Evaluation of the Solar Water Disinfection Process (SODIS) Against *Cryptosporidium parvum* Using a 25-L Static Solar Reactor Fitted with a Compound Parabolic Collector (CPC)

María Fontán-Sainz, Hipólito Gómez-Couso, Pilar Fernández-Ibáñez, and Elvira Ares-Mazás*

Laboratorio de Parasitología, Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, A Coruña, Spain; Plataforma Solar de Almería-Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Tabernas, Almería, Spain

Abstract. Water samples of 0, 5, and 30 nephelometric turbidity units (NTU) spiked with *Cryptosporidium parvum* oocysts were exposed to natural sunlight using a 25-L static solar reactor fitted with a compound parabolic collector (CPC). The global oocyst viability was calculated by the evaluation of the inclusion/exclusion of the fluorogenic vital dye propidium iodide and the spontaneous excystation. After an exposure time of 8 hours, the global oocyst viabilities were $21.8 \pm 3.1\%$, $31.3 \pm 12.9\%$, and $45.0 \pm 10.0\%$ for turbidity levels of 0, 5, and 30 NTU, respectively, and these values were significantly lower ($P < 0.05$) than the initial global viability of the isolate ($92.1 \pm 0.9\%$). The 25-L static solar reactor that was evaluated can be an alternative system to the conventional solar water disinfection process for improving the microbiological quality of drinking water on a household level, and moreover, it enables treatment of larger volumes of water (> 10 times).

INTRODUCTION

The majority of people living in developing countries, especially among rural populations, lack the resources required to gain access to improved sources of drinking water. The lack of water suitable for human consumption is directly related to poverty. At the beginning of this century, the World Health Organization (WHO) reported that approximately one-sixth of the world's population did not have access to clean drinking water, and as a consequence, waterborne diseases constitute one of the most important public health problems in these countries.^{1–3}

Infectious diarrheal illnesses are included in the category of waterborne diseases that cause the highest rates of morbidity and mortality, and it is estimated that 4 billion cases of such diseases and 1.8 million associated deaths occur annually. Diarrhea particularly affects the infant population, and in addition to the high mortality rate (it is estimated that 6,000 children under the age of 5 years every day), it causes malnutrition, with the subsequent repercussions on physical development and susceptibility to other infections.^{3,4}

In geographical areas that receive abundant solar radiation, solar disinfection of drinking water (solar water disinfection process [SODIS]) is an economical and viable alternative for disinfecting water on a small scale. The method consists of filling transparent plastic (polyethylene terephthalate [PET]) bottles with contaminated water and exposing the bottles to the sun for at least 6 hours before consumption of the water.⁵ Numerous studies in the last 15 years have shown the effectiveness of simulated and natural SODIS on the inactivation of viruses, bacteria, fungi, protozoan, and helminth parasites. Among the microorganisms tested are Poliovirus Type II, *Bacillus subtilis*, *Campylobacter jejuni*, *Escherichia coli*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Salmonella thyphimurium*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Candida albicans*, *Fusarium solani*, *Acanthamoeba* spp., *Cryptosporidium parvum*, *Entamoeba invadens*, *Naegleria gruberi*, *Giardia* spp., and *Ascaris suum*.^{6–12} Moreover, studies of the impact on health

of the use of SODIS have shown a significant decrease in the risk of diarrheal diseases among users of the technique (26–75%).^{13–17} Thus, SODIS effectively improves the microbial quality of drinking water for preventing diarrhea. At present, SODIS is one of the recommended methods for disinfection of household drinking water.³ However, there are a number of limitations associated with the SODIS method. It is recommended that (1) the volume of water to be treated should not be more than 2 L; (2) under cloudy conditions, the bottles should be exposed for 2 consecutive days to solar radiation, and in rainy weather, alternative disinfection methods should be used; and (3) the turbidity of the water should not be more than 30 nephelometric turbidity units (NTU).^{5,16,18} Furthermore, only the upper part of the PET bottles is illuminated during exposure to the sun, and therefore, a large fraction of the incident solar radiation does not reach the water in the bottles. Several attempts have been made in recent years to concentrate the radiation by using reflective surfaces.^{19,20} Compound parabolic concentrators (CPCs) are static collectors that concentrate all of the incident solar radiation below a certain angle; therefore, the radiation reflected by the surface of the collectors also reaches the lower part of the reactor, and the entire surface is irradiated almost homogeneously.

Protozoan parasites of the genus *Cryptosporidium* are ubiquitous and significant enteropathogens for a wide range of vertebrates, including humans. They are the cause of the gastrointestinal disease cryptosporidiosis, the main symptom of which is watery diarrhea. Although the disease is normally self-limiting in immunocompetent individuals, persistent infection has been associated with severe and chronic disease in immunocompromised subjects. The infectious form (oocyst) is highly resistant to a wide range of environmental conditions and the chemical agents normally used to disinfect water, and it survives for several days or even months in aquatic environments. Transmission can occur by the fecal/oral route or ingestion of contaminated food or water (the latter serves as a particularly good vehicle of transmission). *Cryptosporidium* has been identified as the cause of the numerous outbreaks of waterborne diseases that affect hundreds or thousands of individuals in developed countries.²¹

In a previous study, we showed the efficacy of the SODIS method in turbid waters experimentally contaminated with *C. parvum* oocysts using PET bottles.²² The aim of present

*Address correspondence to Elvira Ares-Mazás, Laboratorio de Parasitología, Facultad de Farmacia, Campus Universitario Sur, 15782 Santiago de Compostela, A Coruña, Spain. E-mail: melvira.ares@usc.es

study was to evaluate the efficacy of the SODIS process against *C. parvum* using a 25-L static reactor fitted with a CPC as an improvement of the conventional SODIS method.

MATERIALS AND METHODS

***C. parvum*.** *Cryptosporidium* oocysts were collected from a naturally infected neonatal Friesian–Holstein calf by rectal sampling. Concentration (phosphate-buffered saline [PBS], pH 7.2/diethyl ether), purification (discontinuous cesium chloride gradients), and quantification (Neubauer hemocytometer) were performed as previously reported.^{23,24} The oocysts were classified as *C. parvum* by analysis of a fragment of the *Cryptosporidium* oocyst wall protein (COWP) gene.²⁵

Preparation of turbid water samples. Red soil, with a composition that is very similar to the soils in tropical areas,²⁶ was collected close to the Michelin Test Field in Almería, Spain. Turbid water samples were prepared by the addition of the soil to distilled water to achieve the required turbidity levels of 5 and 30 NTU, which was measured with a TN-100 turbidimeter (Eutech Instruments Pte. Ltd., Singapore). Briefly, 0.3 and 3.2 g soil were added to 500 mL distilled water to obtain turbidity levels of 5 and 30 NTU, respectively. The suspensions were shaken for 30 minutes and then allowed to settle for 1 hour. The supernatants were collected, and the turbidity was adjusted to the required level with distilled water. The suspensions were then sterilized by autoclaving for 20 minutes at 120 psi and stored at 4–8°C.²² The turbid water samples were analyzed by the Department of Edaphology and Agricultural Chemistry of the Faculty of Pharmacy of the University of Santiago de Compostela, and chemical properties are summarized in Table 1.

Static SODIS reactor. The SODIS reactor was constructed by placing a methacrylate plastic tube along the length of a north–south-oriented CPC mirror, which was fixed to a metal frame inclined at 37° (equal to the local latitude to recover maximum ultraviolet A [UVA] radiation). The methacrylate tube had an outlet valve at the bottom (for taking samples during the experiments and emptying the disinfection unit after use) and a removable methacrylate port at the top for filling the reactor. The CPC reflector was made of highly reflective anodized aluminum sheeting (MIROSUN® Aluminium GmbH, Ennepetal, Germany) with a concentration factor (CF) of 1 (CF = 1). The reflectivity of the aluminum sheets was 87% for UVA and 90% for the visible and infrared regions of the solar spectrum. The aperture area of the CPC mirror was 0.58 m²; the irradiated length was 92.5 cm, and the irradiated width was 62.5 cm. The length of the tube was 1 m, the external diameter was 20 cm, the thickness was 1 cm, the total volume was 25 L, and the treated volume was 22.5 L (Figure 1).²⁷

Experimental design. All experiments were performed under natural solar radiation at the Plataforma Solar de

FIGURE 1. Photograph showing the 25-L static solar reactor equipped with a CPC filled with a sample of water with a turbidity level of 30 NTU experimentally contaminated with purified *C. parvum* oocysts and exposed to natural solar radiation at the facilities of the Plataforma Solar de Almería.

Almería (PSA) located in the Tabernas Desert (Almería, Spain: latitude, 37°05'54" N; longitude, 2°21'32" W; altitude, 500 m) in June and July of 2009. Water samples with levels of turbidity of 0, 5, and 30 NTU were placed in 30-L containers and spiked with 2.5×10^6 oocysts/L, 6×10^6 oocysts/L, and 10×10^6 oocysts/L of the purified isolate of *C. parvum*, respectively. The samples were shaken for 10–30 seconds, and the reactor was filled with the corresponding sample. The samples were exposed for a maximum time of 8 hours (10:00–18:00 hours local time). During the experiments, the water temperature was monitored every 1 hour with a thermometer (model HI 98509-1; Hanna Instruments, SL, Eibar, Spain). Two samples (50 mL each) were collected from the center of the reactor after 2, 4, 6, and 8 hours, and they were replaced with an equal volume of non-contaminated water with the same level of turbidity and maintained at ambient temperature. The samples were centrifuged at $3,000 \times g$ for 15 minutes, and the sediment obtained was used to evaluate the oocyst viability, which is described below. All tests were performed in duplicate.

Measurement of radiation. Global solar exposure was measured with a pyranometer (model CMP 21; Kipp & Zonen, Delft, The Netherlands) for short-wave global solar radiation measurements in the spectral range from 310 to 2,800 nm during the experiments. Incoming UV radiation (direct plus diffuse radiation from all directions) was measured at between 295 and 385 nm (part of UVA and UVB) with a horizontally placed global UV radiometer (model CUV 3; Kipp & Zonen).

TABLE 1

Analysis of the water samples of different levels of turbidity prepared with the red soil collected in Almería (Spain)

NTU	pH*	DOC† (mg/L)	P-CO ₃ H ⁻ (mg/L)*	P _{inorganic} -CO ₃ H ⁻ (mg/L)*	P _{organic} -CO ₃ H ⁻ (mg/L)*
5	7.01	0.7	0.004	0.000	0.004
30	7.11	3.5	0.017	0.003	0.012

*Parameters were determined in filtered samples.

†Dissolved organic carbon.

Viability assays. The potential viability of *C. parvum* oocysts was determined by inclusion/exclusion of the fluorogenic vital dye propidium iodide (PI; Sigma, Madrid, Spain) and a further modification that includes an immunofluorescence antibody test to verify oocyst identification.^{28,29} Briefly, sediments obtained from the samples were resuspended in 100 μ L Hanks' balanced salt solution (Sigma) and then incubated with 10 μ L PI working solution (1 mg/mL in 0.1 M PBS, pH 7.2) at 37°C for 10 minutes. After PI staining, oocysts were washed two times in PBS at 10,000 $\times$ g at 4°C for 5 minutes and incubated with 30 μ L monoclonal antibodies labeled with fluorescein isothiocyanate (FITC; Aqua-Glo G/C Direct; Waterborne, Inc., New Orleans, LA). Oocysts were first identified under an FITC filter (excitation at 450–480 nm; barrier at 515 nm) before being examined for PI inclusion/exclusion under a PI filter (excitation at 510–550 nm; barrier at 590 nm). The proportions of ruptured (ghost), PI-positive (dead), and PI-negative (viable) oocysts were quantified in an epifluorescence microscope equipped with phase contrast optics, FITC, and PI filters (Eclipse 50i; Nikon Corporation, Tokyo, Japan). The results are shown as the percentage of oocysts that included/excluded the PI dye, which were obtained for each experiment after triplicate counts of more than 100 oocysts.

Taking into account that only intact oocysts were considered in the assays involving inclusion/exclusion of the vital dye PI and that totally or partially spontaneous excysted oocysts are not viable, the global viability of the isolate was calculated as:³⁰

$$\text{Global viability (\%)} = \frac{\text{intact oocysts (\%)} \times \text{PI negative oocysts (\%)}}{100}$$

Statistical analysis. Differences in the percentage of PI-negative oocysts, percentage of totally or partially spontaneous excysted oocysts, global viability, and temperatures reached within the reactor for different turbidity levels and exposure times were compared by pairwise multiple comparison procedures (Student–Newman–Keuls method) and one-way analysis of variance (ANOVA) with GraphPad InStat, version 3.05 statistical software (1992–2000 GraphPad Software, La Jolla, CA). Differences were considered significant at $P < 0.05$.

RESULTS

The days during which the assays were carried out were sunny, with some cloud cover. The mean values for accumulated global and UV irradiances (between 10:00 and 18:00 hours local time) were 36.0 ± 5.0 MJ/m² and $1,370.0 \pm 120.0$ kJ/m², respectively. The maximum values for global and UV radiation were 1,278.0 and 48.2 W/m², respectively (Figure 2). The ambient temperature ranged between a minimum of 20.0°C and a maximum of 39.0°C. The mean temperatures registered inside the reactor and monitored every 1 hour are shown in Figure 3. The temperatures were significantly higher in water samples with turbidity of 30 NTU than in water samples with turbidity levels of 0 and 5 NTU ($P < 0.05$) after 4 hours of exposure until the end of the assays. The maximum temperatures reached in water samples with turbidity levels of 0, 5, and 30 NTU were 52.6°C, 52.5°C, and 57.5°C, respectively, all of which were recorded after 7 hours of exposure (17:00 hours local time).

FIGURE 2. Graphic representation of the mean values of global and UV radiations registered during the solar disinfection studies with the 25-L static solar reactor equipped with a CPC.

The *C. parvum* isolate displayed an initial global viability of $92.1 \pm 0.9\%$ (PI-negative oocysts: $95.4 \pm 0.8\%$; spontaneous excystation: $3.4 \pm 1.8\%$). The percentages of PI-negative oocysts, spontaneous excystation, and global viability obtained in water samples with turbidity levels of 0, 5, and 30 NTU are shown in Figure 4. There was a significant decrease in the percentage of PI-negative oocysts relative to the initial values for the isolate in water with a turbidity level of 0 NTU after 4 hours exposure and until the end of the assays ($P < 0.0001$). In water samples with turbidity levels of 5 and 30 NTU, there was also a significant decrease in the number of PI-negative oocysts ($P < 0.005$) but only after 6 hours exposure. At the maximum exposure time, there were no significant differences between the percentages of PI-negative oocysts in water samples with turbidity levels of 0 and 5 NTU, although there were significant differences between the values at these turbidity levels and those values detected in water samples with a turbidity level of 30 NTU ($P < 0.01$). Moreover, although there was an increase in the percentage of oocysts that underwent spontaneous excystation, the differences were only significant

FIGURE 3. Profiles of the mean temperatures of the water recorded within the 25-L static solar reactor equipped with a CPC containing water samples of different levels of turbidity.

FIGURE 4. Kinetics of inactivation of *C. parvum* oocysts in water samples with different levels of turbidity that were exposed to solar radiation in the 25-L static solar reactor equipped with a CPC.

in relation to the initial values of the isolate in water samples with a turbidity level of 30 NTU ($P < 0.05$).

Finally, there was a significant decrease in the overall viability of oocysts from 4 hours exposure in the water samples with the three levels of turbidity tested ($P < 0.001$), with a similar trend to the observed trend in the inclusion/exclusion of the vital fluorogenic dye PI. At the end of the assays, the global viability in water samples with turbidity levels of 0 and 5 NTU were $21.8 \pm 3.1\%$ (PI-negative oocysts: $24.1 \pm 4.0\%$; spontaneous excystation: $9.4 \pm 5.6\%$) and $31.3 \pm 12.9\%$ (PI-negative oocysts: $35.8 \pm 15.2\%$; spontaneous excystation: $12.3 \pm 4.6\%$), respectively, with no significant differences observed. In the

most turbid water samples, the global viability of the oocysts remained constant from 6 hours exposure, and at 8 hours exposure, it was $45.0 \pm 10.0\%$ (PI-negative oocysts: $57.4 \pm 8.1\%$; spontaneous excystation: $21.9 \pm 6.4\%$); the differences between these values and the global viability observed in water samples with a lower level of turbidity were significant ($P < 0.05$).

DISCUSSION

The results of the present study show that the viability (PI-negative oocysts) of the *C. parvum* in water samples with turbidity levels of 0 and 5 NTU after 8 hours exposure to natural sunlight were similar to those values obtained in a previous study carried out under similar conditions using PET bottles (24.1% versus 22.0% and 35.8% versus 36.7% for water samples with turbidity levels of 0 and 5 NTU, respectively),²² and therefore, the use of the 25-L static solar reactor equipped with a CPC can be a possible alternative to the conventional SODIS method for disinfecting drinking water on a household scale. It also enables treatment of larger volumes of water compared with PET bottles (more than 10 times the amount). Some authors raised the possibility that the volume of the reactor may affect the efficacy of the SODIS method (because the degree to which the radiation penetrates will depend on the diameter of the reactor) and that the changes in temperature are determined by the volume of the water column, although they did not find any significant differences in the dynamics of the inactivation of *E. coli* on exposure of 0.5 and 1.5 L contaminated water to solar light.³¹ In this respect, in a previous study carried out using the same 25-L static solar reactor equipped with a CPC, it was shown that turbid waters contaminated with *E. coli* K-12 were disinfected in 7 hours with water temperatures higher than 50°C . Moreover, no regrowth of bacteria occurred within 24–48 hours of solar disinfection.²⁷

It is recognized that the turbidity of the water restricts the efficacy of the SODIS technique, because it attenuates the penetration of UVA radiation.⁵ This effect was observed when water samples with turbidity levels of 0, 5, 100, and 300 NTU experimentally contaminated with *C. parvum* were exposed to the solar radiation in PET bottles under field conditions.²² Likewise, the results obtained in the present study with the 25-L static solar reactor showed that the turbidity had a negative effect on the efficacy of the process, because the oocyst viability was significantly higher in the most turbid water samples.

Furthermore, one important fact to take into consideration in the application of the SODIS method is the increase in the temperature of the water contained in the reactor as a result of infrared radiation. This thermal effect increases as the turbidity of the water increases.⁵ During the tests, the temperature in the interior of the reactor was registered every 1 hour, because this temperature is also a critical factor to determine the survival of the infective forms of *C. parvum* in the environment.³² Thus, the maximum temperatures were reached in the most turbid water samples (30 NTU), reaching as high as 57°C during the last 2 hours of the assay. In addition, in previous SODIS studies also carried out under field conditions, the phenomenon of spontaneous excystation was observed.²² In this phenomenon, a small percentage of the sporozoites of *C. parvum* may excyst when the oocysts are incubated at 37°C in the absence of any other stimulus, which makes their survival impossible because the environment is different from the

environment provided by the host.³³ In this respect, the highest percentages of spontaneous excystation were observed in the most turbid water samples. However, the global viability values were higher than in water samples with turbidity levels of 0 and 5 NTU.

Because the SODIS method is used under natural conditions, deterioration of the reflective surface material is a potential problem. In a study carried out with the aim of evaluating the effect of degradation of the mirror on the efficacy of the SODIS technique using borosilicate reactors on collectors that had been in use for 3 consecutive years, the authors did not find any significant decrease in the reflectivity of the mirrors.²⁰

However, taking into account that this technology is destined for use in countries with very little economic resources, the costs of the materials, their resistance to environmental conditions, and the maintenance required must all be considered. According to information supplied by the personnel involved in designing these collectors,²⁷ it is estimated that a prototype of the system would cost around US\$200, and assuming a lifespan of 10 years, treatment of 1 L water would cost approximately US\$0.002. The technology obviously involves a higher cost than the use of PET bottles or ceramic filters (< US\$0.001/L). However, it has the same cost per liter as the biosand filter system, and it is cheaper than the coagulant/chlorine system (PuR sachet), which costs > US\$0.01/L.^{3,27,34} In conclusion, the 25-L static solar reactor equipped with a CPC evaluated against *C. parvum* in water can be an alternative system to PET bottles in the improvement of the microbiological quality of drinking water on a household scale in developing regions, and moreover, it enables treatment of larger volumes of water (more than 10 times), although additional studies using other microorganisms are needed to complete the information about the effectiveness of the system.

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Authors' addresses: María Fontán-Sainz, Hipólito Gómez-Couso, and Elvira Ares-Mazás, Laboratorio de Parasitología, Facultad de Farmacia, Campus Universitario Sur, A Coruña, Spain, E-mails: maria.fontan@usc.es, hipolito.gomez@usc.es, and melvira.ares@usc.es. Pilar Fernández Ibáñez, Plataforma Solar de Almería-CIEMAT, Tabernas, Almería, Spain, E-mail: pilar.fernandez@psa.es.

Reprint requests: Elvira Ares-Mazás, Laboratorio de Parasitología, Facultad de Farmacia, Campus Vida, 15782 Santiago de Compostela, A Coruña, Spain, E-mail: melvira.ares@usc.es.

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