH around 11–12, far too high for *E. coli* survival. It is then reasonable to assume that the antibacterial activity detected on STC-SP samples was not induced by the photocatalytic process, but by the silicates, making the inoculum strongly basic. Results from sample coatings without silicate (STC-P) showed no activity after eight hours' irradiation and no activity after 8 h in the dark. Possible explanations are that photogenerated radicals may have reacted with the binder instead of with bacteria or that the inclusion of nanoparticles within the binder may have prevented UV absorption and physical damage by contact. Moreover, the use of TiO_2 powder without a dispersing agent may lead to the formation of aggregates, reducing the surface available for reaction.

Figure 5. Antibacterial activity of SCT-SP coatings under UV irradiation (≈2.5 W/m^2) and in the dark. Mean ± SE, n = 3.

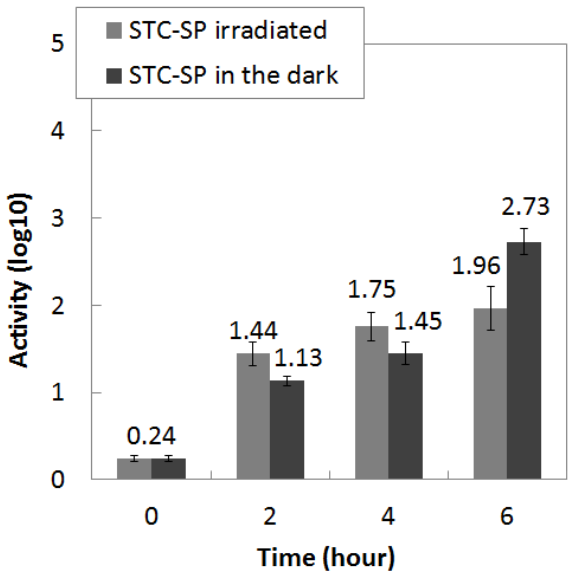
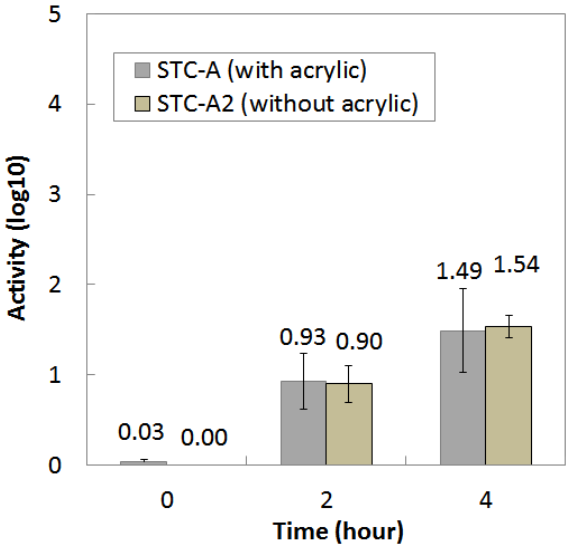


Figure 6 presents the antibacterial activity obtained with STC-A and STC-A2. The activity reaches 1.49 ± 0.47 log for STC-A and 1.54 ± 0.13 log for STC-A2 after four hours' irradiation. The observed increase of antibacterial activity, compared to SCT-P in which no activity was detected, could be attributed to the use of the TiO_2 dispersion. Nanoparticles, stabilized by the dispersing agent, may have provided more active sites for the photocatalytic process. Moreover, the smaller amount of acrylic resin within STC-A and STC-A2 (2%) may have reduced the inclusion of TiO_2 nanoparticles compared to

SCT-P. The similar activities observed on STC-A (with acrylic resin) and on STC-A2 (without acrylic resin) seem to confirm this hypothesis. Further investigations on the formulation of these coatings along with observations of nanoparticle distribution in the binder will be helpful in the development of antibacterial products for building materials.

Figure 6. Antibacterial activity of STC-A and STC-A2 coatings under UV irradiation (≈2.5 W/m^2). Mean ± SE, n = 3.



4. Conclusions

This paper has examined the effect of TiO_2 photocatalyst on *E. coli* in terms of antibacterial activity by carrying out two different tests (a drop deposited on a photocatalytic substrate and a stirring experiment in a TiO_2-bearing suspension).

Some general effects reported in the literature concerning the photocatalytic disinfection process have been observed.

- Prolonged contact (24 h) in the dark leads to significant antibacterial activities, potentially explained by a combination of the direct contact (I) bactericidal effect and the (II) growth inhibiting effect.
- Reducing the distance between nanoparticles and bacteria significantly increases the inactivation of *E. coli* by non-photocatalytic effects (direct contact) and the photocatalysis disinfection process.
- The presence of ions and organic compounds in the suspension during the test delays the inactivation.

In addition, the transparent coatings tested showed significant antibacterial activities under low UV irradiation. The results suggest that improving the formulation, *i.e.*, varying the proportions of the components, could increase the efficiency of coatings. In a broader framework, further experimental investigations will be conducted on the resistance of this coating to fungal proliferation and on the protection it affords against the formation of microbial biofilms.

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Author Contributions

Experimental measurements have been conducted by Thomas Verdier. Analysis and interpretation of the results as well as conclusions have been conducted by all the co-authors. The manuscript has been written by Thomas Verdier with the revision and approval by the others co-authors.

Conflicts of Interest

The authors declare no conflict of interest.

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