Journal of Photochemistry and Photobiology A: Chemistry 202 (2009) 92–98

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Inactivation of *Escherichia coli* on immobilized TiO_2 using fluorescent light

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ARTICLE INFO

Article history:
Received 18 July 2008
Received in revised form 11 November 2008
Accepted 13 November 2008
Available online 21 November 2008

Keywords:
Photocatalysis
TiO_2
UV inactivation
E. coli
Fluorescent light

ABSTRACT

There are many circumstances where it is necessary or desirable to remove or to kill microorganisms found on surfaces. In this paper, we present evidence of the photocatalytic inactivation of *Escherichia coli* (ATCC8739) cells deposited on TiO_2 loaded membrane filters during irradiation with fluorescent light. The TiO_2 selected was the photocatalyst PC105, with loadings ranging from 520 to 15,590 mg m^{-2}. Irradiation was produced by eight 8 W lamps with visible light, and UV (290–400 nm) at 0.05–0.12 W m^{-2} intensity. *E. coli* inactivation as a function of time was monitored for up to 120 min. In the presence of fluorescent light, the inactivation rate of *E. coli* increased with a decrease in the TiO_2 loading, giving the best results at 520 mg m^{-2} loading, with complete inactivation achieved after 2 h of exposure. TiO_2 loading higher than 6236 mg m^{-2} resulted in decreased inactivation. SEM images of photocatalyst and bacteria show that increasing the particle contact with the bacteria enhanced the disinfection process. Thus excess TiO_2 did not enhance the antibacterial effect, once maximum cell–photocatalyst contact had been achieved.

1. Introduction

The photocatalytic chemistry of titanium dioxide has been extensively studied over the last 30 years because of its high stability, lack of toxicity and relatively cheap production cost. Over the last few years, a new range of products has appeared, the aim of which being to apply titanium dioxide (TiO_2) photocatalyst into the surface matrix, which when irradiated with light will decompose dirt and microorganisms. In hospitals, pharma and the food industry, maintenance of hygienic standards is essential. Similarly, facilities such as bathrooms and child care facilities, public conveniences and domestic bathrooms benefit from good hygiene control. Surface hygiene could be improved by the action of fluorescent light on catalytic surfaces, retarding the rate of contamination and saving the cost of cleaning maintenance.

Many variables affect the photoactivity of the TiO_2 photocatalyst, for example intrinsic parameters such as particle size, surface area [1–3], crystal structure and the method of particles preparation, and extrinsic parameters such as temperature of reaction, incident light intensity [4], pH of solution [5,6] and catalyst loading. Crystal structure and particle size [7] are particularly important factors in determining photoactivity; anatase titania nanoparticles have higher activity than the rutile ones [7–9]. TiO_2 photocatalysis depends upon the energy of the incident photons [10]. If only a few photons of energy are required to induce photocatalysis of surface contaminants, then the intensity of UV available from ordinary fluorescent lamp (0.07 W m^{-2}) should be sufficient to inactivate microorganisms. Thus future commercial applications of self-sterilized surfaces in indoor environments with existing lighting could be created.

The mechanism of the photocatalyst process has been extensively studied in the literature and several complex reaction pathways have been reported [11–14]. The photocatalytic process in anatase TiO_2 particles includes chemical steps that produce highly reactive species. Upon irradiation of TiO_2 with photons of wavelength $\leq$385 nm, an electron is promoted from the valence band to the conduction band, thus forming an electron–hole pair [15]. The photogenerated holes and electrons react with water molecules attached to TiO_2 surfaces in the presence of oxygen to form hydroxyl radicals ($^{\bullet}OH$). These are highly reactive for both the oxidation of organic substances and the inactivation of bacteria and viruses [14,16]. The TiO_2 particle may also directly penetrate the cell. Other reactive oxygen species (ROS), such as superoxide ions ($O_2^{\bullet -}$) or ($HO_2^{\bullet}$) are less effective against bacteria, due to the negative charge which prevents them from penetrating bacteria cell membranes [12,17]. These species must be in direct contact with the outer surface of bacteria. Hydrogen peroxide (H_2O_2) is less harmful compared to hydroxyl radicals and superoxide ions, although it can enter into the cell [18,19]. It is still a subject of investigation as to which of these reactive oxygen species are directly involved in the damage to bacteria cells or which species contribute more to the oxidative reactions with organic compounds.

One of the earliest examples of the application of semiconductor photocatalysis as a method of disinfection was the work

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doi:10.1016/j.jphotochem.2008.11.005

of Matsunaga et al. [20]. Their work showed that TiO_2 particles were effective in sensitizing the photokilling of bacteria such as *Lactobacillus acidophilus* and *Escherichia coli* (*E. coli*). Fujishima et al. [10] have reviewed the antibacterial effects and detoxifying actions of TiO_2 photocatalyst on ceramic tiles. They found that titanium dioxide photocatalyst was more effective than any other related antibacterial agent tested, because the photocatalytic reaction worked when cells covered the surface, and while bacteria were actively propagating.

The majority of the work related with TiO_2 has focused on UV light irradiation; only a few papers have demonstrated an antibacterial efficacy in visible light. We have focused our attention on the use of 'indoor' fluorescent light.

The objective of the present study was to investigate the photoinactivation of *E. coli* using TiO_2 powder deposited on a surface during irradiation with fluorescent light.

2. Experimental details

2.1. Materials

2.1.1. Photocatalyst

TiO_2 PC105 was kindly provided by Millennium Inorganic Chemicals (Grimsby, UK). The average diameter crystallite size was 15–25 nm with a nanoanatase phase of 100%. The anatase has an optical E_{bg} of 3.2 V (380 nm). The Brunauer–Emmett–Teller (BET) surface area is 77.9 $m^2\,g^{-1}$.

2.1.2. Test microorganism

E. coli ATCC 8739 was obtained from the American Type Culture Collection (Manasas, VA, USA). To check purity, a loop of culture was streaked onto nutrient agar (NA) (Oxoid Ltd., Hampshire, UK) plate and incubated at 37 °C for 24 h. Broth cultures inoculated from this plate were grown for 18 h in nutrient broth (NB) (Oxoid Ltd., Hampshire, UK) at 37 °C. In order to prepare stock cultures, stored at −80 °C, equal amounts of culture and freezing mix were added together and were incubated at 37 °C for 30–60 min. Samples were dispensed into 1.5 mL sterile plastic screw capped tubes, and frozen. This procedure provides stock cultures that are not attenuated or otherwise altered by successive subculturing [21]. To resuscitate, the culture was defrosted at room temperature and a loop of liquid was inoculated into broth and was incubated at 37 °C for 18 h. The remainder of the culture was returned to the freezer for future use. The freezing mix was made of two solutions. Solution A contained di-potassium hydrogen phosphate (K_2HPO_4) 12.6 g L^{-1}, potassium di-hydrogen phosphate (KH_2PO_4) 3.6 g L^{-1} tri-sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) 0.9 g L^{-1}, ammonium sulphate ($(NH_4)_2SO_4$) 1.8 g L^{-1} and glycerol 300 g L^{-1}. This mixture was autoclaved at 121 °C for 15 min. Solution B consisted of 1.8 g L-1 magnesium sulphate ($MgSO_4{\cdot}7H_2O$). The solution was filter sterilised using a 10 ml Luer-Lok™ syringe (BDH, Poole, UK) and an Acrodisc® filter (32 mm with 0.2 μm non-pyrogenic supar® membrane, Pall Corporation, Cornwall, UK). 1 mL of solution B was added to 100 mL of solution A to produce the final freezer mix. All chemicals used in the freezing mix were obtained from BDH (Poole, UK).

Stock plate cultures were stored at 4 °C. This culture was more readily usable than the frozen stock, and was used to inoculate fresh NA plates for up to 1 month before discarding. In preparation for retention assays, *E. coli* was inoculated onto NA and incubated at 37 °C for 18 h.

2.2. Experimental procedures

2.2.1. Bacterial culture preparation

The method was adapted from Pal et al. [22]. Bacteria were inoculated into 10 mL of Luria Bertani (LB) (LB broth, Invitrogen Ltd., Paisley, UK) broth and incubated aerobically for 18 h at 159 rpm in a shaker at 37 °C. Cells were harvested by centrifugation at 4000 rpm for 5 min before washing twice with sterile 0.9% sodium chloride (NaCl) (Fisher Scientific Ltd., Leicestershire, UK). Following washing, the aqueous phase was discarded and the bacterial pellet was re-suspended in 0.9% NaCl to prevent carryover of nutrient from the original culture medium, and hence any unintentional increase in cell numbers or effect on cell physiology [23]. A cell density of 0.06 at 540 nm was obtained by dilution of cells in 0.9% NaCl. A 10-fold dilution to 10^{-7} was prepared using 0.9% NaCl as diluent (ensuring cells were well suspended by vortex mixing each dilution before transfer). A 50-mL aliquots of each dilution was filtered through a cellulose acetate membrane filter with a diameter of 47 mm (Whatman®, Japan), and an average pore size of 0.45 μm to entrap cells on the filter. In order to determine the optimum concentration for subsequent work (approximately 300 colonies per filter), the filter was plated onto eosin methylene blue (EMB) agar (Oxoid Ltd., Hampshire, UK) since EMB is a selective and differential medium for *E. coli*, and contaminants would be easily visualised. Eosin Y and methylene blue inhibit Gram-positive organisms (*E. coli* is gram negative). *E. coli* produces isolated colonies, 2–3 mm diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light, hence enabling easy visibility [24].

2.2.2. Membrane filter preparation

TiO_2 was suspended in deionised water at different concentrations (Table 1) and sterilised by autoclaving for 15 min at 121 °C. The water employed in all preparations was double distilled Millipore Water (Millipore S.A.S., Molsheim, France).

For the inactivation experiments with TiO_2, 50 mL of the autoclaved TiO_2 suspension at the required concentration was filtered with a 35 mm diameter filter holder, followed by filtration of 50 mL of the approximate bacterial suspension onto the TiO_2-loaded filters. The filter was then placed on an empty sterile Petri dish. The theoretical TiO_2 loading was calculated to be in the range from 520 to 15,590 mg m^{-2}, depending on the initial TiO_2 suspension employed (Table 1).

For the control experiments, 50 mL of the previously prepared bacterial suspension was filtered through an uncoated cellulose acetate membrane filter, before the filter was placed into a sterile Petri dish. The Petri dishes plus contents were then sealed with adhesive tape to prevent drying during light exposure. The light absorbed by the Petri dish lid was measured with a Lambda 40 spectrophotometer (Perkin/Elmer, USA). The Petri dish lid absorbed irradiation below 300 nm, which was outside the range of interest, thus it would not interfere with the photocatalytic effect under investigation.

2.2.3. Bacterial inactivation using fluorescent irradiation

For the photocatalytic experiment, six fluorescent lamps (Silvania, Ontario, Canada) with an energy output of 8 W were fitted

Table 1
Correlation between experimental concentrations in suspension and the calculated surface loading values.

TiO_2 concentration in suspension (g L^{-1})	TiO_2 loading[a] on the membrane filter (mg m^{-2})
0	0
0.01	520
0.03	1,560
0.08	4,157
0.12	6,236
0.20	10,394
0.30	15,592

[a] 50 mL of TiO_2 in suspension on a membrane filter surface of 19.24 cm^2.

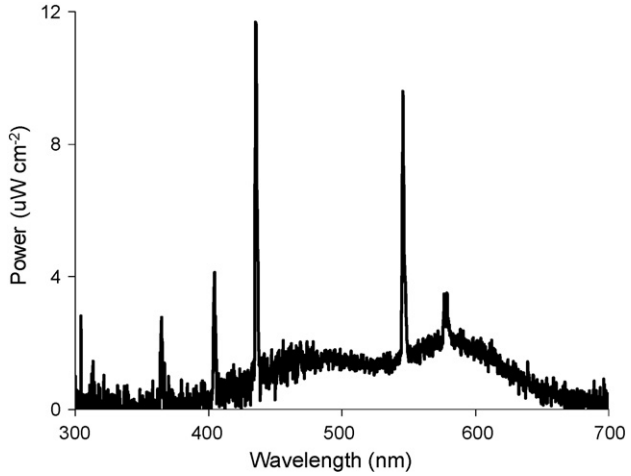

Fig. 1. Power output spectrum of fluorescent light used during the experiment, demonstrating the intensity of the illumination at the sample distance (15 cm).

in an Illuminated Cooled Incubator (Gallenkamp, Loughborough, UK) with a control thermostat set at 20 °C. The wavelength range of 300–700 nm was used as the light source for daylight. The fluorescent lamps were positioned at a distance of 15 cm from the bacteria impregnated filter membrane. Lamps placed on both sides of the cabinet ensured a uniform light energy. A USB4000 Miniature Fiber Optic Spectrometer (Dunedin, USA) was used to determine the UV/Vis power at the sample distance (Fig. 1). Samples were exposed to a portion of UV (290–400 nm) at 0.05–0.12 W m^{-2} intensity, and visible light source (400–700 nm), with a range of intensity 2.70–3.99 W m^{-2} for up to 120 min.

Five irradiation times (0, 15, 30, 60 and 120 min) were employed to examine the efficiency of the photocatalytic inactivation. Three replicates were produced for each experiment, giving 15 filters for each photocatalyst concentration. The experiment was repeated three times. Following irradiation, the filters were immediately removed from the Petri dishes, placed face-down on EMB agar, and incubated at 37 °C for 24 h. The colonies were then counted and recorded the following day.

Two control experiments were carried out; one was conducted in the dark with TiO_2, and the second was conducted with light, but in the absence of TiO_2.

The antibacterial activity (AA) was calculated using Eq. (1)

$$AA = \log\left(\frac{N_{tc}}{N_t}\right) \quad (1)$$

where N_{tc} is the number of CFUs after irradiation of the non-coated test piece at time t and N_t is the number of CFUs after irradiation at the coated photocatalyst filter at time t.

2.2.4. Scanning electron microscopy

A 50-mL aliquot of 0.9% NaCl with an *E. coli* concentration of $3.0 \pm 0.5 \times 10^7$ CFU mL^{-1} was filtered on membrane filters to which six different concentrations of TiO_2 had been applied by filtration. The samples were fixed to stubs for gold sputter coating which was carried out using a Polaron E5100 (Milton Keynes, UK) SEM sputter coater. Samples were sputter coated at a vacuum of 0.0921 mbar, for 3 min, at 2500 V, in argon gas at a power of 18–20 mA. Samples were imaged using a scanning electron microscope (JEOL JSM-5600LV) at 12 kV accelerating voltage.

2.2.5. Photography

Filters with bacteria colonies on the EMB agar were photographed with a digital camera (Cannon Inc., Japan). The photographs were taken with the EMB agar face down on the top of an opaque surface placed over a spotlight to scatter the light through the agar. This method enhanced visibility of dark colonies on the maroon coloured EMB agar.

3. Results

E. coli cells were exposed to different TiO_2 loadings for up to 2 h to assess any bactericidal effect of the photocatalyst PC105 on a filter substrate under fluorescent light. At lower TiO_2 concentrations colony counts decrease with time giving lowest results at a loading of 520 mg m^{-2}, but higher levels of TiO_2 concentrations were not effective (Fig. 2).

The survival ratio was calculated by the difference between the number of CFU before and after exposure to light for every sample. No changes in survival were observed when the TiO_2 coated substrate was stored in the dark (data not shown). In contrast, a decrease in the number of viable cells was observed on the illuminated samples either in absence or presence of catalyst. A typical death curve was observed for bacteria in the light over time, thus it appeared that a drying effect of the light irradiation was causing a decrease in cell viability (Fig. 3). Within the first 15 min of irradiation with the fluorescent light (UV fraction of irradiance of 0.07 W m^{-2}), 43% of the *E. coli* were viable on the TiO_2 free light control, while the number of viable bacteria remained unchanged during 15–120 min. However, there was a significant improvement in the killing efficacy when the TiO_2 was present; around 30% survival for a TiO_2 loading of 15,592 mg m^{-2} and up to 15% survival for a TiO_2 loading of 520 mg m^{-2} during the first 15 min. Bacterial survival decreased exponentially, as expected for a process that depends on the concentration of ROS available at each time.

Eq. (1) was used to calculate the antibacterial activity, which eliminates the effects of drying and lighting observed (Fig. 3). The time needed for microorganism inactivation was shorter at low loadings (Fig. 4). In particular, loadings of 520–4157 mg m^{-2} were extremely effective causing a ~2 log increase in inactivation (99%) within 60 min. When TiO_2 loading was 520 mg m^{-2} (which corresponds to 0.01 g L^{-1} in suspension), total kill was achieved after 2 h of exposure. However, TiO_2 loadings greater than 6236 mg m^{-2} presented a more reduced and slow photocatalytic efficacy, with more than 90% (>1.25 log) of the cells killed after 2 h. Thus it was determined that the optimal loading of TiO_2 used to inactivate *E. coli* under these conditions was 520 mg m^{-2}.

The morphology of bacteria and their interaction with the photocatalyst was investigated by scanning electron microscopy (SEM). This allows an estimation of the particle size and aggregate size of the TiO_2 and visualisation of surface contact with the bacteria. Analysis of the TiO_2 morphology is useful since the photocatalyst oxidation mechanism is explained in terms of surface oriented adsorption of substrates and hydroxyl radicals, hence the inactivation of bacteria is dependent on the surface–cell contact interaction. When a loading of 1560 mg m^{-2} was applied to the filter surface, the underlying filter could still be seen (Fig. 5A) whereas at higher concentrations the total filter area was covered in TiO_2 (Fig. 5B). The bacterial cells are apparent as darker grey elements covering the TiO_2 particles. They are less easy to visualize when directly in contact with the filter.

4. Discussion

There is some controversy in the literature regarding effective inactivation of microoganisms by TiO_2, primarily due to the different experimental conditions (UV/Vis irradiance [4,25,26], length of exposure, photocatalyst presented in suspension or powder [27–30], range of concentration [22,31]) and the different TiO_2 photocatalysts and microorganisms employed, although some effect is generally acknowledged. There is only a modest literature dealing

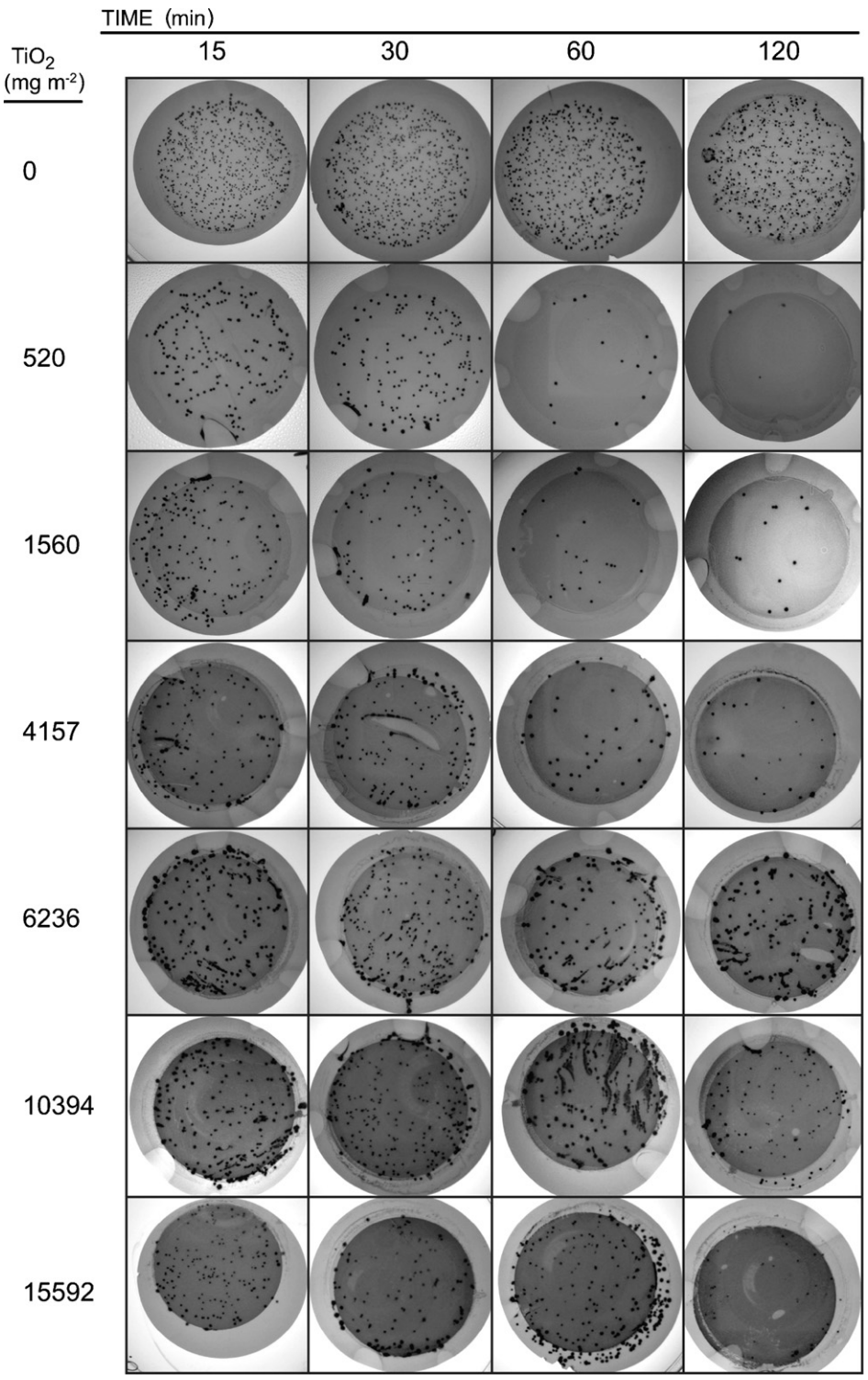

Fig. 2. Photographs demonstrating the effect of TiO_2 loading on the number of CFU following exposure to fluorescent light for 15, 30, 60 and 120 min. At a loading of 520 mg m^{-2} the number of CFU is lowest.

with photoinactivation of microorganisms under fluorescent light [11,25,32–35]. Pal et al. [22] carried out experiments with fluorescent light (0.13 W m^{-2}) and immobilized TiO_2 powder. They found that the inactivation rate increased with TiO_2 loading, and a complete inactivation of *E. coli* K12 was achieved after 30 min of treatment using a loading of 1666 mg m^{-2}. However, in contrast with their results, our study showed an optimum kill was not attained in the first 30 min. This may be due to the slightly lower UV fraction of light (0.07 W m^{-2}) used in our experiment requiring 2 h to achieve kill. It may be suggested that the sensitivity of the photokilling reaction produced by absorbing UV light in a 'daylight' environment is dependent on the amount of UV reaching the photocatalyst surface.

Contradictory reports regarding the decay kinetics of the photocatalytic killing process of microorganisms can be found in the literature. Several papers suggest a first-order kinetics for the bacterial inactivation over time [20,22,26,33,36,37], while other reports propose a two step decay dynamic of the photokilling process [38].

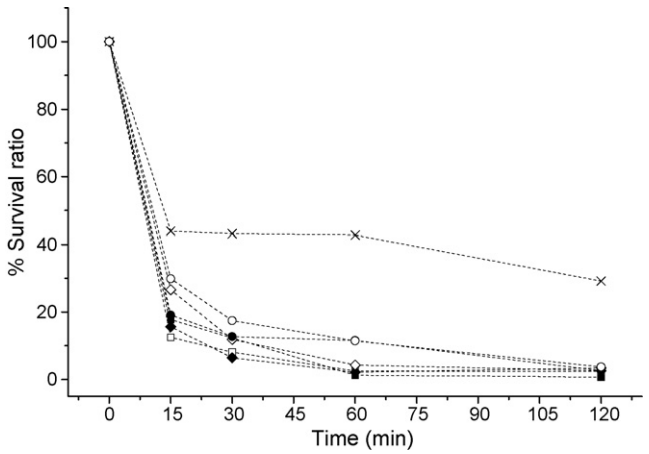

Fig. 3. Plot of the survival ratio of *E. coli* with TiO_2 photocatalyst at different concentrations for samples exposed to fluorescent lamps. TiO_2 loadings are (×) 0 mg m^{-2}, (■) 520 mg m^{-2}, (□) 1560 mg m^{-2}, (♦) 4157 mg m^{-2}, (◊) 6236 mg m^{-2}, (●) 10,394 mg m^{-2}, (○) 15,592 mg m^{-2}. Under fluorescent light, the survival of intact *E. coli* cells dropped as a function of time.

Our results could not be fitted to a decay model as a function of illumination time (data not shown). We suggest that the large number of variables on the photokilling process prevents the decay from being fitted to a simple kinetic model.

It has been generally accepted that the photocatalytic inactivation of microoganisms is mainly due to interaction with highly reactive oxygen species (ROS), particularly hydroxyl radicals. Although many researchers have proposed explanations for the detailed mechanism of TiO_2 [12,26,38,39], the action of the radicals on the bacteria cell membrane remains unclear. In this work, the photocatalytic decomposition caused by an attack of oxidative species on the cell wall was possibly the reason for the killing of bacteria. However, in view of the results presented and the findings in the literature, we propose that the intimate contact possible between the small TiO_2 particles (15–25 nm) and the bacteria (1.5 μm) might cause physical damage to the bacterial cell wall and enhance an antibacterial effect [30].

Our study confirmed that illuminated TiO_2 powder exhibits bactericidal activity, and that photokilling activity increases with longer times of illumination at an optimum TiO_2 loading of 520 mg m^{-2} (which corresponds to 0.01 g L^{-1}). This result is consistent with previous work by Benabbou et al. [36], who found that photocatalyst suspensions with an optimal concentration of 0.25 g L^{-1} with a range of photocatalyst TiO_2 concentration from 0.1 to 2.5 g L^{-1}, improved the inactivation of *E. coli* in the presence of UVA (38 W m^{-2}) irradiation. Although not directly comparable because our work used TiO_2 dried onto a surface instead of suspensions in the presence of 0.07 W m^{-2} irradiation, the underlying principles regarding concentration effectiveness are in agreement.

The uniformity of the TiO_2 coated over the membrane filters was examined using SEM. This allows an estimation of the particle size, aggregate size of the TiO_2 and area of contact with the bacteria. At lower concentrations of TiO_2 (Fig. 5A), the photocatalyst did not completely cover the filter surface; filter pores were visible, some of which entrapped bacteria. The concentration and pattern of deposition of the TiO_2 on the filter surface is of importance in photocatalysis since as the reactive oxygen species ROS (H_2O_2, •OH, O_2^-) is produced by TiO_2, the photons need to be activated to induce the photocatalysis reactions thus killing the bacteria i.e., there is a requirement for light to reach TiO_2, and for TiO_2 to be in contact with bacteria. When the TiO_2 particles fully cover the filter surface (Fig. 5B) and bacteria are deposited on the top of the surface with a high TiO_2 concentration, a thicker layer results, which is less penetrable by light. Thus, the fluorescent light irradiation is less effective in terms of promoting bacterial inactivation. This phenomenon may be due to the effect of the cells themselves, and excess photocatalyst, masking the light from the TiO_2 beneath. Thus the photocatalyst is not activated (Fig. 6). Also, a small fraction of

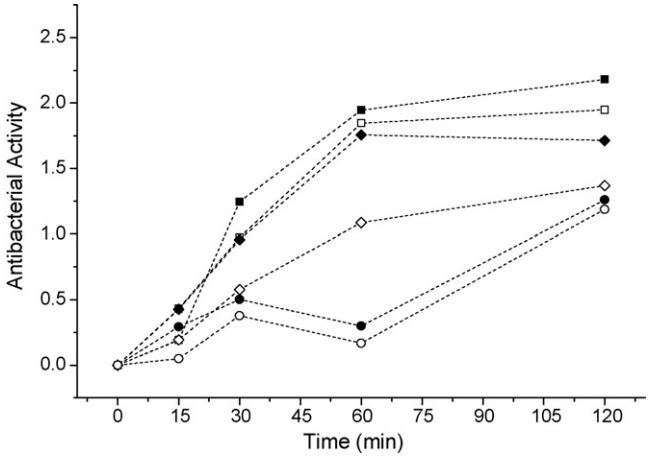

Fig. 4. Plot of the antibacterial activity of TiO_2 photocatalyst at different concentrations in samples exposed to fluorescent lamps. TiO_2 loadings are (■) 520 mg m^{-2}, (□) 1560 mg m^{-2}, (♦) 4157 mg m^{-2}, (◊) 6236 mg m^{-2}, (●) 10,394 mg m^{-2}, (○) 15,592 mg m^{-2}. Filters with less photocatalyst led to faster bacterial inactivation.

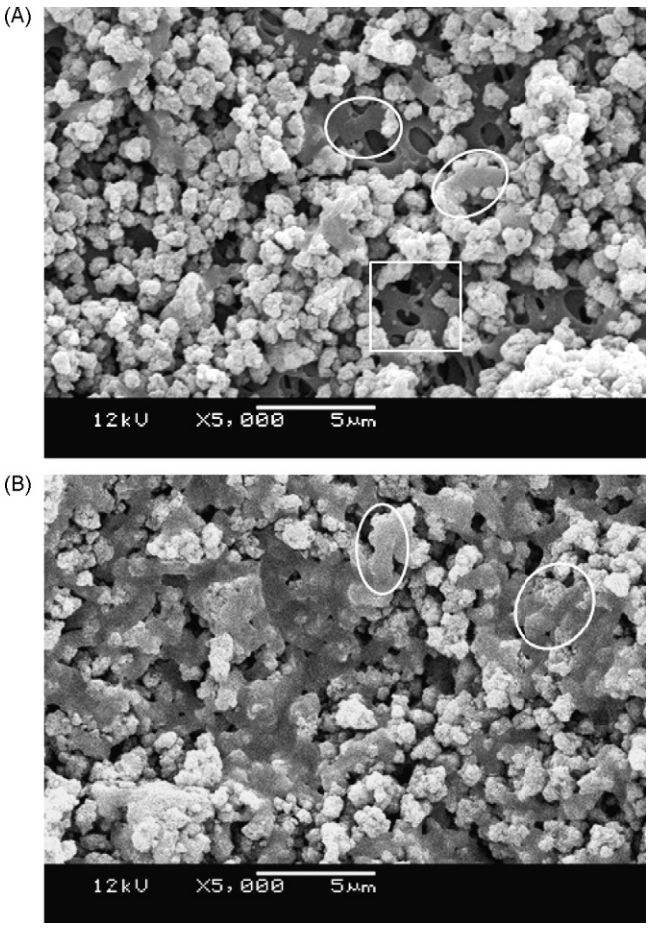

Fig. 5. SEM photographs of agglomerations of nanoparticles TiO_2 and *E. coli* in contact with the aggregates. A loading of (A) 1560 mg m^{-2}, (B) 15,592 mg m^{-2}. Some bacteria are ringed for ease of identification. The exposed filter is apparent in (A), and is indicated by a quadrangle.

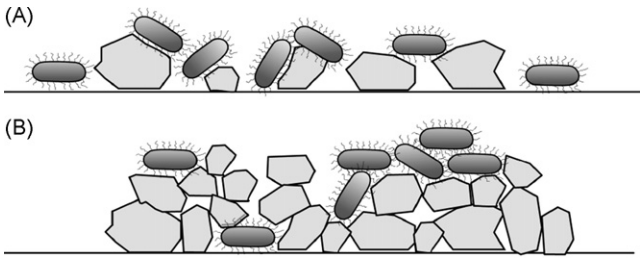

Fig. 6. Schematic illustration of *E. coli* photokilling on TiO_2 on the membrane surface with the cell surface pattern of retention corresponding to (A) low TiO_2 loading, optimum contact between cells and TiO_2 for a good light transmission thus better cell killing rates; and (B) high TiO_2 loading. In this case, light cannot get through the TiO_2 layer or to all cells due to an increased thickness layer and an excess of bacteria across the surface, thus there is less photocatalytic effect.

bacteria could escape UV exposure due to shielding of microorganisms at high bacterial and photocatalyst concentrations. On surfaces with a lower TiO_2 load, bacteria not in contact with photocatalyst can be inactivated when irradiated with light (drying effect). Therefore, on illuminated TiO_2, an important aspect to consider in terms of bacterial inactivation is the relative size and concentration of the titanium particles/aggregates on the surface and their contact with the target cells. Different bacterial cells also vary in sensitivity.

A possible mechanism for bacterial inactivity may be due to the direct contact between bacteria and TiO_2 on the surface, which will increase the probability of attack by $^{\bullet}OH$ as compared to TiO_2 in suspension. We have suggested a model that could justify these results.

In low TiO_2 loading (Fig. 6A), the surface of the photocatalyst is accessible to light and bacteria. This scenario would correspond to a range of loadings 520–4157 mg m^{-2} where nanoparticles are evenly dispersed across the surface. All the UV irradiated light is absorbed by the photocatalyst and the photons can activate the TiO_2. The levels of $^{\bullet}OH$ radicals generated will therefore be sufficient to completely inactivate bacteria. When the TiO_2 loading is higher, 6236–15,592 mg m^{-2}, the photocatalyst is laid down in agglomerate along several layers (Fig. 6B). Recombination of unreacted electron and hole pairs produced by irradiation could be enhanced by the large amount of photocatalyst present. Light will not reach bacteria positioned between or beneath TiO_2 nanoparticles. If the photons cannot reach inner layers of the photocatalyst, no activation will occur. Higher coverage of surface with TiO_2 might also result in piling up of cells onto the catalyst rather than being spread across filter and catalyst, possibly interfering with an effect. The $^{\bullet}OH$ concentration increases with illumination time due to the interaction between light and TiO_2 nanoparticles. When the concentration of TiO_2 is low, there is a balance between the $^{\bullet}OH$ generated and deactivation of bacteria. However, in our experiment, when the TiO_2 loading is higher than 6236 mg m^{-2}, the excessive production of hydroxyl radicals could lead to their self-recombination [30] Eqs. (2) and (3):

$$^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad (2)$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HO_2{}^{\bullet} \quad (3)$$

These reactions lead to the formation of the hydroperoxyl radical $HO_2{}^{\bullet}$ which is less reactive and does not seem to contribute to the oxidative process [40].

It is crucial to take into account the influence of the TiO_2 morphology in the antibacterial properties. Preliminary experiments in suspension reported by Verran et al. [41] demonstrated that the antibacterial activity of nanoparticles pigments was inversely proportional to particle size. Studies on TiO_2 morphology and its effect on the inactivation of cells are currently in progress.

5. Conclusions

This study demonstrates photocatalysis as a viable method of disinfecting bacteria under fluorescent light with immobilized TiO_2. The combinations of photocatalyst which have been tested and illumination from typical light sources such as fluorescent light are a promising direction for future applications of self-disinfection and self-cleaning surfaces.

It was found that the concentration of photocatalyst used influenced the killing of bacteria, with greater activity achieved when the concentration was around 520 mg m^{-2}. Activity was reduced at higher concentrations 15,592 mg m^{-2} since access of light to the particles and access of particles to bacteria was reduced. Using of TiO_2 nanoparticles as opposed to TiO_2 pigment is desirable to allow maximum light activation. Also of importance is contact between TiO_2 photocatalyst and *E. coli*. SEM images show that increased particle contact with bacteria enhanced the photocatalytic effect during the photocatalytic process.

Acknowledgements

The authors would like to thank John Stratton and Claire Bygott from Millennium Inorganic Chemicals for supplying the photocatalyst used in this programme of work and also for partial funding support in this project.

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