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Evaluation of solar photocatalysis using TiO_2 slurry in the inactivation of *Cryptosporidium parvum* oocysts in water

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ABSTRACT

Cryptosporidium is a genus of enteric protozoan parasites of medical and veterinary importance, whose oocysts have been reported to occur in different types of water worldwide, offering a great resistant to the water treatment processes. Heterogeneous solar photocatalysis using titanium dioxide (TiO_2) slurry was evaluated on inactivation of *Cryptosporidium parvum* oocysts in water. Suspensions of TiO_2 (0, 63, 100 and 200 mg/L) in distilled water (DW) or simulated municipal wastewater treatment plant (MWTP) effluent spiked with *C. parvum* oocysts were exposed to simulated solar radiation. The use of TiO_2 slurry at concentrations of 100 and 200 mg/L in DW yielded a high level of oocyst inactivation after 5 h of exposure (4.16 ± 2.35% and 15.03 ± 4.54%, respectively, *vs* 99.33 ± 0.58%, initial value), representing a good improvement relative to the results obtained in the samples exposed without TiO_2 (51.06 ± 9.35%). However, in the assays carried out using simulated MWTP effluent, addition of the photocatalyst did not offer better results. Examination of the samples under bright field and epifluorescence microscopy revealed the existence of aggregates comprising TiO_2 particles and parasitic forms, which size increased as the concentration of catalyst and the exposure time increased, while the intensity of fluorescence of the oocyst walls decreased. After photocatalytic disinfection process, the recovery of TiO_2 slurry by sedimentation provided a substantial reduction in the parasitic load in treated water samples (57.81 ± 1.10% and 82.10 ± 2.64% for 200 mg/L of TiO_2 in DW and in simulated MWTP effluent, respectively). Although further studies are need to optimize TiO_2 photocatalytic disinfection against *Cryptosporidium*, the results obtained in the present study show the effectiveness of solar photocatalysis using TiO_2 slurry in the inactivation of *C. parvum* oocysts in distilled water.

1. Introduction

Enteric protozoan parasites belonging to the genus *Cryptosporidium* are considered of medical and veterinary importance as they infect a wide range of vertebrate hosts, including humans. Cryptosporidiosis severity ranges from asymptomatic or mild to severe with intractable diarrhoea and wasting, depending on immune status, nutrition and age of the affected host. The pathogen is transmitted by the faecal-oral route, either directly by person-to-person or animal-to-person transmission or indirectly *via* ingestion of contaminated water or food [1]. Waterborne route is the most common pathway of cryptosporidiosis transmission. *Cryptosporidium* was identified as the main aetiological agent involved in 60.3% of the waterborne protozoan parasitic outbreaks reported worldwide between 2004 and 2010 [2].

Water/wastewater treatment is based on various mechanical, biological, physical and chemical processes. Unfortunately, some pathogens, such as *Cryptosporidium*, are known to be resistant to these processes, as indicated by their presence in different types of water worldwide, being one of the main pathogenic agents found in wastewater [3–8]. In recent years, advanced oxidation processes (AOPs) have emerged as a means of water/wastewater treatment. AOPs can be defined as aqueous phase oxidation methods based on *in situ* generation of highly reactive oxygen species (ROS), mainly hydroxyl radicals. ROS are powerful oxidant species that can oxidize and mineralize almost any chemical compound, even the most recalcitrant molecules, yielding CO_2 and inorganic ions [9]. Free radicals can also damage microbial cells by attacking the cell wall, cytoplasmic membrane and intracellular structures [10]. Among these processes, heterogeneous solar photocatalysis uses semiconductor catalysts, which can be excited in presence of oxygen and solar radiation (visible and near ultraviolet bands of solar spectrum) to produce ROS. The most commonly used photocatalyst is the semiconductor titanium dioxide (TiO_2), which is nontoxic, chemically stable, available at a reasonable cost, and capable of repeated use without substantial loss of catalytic ability [9]. Numerous studies have demonstrated that TiO_2 solar photocatalysis is effective against a wide range of microorganisms present in water, air and on

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surfaces of certain types of material (Gram-negative and Gram-positive bacteria, unicellular and filamentous fungi, mammalian bacteriophages and viruses, algae and protozoa). However, bacterial endospores, fungal spores and protozoan (oo)cysts are highly resistant to TiO_2 photocatalytic process because they have a robust cell wall [9–11].

Since the recognized resistant nature of infectious forms of *Cryptosporidium* to conventional methods of water disinfection and their reported occurrence in effluents from wastewater treatment plants worldwide [12], the aim of this work is to evaluate the efficacy of solar photocatalysis using TiO_2 slurry in the inactivation of *Cryptosporidium parvum* oocysts in distilled water and simulated municipal wastewater treatment plant (MWTP) effluent under simulated conditions of solar radiation.

2. Materials and Methods

2.1. Cryptosporidium parvum Oocysts

Cryptosporidium oocysts were collected from a naturally infected neonatal Friesian-Holstein calf. Concentration [0.04 M phosphate-buffered saline (PBS) pH 7.2 and diethyl ether], purification (discontinuous caesium chloride gradients), quantification (Neubauer haemacytometer) and molecular characterization were performed as previously reported [13]. Briefly, faeces were collected from a calf by rectal sampling and stored at 5 °C. Faecal material was then homogenised in 10–20 mL of PBS (0.04 M, pH 7.2), filtered through two sieves (mesh sizes 150 and 45 μm), shaken with diethyl ether (2:1, v/v) and concentrated by centrifugation at 2000 ×*g*, 4 °C, for 15 min. The resulting uppermost two layers were removed carefully and discarded, and the sediment was washed with PBS (0.04 M, pH 7.2) by centrifugation at 2000 ×*g*, 4 °C, for 15 min. *Cryptosporidium* oocysts were purified on discontinuous caesium chloride gradients of 1.05, 1.10 and 1.40 g/mL by centrifugation at 2000 ×*g*, 4 °C, for 30 min. Finally, oocysts were counted in a modified Neubauer haemocytometer using 0.16% malachite green solution as counterstain [14,15]. The isolate was identified as *C. parvum* by analysis of a ≈587-bp fragment of the SSU-rDNA gene [16].

2.2. Water Types

Distilled water (DW) [pH 6.1, conductivity < 10 μS/cm, dissolved organic carbon (DOC) <0.5 mg/L] was used as a reference solution for observing and comparing the inactivation kinetics under laboratory conditions, excluding the contribution or interference of any compound containing other types of water. DW was prepared in a water still (Aquatron A4D Bibby Scientific Limited, Stone, United Kingdom) using tap water.

Simulated MWTP effluent was employed as a model of treated discharge from MWTP according to Klamerth et al. [17]. This simulated effluent (pH 7.9, 6.2 NTU, DOC 25 mg/L) was prepared 24 h before use by adding different reagents to distilled water and maintained at 4–8 °C until use (Table 1).

Table 1
Composition of the simulated municipal wastewater treatment plant effluent used to evaluate the inactivation of *C. parvum* oocysts by TiO_2 solar photocatalysis [17].

Reagent	Concentration (mg/L)	Reagent	Concentration (mg/L)
$NaHCO_3$	96	K_2HPO_4	28
NaCl	7	$CaCl_2 \cdot 2H_2O$	4
$CaSO_4 \cdot 2H_2O$	60	Peptone	32
Urea	6	$MgSO_4 \cdot 7H_2O$	2
$MgSO_4$	60	Meat extract	22
KCl	4		

2.3. Simulated Solar Conditions

Solar radiation was simulated using a 1100 W air-cooled xenon arc lamp solar simulator (SUNTEST CPS+, ATLAS Material Testing Technology GmbH, Lisengericht, Germany) fitted with a UV filter (barrier, 290 nm) (Suprax, ATLAS Material Testing Technology GmbH). The intensity of global radiation was adjusted to 500 W/m^2 (from 300 nm to 800 nm) using the irradiance sensor and the microprocessor incorporated in the simulator. This was equivalent to ≈33 W/m^2 of UV radiation (from 290 nm to 390 nm) as measured with a UV radiometer UV34 (PCE Ibérica, S.L., Tobarra, Spain).

The radiometer provides data in terms of incident irradiation (W/m^2), which is defined as the solar radiant energy rate incident on a surface per unit area. Parameter Q_{uv}, which is the accumulative energy per unit of volume (kJ/L) received in a reactor, is calculated according to Eq. (1):

$$Q_{UV,n} = Q_{UV,n-1} + \frac{\Delta t_n \cdot \overline{UV}_{G,n} \cdot Ar}{V_t}; \Delta t_n = t_n - t_{n-1} \quad (1)$$

where $Q_{uv,n}$ and $Q_{uv,n-1}$ represent the UV energy accumulated per litre (J/L) at times t_n and t_{n-1}; Δt_n is the duration of exposure of the sample (s); $\overline{UV}_{G,n}$ is the average incident radiation on the irradiated area (W/m^2); A_r is the illuminated area of collector (m^2); and V_t is the total volume of water treated (L).

2.4. Experimental Design

Samples (25 mL) of TiO_2 suspensions (Degussa P25 Evonik Industries, Essen, Germany) in DW or simulated MWTP effluent (0, 63, 100 and 200 mg/L) were placed in borosilicate glass beakers of 35 mm of diameter (Fisherbrand®, Thermo Fisher Scientific Inc., Waltham, MA, USA), spiked with 15×10^6 purified *C. parvum* oocysts and exposed to simulated solar radiation for a maximum of 5 h. Dark control samples containing 0 and 200 mg/L TiO_2 prepared in the same manner were wrapped in aluminium foil to prevent light falling on the suspension. During the experiments, the samples were covered with borosilicate watch glasses (49 mm of diameter, 1 mm of thickness) and stirred at 350 rpm on a horizontal platform (Cimarec I Scientific Multipoint 6, Thermo Fisher Scientific Inc.). Temperature was monitored every 30 min with a Temp 3JKT thermometer (Eutech Instruments Pte Ltd., Singapore) equipped with a beaded wire probe 3K1200 (OMEGA Engineering, Inc., Stamford, CT, USA), which was placed inside the reactor containing 25 mL of DW and located beside the other samples (Fig. 1).

After intervals of 2.5 h, 5 mL aliquots of the exposed samples were removed and washed with 10 mL of PBS (0.015 M, pH 8.0), by centrifugation at 2000 ×*g*, 15 °C, for 15 min. The supernatants were discarded and the sediments thus obtained were resuspended in 500 μL of PBS (0.015 M, pH 8.0) and used to evaluate the oocyst viability. All tests were performed in duplicate.

2.5. Evaluation of Oocyst Viability

The viability of *C. parvum* oocysts was determined by inclusion/exclusion of the fluorogenic vital dye propidium iodide (PI) (Sigma-Aldrich, Co., St. Louis, MO, USA) and a further modification that includes an immunofluorescence antibody test to verify oocyst identification [18,19]. Briefly, 200 μL aliquots of sediments were incubated with 40 μL of monoclonal antibodies labelled with fluorescein isothiocyanate (FITC) (Aqua-Glo™ G/C Direct, Waterborne, Inc., New Orleans, LA, USA) and 30 μL of PI working solution [1 mg/mL in PBS (0.1 M, pH 7.2)] at 37 °C for 30 min [13]. The samples were washed three times in PBS (0.015 M, pH 8.0) at 10,000 ×*g*, 4 °C, for 5 min. Oocysts were identified first under FITC filter (excitation at 450–480 nm; barrier at 515 nm) before being examined for PI inclusion/exclusion under a PI filter