Photocatalytic inactivation of *Cryptosporidium parvum* on nanostructured titanium dioxide films

O. Sunnotel, R. Verdoold, P. S. M. Dunlop, W. J. Snelling, C. J. Lowery, J. S. G. Dooley, J. E. Moore and J. A. Byrne

ABSTRACT

Control of waterborne gastrointestinal parasites represents a major concern to water industries worldwide. In developed countries, pathogens in drinking water supplies are normally removed by sand filtration followed by chemical disinfection. *Cryptosporidium* spp. are generally resistant to common disinfection techniques and alternative control strategies are being sought. In the current study, the photocatalytic inactivation of *C. parvum* oocysts was shown to occur in buffer solution (78.4% after 180 min) and surface water (73.7% after 180 min). Viability was assessed by dye exclusion, excystation, direct examination of oocysts and a novel gene expression assay based on lactate dehydrogenase 1 (LDH1) expression levels. Collectively, this confirmed the inactivation of oocysts and scanning electron microscopy (SEM) confirmed cleavage at the suture line of oocyst cell walls, revealing large numbers of empty (ghost) cells after exposure to photocatalytic treatment.

Key words | *Cryptosporidium*, photocatalysis, titanium dioxide, viability, water treatment

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INTRODUCTION

Cryptosporidium spp. are waterborne, obligate intracellular protozoan parasites that infect a wide range of vertebrates and undergo endogenous development, culminating in the production of a transmissive, encysted stage (oocyst), which is discharged in the faeces of their host (Caccio 2005). Globally, *Cryptosporidium* is responsible for the majority of gastrointestinal parasitic infections, representing a significant cause of morbidity and mortality in its hosts (Karanis *et al.* 2007). *Cryptosporidium* cannot be cultivated *in vitro* and direct detection and identification of oocysts is notoriously insensitive, without the use of molecular methods. Cryptosporidiosis in humans usually results in self-limited watery diarrhoea in immunocompetent subjects, but has far more devastating effects on immunocompromised patients (e.g. AIDS patients) and can be life-threatening as a result of dehydration caused by chronic diarrhoea (Caccio 2005).

Owing to their tough outer walls, *Cryptosporidium* oocysts are highly resistant to chlorination, and can survive for several months in standing water (Robertson *et al.* 1992) representing a serious problem for large-scale water suppliers and users of private water supplies. In the spring of 1993, a water treatment facility in Milwaukee (Wisconsin, USA) was responsible for a serious *Cryptosporidium* outbreak, infecting over 403,000 people; 93% developed serious clinical symptoms (MacKenzie *et al.* 1995). The total economic cost

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was estimated at US$96.2 million (Corso *et al.* 2003). Surveys in the United Kingdom and the United States have shown that between 50 and 80% of standing water is contaminated with *Cryptosporidium* oocysts (Xiao *et al.* 2001).

To safeguard public health, EU legislation sets a limit of only one *Cryptosporidium* oocyst per 10 litres of treated water (EC 1998). Given the low infectious dose associated with cryptosporidiosis, and oocyst resistance to traditional disinfection and water treatment processes, rigorous analytical tests are required to ensure efficacy of proposed disinfection systems.

Heat treatment is an effective method used to inactivate *Cryptosporidium*; however, this approach is not commercially viable because of the high energy costs. Water treatment based on precipitation and/or flocculation is not sufficient to ensure complete removal of pathogens therefore an additional disinfection step is required. Disinfection using chlorination is effective against many microorganisms but has no significant impact on oocyst viability, at the concentrations currently employed (Betancourt & Rose 2004). Modern drinking water treatment plants use UVC, ozone or ultrafiltration as a tertiary treatment to inactivate or remove priority pollutants including protozoan oocysts. These methods are reliable and effective in controlling waterborne *Cryptosporidium*; however, their relatively high operating costs and inability to deal with seasonal fluctuations in pathogen load, reinforce the need to investigate new water treatment technologies (Betancourt & Rose 2004).

Titanium dioxide (TiO_2) photocatalysis is an advanced oxidation process (AOP) which can be used for water treatment. When irradiated with supra band gap energy, electron-hole pairs are generated within the titanium dioxide particles. The charge carriers can recombine, with the absorbed energy lost as heat, or they can migrate to the surface of the TiO_2 particles where redox reactions with water and dissolved oxygen at the particle solution interface can result in the formation of reactive oxygen species (ROS). ROS formed by photocatalysis include the hydroxyl radical, a powerful indiscriminate oxidant (Staehelin & Hoigné 1985), and superoxide anions, formed by reduction of dissolved oxygen (Ireland *et al.* 1993; Linsebigler *et al.* 1995; Choi & Byung-Woo 2000). Photocatalysis can degrade a wide range of organic compounds and reduce pathogen viability in water (Mills & Le Hunte 1997; Maness *et al.* 1999; Dunlop *et al.* 2002, 2008; Egerton *et al.* 2006).

In this study, we evaluated titanium dioxide photocatalysis as a method for the disinfection of surface water contaminated with *Cryptosporidium* oocysts. In addition to traditional viability assessment, by dye exclusion, excystation and direct examination of oocysts, a novel gene expression assay, based on determination of gene expression levels of the lactate dehydrogenase 1 (LDH1) gene, was used to confirm photocatalytic inactivation.

MATERIALS AND METHODS

Cryptosporidium parvum strain and water source

C. parvum oocysts were obtained from Moredun Scientific Ltd, Penicuik, UK, with an average viability of $>76\%$. Prior to disinfection experiments, stock oocysts were diluted with 1/4 strength Ringer's solution (NaCl 0.9% w/v, KCl 0.042% w/v, $CaCl_2$ 0.025% w/v) or surface water to a final concentration of 5×10^5 oocyst ml^{-1}. The surface water was obtained from Dorisland Drinking Water Treatment Works, Northern Ireland (N54°43′ 18.5″, W5°50′ 58.9″). River water samples were disinfected by filtration through a sterile 0.2 μm syringe filter to remove sediments and microflora and stored at 4°C prior to use. This method was preferred to pasteurizing or autoclaving given the sensitivity of the genetic analysis to bacterial contamination. An aliquot of river water was analysed for salient parameters (BOD $< 1\,mg\,l^{-1}$, pH 7.3, total organic carbon (TOC) $7\,mg\,l^{-1}$, total hardness $112.4\,mg\,l^{-1}$ $CaCO_3$, nitrate as N $0.7\,mg\,l^{-1}$, total phosphate as P $0.02\,mg\,l^{-1}$, sulphate $24\,mg\,l^{-1}$, sodium $13.94\,mg\,l^{-1}$, calcium $24.85\,mg\,l^{-1}$, magnesium $12.22\,mg\,l^{-1}$, iron $0.1711\,mg\,l^{-1}$).

Photocatalysis experiments

Immobilized TiO_2 films were prepared using electrophoretic deposition as described by Byrne *et al.* (1998). Indium-doped tin oxide (ITO) coated borosilicate glass (Instrument Glasses/Donnely Corporation) electrodes (1×1.5 cm) were coated with Degussa P25 to a weight of 1 mg of catalyst per cm^2 of substrate. The catalyst thickness was

measured using scanning electron microscopy and determined to be ~20 μm. Photocatalytic inactivation of viable *Cryptosporidium* oocysts was carried out in a quartz water-jacketed reactor with a volume of 7.5 ml, based on the design by Byrne *et al.* (1998) (Figure 1). A Philips TLD18W black light lamp (Philips, The Netherlands) was used as the irradiation source. The intensity of the lamp (mainline emission 365 nm) was measured using a spectral radiometer (Jobin Yvon Gemini 180) and found to be 24.7 W m^{-2} (300–400 nm) at a distance of 3 cm from the lamp. The TiO_2 sample was positioned in the middle of the reactor, approximately 3 cm from the UV lamp. The oocyst suspension was added to the reactor and continuously stirred using a magnetic stirrer (600 rpm). The solution temperature was maintained at 20 ± 2°C by circulating thermostat controlled water through the reactor jacket. Filtered air (0.2 μm) was sparged into the solution using an aquarium pump at fixed flow rate of 900 cm^3 min^{-1}. The suspension was aerated for 20 min in the dark prior to initiation of the experiment.

Samples were taken for analysis prior to aeration, following aeration ($t = 0$) and at regular intervals during the photocatalytic experiment to assess oocyst viability. At each time point, a 100 μl aliquot was taken and stored at 4°C, prior to viability assessment by dye exclusion staining. Additionally, a 50 μl sample was taken, transferred to liquid nitrogen and stored at −20°C prior to gene expression analysis.

Experiments were carried out with oocysts suspended in either 1/4 strength Ringer's solution or surface water. In order to assess the effect of UVA irradiation on oocyst viability, control experiments were carried out without the TiO_2 sample. Dark control experiments were also carried out. All experiments were performed in triplicate.

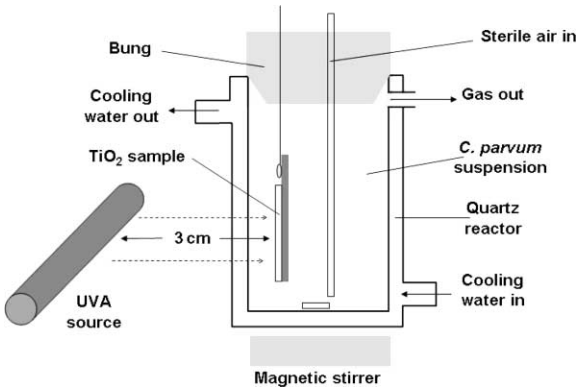

Figure 1 | Photocatalytic reactor.

Following each experiment, the reactor was decontaminated using 7.5 ml H_2O_2 (30% (v/v) Sigma). To ensure complete removal of residual H_2O_2 the reactor was washed five times with sterilized, de-ionized water.

Viability analysis by staining and excystation

Sporozoite nuclei and oocyst viability were assessed using 4-6-diamidino-2-phenyl indole (DAPI; Roche Diagnostics Ltd, Burgess Hill, UK) fluorogenic dye and inclusion/exclusion propidium iodide (PI) vital dye (Sigma), respectively (Bukhari *et al.* 2000). Aliquots (30 μl) of phosphate-buffered saline (PBS; Sigma) containing DAPI (1 mg ml^{-1}) and PI (1 mg ml^{-1}) were added to samples and incubated for 2 min at 37°C, in darkness. Samples were then mounted with glycerol/PBS based anti-bleaching agent AF1 (Citifluor Ltd, London, UK). Oocyst numbers and viability were determined (× 400 magnification) using a Nikon Eclipse E400 microscope. FITC emissions were viewed using a blue filter block (480 nm-excitation, 520 nm-emission), DAPI emissions using a UV filter block (350 nm-excitation, 450 nm-emission) and PI emissions using a UV filter block (560–580 nm-excitation, 600–650 nm emission). Low numbers of doubtful 'oocyst-like' entities were examined at × 1,000 magnification to confirm their identity by the identification of sporozoite nuclei (DAPI), suture lines and, if possible, internal structures (Smith *et al.* 2005).

Excystation was carried out by placing 50 μl sample aliquots in a water bath at 37°C and incubating for 1 h to allow oocysts to release their sporozoites. Excystation rates were estimated by measuring the percentage empty and partially empty oocysts versus intact oocysts.

RNA extraction

Oocyst suspensions were thawed on ice and centrifuged at 10,000 × g for 5 min at 4°C. Most of the supernatant was discarded, leaving a pellet volume of 20 μl, which was centrifuged at 10,000 × g for 2 min at 4°C. The supernatant was removed and the pellet re-suspended in 10 μl Tris-EDTA (TE), pH 7.2. A 50 μl volume of 1% triisopropylnapthalene sulphonic acid (TNS) (Acros Organics, Fair

Lawn, NJ) buffer (1%TNS, 6% p-4 salicylic acid, sodium salt 200 mM Tris-HCl, 25 mM EDTA, 250 mM NaCl, pH 7.8) was added to the sample and vortexed for 5 s (Sharkey *et al.* 2004). The oocyst suspension was subjected to freeze–thaw treatment to rupture oocysts and/or sporozoites, allowing the release of RNA. This involved five cycles of freezing in liquid nitrogen for 30 s followed by thawing at 65°C for 1 min on a heat block. Samples were incubated on ice for 1 h. The sample was centrifuged at 17,000 × g for 2 min at 4°C to remove cellular debris. The supernatant was transferred to a sterile diethyl pyrocarbonate (DEPC; Sigma) treated Eppendorf tube and an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) (60 μl) (Sigma) was added and gently mixed. After centrifuging at 10,000 × g at room temperature for 10 min, the upper aqueous layer was transferred into a fresh tube. The remaining interphase was subjected to another extraction by adding 60 μl 1% TNS buffer. The sample was centrifuged at 10,000 × g at room temperature for 10 min. The upper aqueous layer was collated with the aqueous phase of the first extraction. The RNA was precipitated with two volumes of ice-cold ethanol overnight at −20°C. RNA was pelleted by centrifugation at 20,000 × g for 20 min at 4°C and the remaining alcohol evaporated by leaving the sample on ice for 1–2 h. The pellet was resuspended in 26 μl RNase-free DEPC-treated reagent water (Boehringer Mannheim, Germany) and stored at −20°C.

Ten units of RNase-free DNase I (Boehringer Mannheim, Germany) was used to remove contaminating genomic DNA from total RNA. A 40 μl reaction volume contained 26 μl RNA sample, 6.25 mM $MgCl_2$ and 10 units of DNase I. Incubation was carried out at 37°C for 10 min. DNase I activity was inactivated by addition of 12 μl 20 mM EDTA and incubation at 37°C for 1 min with an extended incubation at 65°C for 10 min. All mRNA samples were checked for the presence of residual DNA by PCR.

Poly-dT-coated magnetic beads were used to extract mRNA from tRNA according to manufacturer's instructions (Dynal Ltd, Bromborough, UK). Briefly, tRNA suspensions were diluted 10-fold with DEPC treated sterile dH_2O and kept on ice. Binding buffer in a 1:1 ratio was added to 100 μl samples before adding the magnetic beads. Capture of mRNA by magnetic beads was established by hybridization with the poly-A tail of mRNAs at room temperature for 5 min on a rotating mixer. Beads were concentrated with a magnet and washed according to manufacturer's protocol. Finally, mRNA was eluted from beads in 10 μl 10 mM Tris-HCl at 70°C for 2 min.

RT-PCR

cDNA was generated using oligo $dT_{12\text{-}18}$ primers (Sigma, Gillingham, UK). Briefly, 1 μl (500 ng) oligo $dT_{12\text{-}18}$ primer was added to 10 μl template RNA, equalling 1,000 oocysts. An initial denaturing step at 65°C for 5 min was carried out, followed by chilling on ice for 3 min. Samples were spun down at 7,000 × g for 30 s prior to adding 4 μl × 5 Strand buffer, 2 μl dithiotreitol (DTT), 1 μl dNTPs (10 mM each) and 1 μl RNA OUT (Sigma, UK). Samples were pre-heated at 42°C for 2 min before adding 1 μl Superscript™ II RNase H^- (Sigma, UK). Amplification of mRNA to cDNA was performed at 42°C for 50 min.

All PCR reagents were obtained from Sigma, UK. Gene expression was measured by targeting the *Cp* LDHI coding region, using forward primer 5′-AGAACATTCATTGCA-CAACA-3′ and reverse primer 5′-CAAAGTAGG-CAGTTCCTGTC-3′, obtaining a 241 bp amplicon.

The PCR reaction mixture contained 2.5 mM $MgCl_2$, 0.2 μl of each primer, 5 μl × 10 PCR buffer, 0.1 μl of each dNTP (10 mM final concentration), 5 μl template cDNA and ddH_2O was added to obtain a final volume of 49.5 μl. A hot start PCR was carried out by adding 0.5 μl *Taq* polymerase after an initial denaturing step at 94°C for 3 min. Forty amplification cycles of 45 s at 94°C, 45 s annealing at 55°C and 1 min elongation at 72°C was followed by a final elongation step for 10 min at 72°C.

Gene expression analysis

PCR products were loaded on a 1.2% (w/v) agarose (Invitrogen, Paisley, UK) with ethidium bromide stain (Sigma, UK) and separated for 1 h at 80 V. Pictures of gels were obtained using an Alpha Imager 2200 (Alpha Innotech, San Leandro, CA). Relative mRNA expression was assessed by measuring the difference in band intensity using Kodak 1D software (New Haven, CT).

Scanning electron microscopy

Oocysts were fixed with 4% formaldehyde and progressively dehydrated with acetone. Critical point drying was carried out to replace acetone with CO_2. Samples were gold coated for 2 min at 20 mA using Polaron SEM Coating unit E5100. Damage to oocyst structures was assessed with FEI Quanta 200 ESEM microscope.

Statistical analysis and controls

Oocyst viability data was analysed using Analyse-it (Analyse-it v. 1.73, Analyse-it Software Ltd, Leeds, UK). Standard deviation and variance within triplicate data sets (viability counting data) was analysed using standard *t*-test. One-way variance analysis (ANOVA) was acquired to measure variance between similar experiments. A two-way ANOVA was performed to compare inactivation trends between the experiments: that is, UV-TiO_2 versus UV only and 1/4 strength Ringer's solution versus surface water.

RESULTS AND DISCUSSION

Photocatalytic disinfection of *Cryptosporidium* oocysts was confirmed using the complementary assays employed. Based on the dye exclusion assay a significant ($p > 0.05$) reduction of *C. parvum* oocyst viability was observed in both Ringer's solution (78.4% after 180 min) and surface water (73.7% after 180 min) (Figure 2). Significant disinfection ($p > 0.05$) was not observed for the dark control or under UVA irradiation alone (in the absence of TiO_2). The presence of organic compounds and trace ions in the surface water had no significant impact on the photocatalytic inactivation of the oocysts in comparison to that in Ringer's solution. Catalyst fouling was not observed during repeated analysis in surface water. However, further work should be carried out using non-filtered surface water to fully assess the potential of catalyst fouling from the suspended solid content of the surface water.

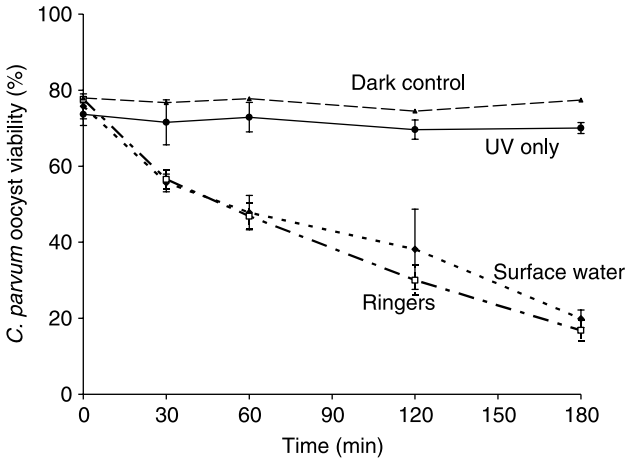

Figure 2 | Photocatalytic inactivation of *C. parvum* oocyst suspended in Ringer's solution and surface water.

Previous studies have demonstrated photocatalytic disinfection of *Cryptosporidium* oocysts using vital dye exclusion and excystation assays. Otaki *et al.* (2000) reported photocatalytic inactivation of oocysts, using TiO_2 (Ishihara ST-K03) immobilized onto the bottom of a glass beaker, and UVA and UVC irradiation (7.0 mW cm^{-2} at 365 nm and 0.2 mW cm^{-2} at 254 nm, respectively). Oocyst inactivation was significantly faster under UVC irradiated TiO_2 in comparison with UVA photocatalysis, suggesting a synergistic disinfection mechanism. Curtis *et al.* (2002) reported a 26% reduction in *C. parvum* oocysts viability following 60 min treatment using an electric field enhanced (EFE) photoreactor comprising a 5 cm × 5 cm Ti/TiO_2 electrode irradiated using 2 × 8W UVA lamps. The initial oocyst inactivation rates reported in the present study (without EFE) are comparable to those reported by Curtis *et al.* (2002). However, the oocyst stock used in that study was low in initial viability (<70%), indicating ageing oocysts, which have been shown to be more susceptible to disinfection treatments (Li *et al.* 2004).

In order to gain further understanding of the mechanism of ROS induced oocyst damage, analysis of the DAPI and PI staining procedures and additional viability assays were undertaken. DAPI staining is generally used in routine diagnosis of *Cryptosporidium* by the UK Drinking Water Inspectorate and the water industry to visualize the sporozoite nuclei within the oocyst shell. PI-staining is widely employed to assess *Cryptosporidium* viability; however one should be cautious as it can overestimate the infectivity of oocysts (Bukhari *et al.* 2000). Loss of membrane integrity allows PI to enter the nucleus and stain nucleic acids. Low sporozoite counts and a high percentage of ghost cells were observed following photocatalytic treatment, suggesting sporozoites release by loss of oocyst cell wall integrity.

Upon release, sporozoites were likely to be degraded as a result of ROS attack, as demonstrated by the lack of 'free' sporozoites observed post photocatalytic treatment.

Excystation experiments were undertaken to assess the ability of the treated oocysts to release their sporozoites, a transition step required for host cell invasion. A relatively low excystation rate of 3–6% was observed following treatment in comparison to oocyst viability assessed by dye staining (16–20%). This suggests that damage occurring to the oocyst cell wall is sufficient to prevent sporozoite excystation, in cases where sporozoites have not been lost during photocatalytic disinfection. We propose that oxidative damage to sporozoites surface receptor function, a vital stage required for the initiation of the cell invasion process, may prevent excystation. Structural alterations and loss of cellular motility were also confirmed by microscopy. SEM images prior to (Figure 3a) and following (Figure 3b) photocatalytic treatment of the surface water were undertaken to confirm the findings of DAPI-PI and excystation analysis. Significant shrinkage, presumably due to sporozoite loss, was observed in the treated oocysts (Figure 3b). SEM analysis showed a large number of empty (ghost) oocysts following treatment confirming rupture of the oocyst wall suture line. The appearance of blebs of approximately 0.2 μm uniformly distributed on the oocyst surface indicated further oocyst wall damage had occurred. The mechanism of photocatalytic disinfection is complex and still under investigation. The ROS generated at the TiO_2 surface have been proposed to be the responsible agent for the disruption of cell membranes leading to loss of respiration and microbial inactivation (Maness *et al.* 1999). Irreparable DNA damage has also been reported following exposure of cells to super oxide anions and/or hydroxyl radicals (Su *et al.* 2006).

The ROS produced by photocatalysis resulted in a significant decrease in oocyst viability as confirmed by DAPI-PI viability staining and excystation. Although *Cryptosporidium* possesses an NADPH-dependent H_2O_2 scavenging system, low or no levels were observed for most anti-oxidant enzymes, including superoxide dismutase (SOD) when exposed to hydrogen peroxide (Entrala & Mascaro 1997). Expression profiles observed in closely related *Giardia* suggested that free radicals were quenched by NADH oxidase and NADPH peroxidase, rather than

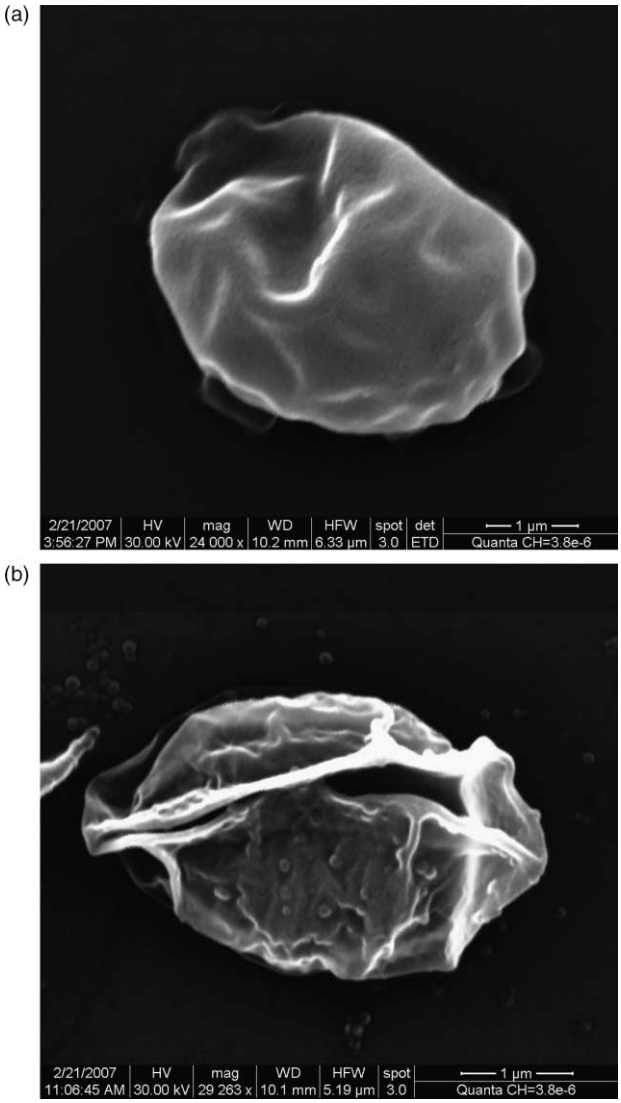

Figure 3 | SEM images of untreated intact oocyst (a) and empty shell of oocyst following photocatalytic treatment (b).

conventional anti-oxidant enzymes such as superoxide dismutase, catalase or peroxidase (Brown *et al.* 1995). As homologues of these genes are present in the *Cryptosporidium* genome, a similar radical scavenging mechanism is likely to be present. However, it is not sufficient to prevent ROS damage induced by photocatalytic treatment. Morita *et al.* (2002) reported the repair of UVC disinfected *Cryptosporidium* oocysts via dark repair and photoreactivation processes, confirming the presence of UV repair mechanisms (Shin *et al.* 2001; Rochelle *et al.* 2004). The use of low intensity UVA appears to have no

significant effect on oocyst viability, nuclear membrane or mRNA integrity.

Previous studies using dye exclusion and excystation assays have demonstrated the efficacy of ROS for disinfection of *Cryptosporidium* in buffer solution and distilled water (Curtis *et al.* 2002; Lee *et al.* 2003; von Gunten 2003; Kim *et al.* 2007). Kniel *et al.* (2004) reported a significant reduction in *in-vitro* viability by exposing *Cryptosporidium* oocysts to hydroxyl radicals, using hydrogen peroxide as a precursor. Otaki *et al.* (2000) described the photocatalytic and UV inactivation of *C. parvum* oocysts. The presence of TiO_2 increased the rate of oocyst inactivation during UVA or UVC irradiation (7.0 mW cm^{-2} at 365 nm and 0.2 mW cm^{-2} at 254 nm, respectively). Curtis *et al.* (2002) reported the disinfection of *C. parvum* oocysts using an electric field enhanced (EFE) photoreactor irradiated using 2 × 8W UVA lamps. The EFE reactor was used to enhance radical formation during TiO_2 photocatalysis with a 26% reduction in viability observed in 60 min. Méndez-Hermida *et al.* (2007) reported the photocatalytic disinfection of C*ryptosporidium parvum* oocysts using solar radiation. The rate of disinfection was significantly increased in the presence of the photocatalyst.

Gene expression of *Cp* LDH1 was carried out as a novel and relatively quick viability assay. RT-PCR analysis of the *Cp* LDH1 gene following photocatalytic treatment revealed a decrease in intensity with increased treatment time, culminating in a complete loss of signal at 180 min (Figure 4). mRNA degradation followed a similar pattern to loss of viability observed in the DAPI-PI staining, however mRNA was not detected at 180 min of treatment, while ~25% of oocysts appeared viable by staining. No decrease in intensity was observed in the control gels (Figure 4). RT-PCR analysis of the *Cp* LDH1 transcripts is extremely sensitive and can routinely detect as low as 10 viable oocysts (data not shown). The observed difference between dye staining and RT-PCR analysis may result from increased mRNA sensitivity to ROS in comparison to the structural components of the oocysts and sporozoites.

The range of complementary viability assessment techniques used in this study conclusively demonstrated the photocatalytic efficacy of immobilized TiO_2 towards the inactivation of *Cryptosporidium*, in real surface water (not distilled water). We show for the first time that RT-PCR can be used to accurately assess oocyst viability following photocatalytic disinfection. Molecular assays may ultimately replace systems based on microscopy due to the greater ease of automation and the inherent sensitivity of systems built around amplification reactions.

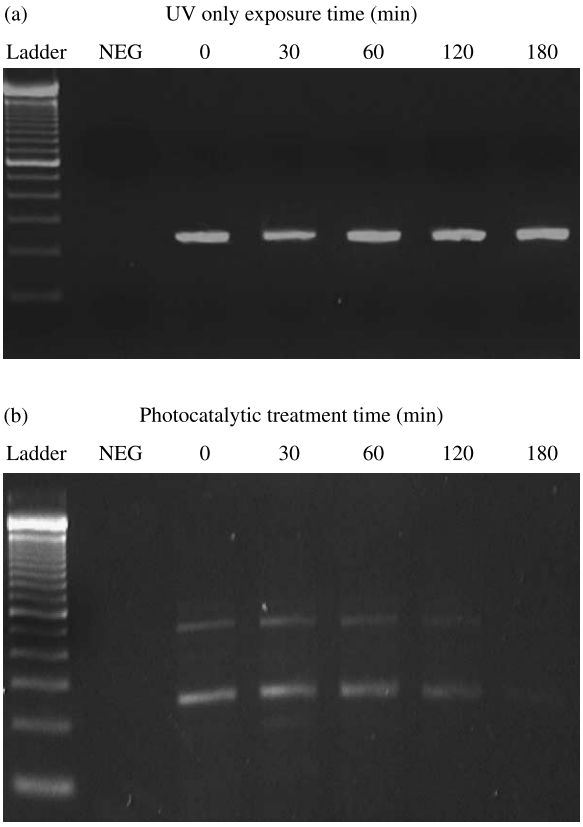

Figure 4 | Gene expression of *Cp* LDH1 for (a) UV only; and (b) photocatalysis.

CONCLUSION

In the present study, titanium dioxide photocatalysis was shown to inactivate *Cryptosporidium parvum* oocysts suspended in Ringer's solution (78.4% after 180 min) and surface water (73.7% after 180 min). Significant disinfection ($p > 0.05$) was not observed for the dark control or under UVA irradiation alone (in the absence of TiO_2).

Photocatalysis is unlikely to be adopted for large-scale water treatment in the developed world due to volume and treatment time constraints. A more realistic opportunity to use the technology may exist in small point-of-use units where potable water could be treated for disinfection

of pathogens or trace priority pollutants remaining in (or entering) the water distribution network following conventional treatment: for example, chlorine-resistant pathogens, endocrine-disrupting chemicals (EDCs) or pharmaceuticals and personal care products (PPCPs). Photocatalytic disinfection of water for potable use may also find application in developing countries where solar excitation is a possibility.

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