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Photocatalytic water disinfection of Cryptosporidium parvum and Giardia lamblia using a fibrous ceramic TiO₂ photocatalyst
Sergio Navalon, Mercedes Alvaro, Hermenegildo Garcia, Daniel Escrig and Víctor Costa

ABSTRACT
Cryptosporidium parvum and Giardia lamblia are two of the most chlorine resistant microorganisms with notable adverse effects on humans. Our study shows that waters containing these two protozoa at low concentrations can be efficiently disinfected in continuous flow by using a commercial fibrous ceramic TiO₂ photocatalyst. The efficiency of the photocatalytic disinfection is largely enhanced by adding a small concentration of chlorine. In this way, the residence time on the photoreactor can be considerably shortened. In contrast, under the same conditions and radiance power, UV light without any photocatalyst is significantly less efficient, particularly for G. lamblia. These results exemplify the advantages of the photocatalytic process for safe and complete water disinfection.

Key words | Cryptosporidium parvum, Giardia lamblia, photocatalysis, water disinfection

INTRODUCTION
Water disinfection to ensure the absence of pathogen microorganism noxious for the human health is one of the main issues in water treatment (Orme et al. 1990; Pontius 2002; Betancourt & Rose 2004). Among the different protozoa relevant for water disinfection tests, Cryptosporidium parvum and Giardia lamblia are two microorganisms of large importance since they are known to be remarkable resistant to disinfection by chlorination (Rice et al. 1982; Betancourt & Rose 2004). UV irradiation is a general method for water disinfection (Hijnen et al. 2006; Linden et al. 2007). However C. parvum and G. lamblia are also notoriously resistant to UV light and for these two protozoa remarkable long exposition times compared to aerobic bacteria are needed in order to have a large reduction on the microorganism count (Hijnen et al. 2006). With these precedents in mind and choosing C. parvum and G. lamblia as test protozoa, in the present work we want to report the disinfection activity of a novel silica-supported TiO₂ ceramic photocatalyst.

TiO₂ is a widely used photocatalyst (Fujishima et al. 2000) that upon excitation generates a charge separated state consisting on electrons in the conduction band and positives holes in the valence band. According to the electrochemical values, electrons in the conduction band are mildly reducing species, but in any case able to reduce molecular dioxygen into O₂² superoxide. In contrast, holes in the valence band are strongly oxidizing agents able to oxidize water to highly aggressive hydroxyl radicals. These OH radicals as well as superoxide and other active oxygen species are responsible for disinfection in water by attacking cellular membranes as well as cytoplasmatic proteins (Rincón & Pulgarin 2003, 2004).
One general problem of TiO$_2$ is how to design a photochemical reactor for continuous flow operation (Pozzo et al. 1997). Most of the reported work on photocatalytic disinfection has been performed with small volumes under batchwise conditions (Mendez-Hermida et al. 2005) and using suspended TiO$_2$ powders (Sökmen et al. 2007; Ryu et al. 2008). In this context, TiO$_2$ powders suspended in water are difficult to be used in continuous flow operation, supported titania photocatalyst being much more suitable for this purpose. This issue of how to present the photocatalyst is particularly important for continuous flow experiments, since immobilization of the semiconductor can lead to a decrease of the disinfection efficiency compared to the use of stirred TiO$_2$ powders. Therefore, the recently reported titania-containing silica fibers are promising because they can serve to prepare non-woven porous fabrics that can be permanently exposed to UV light in a photochemical reactor without leaching titania (Toshihiro et al. 2002). A continuous flow can go through this photocatalytic fabric, thus ensuring an optimal photocatalytic activity of the system by contacting the water with the light exposed photocatalyst. In the present work we report the disinfection efficiency of a silica-supported titania fabric (Toshihiro et al. 2002) for C. parvum and G. lamblia protozoa. Compared to related precedences on photocatalytic disinfection (Sökmen et al. 2007; Ryu et al. 2008) our study presents as important features the use of contaminated solutions prepared at low protozoa counts and using real water that contains significant concentrations of carbonate and bicarbonate, inorganic salts and dissolved organic matter (DOM). This situation, low protozoa count and natural freshwater with a high content in inorganic salts and DOM makes more real the present study in comparison to the use of model solutions with purified water (Lisle & Rose 1995). We will present data showing that photocatalytic irradiations using TiO$_2$ ceramics is much more efficient for water disinfection than exclusive UV irradiation.

**METHODS**

**Materials**

C. parvum ($10^7$ oocysts in 1 ml filtered PBS) and G. lamblia ($10^6$ cysts in 1 ml filtered PBS) were supplied by BTF Company. Sodium hypochlorite ($10–13\%$) and sodium thiosulfate were of analytical grade and supplied by Sigma-Aldrich.

Supported titania photocatalysts were supplied by UBE Corporation Europe and it is commercially available (http://www.ube.es). Basically this photocatalyst consists in a non-woven fabric of silica–titania fibers (Toshihiro et al. 2002). The photocatalytic ceramic TiO$_2$ fibers were prepared by thermal decomposition at high temperature of 50/50 wt mixtures of polycarbosilane and titanium butoxide (Toshihiro et al. 2002). During the thermal treatment at 1,200°C formation of a nanocrystalline TiO$_2$ particles embedded into the ceramic matrix occurred while the different density makes the TiO$_2$ phase to migrate predominantly to the upper part of the ceramic material (see Figure 1). According to XRD diffraction applying the Scherrer’s equation the average particle size of TiO$_2$ was 8 nm. The TiO$_2$ nanocrystals are predominantly anatase (Figure 1b). The composite fibre has an average diameter of 10 μm. The tensile strength of the fibre was about 2 GPa. Figure 1 shows a pictorial illustration of the structure of the

![Figure 1](image_url)  
Figure 1 | (a) Cartoon showing the inhomogeneous structure of the fibrous ceramic UBE photocatalyst. (b) XRD of the titania phase in UBE photocatalyst corresponding to anatase.
fibrous ceramic photocatalyst in where the surface enriched TiO$_2$ nanocrystals has been indicated.

**UV irrations**

150 liters of model water contaminated with *C. parvum* and *G. lamblia* were prepared from potable water by dechlorination with an excess of thiosulfate and subsequently was mixed with 1 L solutions of the protozoa. Prior to contamination the absence of chlorine was tested by the N,N$^0$-diethyl-1,4-phenylenediamine (DPD) method (ISO 7393-2:1985). The contaminated water was contained in a reservoir (500 L) and was recirculated at 500 L/h through a homemade (see Figure 2) photoreactor consisting of a low pressure mercury UV lamp (765 mm length, 254 nm, 10 mW/cm$^2$) of 40 W nominal power. The total fluence energy exiting the photocatalyst was 11.4 W. Although the photoreactor is designed to have two filters (20 and 5 μm, respectively) in series before reaching the photocatalyst chamber, the experiments reported here were carried out in the absence of any filter in order to avoid protozoa retention in the system. Prior to irradiation the contaminated water was circulated through the system for at least 30 min. The irradiation time was 0.63 min per cycle. The system was operated under continuous flow (500 L/h). Recirculating the contaminated water from the reservoir through the photoreactor and back into the tank. Sampling immediately prior to irradiation and at the required time were carried out by collecting 25 L from the photoreactor outlet.

**Photocatalytic irrations**

The UBE photocatalyst shaped as truncated cones (I.D. 50 to 45 cm, 140 mm height) was fit inside a quartz photoreactor around the UV lamp (see Figure 2b). To cover the lamp length a set of four cones were placed coaxially inside the photoreactor. The water flows parallel to the lamp from the bottom to the top of the photoreactor. For those experiments in which hypochlorite was added, first the potable water was dechlorinated and when the small ClO$^-_2$ concentration required was added. The ClO$^-_2$ concentration indicated in Table 1 refers to the initial concentration that decreases during the course of the photocatalytic irradiation. The ClO$^-_2$ concentration during the course of the photocatalytic experiments in the presence of this disinfectant agent was monitored periodically and the value was reset to the initial concentration when measurements indicated that ClO$^-_2$ amount was below 0.9 ppm. Experiments were carried out in duplicate maintaining the initial count of protozoa as similar as possible. No significant differences in the results of the irradiations were observed in the replicates.

**C. parvum and G. lamblia analysis**

Analyses were carried out in Valencia (Spain) by GAMA-SER S.A. an independent certified laboratory. The applied protocol was based on the EPA method 1623 ([US EPA 1999](#)): *C. parvum* and *G. lamblia* in water were analyzed by
filtration, immunomagnetic separation (IMS) and immuno-fluorescence assay (FA) microscopy. This method does not identify the *C. parvum* or *G. lamblia* stain, nor can it determine the viability or infectivity of detected oocysts and cysts (EPA method 1623).

The steps to estimate the *C. parvum* and *G. lamblia* concentration, described in the EPA method, are the following:

**a. Filtration, elution and separation**

20 L of the water sample was filtered through a cartridge (Envirocheck de Pall, 1 μm) to retain the oocysts or cysts of *C. parvum* and *G. lamblia*, respectively. Materials on the filter are eluted with a buffer (containing Laurent12, Antifoam, Tris and EDTA) and the eluate is centrifuged to separate the oocysts and cysts, and the supernatant fluid is removed. The oocysts and cysts are magnetized by attachment of magnetic beads conjugated to anti-*C. parvum* and anti-*G. lamblia* antibodies. The magnetized oocysts and cysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts by treatment at pH 5.

**b. Oocyst and cyst count**

The oocysts and cysts are stained on well slides with fluorescently labeled monoclonal antibodies and 4,6-diamidino-2-phenylindole (DAPI) (see Figure 3b and 4b). The stained samples are examined using fluorescence (fluorescein-5-isothiocyanate, FITC) (see Figure 3a and 4a) and differential interference contrast (DIC) microscopy. *C. parvum* oocyst contains four banana-shape sporozoites

<table>
<thead>
<tr>
<th>Entry</th>
<th>Irradiation time (min)</th>
<th>ClO(^{-}) (ppm)</th>
<th>C. parvum (oocyst/20L)</th>
<th>G. lamblia (cysts/20L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>0</td>
<td>51</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21.5%</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>97.2%</td>
<td>86.2%</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>0.15</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.92%</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0</td>
<td>19</td>
<td>4</td>
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<td></td>
<td></td>
<td></td>
<td>78.9%</td>
<td>86%</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>0</td>
<td>72</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>98.6%</td>
<td>94.3%</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>0</td>
<td>29</td>
<td>0</td>
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<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>95.1%</td>
</tr>
<tr>
<td>7</td>
<td>4.5</td>
<td>0.15</td>
<td>12</td>
<td>4</td>
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<td></td>
<td></td>
<td></td>
<td>66%</td>
<td>72%</td>
</tr>
<tr>
<td>8</td>
<td>8.5</td>
<td>0.15</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>100%</td>
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<tr>
<td>9</td>
<td>30</td>
<td>0.15</td>
<td>52</td>
<td>52</td>
</tr>
</tbody>
</table>

Table 1 | Results of the disinfection efficiency (in %) given below the count number for *C. parvum* and *G. lamblia* in the different experiments. The data correspond to the average of two experiments showing similar results. Photocatalytic conditions: 500 L/h; Volume system = 150 L; UV mercury lamp (254 nm, 40 W)
therein positive count is considered when one or more sporozoites are observed by microscope (DAPI stain). *G. lamblia* has four nuclei and in this case a positive count requires the observation of at least one nucleus by microscope (DAPI stain).

**RESULTS AND DISCUSSION**

The preparation and characteristics of the titania containing silica fibers have been reported elsewhere ([Toshihiro et al. 2002](#)). Basically this photocatalyst is prepared by thermal treatment of a mixture of polycarbosilane and titanium butoxide (50% weight) at temperature of 1,200°C. Under these conditions the organic components pyrolyzed, giving rise of the formation of silica and titania phases and at this temperature titania domains migrate towards the surface of the fiber (see Figure 1) making a titania enriched functional layer of a maximum thickness of 500 nm. This exposed titania phase photocatalytically active is accessible to substrates and microorganism present in water. The most important property of these fibers is the complete absence of leaching and migration of the titania particles from the solid to the solution. The absence of leaching is very important to ensure the stability and durability of the photocatalyst under continuous flow. These fibers, approximately of 7 µm diameter can be compressed to form porous mats that allow mass transfer from one side to the other of the photocatalyst fabric.

The mechanical resistance and the hardness on this fabric allows the design of a cylindrical photoreactor in which a 254 nm low pressure mercury lamp located at the cylinder axis illuminates the internal face of the TiO₂ containing fiber. The water flows from the central part of the cylindrical photoreactor to the external part of the cylinder crossing the illuminated photocatalyst. Figure 2 shows a schematic of the system used. The experiments were conducted at a flow of 500 L/h under atmospheric pressure giving a residence time in the photoreactor of 0.63 min per cycle. The system allows recirculation of the water through the photoreactor. For the experiments, potable water from the public network free from *C. parvum* and *G. lamblia* was dechlorinated and then purposely contaminated simultaneously with these protozoa. It is very common in real waters ([Bukhari et al. 1997; Hancock et al. 1998](#)) to have simultaneously more than one microorganism of which one could be more sensitive to disinfection than others. For the present study and in order to mimic more closely real conditions we added to the water the two protozoa. Photocatalytic disinfection tests combine simultaneously counts for oocysts and cysts of *C. parvum* and *G. lamblia*, respectively. This procedure was found the most convenient because the population of *C. parvum* and *G. lamblia* can be determined simultaneously ([EPA method 1623](#)) by counting the oocysts/cysts of these two protozoa by epifluorescence in a microscope (Figure 3 and 4). Contamination was accomplished by mixing standard commercial vials of the corresponding protozoa into the tank. The initial protozoa population was determined by taking a sample before switching the photoreactor on.

The resulting water contaminated with *C. parvum* and *G. lamblia* was stirred and recirculated for a sufficiently long time to ensure good dispersion of protozoa in the system. With this type of contaminated samples we performed four series of disinfection test using: 1) exclusively UV light (low pressure mercury lamp, 254 nm); 2) UV light (same source as before) and UBE photocatalyst, 3) UV...
light and low concentration of hypochlorite and 4) UV light (254 nm), UBE photocatalyst and a low concentration of hypochlorite. The reasons to perform this series of experiments were because, on one hand, we wanted to have a blank control in the absence of any photocatalyst to determine the percentage of UV light disinfection that will be overimposed on the photocatalytic effect. On the other hand, we also wanted to establish whether the presence of a small concentration of chlorine typically present in water enhances the efficiency of the photocatalytic process. An additional reason why it is of interest to perform the photocatalytic study in the presence of chemical disinfection agents is because most legal regulations oblige water to contain a minimum concentration of any of these disinfection agents, preferably chlorine. We have also made a control in the dark when the disinfection agent is present.

The results obtained in the photocatalytic study of the samples contaminated by C. parvum and G. lamblia are summarized in Table 1.

From the results presented in Table 1, it can be seen that UV irradiation in the absence of photocatalyst is very inefficient to promote at short irradiation times a measurable percentage of reduction in the number of counts. Our results are in line with well established literature data that shows the low efficiency of UV light to effect the disinfection of C. parvum and G. lamblia (Morita et al. 2002). As a matter of fact, we selected these two protozoa for the present study because we wanted to demonstrate the activity of UBE photocatalyst for disinfection of the most reluctant microorganisms. Only when the irradiation time was sufficiently long (entry 2 in Table 1), a significant decrease in the population of the two protozoa was observed. Even at these long irradiation times, G. lamblia was still remarkably resistant and about 14% of the initial population survives to this treatment. Considering that increasing the UV light exposure time to longer than 30 min will not make viable the photochemical disinfection method, no further experiments were pursued.

In contrast to the low activity of UV light to effect disinfection, Table 1 shows that the photochemical process implemented with UBE photocatalyst becomes considerably more efficient. As it could be easily anticipated, the percentage reduction of the number of counts increases gradually along the time of exposure. Thus, the percentage of population reduction using UV light combined with UBE photocatalyst becomes already high at 10 min exposure, becoming almost complete at 18 min (Table 1 entry 5). Particularly notable is the efficiency with respect to G. lamblia that appears to be the most resistant microorganism to the UV light. As commented previously for the UV irradiations illumination times longer than 30 min were not considered based on its lack of industrial viability.

Thinking in real applications of the photocatalytic system and considering that the water to be treated should contain some hypochlorite due to the current legal regulations (EU. 1998, it was of interest to test the biocide activity of the photochemical reactor implemented with the fibrous ceramic photocatalyst for contaminated water containing a small percentage of hypochlorite. The idea was to determine the influence that the presence of chlorine, even in small concentrations, plays promoting the photocatalytic disinfection.

As it can be seen in Table 1, we have found a positive synergism between hypochlorite and photocatalysis. Thus, even at very short exposure times (entry 7 in Table 1), the population reduction was significant and it was complete for less than 9 min of irradiation (entry 8 in Table 1). These results compare very favourably with the situation in which hypochlorite was absent. In this regard, a few comments should be made. First, the two protozoa under study are known to be highly resistant to disinfection by chlorine alone (Betancourt & Rose 2004) and no biocide activity occurs at the low hypochlorite concentrations tested as it was proved by a blank control (see Table 1, entry 9). The second comment is that, a blank control in which UV light was employed for disinfection of a solution containing 0.15 ppm hypochlorite was notable inefficient to effect population reduction of the two protozoa. These results point out again the role played by the titania photocatalyst in the disinfection. The third comment refers to the practicality of the system under the conditions employed in Table 1, entries 7 and 8. In fact, typically high hypochlorite concentration, in the 10 ppm range, is used for waters suspicious to contain C. parvum and G. lamblia. This high hypochlorite concentration causes adverse effects and nuisance in humans in contact with these waters containing high hypochlorite concentrations. One example of this is swimming pool waters (Hamence 1980; Scotte 1984).
in which over-chlorination is a common practice to minimize sanitary problems. In these cases, the use of the photocatalytic system described here will be highly valuable because it could allow using very low chlorine concentrations while ensuring the absence of bacteria and other noxious microorganisms.

CONCLUSIONS

In summary, the present results show that a UV photo-reactor implemented with a fibrous ceramic TiO₂ is highly efficient to promote the continuous flow water disinfection of resistant germs as *Cryptosporidium parvum* and *Giardia lamblia*. The action of this photocatalytic system is remarkably enhanced by the presence of hypochlorite in a very low concentration. This system has practical use for public recreational aquatic resorts having high biological contamination risk.

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