

## Photoinactivation of *Escherichia coli* on acrylic paint formulations using fluorescent light

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### ARTICLE INFO

#### Article history:

Received 10 August 2009

Received in revised form

10 November 2009

Accepted 2 December 2009

Available online 30 December 2009

#### Keywords:

Photocatalysis

Paint

TiO<sub>2</sub>

UV inactivation

*E. coli*

Fluorescent light

### ABSTRACT

The composition of acrylic paints containing TiO<sub>2</sub> photocatalyst was evaluated in terms of bacterial inactivation using *Escherichia coli* (ATCC8739), during irradiation with fluorescent light. Paint formulations containing photocatalyst and photocatalyst plus extenders were prepared as films. Complete bacterial inactivation was obtained within 96 h for simple paint formulations containing photocatalyst at different loadings (15–80% of total pigment volume concentration). The addition of extenders such as silica and talc to the paint formulation did not affect the efficacy of bacterial inactivation. However, the use of CaCO<sub>3</sub> as extender reduced the photocatalytic activity of the paint. This is attributed to reduced contact between the microorganism and the nanoparticles when CaCO<sub>3</sub> is present, especially at concentrations higher than 40%. Results show the importance of careful paint formulation to enable activation of photocatalyst for *E. coli* killing under fluorescent light.

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### 1. Introduction

Titanium dioxide and titania based composites are the whitest and brightest of the commercial white pigments used in everyday products [1], and as such they are widely used. Materials such as cement, wood, plastics, ceramics and paints may become colonized and degraded by microorganisms and numerous adverse health effects have been associated with microbial growth on building surfaces [2,3]. Surface hygiene could be improved by the application of TiO<sub>2</sub> photocatalysis to create self-cleaning building materials, thus retarding surface contamination and saving on the cost of cleaning maintenance. Titanium dioxide is a strong absorber of UV light. Upon absorption of photons of wavelength  $\leq 385$  nm by TiO<sub>2</sub>, an electron is promoted from the valence band to the conduction band, forming an electron–hole pair. The photogenerated holes react with water molecules attached to TiO<sub>2</sub> surfaces to form hydroxyl radicals ( $\cdot\text{OH}$ ) while photogenerated electrons react with molecular oxygen to give superoxide radical anions ( $\text{O}_2^{\cdot-}$ ) [4,5]. These radicals are highly reactive for both the oxidation of organic substances and the inactivation of bacteria and viruses. Over the last 25 years, continued research efforts in the area of anti-bacterial photocatalysis have led to significant developments in this field. Coatings containing anti-bacterial properties have been available

for products such as glass, window blinds, wallpaper and tiles for many years. Fujishima et al [1,6] reviewed the anti-bacterial effects and detoxifying actions of TiO<sub>2</sub> as a photocatalyst on ceramic tiles applied on several hospital rooms. They found a decrease in anti-bacterial counts within a period of 1 hour accompanied by a decrease in bacterial counts even in the surrounding air.

In recent years, the development of paints with antimicrobial properties has been of enormous interest for the industry. Applications of TiO<sub>2</sub> photocatalysis on coatings are described in the literature [7–10], but only a few papers give information on the photocatalytic interaction between TiO<sub>2</sub> and building materials such as plaster or paints [11,12]. The thermal and photochemical activity of nano and micron particle grade anatase and rutile TiO<sub>2</sub> pigments has already been assessed in polyethylene and alkyd based paint films in our laboratory [13,14]. Pigment particle size and surface area play an important role in any polymer–pigment interaction, because pigments can affect the thermal and photochemical stability of the polymer material. Further, photooxidation studies have shown a clear demarcation between paint films containing nanoparticle and pigmentary grade titanium dioxide, with the former being more active. The use of rutile pigment, which is considered to be less active than pigmentary anatase in the photodegradation of titania-coating surfaces [15], in conjunction with nanoparticle anatase grade, is a viable option for the development of self-cleaning paints and antimicrobial surfaces [16–18]. For self-cleaning paints it is necessary to effect a compromise between controlling the surface activity of the paint whilst allowing only

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microlayer shedding of matrix. Thus, the formulation of such paints requires considerable care.

Paint coatings are composed of an organic phase (binder and solvents) and a mineral phase (binder and extenders). An organic binder (styrene acrylic emulsion), representative of an indoor paint, was selected for our study. The binder is responsible for the general mechanical properties of the film. The mineral phase of the coating is mainly composed of extenders, which give volume to the paint, and of pigments which provide white colour.

In this study we have focused on a series of anti-bacterial acrylic paint formulations of increasing complexity, containing TiO<sub>2</sub> anatase nanoparticles (PC105), intended for use in an indoor living environment under illumination from fluorescent light. The aim was to obtain a better understanding of any interactions occurring between the additives of the paint and the nanoparticles TiO<sub>2</sub>, and also any potential effect on the antimicrobial properties of the system.

## 2. Materials and methods

### 2.1. Paint formulation

The formulation of paints used in this study is shown in Tables 1 and 2. The formulation of the first, simpler, paints tested (Table 1) was followed with the addition of extenders (Table 2). The formulations were prepared in the laboratories of Millennium Inorganic Chemicals (Grimsby, UK). TiO<sub>2</sub> PC105 has an average diameter crystallite size of 15 nm–25 nm with a nanoanatase phase of 100%. Extenders were added based on the pigment volume concentration (PVC) which is a measure of the amount of a particular pigment that can be added to the styrene/acrylic copolymer of the coating to create a protective coating. Extender CaCO<sub>3</sub> and silica show bimodal particle sizes distribution. Major peaks are positioned at 0.2 µm and 1.6 µm for the CaCO<sub>3</sub> and at 2 µm and 6 µm for silica. Talc has a uniform particle size distribution of 7 µm. Particle size distribution was measured with the Malvern Particle Sizer.

The paint coatings were spread onto polyester (PS) sheets by means of a spiral wound rod bar (8) graduated to provide a wet film thickness of the coating of approximated 100 µm. The films were allowed to cure under ambient air conditions for 15 days prior to testing to let any trapped solvent evaporate. A transparency sheet (Impega®, A4) constituted mainly of PS, with a film thickness of 104 µm was used as the control. PS sheets were used as support due to their flexibility, facilitating manipulation in the experiments.

### 2.2. Test microorganism

*Escherichia coli* (*E. coli*) ATCC 8739 was obtained from the American Type Culture Collection (Manassas, VA, USA). To check purity, a loop of culture was streaked onto nutrient agar (NA) (Oxoid

**Table 1**

Photocatalyst PVC values. Samples 1A–1G are comprised of a styrene acrylic emulsion, solvent, antifoaming, dispersant, water and TiO<sub>2</sub> photocatalyst at various loadings.

Samples tested	% PVC <sup>a</sup>	% total PVC <sup>b</sup>
	Photocatalyst (PC)	
1A	15	15
1B	30	30
1C	40	40
1D	50	50
1E	60	60
1F	70	70
1G	80	80

<sup>a</sup> PVC is calculated from the total sum by volume of all pigments and extenders divided by the total sum of volume of all pigments, extenders and polymer.

<sup>b</sup> The difference between 100% and the PVC being the acrylic polymer.

**Table 2**

Photocatalyst and extenders PVC values. Samples 2A–2L are comprised of a styrene acrylic emulsion, solvent, antifoaming, dispersant, water and various loadings of TiO<sub>2</sub> photocatalyst and extender (CaCO<sub>3</sub>, silica or talc).

Samples tested	% PVC <sup>a</sup>				% total PVC <sup>b</sup>
	Photocatalyst (PC)	Extenders			
		CaCO <sub>3</sub>	Silica	Talc	
2A	15	15	—	—	30
2B	15	—	15	—	30
2C	15	—	—	15	30
2D	30	30	—	—	60
2E	30	—	30	—	60
2F	30	—	—	30	60
2G	10	20	—	—	30
2H	10	30	—	—	40
2I	10	40	—	—	50
2J	10	50	—	—	60
2K	10	60	—	—	70
2L	10	70	—	—	80

<sup>a</sup> PVC is calculated from the total sum by volume of all pigments and extenders divided by the total sum of volume of all pigments, extenders and polymer.

<sup>b</sup> The difference between 100% and the PVC being the acrylic polymer.

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) at 37 °C. In order to prepare stock cultures for storage at –80 °C, equal amounts of culture and freezing mix [19] 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 sub culturing [20]. To resuscitate, the culture was defrosted at room temperature and a loop of liquid was inoculated into broth and incubated at 37 °C for 18 h. Stock plate cultures were stored at 4 °C for up to one month before discarding. In preparation for retention assays, *E. coli* was inoculated onto NA and incubated at 37 °C for 18 h.

### 2.3. Anti-bacterial experimental procedure

To determine the survival of bacteria on the test surfaces, samples were analysed using a method adapted from the Japanese Industrial Standard JIS Z 2801:2000 (E) [21].

*E. coli* was used as a model microorganism for all inactivation studies. *E. coli* cells were inoculated into 10 mL of NB and incubated aerobically for 18 h at 160 rpm in a shaker at 37 °C. Thirty µL aliquots were transferred to 100 mL of NB, and then cells were grown for 4 h under the same conditions. Bacterial cells were harvested by centrifugation at 3000 rpm (vortex Sigma 204, Sigma Laborcentrifugen, Germany) for 10 min. The bacterial pellet was re-suspended in 100 mL of sterilized quarter strength ringer solution (Oxoid). An optical density of 0.060 at 540 nm was obtained to achieve an initial concentration of bacteria of  $1.4 \pm 0.2 \times 10^7$  CFU mL<sup>-1</sup>. One hundred µL aliquots of the suspension were placed on the surface of both the test paint coating and the PS control film in a Petri dish (35 mm diameter × 10 mm height) with the lid on. The area of the films surface was 25 mm × 25 mm. To spread out the liquid, polyethylene film (PE) was gently pressed onto the drop. The individual suspensions were held in intimate contact with the film. The lid was placed on the dish, thirty round Petri dishes plus contents were placed per large square Petri dish (2450 mm × 2450 mm × 250 mm) which contained sterilized moistened tissues, to prevent drying during light exposure. The light absorbed by the Petri dish lids were measured with a Lambda 40 spectrophotometer (Perkin/Elmer, USA). Both Petri dish lids absorbed irradiation below 300 nm, which was outside the range of interest, thus would not interfere with the photocatalytic effect under investigation.

The efficiency of photocatalytic inactivation was examined at four irradiation times (0, 24, 48 and 96 h). Cells on the coatings and in any remaining suspension were collected from the small Petri dish by swabbing. Swab and films were transferred into 10 mL of neutralising solution [20 g L<sup>-1</sup> Soya Lethicin (Holland & Barret, USA) and 30 g L<sup>-1</sup> Tween 80 (Sigma–Aldrich, USA)] and vortex-mixed. This neutralising medium is considered suitable for protecting cells and maintains viability during the recovery of cells from a coated surface [22,23]. The viable population associated with these samples was enumerated by plated serials dilutions (to 10<sup>-4</sup>) onto nutrient tryptone soya agar (TSA) (Oxoid), and incubating at 37 °C for 24 h. At the end of the incubation period colonies on the agar plates were counted and the number of colony forming units (CFU) per mL was calculated. Counts were used from agar plates showing 15–300 colonies.

Two control experiments were carried out; one in the dark, and the second with light, but in the absence of paint (uncoated film). All test apparatus was sterilized using pressurize steam as it was important to maintain aseptic technique when manipulating the microorganisms to prevent cross contamination. Coatings were wiped with ethanol and air dried prior to experiments.

The bacterial inactivation (BI) was calculated using equation (1).

$$\%BI = \left( \frac{(N_{tc} - N_t)}{N_{tc}} \right) \times 100 \quad (1)$$

Where;  $N_{tc}$  is the number of CFUs after irradiation of the non-coated test piece (PS film) at time  $t$ ,

$N_t$  is the number of CFUs after irradiation of the coated paint containing photocatalyst at time  $t$ .

Three replicate samples were produced for each experiment, resulting in twelve paint films for each paint formulation. Every experiment was repeated three times under the identical conditions and the average concentration with the statistical deviation was used for the data analysis.

The statistical two way analysis of variance test was carried out using Microsoft Office Excel 2007, to determine the significance of two effects; the time of irradiation of the samples and the presence of photocatalyst in the samples. A  $P$  value of less than 0.05 was considered significant.

#### 2.4. Fluorescent irradiation

For the photocatalytic experiment, six fluorescent lamps (Silvania, Ontario, Canada) with an energy output of 8 W were fitted into an Illuminated Cooled Incubator (Gallenkamp, Loughborough, UK) with a control thermostat set at 20 °C (Fig. 1). Fluorescent lamps were turned on for 30 min prior to the experiment to stabilize the intensity of the light, and remained on until paint films were removed. Bacteria impregnated paint films were placed on platforms that were vertically adjustable to give a distance of 150 mm from the fluorescent lamps. Lamps placed on both sides of the cabinet ensured uniform light energy. A wavelength range of 300–700 nm was used to reproduce daylight. A USB4000 Miniature Fiber Optic Spectrometer (Dunedin, USA) was used to determine the UV/Vis power at the sample distance. Samples were exposed to a portion of UV light (290–400 nm) at 0.05–0.12 W m<sup>-2</sup> intensity, and visible light source (400–700 nm), with a range of intensity 2.70–3.99 W m<sup>-2</sup> for up to 96 h [19].

#### 2.5. Scanning electron microscopy

Samples of paint films prepared as described above were desiccated and fixed to stubs for gold sputter coating using a Polaron E5100 (Milton Keynes, UK) SEM sputter coater at

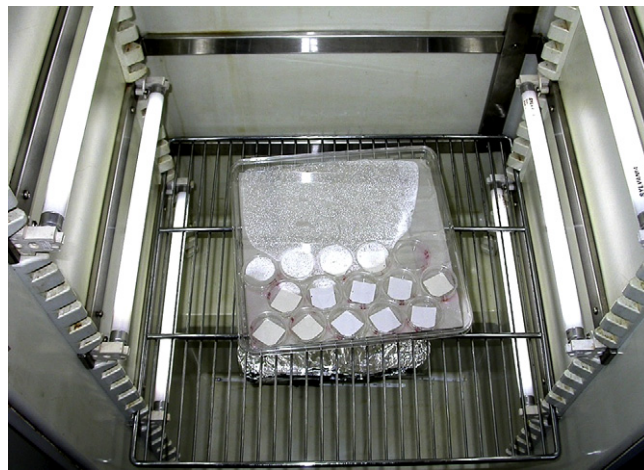


Fig. 1. Paint films exposed to the illumination system. On the upper shelf, paint samples were placed in an uncovered square Petri dish under illumination. On the lower shelf, a square Petri dish covered in aluminium foil contains paint samples with light excluded.

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 in order to obtain the excitation of all the elements.

### 3. Results and discussion

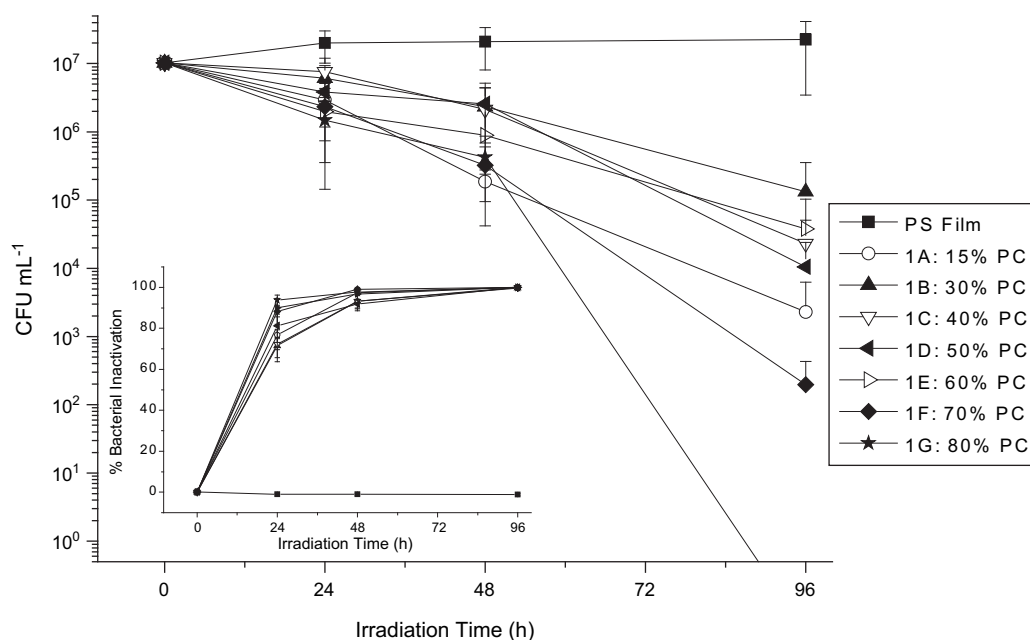
Three acrylic formulations were tested to evaluate the effect of extender on the activity of the paint formulations. Essentially PC105 mixed into the paints displayed its ability to inactivate bacteria under fluorescent light irradiation. During parallel control studies performed under identical conditions in the dark, viable cell counts were almost identical to initial conditions (data not shown), indicating that the photocatalytic paint has no bactericidal activity in the absence of light.

#### 3.1. Effect of photocatalyst concentration on anti-bacterial activity

The effect of photocatalyst concentration on anti-bacterial activity was evaluated in the first set of paints (Table 1) under fluorescent light (Fig. 2). Samples containing photocatalyst showed a statistically significant reduction when compared to the control ( $P < 0.05$ ). Total inactivation of *E. coli* (initial concentration 10<sup>7</sup> CFU mL<sup>-1</sup>) occurred after 96 h when 80% TiO<sub>2</sub> (total PVC) was used. A cell concentration of 10<sup>3</sup>–10<sup>6</sup> CFU mL<sup>-1</sup> remained after the same irradiation time period on surfaces with lower TiO<sub>2</sub> concentrations.

In general, a higher initial photocatalyst concentration resulted in a more efficient antimicrobial effect. This may be due to an increase of photocatalyst present on the surface of the film resulting in an increased in nanoparticles-cell contact. However, the sequence of inactivation did not necessarily increase linearly with TiO<sub>2</sub> concentration; this was because although the catalytic TiO<sub>2</sub> nanoparticles were uniformly dispersed in the solubilised paint suspension, the concentration of photocatalyst on the top layer of the surface could be complicated by a number of factors (such as titania being shielded beneath other components or being piled upon itself) when the paint was prepared in films.

From the bacterial survival results, calculated using equation (1) (Inset in Fig. 2), an effective anti-bacterial activity was observed for all PC concentrations. Even paints with a lower percentage of photocatalyst were found capable of decomposing bacteria under



**Fig. 2.** Photocatalytic inactivation of *E. coli* on paint films containing a styrene acrylic copolymer base and TiO<sub>2</sub> photocatalyst at different concentrations (15–80% of the total PVC) when exposed to light from fluorescent lamps for up to 96 h. Inset: Percentage of bacterial inactivation of *E. coli* cells as a function of illumination time. When irradiated, the paints reduced the viability of *E. coli*. ( $n = 3$ , bar = standard error).

irradiation exposure. There is also a competitive effect between surface particle concentration and depth of penetration by light activation. Thus, at lower TiO<sub>2</sub> particle concentration the light is able to penetrate deeper into the paint film where further reactions may occur giving rise to greater activation, i.e., more release of active radicals and singlet oxygen (<sup>1</sup>O<sub>2</sub>). The photocatalytic effect of the titania in paint films will be due to photogenerated <sup>1</sup>O<sub>2</sub> producing hydroperoxide radicals (HO<sub>2</sub><sup>•</sup>) via a reaction with moisture [16]. Chemical steps of the photocatalytic process produce reactive species (•OH, O<sub>2</sub><sup>•-</sup>, HO<sub>2</sub><sup>•</sup>) which are implicated in the killing of *E. coli* [24,25]. No decrease in the number of CFUs was observed in irradiated control samples uncoated film (i.e. in the absence of TiO<sub>2</sub>).

The efficiency of the photocatalyst TiO<sub>2</sub> is important as the amount used in paint products affects their final price. Further, the application of the paint can be limited since the requirement of a high photocatalyst concentration can influence the durability and overall quality of the paint. For these reason, TiO<sub>2</sub> particles with photocatalytic activity should not be present in higher amounts than 20% tPVC, preferably 1–15%. If the TiO<sub>2</sub> content is greater than 30% of the total PVC, the paint will exhibit an increased amount of chalking and degradation of the organic binder thus resulting in a problem with long-term durability [12,26,27]. Visual observations during sampling at each photocatalyst concentration indicated that at higher concentrations more cracking and flaking was observed on the surface, reducing the quality of the product. This reduction of quality was due to mechanical properties, as it was observed before the irradiation experiment.

### 3.2. Effect of extenders and photocatalyst concentration on anti-bacterial activity

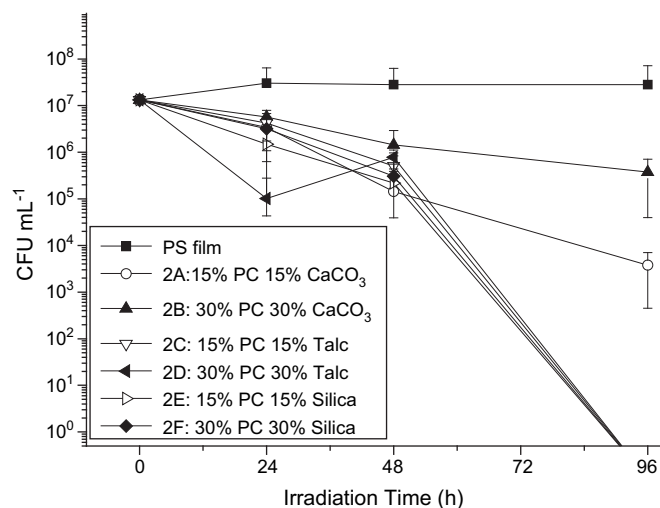
The effect of paints with photocatalyst and extenders (Table 2) on *E. coli* viability under fluorescent light irradiation (Fig. 3) was studied. Complete inactivation was observed after 96 h when silica and talc were used as extenders.

The effectiveness of bacterial killing was lower when CaCO<sub>3</sub> was used as the extender; a 4 log CFU mL<sup>-1</sup> reduction was achieved when the paint contained 15% of CaCO<sub>3</sub>, whereas at 30% CaCO<sub>3</sub>

a 2 log CFU mL<sup>-1</sup> reduction was observed. Although such decrease was still significantly different from the control ( $P < 0.05$ ), the use of a higher CaCO<sub>3</sub> concentration was less effective at achieving kill of bacteria.

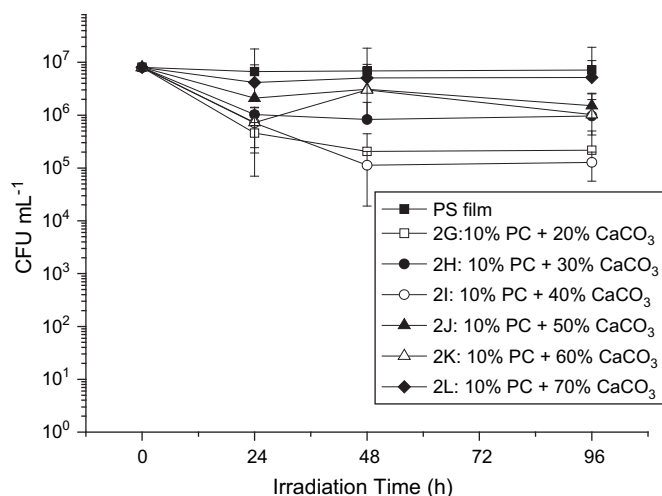
### 3.3. Effect of calcium carbonate concentration on anti-bacterial activity

The addition of calcium carbonate (CaCO<sub>3</sub>) as an extender and its influence on the photocatalytic activity of 10% of TiO<sub>2</sub> was evaluated by increasing the percentage of CaCO<sub>3</sub> added to the paint (Fig. 4). The paint which contained lower CaCO<sub>3</sub> concentrations (up to 40% of the total PVC) demonstrated a significant decrease in *E. coli* numbers after 96 h (2 log), whereas there was no significant differences in anti-bacterial activity of paints with concentrations



**Fig. 3.** Photocatalytic inactivation of *E. coli* on paint films containing an extender (CaCO<sub>3</sub>, talc or silica), a styrene acrylic copolymer base and TiO<sub>2</sub> photocatalyst when exposed to fluorescent lamps for up to 96 h. ( $n = 3$ , bar = standard error).





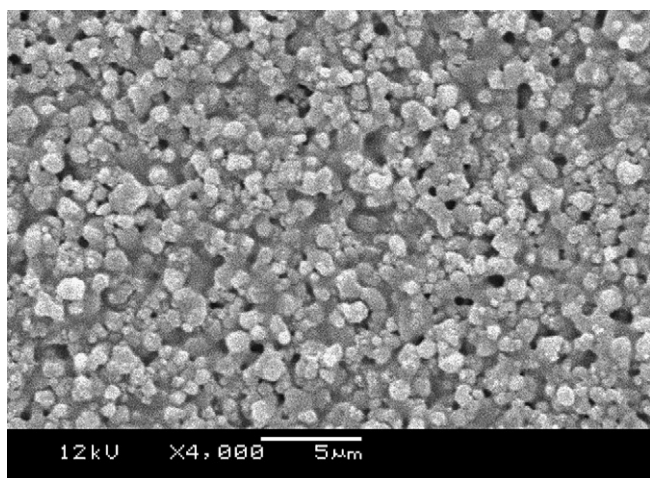
**Fig. 4.** Photocatalytic inactivation of *E. coli* on paint films containing a styrene acrylic copolymer base, 10% of  $\text{TiO}_2$  photocatalyst and  $\text{CaCO}_3$  as an extender at different concentrations between 20% and 70% of the total PVC in samples when exposed to fluorescent lamps for up to 96 h. ( $n = 3$ , bar = standard error).

higher than 40%  $\text{CaCO}_3$  when compared with the control ( $P > 0.05$ ), where  $< 1$  log decrease was achieved. This again indicates that the use of more  $\text{CaCO}_3$  resulted in lower bacterial inactivation. If the concentration of  $\text{CaCO}_3$  is higher than 40%, this could, in effect, ‘swamp’ the  $\text{TiO}_2$  nanoparticles, reducing contact between the microorganism and the photocatalyst. Therefore, when a minimum amount of UV energy is used for activation of the  $\text{TiO}_2$ , as in this experiment, an increase in the extender concentration in the paints could lead to a decreased microbial inactivation.

### 3.4. Morphology of paint films

SEM images of the paint samples were examined in order to assess the state of dispersion of the nanoparticles within the acrylic polymer matrix. Analysis of the photocatalyst accessibility by microscopy is important since the photocatalyst oxidation mechanism is explained in terms of surface-orientated adsorption of substrates and hydroxyl radicals, hence the inactivation of bacteria is dependent on the surface – cell contact interaction.

A representative image of the paint containing  $\text{TiO}_2$  at 40% of the total PVC is shown in Fig. 5.  $\text{TiO}_2$  particles (bright white particles)

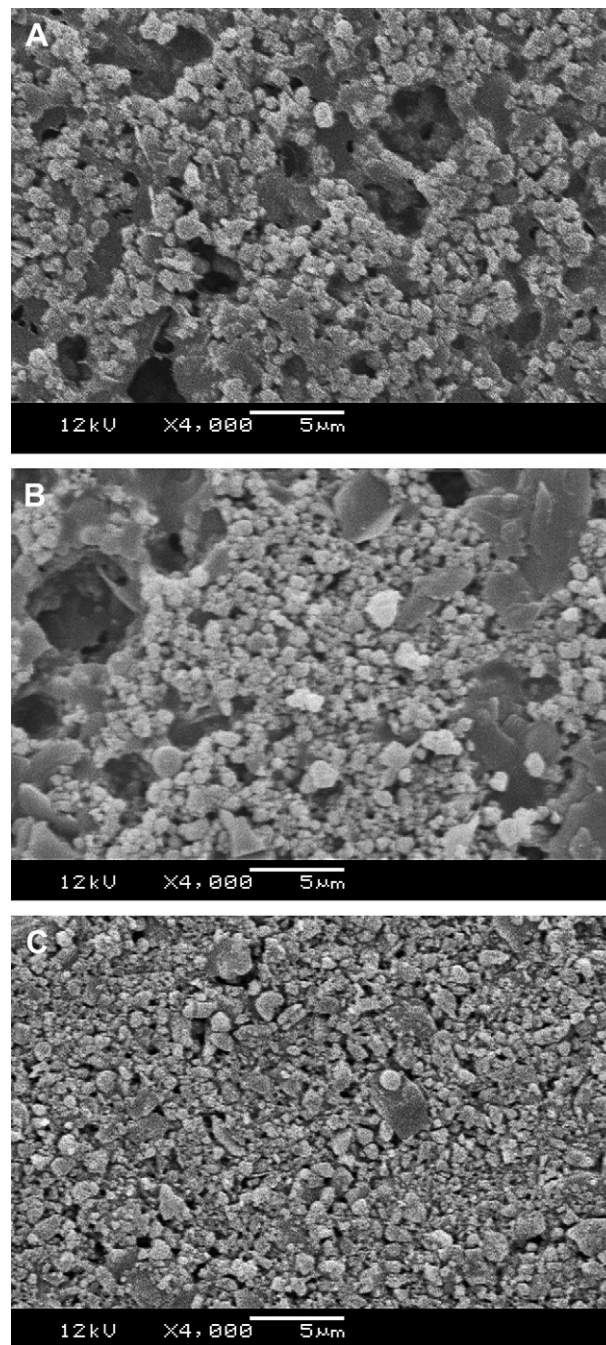


**Fig. 5.** SEM image of paint at 40% photocatalyst from the total PVC (The bright spots are the nanoparticles).

appear homogeneously dispersed all over the surface embedded in the organic binder of the paint (grey shadows in between the particles). In this paint sample bacteria cells would have direct contact with the photocatalyst leading to complete bacterial inactivation.

SEM images of paints containing photocatalysts and extenders are shown in Fig. 6. In these cases, the photocatalyst is more widely dispersed, often obscured by additional components as the formulation of the paint becomes more complex.

Fig. 6A and B (silica and talc as extenders, respectively) show aggregates of the photocatalyst surrounded by a porous structure. This might be due to the different sizes of the (darker) extender and (pale)  $\text{TiO}_2$  nanoparticles.  $\text{TiO}_2$  particles remain on the surface



**Fig. 6.** SEM images of paints with extenders coatings at A. 30% PC and 30% Silica, B. 30% PC and 30% Talc, C. 10% PC and 50%  $\text{CaCO}_3$  from the total PVC.

above the talc and silica forming pores of sizes larger than 3  $\mu\text{m}$ . Consequently, the photocatalyst activation can occur without difficulty, encouraging the interaction with microorganisms either on the surface or inside the pores.

On the other hand, in Fig. 6C  $\text{TiO}_2$  nanoparticles are very difficult to differentiate from the similarly sized  $\text{CaCO}_3$ , although the shape of the  $\text{CaCO}_3$  particles is less regular. Since both particles have similar size and weight, the photocatalyst appeared to be completely mixed with the  $\text{CaCO}_3$ . Thus the  $\text{TiO}_2$  would be present in, on and beneath the surface, and therefore there would be a reduced fraction of photocatalyst available to the microorganism.

The strong oxidation power of photogenerated holes on the catalyst surface enables it to react with physisorbed water at the catalyst/water interface [28]. This reaction generates the highly reactive hydroxyl radicals which are the main species responsible for the inactivation of bacteria. Water present in the medium may play a role of mediator in the process, by transporting the hydroxyl radicals. Gumy et al. [29] estimated the diffusion length of OH radicals in a suspension of  $\text{TiO}_2$  and *E. coli*. However, other authors suggest that the reactions responsible for bacterial inactivation occur mainly at the surface of the photocatalyst via photogenerated holes rather than by free diffusion of radicals into the homogeneous phase [30]. Thus it is not clear in the literature whether sufficient OH radicals are transported through the aqueous interphase from  $\text{TiO}_2$  to bacteria to enable inactivation. Additionally, the potential effect of the water interphase as carrier of OH radicals could be assumed to be similar in all our samples, thus this effect would not be significant in this instance.

Results previously obtained in the antimicrobial work showed complete inactivation of bacteria for paint containing photocatalyst, and paints containing photocatalyst and extenders silica and talc. In contrast, when the extender added to the paint was  $\text{CaCO}_3$  the anti-bacterial effect was reduced. SEM images validate this result since the more nanoparticles present on the top of the surface, the easier is the contact between nanoparticles and bacteria.

Experiments previously undertaken in our laboratory have shown that not only were atmospheric pollutant gases such as nitrogen oxides ( $\text{NO}$ ,  $\text{NO}_2$ ) reduced with increasing titania doping but also with increasing levels of  $\text{CaCO}_3$  in polysiloxane photocatalytic paint. This was due to an increase in the porosity of the paint with increased concentrations of calcium carbonate and titania, so that the access of moisture and oxygen to the paint were enhanced [12,17]. On the contrary, in this paper we describe a reduction in the photocatalytic activity against bacteria when adding increasing concentrations of  $\text{CaCO}_3$ . Scanning electron micrographs of the paint films revealed pores of approximately 0.5  $\mu\text{m}$  (Fig. 6C), which are considerably smaller than typical *E. coli* cells (approximately 1.5  $\mu\text{m}$  length  $\times$  0.9  $\mu\text{m}$  diameter). Thus, pores created by the combination of  $\text{CaCO}_3$  and  $\text{TiO}_2$  in the paint could reduce contact between the *E. coli* and the  $\text{TiO}_2$  and as a result reduce bacterial inactivation. However, the larger pores created by extenders silica and talc facilitate the access of bacteria to the pores where bacterial inactivation can also occur.

To the best of our knowledge this is the first published study to demonstrate how the components added to a paint formulation affect photocatalytic activity and bactericidal activity. Most of the work carried out using anti-bacterial paints is described in patent form, thus there is a lack of comprehensive data to enable comparison. Our results serve as a basis for future investigations.

#### 4. Conclusions

This study was aimed at understanding the influence of photocatalyst paint formulations on anti-bacterial ability when activated under fluorescent light. Results show that the concentration

of photocatalyst and the extenders present in the paint affected anti-bacterial activity, with higher activity with a simple paint formulation containing only photocatalyst and polymer. The use of 15% photocatalyst concentration in paints was sufficient to induce bacterial inactivation with the exception of paints containing  $\text{CaCO}_3$ , where a significant blocking of the photocatalyst effect was observed. SEM images showed the photocatalyst being embedded within  $\text{CaCO}_3$  leading to limited access to light and thus reduced bacterial inactivation. In contrast, the particle size of extenders silica and talc is larger. In this case, the photocatalyst lies on the surface of the film surrounded by pores ( $>3 \mu\text{m}$ ) created by these extenders. Consequently, photocatalyst activation occurred without difficulty, as the microorganisms can interact with the photocatalyst either on the surface or inside the pores.

Therefore, it is of prime importance to ensure contact efficiency between the photocatalyst and the target which is to be oxidized and decomposed. An improvement in the paint formulation design for intended application seems to be a desirable direction for further investigations.

#### Acknowledgements

The authors are grateful to Millennium Inorganic Chemicals (MIC) for supplying the paint samples used in this programme of work and also for partial funding support in this project, with special thanks to John Stratton and Claire Hill.

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