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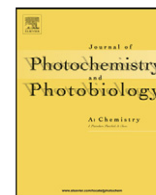

Photobactericidal effects of TiO_2 thin films at low temperatures—A preliminary study

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ABSTRACT

The efficacy of TiO_2 photocatalysis for the destruction of pathogenic bacteria has been demonstrated by a number of groups over the past two decades. Pathogenic bacteria represent a significant hazard for the food and drink industry. Current practices in this industry dictate that rigorous sanitizing regimes must be regularly implemented resulting in lost production time. The incorporation of a TiO_2 antibacterial surface coating in this setting would be highly desirable. In this paper we report a preliminary study of the efficacy of a TiO_2 coating, doped with the lanthanide, neodymium, at low temperature conditions such as those utilised in the food and drink sector. The rapid destruction of *Staphylococcus aureus*, a common foodborne pathogen, was observed using TiO_2 films coated to glass and steel substrates.
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1. Introduction

Foodborne disease caused by bacteria such as *Staphylococcus aureus*, *Salmonella* spp. and *Campylobacter* spp. represents a significant worldwide health problem [1]. The microbiological safety of food products is of utmost importance to the food and drink industry hence cleaning and sanitizing procedures are paramount. The objective of this regime is twofold; firstly to physically remove all food soil which provide nutrients for bacteria to grow and secondly to kill those bacteria which may be present.

Current practices within the food and drink industry dictate that detailed procedures for cleaning and sanitizing all food product contact surfaces must be in place. Surfaces are generally cleaned using chemical disinfectants such as hypochlorite and quaternary ammonium salts. A disinfectant can be defined as a chemical agent that reduces the number of viable microorganisms; hence sterilization is not always achieved. Furthermore, surfaces treated with a disinfectant are only effectively clean or perhaps sterile for a short period after cleaning. This means that the regime must be implemented on a regular basis, resulting in significant “down time” i.e. lost production time, several times a day. Other methods employed by the food and drink industry to sterilise the working environment include the process of treating with ozone or hydrogen peroxide following food poisoning incidents/food scares. These methods, however, as well as being ineffective in buildings with high ceilings, require ventilation of the treated environment following the procedure.

The deployment of an antimicrobial surface coating in this setting, in conjunction with good hygienic practices, may prove to be an effective mechanism to reduce the incidence of foodborne infection and intoxication. Currently, a major obstacle preventing the widespread adoption of antimicrobial surfaces/coatings in the food and drink industry is the lack of activity exhibited by antimicrobial materials at low temperatures. Many environments in the food and drink industry are kept at temperatures of 10 °C or less (chill rooms) to minimise the proliferation of pathogens. Most antimicrobial materials, however, rely upon the diffusion of active agents, and such low temperatures prevent effective diffusion. Generally, organisms that cause infection in humans are mesophiles i.e. they have an optimum growth temperature of around 37 °C. All microorganisms, however, exhibit a range of temperature over which they can grow and mesophiles like *S. aureus* and *Escherichia coli* have a minimum growth temperature of around 10 °C. This is the reason why such strict cleaning and sanitizing regimes are required in the food and drink industry. Even more problematic are organisms like *Listeria monocytogenes* which has a minimum growth temperature of 1 °C and *Pseudomonas maltophilia* which can grow at 4 °C.

Titanium dioxide photocatalysis has been shown to be an effective mechanism for killing pathogenic bacteria [2–6]. The photocatalyst may be used in the form of an aqueous suspension or

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on a solid support. The use of TiO_2 films for the destruction of pathogenic bacteria has been reported by several authors and found to be as effective as the suspended form [7–10]. This approach overcomes the disadvantage of having to separate catalyst from liquid following treatment. It also presents an excellent opportunity to develop an antimicrobial surface coating. Doping TiO_2 films with lanthanide ions as a means of increasing photocatalytic activity, has been reported previously [11–15]. The incorporation of such ions into a TiO_2 matrix is thought to provide a means of concentrating organic pollutants at the semi-conductor surface thereby enhancing the photoactivity of the TiO_2 layer.

The use of a TiO_2 antimicrobial coating would be particularly appropriate in the food industry setting since the technology does not introduce toxins or residues into the disinfection process. Moreover, it would not alter the chemical composition, taste, odour or pH of food being prepared on the target surface. This paper describes a preliminary study of the ability of a thin TiO_2 film to photocatalytically degrade *S. aureus* at chill room temperature. The objectives were to identify a formulation for a TiO_2 coating with strong photocatalytic activity and to assess the antibacterial efficacy of this coating at chill room temperature (10 °C) compared to standard room temperature (25 °C). We also report on the use of a novel laser annealing process to cure TiO_2 thin films.

2. Materials and methods

2.1. Preparation of rare earth doped and undoped TiO_2 films

The sol–gel formulations were produced following a modified method by Mills et al. [16]. 4.65 g (4.43 mL) of glacial acetic acid was added to 20 mL of titanium isopropoxide. To this solution 120 mL of 0.1 mol L^{-1} nitric acid was added before heating the mixture at 80 °C for 8 h in a water bath. The resulting opaque solution was then filtered through a 0.45 μm filter to remove any aggregated particles. To produce the doped titania films, individual rare earth metals, in the form $X(NO_3)_3{\cdot}6H_2O$ (where X = Gd, Nd or Er), were dissolved in the 120 mL of 0.1 mol L^{-1} nitric acid before adding the solution to the titanium isopropoxide and acetic acid solution. The initial concentration chosen was 0.5 wt.% (0.6 g), which was found to be the optimum concentration of dopant by Xu et al. [12]. The glass slides were then dip-coated twice at 3 cm s^{-1}, allowed to dry at 45 °C before being calcined in a high temperature chamber furnace (Carbolite, UK) at 450 °C for 30 min. Detailed information on the preparation and characterisation of the films has been reported previously [17].

2.2. Preparation of polyethylene glycol coated titanium dioxide films

The filtered sol–gel formulation was concentrated down to reduce the volume by half at 95 °C for 1.5 h. To the concentrated sol, 14.25 g of polyethylene glycol (PEG) 6000 was added. The gel-like sol was placed in a 70 °C water bath for 1.5 h and became more viscous again after stirring. The glass slides were then dip-coated, once, as described above and annealed in the furnace at 450 °C for 30 min.

An alternative laser annealing system was also employed for some of the coatings. In this case TiO_2 coated steel samples were laser annealed with a pulsed KrF excimer laser (EX350, GAM laser Inc.), emitting at 248 nm with 24 ns pulses. The samples were raster scanned with laser pulses of 190 mJ energy at a pulse rate of 5 Hz. The scanning speed (17.21 mm/s) was adjusted to provide sufficient overlap of the subsequent laser pulses on the sample surface to ensure uniform annealing.

2.3. Methylene blue degradation

A 100 mL volume of a 1×10^{-5} mol L^{-1} methylene blue (Fisher, UK) solution was placed in a custom built UV transparent batch reactor. TiO_2 coated glass slides were placed vertically into the methylene blue solution and irradated from the side, at a distance of 20 cm, with a 500 W xenon lamp (Dr Hönle, UK). The reaction vessel was covered with a loose fitting lid and stirred throughout. Samples of the solution were taken in triplicate every 15 min and the peak absorption of methylene blue (664 nm) was analysed using a UV–Vis absorption spectrophotometer (Perkin Elmer, lambda950).

2.4. Bacteria and culture conditions

S. aureus NCTC 6571 was purchased from the National Collection of Type Cultures (London, UK). This was sub-cultured and maintained on nutrient agar. To prepare the bacterial culture for photocatalysis experiments, three to five well isolated colonies of the same morphological type were lifted from the nutrient agar plate (NCCLS, 2000), inoculated into 100 mL nutrient broth and placed in an orbital incubator set at 37 °C and 100 rpm (IOX400.XX2.C; Sanyo Gallenkamp PLC, Loughborough, UK). After 18 h incubation, bacterial cells were harvested by centrifugation (MSE Centaur 1; Fisons, Loughborough, UK) at 4000 rpm for 10 min, then washed and re-suspended in sterile distilled water and finally adjusted to give a cell density of 1×10^4 colony formining units (cfu)/mL.

2.5. Photocatalytic destruction of *S. aureus* at standard room temperature and chill room temperature

Antibacterial activity of the UV-illuminated coatings was assessed using a variation of the method described by Sunada et al. [9]. Humidity chambers were prepared by placing a petri dish, containing 50 mL of sterile distilled water, into a sterile glass trough and pipetting an additional 20 mL of sterile distilled water into the base of the trough. Three slides (coated or uncoated) were placed on top of each petri dish. A glass ring cell was aseptically placed onto the centre of each slide and 300 μL of bacterial suspension was carefully pipetted into each ring cell. The glass troughs were covered with cling film and placed 15 cm beneath a 6 × 8 W UV-A black light (spectral output 311–415 nm peaking at 368 nm; Philips TL 8W/08 F8 T5/BLB) which had been switched on 15 min prior to use to allow the bulbs to reach a standard light intensity. The first set of experiments were performed at standard room temperature (25 °C). Later experiments were performed at chill room temperature (10 °C) using a cooled incubator (Jencons Model 3SE 451). Samples were collected at hourly intervals in the following way. A slide was removed from the humidity chamber and the bacterial suspension was pipetted into a microcentrifuge tube, this was then made up to a total volume of 1 mL with sterile distilled water. The glass ring was then aseptically removed with forceps, a sterile cotton tipped swab was then moistened with the suspension in the microcentrifuge tube and run over the surface of the slide to collect any additional bacteria. The tip of the swab was then broken off into the microcentrifuge tube and this was vortexed three times (10 s each time). A 10-fold dilution series of the resulting bacterial suspension was prepared in sterile distilled water and samples of each dilution were plated onto nutrient agar. Colonies were counted after 17 h incubation at 37 °C and again after 24, 40 and 72 h incubation, to check for additional bacterial growth, and viable counts were calculated. Control samples were processed as above except that for UV only controls, uncoated glass slides were used and for dark controls the humidity chambers containing TiO_2 coated slides were wrapped in aluminium foil and placed on the top shelf of the chill incubator away from the UV light source. Humidity

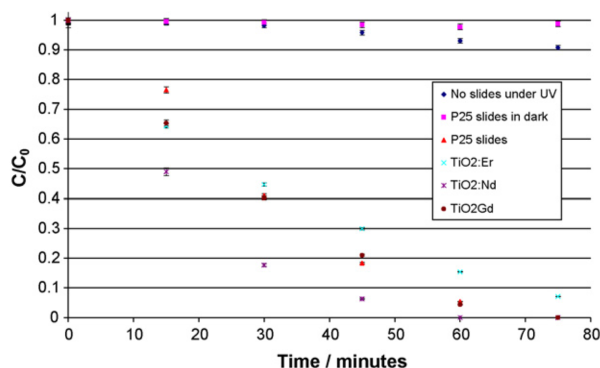

Fig. 1. Comparison of methylene blue degradation by TiO_2 rare earth doped and undoped coated glass slides.

chamber experiments were also performed in glass troughs which were not covered with cling film.

3. Results and discussion

3.1. Development of TiO_2, $NdNO_3$ and PEG coatings

Initially the TiO_2 thin films were assessed using a simple methylene blue degradation assay, which has been well characterised as an indicator of photocatalytic activity [14,15,18,19]. PEG was incorporated into the coating as it has been reported to result in a more durable coating with greater bending/flexing properties [20] although the incorporation of PEG has been reported to result in pore formation [21]. This is obviously an undesirable effect in this case thus in order to address this problem, future work will focus on improving both the durability and the smoothness of the films. Since the formulation containing PEG was more viscous than the one without, the slides only had to be dip-coated once in order to produce a homogeneous thin film.

Data from Fig. 1 shows that when TiO_2 coated glass slides were doped with either neodymium, gadolinium or erbium a significant improvement in methylene blue degradation was observed compared to undoped TiO_2. Neodymium exhibited the greatest amount of methylene blue degradation and was therefore chosen as the dopant for all subsequent TiO_2 films. Furthermore, the neodymium doped films exhibited a more efficient methylene blue degradation than Degussa P25 coated slides. The use of rare earth dopants to enhance the photocatalytic activity of TiO_2 has been widely reported. There is however some variation in the literature with regards to the most efficient rare earth metal in terms of photocatalytic activity. For example, Štengl et al. [13] showed that Nd^{3+} exhibited the best photocatalytic properties in visible light activated TiO_2 nanoparticles doped with a range of rare earth metals however in other studies Gd^{3+} [14] and La^{3+} have been shown to be more effective [15]. In the current study it is unclear why the neodymium doped films showed the best rate of methylene blue degradation, the reasons for this observed effect require greater investigation.

3.2. Photocatalytic destruction of *S. aureus* at standard room temperature and chill room temperature

Results presented in Fig. 2a show that *S. aureus* cells on TiO_2 coated slides are killed after 4 h of UV illumination when the experiment is performed at standard room temperature. Destruction of *S. aureus* on the uncoated slides, by comparison, occurred after 6 h illumination (Fig. 2a). This indicates that the TiO_2 photocatalytic process results in a more efficient bacterial destruction than UV photolysis alone. Data from the dark control shows that

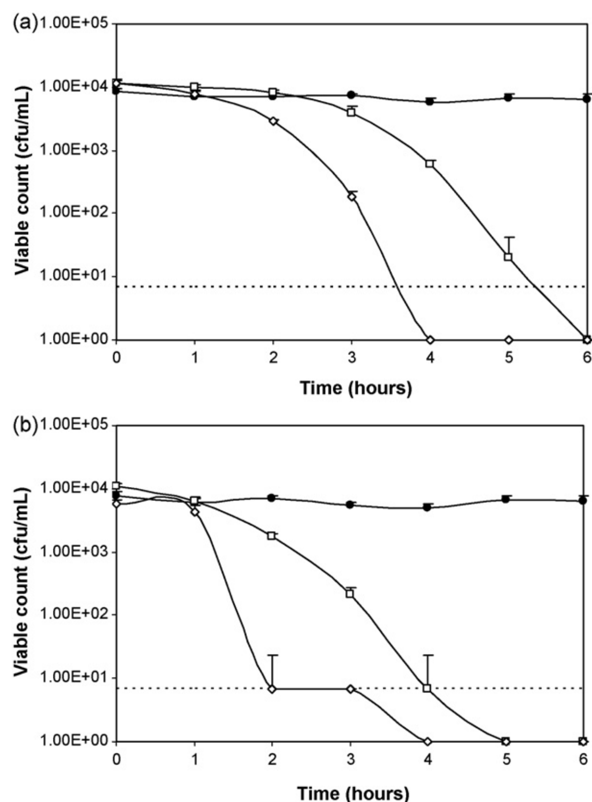

Fig. 2. Antibacterial activity of coatings at (a) 25 °C and (b) 10 °C using the humidity chamber method with cling film. ◊: UV-illuminated TiO_2, $NdNO_3$ and PEG coated glass slides; □: UV-illuminated uncoated glass slides; ●: uncoated glass slides in dark; - - -: minimum detectable number of cfu/mL (error bars represent standard error of the mean of ten 20 μL samples).

these bacteria maintained excellent viability for the duration of the experiment.

When UV-illuminated TiO_2 coated slides were tested at chill room temperature instead of standard room temperature, no decrease in their antibacterial activity was detected (Fig. 2b). As before, complete destruction of the bacterial population occurred within 4 h. The initial rate of bacterial killing at the decreased temperature actually appeared to be slightly improved, with bacterial numbers being reduced to the minimum detectable level within just 2 h. For bacteria on the UV-illuminated uncoated slides, the kill time decreased from 6 h (Fig. 2a) to just 5 h (Fig. 2b) at the reduced temperature. This is likely to be attributable to the combined stress of low temperature and UV on the bacteria. There are two mechanisms by which this may have occurred. Firstly, it is known that bacteria exposed to UV light synthesise a number of proteins including the enzyme alkyl hydroperoxide reductase to protect themselves from UV killing [22]. At low temperatures, translation of mRNA to protein is compromised [23] and it may be that synthesis of alkyl hydroperoxide was inhibited. Alternatively, the decrease in kill time may be due to inhibition of a bacterial process called the cold shock response. A temperature drop from 37 to 10 °C, by itself, does not significantly affect the viability of *S. aureus* cells [23] but it is sufficient to induce the cold shock response. This results in increased transcription and translation of a number of cold shock genes including lrgA and lrgB, which are believed to counteract the cell's programmed cell death machinery [23,24]. Treating bacteria with UV results in inhibition of protein synthesis due to tRNA damage [25], and inhibition of protein synthesis from genes such as lrgA and lrgB may have contributed to loss of bacterial viability. Data from the dark control, i.e. TiO_2 coated slides kept in the dark, showed that no bacterial destruction took place.

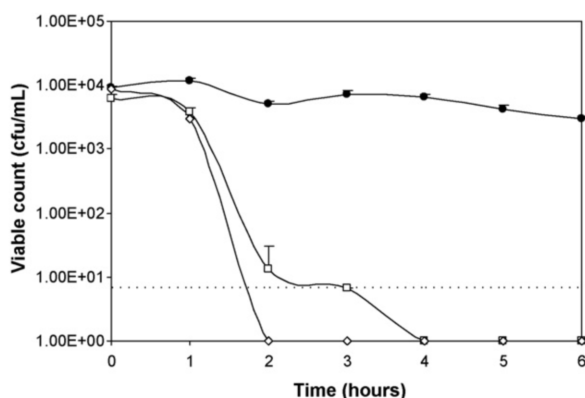

Fig. 3. Antibacterial activity of coatings at 10 °C using the humidity chamber method without cling film. ◊: UV-illuminated TiO_2, $NdNO_3$ and PEG coated glass slides; □: UV-illuminated uncoated glass slides; ●: uncoated glass slides in dark; - - -: minimum detectable number of cfu/mL (error bars represent standard error of the mean of ten 20 μL samples).

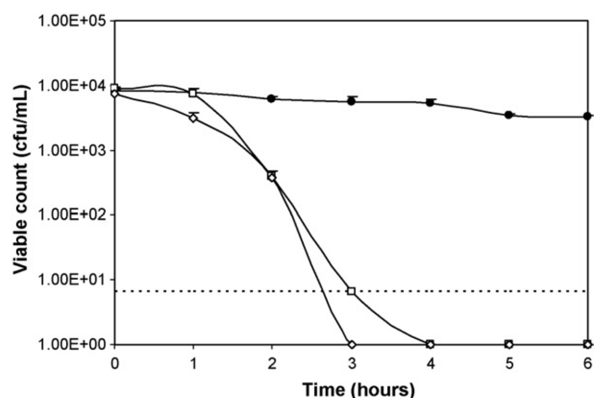

Fig. 4. Antibacterial activity of laser annealed coatings at 10 °C using the humidity chamber method without cling film. ◊: UV-illuminated TiO_2, $NdNO_3$ and PEG coated steel slides; □: UV-illuminated uncoated steel slides; ●: uncoated steel slides in dark; - - -: minimum detectable number of cfu/mL (error bars represent standard error of the mean of ten 20 μL samples).

An observation made during the cold temperature experiments was the buildup of condensation on the cling film of chilled humidity chambers. The purpose of the cling film was to prevent the bacterial inocula from drying out, a common problem when experiments are performed at standard room temperature. In recognition of the possibility that condensation might be reducing the amount of UV light reaching the slides, it was decided to repeat the cold temperature experiment without cling film. This modified setup, it was rationalised, would more closely resemble the conditions under which the TiO_2 films would be used in industry.

3.3. Photocatalytic destruction of S. aureus at chill room temperature using the modified humitidy chamber method

When photocatalysis experiments were performed without cling film, the kill time of the UV-illuminated TiO_2 coated slides was reduced from 4 h (Fig. 2b) to just 2 h (Fig. 3). This represents a major improvement on the kill time. Clearly, the buildup of condensation had been reducing the amount of light reaching (and activating) the TiO_2 thin films in the previous experiment. A decrease in kill time was also observed for the UV-illuminated uncoated slides; UV alone was found to kill the bacteria after 4 h (Fig. 3) instead of 5 h (Fig. 2b). All subsequent experiments were performed using the modified humidity chamber method.

3.4. Photocatalytic destruction of S. aureus on laser annealed coatings at chill room temperature

Following the results with the furnace annealed coatings, it was decided to develop a laser curing technique in an attempt to produce a more robust and durable coating. Steel was chosen as the target material for this experiment as this type of surface is commonly used in the food industry. In Fig. 4a it can be clearly seen that *S. aureus* cells applied to uncoated steel slides and illuminated with UV light at 10 °C were killed after 4 h. *S. aureus* cells on TiO_2 coated steel slides which had been laser annealed, at a laser pulse rate of 5 Hz, were killed after 3 h of UV illumination (Fig. 4). This result shows that the laser annealed coatings have antibacterial activity when illuminated with UV light and are also effective at low temperatures.

When the laser annealed coatings were examined following the photocatalysis experiments some damage was detected; the coating appeared to have been partially rubbed off (data not shown). This observation suggests that the current laser annealing process has not produced a robust, durable coating. This may explain why the kill time was slightly longer for laser annealed coatings (Fig. 4) than the furnace annealed coatings tested previously (Fig. 3). To address this problem, the pulse rate of the laser will be increased from 5 to 50 Hz for future coatings and this should produce a more robust film.

4. Conclusions

The development of a TiO_2 surface coating with antimicrobial activity at chill room temperature has been investigated. Furnace annealed TiO_2 coatings doped with $NdNO_3$ and containing PEG were produced. A humidity chamber method was developed and photocatalytic disinfection experiments were performed in a cooled incubator at 10 °C, to simulate conditions akin to those used in the food industry. It was established, using this method, that photocatalytic degradation of *S. aureus* at 10 °C could take place within 2 h. Laser cured TiO_2 coatings were then developed in an attempt to produce a more robust and durable coating. Preliminary data attained in this study indicate that, while these coatings do show antibacterial activity at low temperatures, the coating is not sufficiently robust. Future work will focus on modifying the laser parameters to produce an enhanced coating process.

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