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Solar and photocatalytic disinfection of protozoan, fungal and bacterial microbes in drinking water

J. Lonnen[a], S. Kilvington[a], S.C. Kehoe[b], F. Al-Touati[c], K.G. McGuigan[c,*]

[a]*Department of Infection, Immunity & Inflammation, Maurice Shock Building, School of Medicine, University of Leicester, Leicester LE1 9HN, UK*

[b]*Department of Surgery, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland*

[c]*Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, 123 St Stephen's Green, Dublin 2, Ireland*

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Abstract

The ability of solar disinfection (SODIS) and solar photocatalytic (TiO_2) disinfection (SPC-DIS) batch-process reactors to inactivate waterborne protozoan, fungal and bacterial microbes was evaluated. After 8 h simulated solar exposure (870 W/m^2 in the 300 nm–10 μm range, 200W/m^2 in the 300–400 nm UV range), both SPC-DIS and SODIS achieved at least a 4 log unit reduction in viability against protozoa (the trophozoite stage of *Acanthamoeba polyphaga*), fungi (*Candida albicans*, *Fusarium solani*) and bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*). A reduction of only 1.7 log units was recorded for spores of *Bacillus subtilis*. Both SODIS and SPC-DIS were ineffective against the cyst stage of *A. polyphaga*.

Keywords: Solar; Disinfection; Photocatalyst; TiO_2; Protozoa; Fungi; Bacteria; Cysts; Spores

1. Introduction

Batch-process solar disinfection (SODIS) has been shown to be an effective treatment for drinking water contaminated with bacterial (McGuigan et al., 1999; Smith et al., 2000) and viral (Sommer et al., 1997) pathogens, especially in those developing countries where safe and reliable drinking water is not available all year round (Conroy et al., 1996, 1999, 2001). However, the efficacy of SODIS against protozoan pathogens such as *Cryptosporidium* or *Acanthamoeba* is, as yet, untested. This is a fundamental issue since many protozoan cysts are thermo-tolerant and thought to be highly resistant to standard UV-disinfection techniques (Morita et al., 2002). *Acanthamoeba* is an ideal model in this respect as the trophozoite stage can be cultured under controlled conditions and used to produce large numbers of highly resistant cysts (Hughes et al., 2003a). The photocatalyst TiO_2 has been successfully used within SODIS reactors to enhance and accelerate the inactivation rate of bacterial pathogens (Block et al., 1997; Blake et al., 1999; Herrera Melian et al., 2000; Salih, 2002; Rincón and Pulgarin, 2003) in a process named solar photocatalytic disinfection (SPC-DIS).

Abbreviations: AOP, Advanced oxidative process; CFU, Colony forming units; OHP, Overhead projector; PBS, Phosphate buffered saline; DPBST, Dulbecco's PBS + 0.05% (weight/volume) Tween 80; PET, Poly(ethylene terephthalate); SODIS, Solar disinfection; SPC-DIS, Solar photocatalytic disinfection; TiO_2, Titanium dioxide

*Corresponding author. Tel.: +353 1 4022207; fax: +353 1 4022168.

E-mail address: kmcguigan@rcsi.ie (K.G. McGuigan).

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The primary objective of this study was to determine if batch-process SODIS and SPC-DIS is effective against protozoa using the model organism *Acanthamoeba polyphaga,* a pathogenic free-living amoeba characterised by a life cycle of feeding and replicating trophozite and highly resistant cyst stage (Kilvington and White, 1994). In addition, the efficacy of the SPC-DIS system against other microbes such as fungal and bacterial organisms which might be expected to be more resistant to SODIS was also compared. In order to provide SPC-DIS as an appropriate technology for people living in poor socio-economic circumstances, a further aim was to produce the TiO_2 coatings using materials and equipment that would be available in developing countries.

2. Experimental

2.1. Immobilisation of TiO_2 onto acetate sheets

A 5 mm diameter cylindrical "bead" of black silicone adhesive bathroom sealant (Evo-Stick, Evode Industries, Dublin, Ireland) was laid down one side of an A4 size overhead projector (OHP) acetate transparency sheet (5star Ltd. Cambridge, UK). A reasonably reproducible coating of adhesive was accomplished by using a rectangular, tooth-edged, tiling trowel to spread the adhesive across the OHP acetate surface in straight, parallel lines. Each sheet was then completely covered with Degussa P-25 TiO_2 powder and allowed to dry for 24 h (Salih, 2002). Any excess TiO_2 powder was shaken off and retrieved, before the coated acetate was washed with distilled water and allowed to dry overnight at room temperature. The coated acetate insert was then trimmed, rolled up and inserted through the neck opening of a 100 ml Duran bottle before being unfolded such that if the bottle was laid horizontally on its side, the sheet covered the lower half of the internal surface with the TiO_2 coating facing the central axis of the bottle. The finished sheet had an approximate TiO_2 coverage of 25 mg/cm^2 (McGuigan, 2004).

2.2. Photochemical experiments

Solar irradiation was simulated using a commercial 1000W Diverging Beam, Xenon Arc Solar Simulating Lamp (Oriel Corp. Stratford, CT, USA) fitted with an Air Mass 1.0 solar filter (Oriel Corp. Stratford, CT, USA). Optical irradiances were monitored using a calibrated optical power meter (Coherent, Cambridge, UK, model 200/10+). Sample bottles were laid on their sides under the lamp such that the TiO_2-coated insert was nearest the floor and facing upwards to the light. Each test sample was exposed to global irradiances of 870 W/m^2 in the 300 nm–10 μm range (200 W/m^2 in the 300–400 nm UV range), which corresponds to strong equatorial sunshine (Joyce et al., 1996).

2.3. Test organisms

A. polyphaga strain Ros was isolated from unpublished cases of acanthamoeba keratitis in the UK. Trophozoites were adapted to growth in tissue culture flasks (Nunc Life Technologies, Paisley, UK) at 32 °C in a semi-defined axenic culture medium. The medium (♯6) comprised 20 g Biosate peptone (BBL, Beckton Dickinson, UK), 5 g glucose, 0.3 g KH_2PO_4, 10 mg Vitamin B_{12}, 15 mg methionine per litre of double distilled water and the pH adjusted at 6.5–6.6 with 1 M NaOH. The medium was sterilised by autoclaving at 120 °C for 15 min and penicillin and streptomycin (100 U/ml and 100 μg/ml, respectively) added before use. The complete medium was stored at 4 °C for use within 1 month.

Cysts were prepared from late log phase trophozoite cultures using the #6 culture medium supplemented with 50 mM $MgCl_2$ (Khunkitti et al., 1998; Hughes et al., 2003b). Trophozoites were harvested by centrifugation at 1000*g* for 10 min and adjusted to approximately 2×10^5/ml in 100 ml of encystment medium in a 175 cm^2 tissue culture flask (Nunc Life Technologies, Paisley, UK). The flask was then placed upright in a shaking incubator at 100 rev/min at 32 °C for 7 days after which microscopic examination would show >90% mature cysts. The cysts were harvested by centrifugation at 1000*g* for 10 min and washed three times with $\frac{1}{4}$ strength Ringer's solution. The final pellet was resuspended in a few ml of $\frac{1}{4}$ strength Ringer's solution and sonicated before use with three pulses at 50% amplitude each for 5 s (Ultrasonic Engineering Ltd., London, England). Previous experiments had shown that this removed cyst clumps without affecting viability. Cysts were counted using a Modified Fuchs Rosenthal haemocytometer, adjusted to 5×10^6 cells/ml and stored at 4 °C for use within 2 weeks.

Pseudomonas aeruginosa (ATCC 9027), *Candida albicans* (ATCC 10231) and *Fusarium solani* (ATCC 36031) were obtained from the American Type Culture Collection, Rockville, USA. *Escherichia coli* (DH5α) was an in-house strain of the widely available molecular cloning host and was selected as a reference coliform enteric bacterium. All strains were cultured on appropriate agar media as detailed elsewhere (International Organization for Standardization: ISO/CD 14729, 2000). *Bacillus subtilis* (laboratory strain) spores were obtained from culture on a defined sporulation medium for 6 days in air at 37 °C (Penna et al., 2001). Spores were washed off plates and re-suspended in $\frac{1}{4}$ strength Ringer's solution to a concentration of 1×10^7–1×10^8/ml. *F. solani* was grown on potato dextrose agar for 4