**Variables to be considered when assessing the photocatalytic destruction of
bacterial pathogens**

T.P. Tim Cushnie ^a

Peter K.J. Robertson ^a

Simon Officer ^{a,b}

Pat M. Pollard ^a

Cathy McCullagh ^a

Jeanette M.C. Robertson ^{b,*}

^a Centre for Research in Energy and the Environment (CRE+E), The Robert Gordon University, Schoolhill, Aberdeen. United Kingdom. AB10 1FR.

^b School of Pharmacy and Life Sciences, The Robert Gordon University, St. Andrew Street, Aberdeen. United Kingdom. AB25 1HG.

* Corresponding author

E-mail j.robertson@rgu.ac.uk

Telephone +441224 262800

Fax +441224 262828

Abstract

The current study sought to assess the importance of three common variables on the outcome of TiO₂ photocatalysis experiments with bacteria. Factors considered were (a) ability of test species to withstand osmotic pressure, (b) incubation period of agar plates used for colony counts following photocatalysis and (c) chemical nature of suspension medium used for bacteria and TiO₂. *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* ser. Typhimurium and *Pseudomonas aeruginosa* were found to vary greatly in their ability to withstand osmotic pressure, raising the possibility that osmotic lysis may be contributing to loss of viability in some photocatalytic disinfection studies. Agar plate incubation time was also found to influence results, as bacteria treated with UV light only grew more slowly than those treated with a combination of UV and TiO₂. The chemical nature of the suspension medium used was found to have a particularly pronounced effect upon results. Greatest antibacterial activity was detected when aqueous sodium chloride solution was utilised, with $\sim 1 \times 10^6$ CFU mL⁻¹ *S. aureus* being completely killed after 60 min. Moderate activity was observed when distilled water was employed with bacteria being killed after 2 h and 30 min, and no antibacterial activity at all was detected when aqueous tryptone solution was used. Interestingly, the antibacterial activity of UV light on its own appeared to be very much reduced in experiments where aqueous sodium chloride was employed instead of distilled water.

Keywords: Titanium dioxide; antibacterial; incubation time; suspension medium; bacterial species; osmolarity

1. Introduction

Microorganisms are responsible for millions of deaths and many millions of cases of disease and disability each year (WHO, 2002). The ability to control or destroy microorganisms is therefore of enormous importance to many organisations and industries e.g. healthcare, food and drink, water treatment and military. This challenge is compounded by the ever-increasing problem of antibiotic resistant hospital (Gould, 2006) and community acquired infections (Moellering, 2006), the development of resistance to common disinfectants (Ben Saida et al., 2008), health concerns regarding the use of existing disinfectants (Gopal et al., 2007) and the threat of biological terrorism (Wallin et al., 2007; Moran et al., 2008). Hence, alternative methods of controlling the spread of and/or eradicating microorganisms are urgently being sought.

Since it was first reported over 20 years ago (Matsunaga et al., 1985), the photocatalytic destruction of microorganisms has been a subject of much research interest (McCullagh et al., 2007). There are many potential applications for this antimicrobial activity including the disinfection of drinking water supplies (Paleologou et al., 2007), air (Guillard et al., 2008) and surfaces in healthcare and industrial environments (Evans and Sheel, 2007). Unfortunately, the antibacterial activity of TiO₂ photocatalysis has been investigated under a wide range of experimental conditions which makes it difficult for results from different studies to be properly compared. Variables already found to affect experimental outcome include photocatalyst concentration, UV light intensity, microbial starting concentration, temperature, pH, aeration (McCullagh et al., 2007), organism type (Kühn et al., 2003), the presence of inorganic ions and dihydroxybenzenes (Rincón

and Pulgarin, 2004), size and structure of photocatalyst particle, stirring (Verran et al., 2007) and photocatalytic reactor geometry (Josset et al., 2007).

The aim of the present study was to identify other common, but previously untested, variables likely to affect results from photocatalytic disinfection experiments. The first variable to be assessed was the ability of test bacteria to withstand osmotic pressure. Currently, a range of different species e.g. *Staphylococcus aureus* (Kühn et al., 2003; Vohra et al., 2006), *Escherichia coli* (Ibáñez et al., 2003; Evans and Sheel, 2007; Nakamura et al., 2007), *Salmonella* ser. Typhimurium (Ibáñez et al., 2003) and *Pseudomonas aeruginosa* (Ibáñez et al., 2003; Kühn et al., 2003) are being used in photocatalysis studies, often in liquids that are hypotonic e.g. water (Ibáñez et al., 2003; Vohra et al., 2006; Evans and Sheel, 2007). The second variable to be tested was the length of time samples are incubated on agar for before colony counts are performed. Typically in photocatalytic disinfection studies, agar plates are incubated for 24 h before viable counts are determined (Ibáñez et al., 2003; Kim et al., 2003; Fu et al., 2005; Sichel et al., 2007; Hu et al., 2007), but incubation times as short as 16 h have been reported (Kühn et al., 2003). The third and final variable to be tested was the chemical nature of the suspension medium used for photocatalytic disinfection experiments. Currently, there is quite a broad range of media being used and recommended. These include distilled (Sichel et al., 2007) and deionised water (Huang et al., 2000; Verran et al., 2007), saline based media such as aqueous 0.9% (w/v) sodium chloride solution (Kühn et al., 2003; Hu et al., 2007) and 1/4 strength Ringers solution (Dunlop et al., 2002), and peptide rich media such as Luria-Bertani broth (Wong et al., 2006), 1/500 nutrient broth (Sunada et al., 2003), aqueous tryptone solution (Rincón and Pulgarin, 2003) and aqueous tryptone solution with sodium chloride (BSI, 1997).

2. Materials and methods

2.1 Chemicals

Tryptone T, nutrient broth and nutrient agar were purchased from Oxoid Ltd. (Basingstoke, UK.). Titanium dioxide (P25) was from Degussa-Hüls AG (Frankfurt, Germany) and sodium chloride (general purpose grade) was from Fisher Scientific UK Ltd. (Loughborough, UK.).

2.2 Bacteria and culture conditions

Staphylococcus aureus NCTC 6571, *S. Typhimurium* NCTC 74 and *Ps. aeruginosa* NCTC 10662 were purchased from the National Collection of Type Cultures (London, UK.) and *E. coli* ATCC 10536 was purchased from the National Collection of Industrial Marine and Food Bacteria (Aberdeen, UK.). All bacterial cultures were sub-cultured and maintained on nutrient agar. To prepare bacterial cultures for experiments, three to five well isolated colonies of the same morphological type were removed from these plates (NCCLS, 2000), inoculated into 100 mL nutrient broth and incubated at 37 °C in an orbital incubator set at 100 rpm (IOX400.XX2.C; Sanyo Gallenkamp PLC, Loughborough, UK.). After 18 h incubation, the cells were harvested by centrifugation (MSE Centaur 1; Fisons, Loughborough, UK.) at 4000 rpm for 10 min, then (unless otherwise stated) washed and re-suspended in sterile distilled water.

2.3 Assessment of the viability of different bacterial species in distilled water

Three sterile 150 mL glass (Boro 3.3) beakers (Fisherbrand) containing 99 mL sterile distilled water (pH 7.0 and 21±1°C) were inoculated with 1 mL of ~1 x 10⁸ CFU mL⁻¹ washed bacteria and magnetically stirred at room temperature. Samples

were taken after 0, 1, 2, 3 and 4 h. Serial dilutions were performed in sterile distilled water and ten 20 μL drops of each dilution (including neat sample) were pipetted onto nutrient agar plates (resulting in a minimum detection limit of $\sim 5 \text{ CFU mL}^{-1}$). After 24 h incubation at 37 °C, colony counts were performed and viable counts were determined. Three populations of the same bacterial species were tested per experiment to facilitate the detection of any intra-experimental variation and each experiment was repeated to verify the reproducibility of results. Studies were performed with *S. aureus* NCTC 6571, *E. coli* ATCC 10536, *S. Typhimurium* NCTC 74 and *Ps. aeruginosa* NCTC 10662. Bacterial species which showed poor viability in distilled water were retested in iso-osmotic 0.9% (w/v) sodium chloride solution.

2.4 Assessment of the effect of agar plate incubation time on results from photocatalysis time-kill studies

Photocatalysis experiments were performed in sterile 150 mL glass beakers containing 99 mL of sterile distilled water with or without 0.1 g of TiO_2 and 1 mL of $\sim 1 \times 10^8 \text{ CFU mL}^{-1}$ *S. aureus* NCTC 6571. UV-illuminated beakers were positioned at a distance of 10 cm from a 6 x 8 W UV-A lamp (spectral output 311-415 nm peaking at 368 nm; Philips TL 8W/08 F8 T5/BLB). The photonic output of the lamp was determined to be $6.8 \times 10^{-5} \text{ Einsteins s}^{-1}$ using ferrioxalated actinometry. Reaction mixtures were magnetically stirred throughout the experimental period. Samples were taken after 0, 0.5, 1.0, 1.5, 2.0 and 2.5 h and processed as described previously. Colony counts were performed after 17, 24 and 40 h incubation at 37 °C, and viable counts were determined. Statistical differences between colony counts from agar plates incubated for 17, 24 and 40 h were assessed by one way analysis of

variance (ANOVA) using the minitab statistical package (Minitab, State College, PA, USA). This work was repeated to verify the reproducibility of results.

2.5 Assessment of the effects of different suspension media on results from photocatalysis time-kill studies

This was performed as described in section 2.4 with the exception that the *S. aureus* cells were washed in aqueous 0.9% (w/v) sodium chloride solution, and experiments were performed in aqueous 0.9% (w/v) sodium chloride (pH 7.0 and $21\pm1^\circ\text{C}$) or aqueous 0.1% (w/v) tryptone and 0.85% (w/v) sodium chloride solution (pH 7.0 and $21\pm1^\circ\text{C}$). As was the case in the previous two sections, the minimum detectable number of bacteria in these experiments was $\sim 5 \text{ CFU mL}^{-1}$ [a reflection of the fact that 200 μL of neat sample (10 x 20 μL drops) was plated out from each reaction vessel].

3. Results and discussion

3.1 Viability of different bacterial species in distilled water

From Fig. 1a, it can be seen that some bacterial species are better adapted to survival in water than others. The Gram positive bacterium *S. aureus* consistently maintained excellent viability in this hypotonic environment for the duration of these experiments, while the viability of Gram negative bacteria *E. coli* and *S. Typhimurium* was quite erratic (with standard deviation exceeding mean viable count on several occasions). These results are likely to be attributable to the fact that the Gram positive cell wall is thicker (30 to 100 nm) than the Gram negative cell wall (20 to 30 nm) (Singleton, 1999). In hypotonic liquids, water enters bacterial cells by

osmosis and if the cell wall of the bacterium is not sufficiently strong, lysis occurs and viability is lost. This explanation is supported by the fact that both of these Gram negative species consistently maintained excellent viability in iso-osmotic 0.9% (w/v) sodium chloride solution (Fig. 1b). In the case of *Ps. aeruginosa*, this Gram negative bacterium was found to be capable of maintaining viability in distilled water (Fig. 1a). However, the cell wall of *Ps. aeruginosa* has very low permeability (Lambert, 2002), and this may allow it to survive in hypotonic environments more readily than other bacteria in its class. These findings show that distilled water should be avoided as a suspension medium for Gram negative bacteria such as *E. coli* or *S. Typhimurium*, in photocatalytic disinfection experiments.

3.2 The effect of agar plate incubation time on results from photocatalysis time-kill studies

Results presented in Fig. 2 indicate that cells of *S. aureus* illuminated with UV light for 2 h in the absence of TiO₂ generate colonies more slowly than those that are treated with TiO₂ only or those that are illuminated with UV for the same period of time in the presence of 1 g L⁻¹ TiO₂. When colony counts were performed after 17 h incubation at 37 °C, there was no significant difference ($p = 0.190$) between the number of bacteria surviving in the UV and TiO₂ treatment group and the UV only treatment group, suggesting that both of these methods of disinfection were equally effective. However, when colony counts were performed after 24 and 40 h incubation at 37 °C, the difference in bacterial numbers between the two groups was highly significant ($p = 0.000$ and $p = 0.000$ respectively) with greater bacterial destruction occurring in the UV and TiO₂ treatment group. The presence of slow growing bacteria in the UV only treatment group correlates well with results from previously

reported work (Robertson et al., 2005) and supports the suggestion that the antibacterial mechanism of action of UV (photolysis) differs from that of UV and TiO₂ (photocatalysis). With regard to the cause of the growth delay, this has been attributed to UV-induced membrane damage, photo-damaged tRNA and elicitation of the stringent response (Pizarro, 1995), which is involved in guanosine 5'-diphosphate 3'-diphosphate synthesis and inhibits stable RNA synthesis (Kramer et al., 1988).

This study is the first to report the importance of having an adequate period of agar plate incubation prior to performing colony counts. In previous investigations of photocatalytic disinfection, colony counts were typically performed after 24 h incubation but our results show that even this is too early for UV only treated bacteria and that counting colonies of surviving bacteria, following UV treatment, at this time results in the production of erroneous results. This finding is particularly significant considering that UV photolysis is commonly employed as a method of water disinfection and shows that great care needs to be taken when reporting the effectiveness of this technique. There was no difference in colony counts at any of the three time points in the dark control experiment (TiO₂ only).

3.3 The effects of different suspension media on results from photocatalysis time-kill studies

When bacteria were suspended in aqueous 0.9% (w/v) sodium chloride solution instead of distilled water, a significant increase in antibacterial activity was observed (Figs. 3a and 3b). Complete destruction of $\sim 1 \times 10^6$ CFU mL⁻¹ *S. aureus* took place in just 60 min as opposed to 2 h and 30 min when using distilled water. There are a number of hypotheses which may explain this effect. It is possible that the sodium chloride is increasing adsorption of bacteria onto the photocatalyst, an effect which

has been positively correlated to bactericidal activity (Gogniat et al., 2006).

Alternatively, it may be that Cl^- ions are altering the permeability of bacterial cells, or that the photocatalyst is oxidising the Cl^- ions and generating hypochlorite which has disinfectant properties (Singleton, 1999). The failure of the UV-illuminated TiO_2 to cause any decrease in viable count between 1 h 30 min and 2 h in Fig. 3a was slightly unexpected but this may simply be due to 'persistence', an acknowledged phenomenon in time-kill studies with bacteria (Lorian, 1996). Importantly, findings from these experiments highlight how the chemical nature of the bacterial suspension medium can significantly influence the results of photocatalytic disinfection experiments, and may go some way to explaining the differences in disinfection rates reported in the literature.

An interesting observation made during this study was that the antibacterial activity of UV light alone actually decreased when bacteria were suspended in 0.9% (w/v) sodium chloride instead of distilled water (Figs. 3a and 3b). This finding is in sharp contrast to the results obtained with UV illuminated TiO_2 , and lends additional support to the suggestion that the antibacterial mechanism of action of UV differs from that of UV illuminated TiO_2 . A likely explanation for this result is that bacterial cells with UV-damaged cytoplasmic membranes are capable of surviving in iso-osmotic environments such as 0.9% (w/v) sodium chloride but that they swell and burst in hypotonic environments such as distilled water. Caution should therefore be exercised when comparing data from studies employing 0.9% (w/v) sodium chloride as a suspension medium and those using distilled water, as the former is likely to result in a more exaggerated difference between the observed antibacterial activity of the photocatalysis and photolysis reactions.

When bacteria were suspended in aqueous 0.1% (w/v) tryptone and 0.85% (w/v) sodium chloride solution instead of distilled water for disinfection experiments, no antibacterial activity was detected in any treatment group (Fig. 3c). These results indicate that peptides are capable of inhibiting the antibacterial activity of UV-illuminated TiO₂. This is likely to be at least partially attributable to their absorption of UV light. Tryptone was found to absorb light in the range 308 to 453 nm (peaking at 334 nm) when examined by scanning spectroscopy (Biomate 5; Thermo Spectronic, Cambridge, UK.). Another factor likely to be contributing to the loss of antibacterial activity is competition between peptides and bacteria for •OH radicals (Rincón and Pulgarin, 2004). These findings show that peptide rich suspension media such as tryptone solution and Luria-Bertani broth are not suitable media for performing photocatalytic disinfection experiments in and that data from studies employing this type of medium require careful interpretation.

4. Conclusions

Previously unexplored experimental parameters affecting the outcome of photocatalytic disinfection experiments have been assessed. It was established that different bacterial species commonly used in photocatalysis studies vary greatly in their ability to withstand osmotic pressure and that Gram negative bacteria such as *E. coli* and *S. Typhimurium* should not be tested in distilled water. In addition, the length of the incubation period for agar plates on which colony counts are performed was found to have a significant effect on experimental outcome. Bacteria treated with UV only grew more slowly than those treated with TiO₂ and UV, often taking in excess of 24 h to produce visible colonies. The chemical nature of the suspension

medium in which bacteria are suspended for photocatalytic disinfection experiments was found to have a profound effect upon the kill times obtained. In the current study, greatest antibacterial activity was observed when aqueous sodium chloride solution was used as the suspension medium. Moderate antibacterial activity was detected when distilled water was used and no antibacterial activity was detected when aqueous tryptone solution was used. Furthermore, the use of sodium chloride as a bacterial suspension medium affected the ability of UV light alone to destroy bacteria. These findings emphasise the complex nature of photocatalytic studies using microorganisms as opposed to chemical pollutants, and add to the plethora of variables which are already known to affect experimental outcome (Kühn et al., 2003; Rincón and Pulgarin, 2004; McCullagh et al., 2007; Verran et al., 2007; Josset et al., 2007). Our findings reinforce the need for rigorous testing and careful interpretation of results reported in the literature.

Acknowledgements

This project was supported by funding from the Scottish Executive under the Proof of Concept Programme. The authors are grateful to Dr. Andrew Lamb and Miss Vivienne Hamilton for useful advice and discussions.

References

- Ben Saida, N., Marzouk, M., Ferjeni, A., Boukadida, J., 2008. A three-year surveillance of nosocomial infections by methicillin-resistant *Staphylococcus haemolyticus* in newborns reveals the disinfectant as a possible reservoir. Pathol. Biol. doi:10.1016/j.patbio.2008.02.019
- BSI, 1997. BS EN 1040:1997 Chemical Disinfectants and Antiseptics - Basic Bactericidal Activity - Test Method and Requirements (Phase 1). British Standards Institute, London.
- Dunlop, P.S.M., Byrne, J.A., Manga, N., Eggins, B.R., 2002. The photocatalytic removal of bacterial pollutants from drinking water. J. Photoch. Photobio. A 148, 355-363.
- Evans, P., Sheel, D.W., 2007. Photoactive and antibacterial TiO₂ thin films on stainless steel. Surf. Coat. Tech. 201, 9319-9324.
- Fu, G., Vary, P.S., Lin, C.T., 2005. Anatase TiO₂ nanocomposites for antimicrobial coatings. J. Phys. Chem. B 109, 8889-8898.
- Gogniat, G., Thyssen, M., Denis, M., Pulgarin, C., Dukan, S., 2006. The bactericidal effect of TiO₂ photocatalysis involves adsorption onto catalyst and the loss of membrane integrity. FEMS Microbiol. Lett. 258, 18-24.
- Gopal, K., Tripathy, S.S., Bersillon, J.L., Dubey, S.P., 2007. Chlorination byproducts, their toxicodynamics and removal from drinking water. J. Hazard. Mater. 140, 1-6.
- Gould, I.M., 2006. Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and its control. Int. J. Antimicrob. Ag. 28, 379-384.

- Guillard, C., Bui, T.-H., Felix, C., Moules, V., Lina, B., Lejeune, P., 2008. Microbiological disinfection of water and air by photocatalysis. *C. R. Chim.* 11, 107-113.
- Hu, C., Guo, J., Qu, J., Hu, X., 2007. Efficient destruction of bacteria with Ti(IV) and antibacterial ions in co-substituted hydroxyapatite films. *Appl. Catal. B-Environ.* 73, 345-353.
- Huang, Z., Maness, P.-C., Blake, D.M., Wolfrum, E.J., Smolinski, S.L., Jacoby, W. A., 2000. Bactericidal mode of titanium dioxide photocatalysis. *J. Photoch. Photobio. A* 130, 163-170.
- Ibáñez, J.A., Litter, M.I., Pizarro, R.A., 2003. Photocatalytic bactericidal effect of TiO₂ on *Enterobacter cloacae*: Comparative study with other Gram (-) bacteria. *J. Photoch. Photobio. A* 157, 81-85.
- Josset, S., Taranto, J., Keller, N., Keller, V., Lett, M.-C., Ledoux, M.J., Bonnet, V., Rougeau, S., 2007. UV-A photocatalytic treatment of high flow rate air contaminated with *Legionella pneumophila*. *Catal. Today* 129, 215-222.
- Kim, B., Kim, D., Cho, D., Cho, S., 2003. Bactericidal effect of TiO₂ photocatalyst on selected food-borne pathogenic bacteria. *Chemosphere* 52, 277-281.
- Kramer, G.F., Baker, J.C., Ames, B.N., 1988. Near-UV stress in *Salmonella typhimurium*: 4-thiouridine in tRNA, ppGpp, and ApppGpp as components of an adaptive response. *J. Bacteriol.* 170, 2344-2351.
- Kühn, K.P., Chaberny, I.F., Massholder, K., Stickler, M., Benz, V.W., Sonntag, H.-G., Erdinger, L., 2003. Disinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light. *Chemosphere* 53, 71-77.
- Lambert, P.A., 2002. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *J. Roy. Soc. Med.* 95 Suppl 41, 22-26.

- Lorian, V. (Ed.), 1996. Antibiotics in Laboratory Medicine. Williams and Wilkins, London.
- Matsunaga, T., Tomoda, R., Nakajima, T., Wake, H., 1985. Photoelectrochemical sterilization of microbial cells by semiconductor powders. FEMS Microbiol. Lett. 29, 211-214.
- McCullagh, C., Robertson, J.M.C., Bahnemann, D.W., Robertson, P.K.J., 2007. The application of TiO₂ photocatalysis for disinfection of water contaminated with pathogenic micro-organisms: A review. Res. Chem. Intermediat. 33, 359-375.
- Moellering Jr., R.C., 2006. The growing menace of community-acquired methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. 144, 368-370.
- Moran, G.J., Talan, D.A., Abrahamian, F.M., 2008. Biological terrorism. Infect. Dis. Clin. N. Am. 22, 145-187.
- Nakamura, H., Tanaka, M., Shinohara, S., Gotoh, M., Karube, I., 2007. Development of a self-sterilizing lancet coated with a titanium dioxide photocatalytic nano-layer for self-monitoring of blood glucose. Biosens. Bioelectron. 22, 1920-1925.
- NCCLS, 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Guideline (M7-A5). National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania.
- Paleologou, A., Marakas, H., Xekoukoulotakis, N.P., Moya, A., Vergara, Y., Kalogerakis, N., Gikas, P., Mantzavinos, D., 2007. Disinfection of water and wastewater by TiO₂ photocatalysis, sonolysis and UV-C irradiation. Catal. Today 129, 136-142.
- Pizarro, R.A., 1995. UV-A oxidative damage modified by environmental conditions in *Escherichia coli*. Int. J. Radiat. Biol. 68, 293-299.

- Rincón, A.G., Pulgarin, C., 2003. Photocatalytical inactivation of *E. coli*: Effect of (continuous-intermittent) light intensity and of (suspended-fixed) TiO₂ concentration. *Appl. Catal. B-Environ.* 44, 263-284.
- Rincón, A.-G., Pulgarin, C., 2004. Effect of pH, inorganic ions, organic matter and H₂O₂ on *E. coli* K12 photocatalytic inactivation by TiO₂: Implications in solar water disinfection. *Appl. Catal. B-Environ.* 51, 283-302.
- Robertson, J.M.C., Robertson, P.K.J., Lawton, L.A., 2005. A comparison of the effectiveness of TiO₂ photocatalysis and UVA photolysis for the destruction of three pathogenic micro-organisms. *J. Photoch. Photobio. A* 175, 51-56.
- Sichel, C., Tello, J., de Cara, M., Fernández-Ibáñez, P., 2007. Effect of UV solar intensity and dose on the photocatalytic disinfection of bacteria and fungi. *Catal. Today* 129, 152-160.
- Singleton, P., 1999. *Bacteria in Biology, Biotechnology and Medicine*. John Wiley and Sons Ltd., Chichester.
- Sunada, K., Watanabe, T., Hashimoto, K., 2003. Bactericidal activity of copper-deposited TiO₂ thin film under weak UV light illumination. *Environ. Sci. Technol.* 37, 4785-4789.
- Verran, J., Sandoval, G., Allen, N.S., Edge, M., Stratton, J., 2007. Variables affecting the antibacterial properties of nano and pigmentary titania particles in suspension. *Dyes Pigments* 73, 298-304.
- Vohra, A., Goswami, D.Y., Deshpande, D.A., Block, S.S., 2006. Enhanced photocatalytic disinfection of indoor air. *Appl. Catal. B-Environ.* 65, 57-65.
- Wallin, A., Luksiene, Z., Zagminas, K., Surkiene, G., 2007. Public health and bioterrorism: Renewed threat of anthrax and smallpox. *Medicina (Kaunas)* 43, 278-284.

- Wong, M.S., Chu, W.C., Sun, D.S., Huang, H.S., Chen, J.H., Tsai, P.J., Lin, N.T., Yu, M.S., Hsu, S.F., Wang, S.L., Chang, H.H., 2006. Visible-light-induced bactericidal activity of a nitrogen-doped titanium photocatalyst against human pathogens. *Appl. Environ. Microb.* 72, 6111-6116.
- WHO, 2002. Communicable Diseases Progress Report 2002 Global Defence against the Infectious Disease Threat. World Health Organization, Geneva.

Figure captions

Fig. 1 Assessment of the viability of different bacterial species in (a) distilled water and (b) aqueous 0.9% (w/v) NaCl solution (error bars represent standard deviation from mean for three populations). ●: *S. aureus*; □: *E. coli*; ▲: *S. Typhimurium*; ◇: *Ps. aeruginosa*

Fig. 2 Viable counts determined for different populations of *S. aureus* following various periods of incubation (error bars represent standard deviation from mean for ten 20 µL samples). □: 17 h; ■: 24 h; ■: 40 h

Fig. 3 Data from photocatalysis experiments in which *S. aureus* NCTC 6571 was suspended in (a) distilled water, (b) aqueous 0.9% (w/v) NaCl solution or (c) aqueous 0.1% (w/v) tryptone and 0.85% (w/v) NaCl solution. ◇: UV and TiO₂; □: UV only; ▲: TiO₂ only; ●: No UV or TiO₂; ----: minimum detectable number of CFU mL⁻¹

Fig. 1 Assessment of the viability of different bacterial species in (a) distilled water and (b) aqueous 0.9% (w/v) NaCl solution (error bars represent standard deviation from mean for three populations). ●: *S. aureus*; □: *E. coli*; ▲: *S. Typhimurium*; ◇: *Ps. aeruginosa*

Fig. 2 Viable counts determined for different populations of *S. aureus* following various periods of incubation (error bars represent standard deviation from mean for ten 20 µL samples).
□: 17 h; ■: 24 h; ■: 40 h

Fig. 3 Data from photocatalysis experiments in which *S. aureus* was suspended in (a) distilled water, (b) aqueous 0.9% (w/v) NaCl solution or (c) aqueous 0.1% (w/v) tryptone and 0.85% (w/v) NaCl solution. ◇: UV and TiO₂; □: UV only; ▲: TiO₂ only; ●: No UV or TiO₂; ----: minimum detectable number of CFU mL⁻¹