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Elimination of water pathogens with solar radiation using and automated sequential batch CPC Reactor

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Keywords: Solar disinfection; compound parabolic collector (CPC); *E. coli*.

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Abstract

Solar disinfection (SODIS) of water is well known, effective process which is practiced at household level in many developing countries. However, this process is limited by the small volume treated and the lack of indication of treatment efficacy. Low cost glass tube reactors together with compound parabolic collectors (CPC) technology, has been shown to significantly increase the efficiency of solar disinfection. However, these reactors still require user input to control each batch SODIS process and there is no feedback that the process is complete. Automatic operation of the batch SODIS process, controlled by UVA-radiation sensors, can provide information on the status of the process can ensure the required UVA dose to achieve complete disinfection received and reduces user work-load through automatic sequential batch processing. In this work, an enhanced CPC photo-reactor with a concentration factor of 1.89 was developed. The apparatus was fully automated to allow exposure to a threshold UVA dose, with treated water subsequently dispensed into a reservoir. The reactor was tested using *Escherichia coli* as a model pathogen in natural well water. A 6-log inactivation of *E. coli* was achieved following exposure to the minimum uninterrupted lethal UVA dose. The enhanced reactor decreased the exposure time required to achieve the lethal UVA dose, in comparison to a CPC system with a concentration factor of 1.0. Doubling the lethal UVA dose prevented the need for a period of post exposure dark inactivation and therefore significantly reduced the overall SODIS treatment time. Using this reactor, SODIS can be automatically carried out at an affordable cost, with reduced exposure time and minimal user input.

Keywords: Solar disinfection, *Escherichia coli*, photoreactor, compound parabolic collector.

1. Introduction

Lack of access to a reliable and safe source of potable water is a significant problem in developing countries. Each year, there are approximately 4 billion cases of diarrhoea resulting in an estimated 1.8 million fatalities. Every day approximately 4500 children die of dehydration due to diarrhoea [1]. Water treatment processes which are robust, easy to use and low cost could be readily deployed for point-of-use and may also find application in emergencies situations where access to safe potable water is a primary concern.

Solar disinfection (SODIS) is a water treatment method suitable for use at household level. Normally SODIS is carried out by placing water in transparent containers (usually ≤ 2 L plastic bottles) and exposing to sunlight (≥ 6 h) [2, 3]. The synergistic effect of mild thermal heating and solar UV radiation is responsible for the inactivation of pathogens in the water. The inactivation rate depends on temperatures reached during the process and also on the type of microorganism present in the water [4,5]. This SODIS practice has significant limitations which include, a) the recommended time for SODIS treatment is six hours in full sunshine or, two consecutive days in cloudy conditions; b) the volume of water treated is small, typically 1.5 to 2 L in bottles; and c) the user has no feedback indicating treatment efficacy or completion.

SODIS in glass tube photo-reactors (with and without photocatalyst), incorporating compound parabolic collectors (CPC's), has been shown to be effective for the inactivation of a range of microorganisms, including bacteria (*E. coli*) and fungi (*Fusarium spp*) [6,7,8]. Our recent contribution shows a new low cost CPC SODIS reactor for purifying 25 L-batches of untreated water. This concept is based on CPC enhancement, low cost materials and increased volume of treated water. This system was tested for six months under natural sunlight and was demonstrated to be efficient against *E. coli* [9].

Even with improvements in reactor efficiency, the SODIS process is both dependant upon, and controlled by users i.e., a person must check that treatment is carried out under recommended operational conditions for the minimum treatment time of 6 h. For example, the user must pay attention to the local weather, note the exposure time and trust that process will improve the microbiological safety of the treated water. These limitations may contribute to low levels of compliance in the use of SODIS. As the treatment time is completely dependent

on the ambient solar irradiance there is a need for measurement of the UVA dose to simply indicate treatment completion, or preferably, provide feedback control loop that controls the process. The UVA dose can be calculated as follows:

$$Dose(J\ m^{-2}) = \int UVA(W\ m^{-2}) \cdot dt(s) \cdot C \quad (\text{Eq. 1})$$

Where, UVA is the solar irradiance (320 - 400 nm) incident upon the reactor; dt is the exposure time; and C is the concentration factor of the mirror [6]. C is a dimensionless number that defines the multiplication factor by which sunlight is concentrated at the absorber/receiver. In this case, the absorber is the glass tube of the photo-reactor.

We have recently demonstrated that SODIS relies upon the receipt of a minimum and uninterrupted UVA dose, defined as the “lethal UVA dose”. For 10^6 CFU mL^{-1} of *E. coli* K-12 in 2.5 L of well-water in a CPC reactor with $C = 1$, this dose was found to be $\geq 108\ \text{kJ/m}^2$. The lethal dose depends on the total amount of water treated per batch. This means that the amount of solar UVA energy per unit of volume that has to be delivered uninterruptedly into the system is $8.6\ \text{kJ/L}$ (where we considered the irradiated collector surface, $0.2\ \text{m}^2$; and the total volume, 2.5L). This lethal dose also depends microbiological contamination and on the physical and chemical properties of the water. For example, and like for all water treatments, the more resistant is the microorganism the more amount of energy will be required to disinfect the water. And for this reason, it must be experimentally determined for very different real water sources like river water, underground water or rain water. The lethal UVA dose was also demonstrated to be independent on UVA irradiance, for solar UVA irradiance between 14 and $40\ \text{Wm}^{-2}$ [10]. CPC enhanced SODIS reduced the time needed for complete inactivation (until detection limit) of bacteria on both cloudy and sunny days. However, following receipt of the lethal UVA dose, a period of approximately 2 h post-exposure was necessary before complete disinfection (i.e. 6-log unit reduction) [10]. For example, a 3-log kill was observed if the water was tested immediately following the lethal dose (1 h in sunny conditions), but a 6-log kill was observed if the water was left to stand for 2 h following exposure, before being tested. Therefore, the total treatment time for a 6-log kill was 3 h.

In an attempt to address the practical problems associated with SODIS, a novel sequential batch photo-reactor was designed with the aim of decreasing the treatment time required and

reduces user-dependency. The new photoreactor incorporated two major improvements over traditional CPC photo-reactors. Firstly, to reduce the solar exposure time required to receive the lethal UVA dose, the C of the CPC was increased from 1.00 to 1.89, i.e. the glass tube receives almost twice the quantity of UV solar radiation in comparison to a $C = 1$ CPC system. Secondly, the treatment time was automatically controlled by an electronic UVA sensor. The feedback sensor system controlled the gravity-filling of the reactor from an untreated water reservoir, and controlled the discharge of the treated water into a clean reservoir following receipt of the pre-defined UVA dose. The full sequence was then automatically repeated for as many times as permitted by the solar UVA intensity during daylight hours. The reactor was tested using *E. coli* as the model pathogen in well water under real sun conditions.

2. Materials and methods

2.1 Sequential batch photo-reactor

The sequential batch photo-reactor consisted of a glass tube positioned at the focus of a CPC mirror; two 25 L reservoir tanks (the untreated water tank (UWT) and the treated water tank (TWT)) and a control system consisting of a UVA photodiode, electronic valves to control fluid flow and the necessary hardware/software to automate the device (figure 1). The electronic control system measured the solar intensity and calculated the solar UVA dose. When the pre-programmed dose had been acquired, a series of electronic valves opened to dispense the treated water into the TWT. The tube was subsequently refilled from the UWT and the treatment cycle automatically re-started. The system also included water level sensors in the UWT and TWT. These sensors were incorporated to stop the cycle if the UWT level was too low or the TWT level was too high.

The photoreactor tube (1.50 m length, 0.05 m outer diameter, 1.8 mm wall thickness, and 2.5 L illuminated volume) was made of borosilicate glass (Schott-Duran, Germany). The glass had a transmittance of 89-90% in the UVA range. The tube was sealed with PTFE (Polytetrafluoroethylene) end caps connected to two electronic valves (Betavalve, UK), which were regulated by the control system.

The CPC mirrors were made from highly reflective aluminium sheets (type 320G ALANOD anodized aluminium of 0.5 mm thickness, Alanod Aluminium GmbH, Ennepetal, Germany).

The manufacturer reports a reflectivity of 82% for the UV and 85% for the rest of the solar spectrum. CPC mirrors with $C = 1.00$ and $C = 1.89$ were used in these experiments.

A major advantage of CPC systems is that the concentration factor remains constant for all values of sun zenith angle within the acceptance angle limit, whereas conventional parabolas or flat mirrors require sun tracking to maintain the same concentration factor. On the other hand, CPC mirrors requires almost 2 to 4 times the reflective area of a conventional parabola. Due to the inherent characteristics of non-imaging optics used by CPC reflectors, the area of reflectors can be truncated to almost 50% of their actual length with a loss of less than 10% in the concentration ratio [114]. In this way, the total reflector area is reduced to half it's original length and there is very little loss in radiation concentration.

For the case of $C=1$, the acceptance angle is $\theta_c=90^\circ$ and the result is an involute reflector with an aperture width of 15.70 cm which is shown in figure 2(a). For the case of $C=1.89$, the acceptance angle is $\theta_c=30^\circ$. Replacing this value in eq (2) yields $C=2$ and a total reflector height of 36.13 cm and 31.4 cm of aperture width. For the sake of easy manufacturing and to lower reflector area, the CPC was truncated to almost half it's height, yielding a height of 19.37 cm, an aperture width of 29.70 cm and $C=1.89$, as can be seen in figure 2(b). Hence, reducing the mirror area by nearly 50%, diminished the concentration factor by only 5%.

As mentioned earlier, only sun rays with an incidence angle lower than the acceptance angle will be useful for concentration purposes. In the case where $C=1$, $\theta_c=90^\circ$, and the concentrator accepts all sun rays from sunrise till sunset. In the case where $C=1.89$, only sunrays with $\theta < 30^\circ$ will be accepted. For the fixed and inclined system used in this work, such values of incidence angles can be obtained approximately ± 2 h from solar noon, yielding approximately, between 4 and 7 h of useful concentrated sunlight in different seasons of the year. In the case of fixed systems (non-tracking) equipped with CPC mirrors, the available hours of sun within the acceptance angle diminishes as the concentration factor rises. The mathematical relationship between the available hours of sunshine and the acceptance angle of CPC concentrators are beyond the scope of this work and are thoroughly explained in Rabl 1976 [124].

Figure 3

The UVA dosage is determined only by exposure time (t , s) and irradiance (UVA, W m^{-2}), as explained in the introduction (eq. 1). The size of the reactor is affected by two design parameters: 1) concentrating factor of the solar mirror, and 2) total volume of treated water. In this study, we used two solar systems, one with a concentration factor of 1.0 and the other with 1.89. The total volume and irradiated volume in both reactors was the same. That means that UVA irradiance collected by the mirror and delivered to the water only depends on exposure time and concentration factor. The experiments were started at different local times so the system received different UVA dosages during irradiation.

2.2 Measurement of solar radiation

Solar UVA radiation was measured with a global UVA radiometer described elsewhere [9]. The radiometer had the same inclination as that of the platform where experiments were conducted.

UVA irradiance was measured outside the tube, but inside the reactor there are losses of photons due to absorption and scattering effects inside the reactor water containing molecules and bacteria. Quantification of efficient radiation inside the reactor cannot be taken into account easily, which is matter of an independent (theoretical and experimental) study. Nevertheless, our studies supporting the lethal dose concept are based on UVA dose measurements done also outside the tube with same type of well water (equal turbidity and bacterial load), therefore the correlation between UVA dose received and disinfection result, determined in our previous work [10], can be considered as valid for the present study.

Calibration of UVA control sensor within the sequential batch system

The UVA photodiode (TW30SX, Sg-lux, Germany) and control electronics were calibrated against a spectral radiometer (Gemini 180, Yobin Yvon, UK) using a 1 kW Xenon source fitted with AM1 filter. A linear response was observed within a UVA range of 5 to 60 W m^{-2} described by the following relationship: Output voltage (V) = $0.0069 \times \text{UVA irradiance (W m}^{-2}) + 0.0045$; $R^2 = 0.999$.

The sensor's response was also validated against global solar UVA radiation at PSA using the global UVA radiometer (295-385 nm, Model CUV3, Kipp & Zonen, Netherlands). Figure 4 shows the response observed during full sun (27th February 2008) and during cloudy weather (8th April 2008). In both weather conditions the response was accurate, however, when the

sun was at a low angle (early morning, late afternoon) shading of the sensor's active area by the diode casing occurred and the accuracy decreased slightly. Therefore, the sensor was calibrated between 11.00 and 16.00 hours local time.

2.3 Solar Disinfection Experiments

In a typical experiment, the UWT was filled with 25 L of well water inoculated with *E. coli* to give an initial bacterial loading of 10^6 colony forming units per mL (CFU mL⁻¹). The control cycle was initialised which filled the photoreactor with 2.5 L. Following exposure to the pre-defined UVA dose, the system automatically discharged the photoreactor into the TWT. Samples were taken from the UWT and the TWT for bacterial analysis. Water temperature and UVA irradiance were monitored during the experiments.

2.4 Well water

In order to simulate naturally contaminated water and to avoid osmotic stress on the bacteria, natural well-water was used for the experiments. Water was collected from a well situated on the PSA site at a depth of approximately 200 m. A single batch of well water (approximately 100 L) was withdrawn to ensure the same stock of water was used for all the experiments. Table 1 shows the values of water quality parameters of the well water. To preserve the chemical integrity of the well water it was not autoclaved before each experiment. The concentration of naturally occurring organisms was determined by plate count enumeration technique using both LB agar and Endo agar and was found to be less than the detectable limit (DL) of 4 CFU mL⁻¹. Turbidity measurements were performed using a turbidimeter (model 2100N, Hach, USA). For all experiments turbidity values between 1 and 2 NTU were obtained. Iron was not present in the water (UV-VIS measurements, DL 0.05 mg/L), however, a high concentration of HCO₃⁻, ~ 500 mg/L, was determined (5050A TOC analyser, Shimadzu, Japan). The ions present in the water were analyzed with ion chromatography (Dionex DX-600, USA). This well water has been used in previous solar disinfection research [7, 9, 10].

2.5 Bacterial strain and quantification

E. coli K12 (ATCC 23631) was generated and grown as described elsewhere [9]. All disinfection experiments were done in by adding bacterial stock to water in the solar photoreactor to obtain an initial concentration of 10^6 CFU mL⁻¹. Samples were taken at different time intervals over 4 or 5 h total experiment time. Samples were diluted in PBS (Phosphate

Buffer Solution), enumeration of bacteria was carried out using the standard plate count method. Volumes of 20 μL were plated onto LB agar plates, incubated at 37°C overnight and counted the following day. To determine the initial bacterial concentration in the reactor, a sample of water was taken for bacterial enumeration before the system was exposed to sunlight. This sample was maintained in the dark at laboratory temperature (25°C) for the duration of the solar exposure experiment (“no treatment control”) and the bacterial concentration re-determined as described above. Volumes of 250 μL of undiluted samples were plated when bacterial concentration was expected to be below 1 CFU per plate; therefore, the DL for this quantification method was 4 CFU mL^{-1} . Analysis for bacterial re-growth was undertaken for all experiments by leaving the last two samples taken from the reactor at room temperature for 24 h and 48 h. Bacterial concentration was determined using the plate count method described above with samples plated onto both LB agar and Endo agar (Sigma-Aldrich, USA) plates with samples taken after 24 and 48 h. All experiments were conducted in triplicate, and each bacterial sample was plated in triplicate.

Statistical data analysis was done as described in ref. [9]. Data points in figures represent the average of data analysis and the error bars show the standard deviation.

3. Results and discussion

3.1 Comparison of SODIS in CPC 1.00 and CPC 1.89

SODIS experiments using the CPC photo-reactor equipped with either $C = 1.00$ or $C = 1.89$ were carried out under real sunlight conditions using 2.5 L of well water containing $1 \times 10^6 \text{ CFU mL}^{-1}$ *E. coli*. The reactor with CPC 1 was exposed to sunlight at 10.30-12.30 local time receiving 229 kJ m^{-2} of solar UVA; and CPC 1.89 was exposed at 12:00-13:00 to achieve 245 kJ m^{-2} of UVA dose. Both were covered after exposure to examine post-treatment inactivation in the dark. Samples (10 mL) were taken at regular intervals for bacterial analysis during SODIS treatment and also during the post-exposure period.

In the $C = 1.00$ CPC (Figure 5a) a 3 log kill was observed after 60 min exposure, and complete bacterial inactivation (until detection limit) was achieved after 2 h exposure. Therefore, the total treatment time to achieve a 6-log inactivation was 2 h. For $C = 1.89$ CPC a 6 log kill was observed after 60 min exposure and during the dark period bacterial regrowth

was not detected (Figure 5b). As expected, the total treatment time observed for CPC 1 was halved when the CPC 1.89 was used, i.e. the time required to receive a similar UVA dose in the CPC 1 is almost twice the time needed for CPC 1.89. Therefore, the system with a CPC = 1.89 will permit treatment of double the volume of water in comparison to that of with a CPC = 1.

Figure 5 ('a' and 'b').

It is considered that photolytic bacterial inactivation proceeds via photon damage followed by subsequent reactions leading to cell death [10]. The sequence of disruption to normal bacterial cell function during solar disinfection has been described by Berney et al. (2006) [13]. One of the important effects observed during irradiation of cells is the damage of DNA, where interaction with UV-radiation produces cyclobutane dipyrimidine dimers preventing mRNA translation and cell reproduction. Bacteria have evolved a number of defence mechanisms and can initiate a complex enzyme system to repair genetic damage [14]. Bohrerova and Linden examined the DNA photo-repair rate of *E. coli* during exposure to four different fluorescent lamps and natural sunlight [15]. During studies using fluorescent lamps photo-repair was observed, however, they concluded that the initiation of the photo-repair process in *E. coli* did not take place above a critical level of exposure to solar radiation [15]. Recent contribution of Bosshard et al. showed that the first targets on the way to cell death were found to be the respiratory chain and even the the cells' potential to generate ATP were inhibited [16].

3.2 Increasing the lethal UVA dose

Our previous results [10] demonstrated that “an uninterrupted minimum lethal UVA dose” of 108 kJ m^{-2} , was necessary to disinfect 2.5 L of well-water polluted with *E. coli* K-12 (initial concentration $\sim 10^6 \text{ CFU mL}^{-1}$) in the $C = 1.00$ solar CPC reactor. Nevertheless, we observed a 3-4 log kill during solar exposure and complete inactivation 2 h after treatment when the reactor was kept in the dark. A similar result was observed in Figure 6(a), where the CPC 1.89 system received 108 kJ m^{-2} (corresponding to 35 min of solar exposure). This graph shows a 2 log decrease under illumination with complete disinfection attained following 2 h of dark treatment. Treatment following receipt of the minimum lethal UVA dose therefore resulted in a total batch treatment time of 2 h and 35 min for 2.5 L of water.

In order to remove the need for a dark inactivation period, and allow faster batch processing, the solar exposure can be lengthened thereby increasing the UVA dose. The effect of increasing the UVA dose upon the total treatment time required for complete disinfection was investigated in the CPC 1.89 photo-reactor.

Complete disinfection (3×10^6 CFU mL⁻¹ to DL) was observed within 1 hour of solar exposure without the need of post-exposure dark treatment (Figure 5b) (UVA dose equal to 245 kJ m⁻²). The water temperature in the reactor remained below 35°C at all times, therefore inactivation of bacteria cannot be attributed thermal effects but to the synergistic effects of mild heat and UVA light observed during SODIS [171716] where the main photo-inactivation mechanism of bacterial depend on the reactive oxygen species (ROS) generation [10]. These results demonstrate that exposure to a UVA dose of approximately double the minimal lethal UVA dose halves the total treatment time required to process 2.5 L in the CPC 1.89 photo-reactor. In addition, potential health risks associated with bacterial recovery in the dark are significantly reduced.

Figure 6 ('a' and 'b').

These findings support our initial results, where following receipt of the minimum uninterrupted lethal UVA dose the concentration of viable *E. coli* K-12 cells decreased to the detection limit. In addition, bacterial re-growth was not evident 24 or 48 h following SODIS treatment, indicating that photo-repair mechanisms had not been activated and/or were not effective.

3.3 Sequential batch processing

In order to treat water using SODIS in sequential batches, complete disinfection must be observed before the treated water can be dispensed into the treated water tank. If a post-exposure dark inactivation period is required in the photo-reactor, this would significantly increase the total treatment time. The results in figure 5b confirm that solar exposure corresponding to UVA dose equal to 245 kJ m⁻² (received in approximately 1 hour in the CPC 1.89 system) is sufficient to ensure complete bacterial inactivation and therefore permit sequential batch processing based upon receipt of that UVA dose.

The location where this study was carried out, South of Spain, receives an average UVA irradiation of $(1180 \pm 20) \text{ kJ m}^{-2}$ (yearly average of 2007 to 2010), which would permit treatment of 6 batches of water per day. Standard sunny days in this area have an average UVA irradiance of 30 W m^{-2} . Therefore, the use of an automated $C = 1.89$ CPC photo-reactor would permit processing of 6 sequential batches of 2.5 L each day, with the single tube photo-reactor producing 15 L of solar purified water each per day. The sequential batch system is modular and could be scaled up to allow several CPC photoreactors to be used under the control of a single UVA sensor. For example, six $C = 1.89$ CPC modules could theoretically produce around 90 L of potable water per day, which would be a suitable volume of drinking water for several households. Allowing for maintenance and non-optimal solar conditions, each 6-tube system could produce approximately 31,500 L during a typical year.

A preliminary cost-based analysis, using parameters previously described by Clasen et al., indicated that a 6-tube automated sequential batch system, with a predicted life span of ten years, could provide solar disinfected water at a total treatment cost equivalent to \$0.23 per 100 L [181817]. This compares favourably with commonly used point-of-usewater treatment processes, such as chlorine solutions and P&G PUR® sachets, which have been estimated to cost \$0.045 and \$1.00 per 100 L respectively [181817]. Research is ongoing to further reduce the initial cost of the automated SODIS system through the use of alternative materials for CPC's and low power electronics in the control apparatus.

4. Conclusions

The use of a CPC photo-reactor with a C of 1.89 approximately halves the time taken to acquire the lethal UVA dose, in comparison to a CPC with a C of 1.00. However, a dark inactivation period, following the solar exposure, is required to achieve a 6-log kill. This dark inactivation period introduces uncertainty in relation to the SODIS treatment and increases the total treatment time. Doubling the UVA dose was demonstrated to give a 6-log kill without need for a dark inactivation period, permitting batch treatment in approximately 1 hour (under typical solar conditions). The addition of simple, low cost electronic control apparatus to SODIS photo-reactors allows sequential processing of batch SODIS. The system described has a number of advantages including 1: ensuring that double the lethal dose is received 2: providing feedback to the user during the treatment process (i.e. process not complete), and 3: removing user input with respect to control of the SODIS process. Cost-based analysis of the

sequential batch CPC solar disinfection reactor shows that it compares favourably with other point-of-use water purification systems.

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FIGURE CAPTIONS

Figure 1. Schematic of the sequential batch system.

Figure 2. Diagram of CPC mirrors with concentration factor 1 (a) and 1.89 (b).

Figure 3. CPC collector diagram in relation with acceptance angle (θ_c) and aperture.

Figure 4. Response of the Sg-lux sensor (dashed lines) and global UVA radiometer at PSA (solid line) during sunny (case 1) and cloudy weather (case 2).

Figure 5. Inactivation of *E. coli* in well water during natural sunlight exposure using the sequential batch reactor (a) C of 1.00 (UVA dose = 229 kJ m^{-2}); (b) C of 1.89 (UVA dose = 245 kJ m^{-2}).

Figure 6. Inactivation curve of *E. coli* in well water during natural sunlight exposure using the sequential batch reactor with $C = 1.89$ and dark post-irradiation effect after deliver 108 kJ m^{-2} (a) and 245 kJ m^{-2} (b).

Table 1: Summary of physical and chemical properties of the well-water batch used for the experiments.

<i>Natural well-water at PSA</i>			
Cl ⁻	285 ± 2 mg/L	Na ⁺	501.1 ± 0.8 mg/L
NO ₃ ⁻	8.2 ± 0.5 mg/L	NH ₄ ⁺	ND
SO ₄ ²⁻	205.0 ± 0.5 mg/L	K ⁺	9.4 ± 0.3mg/L
F ⁻	0.9 ± 0.3 mg/L	Mg ²⁺	64.5 ± 0.6 mg/L
Br ⁻	ND	Ca ²⁺	79.1 ± 0.5 mg/L
PO ₄ ³⁻	ND	HCO ₃ ⁻	495 ± 15 mg/L
pH	7.8	Conductivity	2805 µS/cm
Turbidity	1.5 NTU	Bacteria	0 CFU mL ⁻¹
TOC	5 mg/L	COD	45 mg/L

Figure 1.

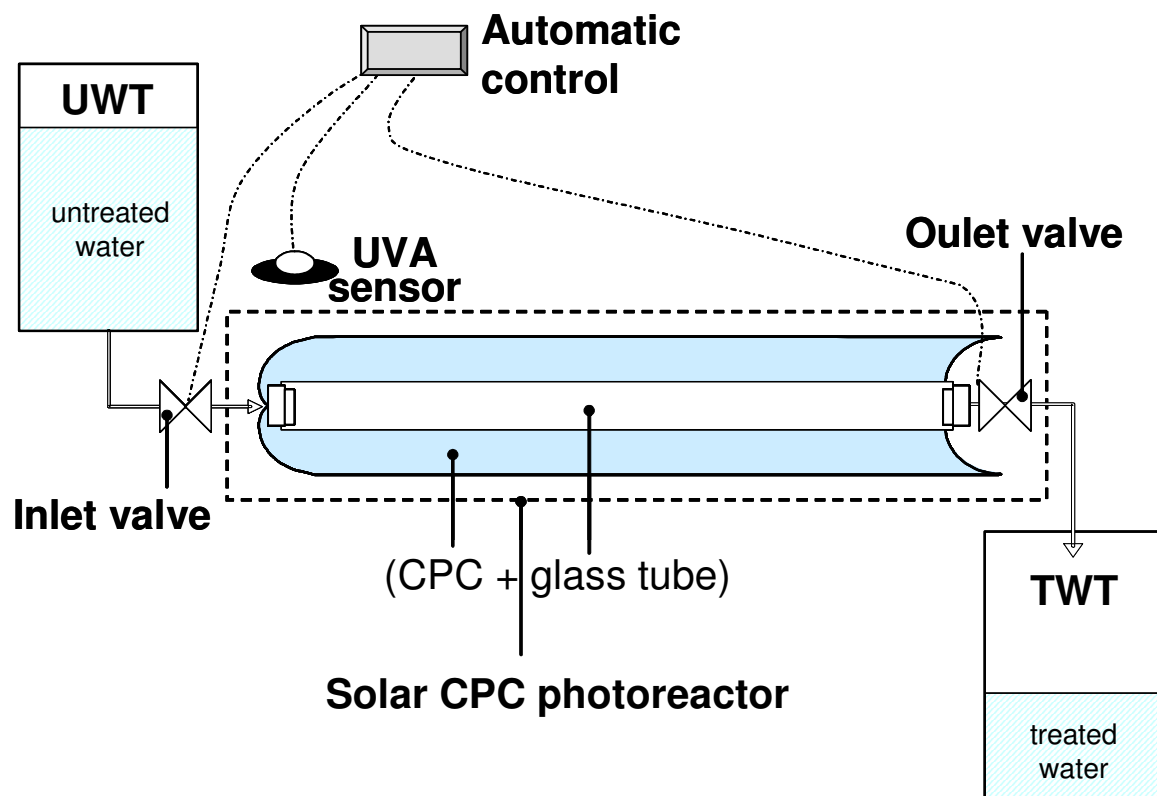


Figure 2.

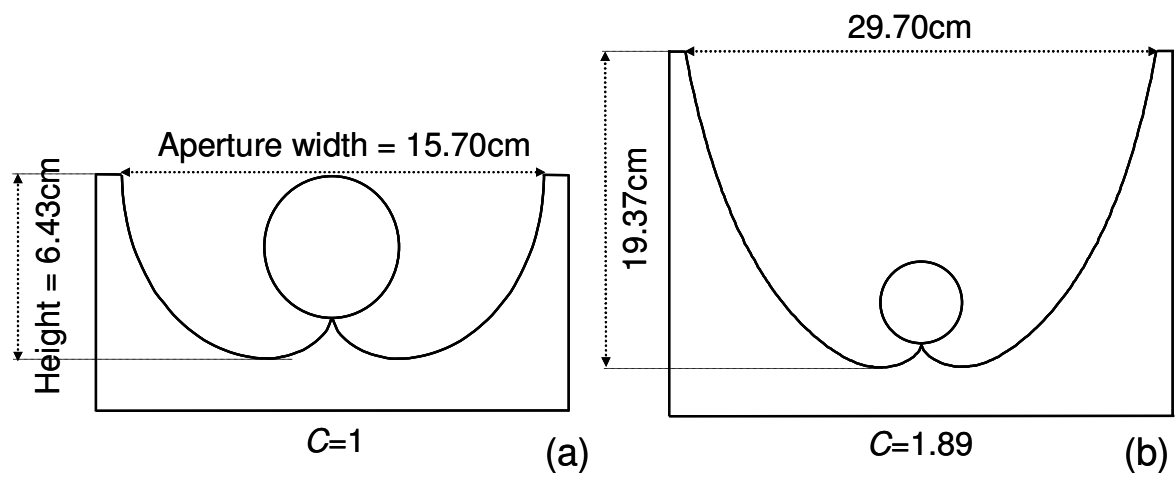


Figure 3.

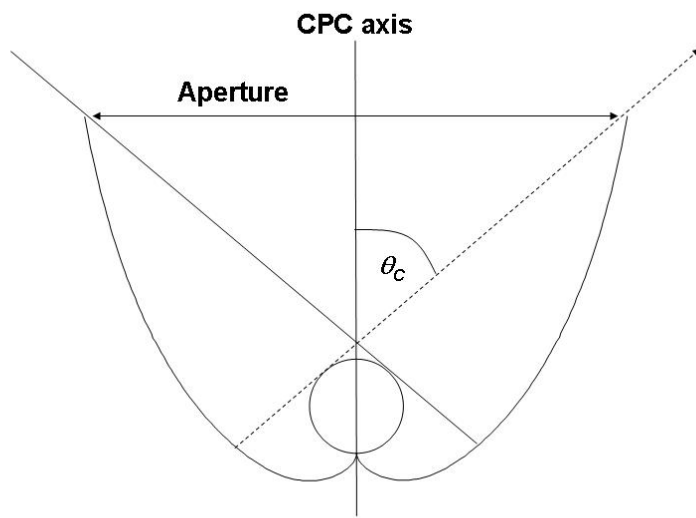


Figure 4.

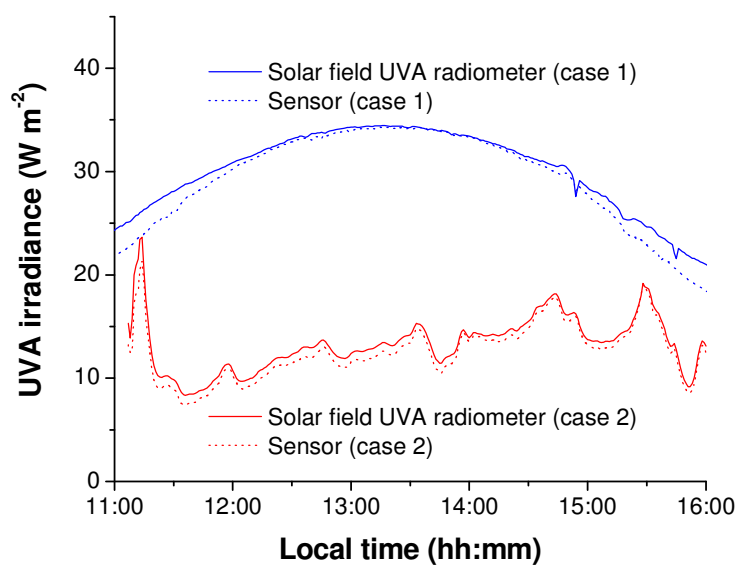
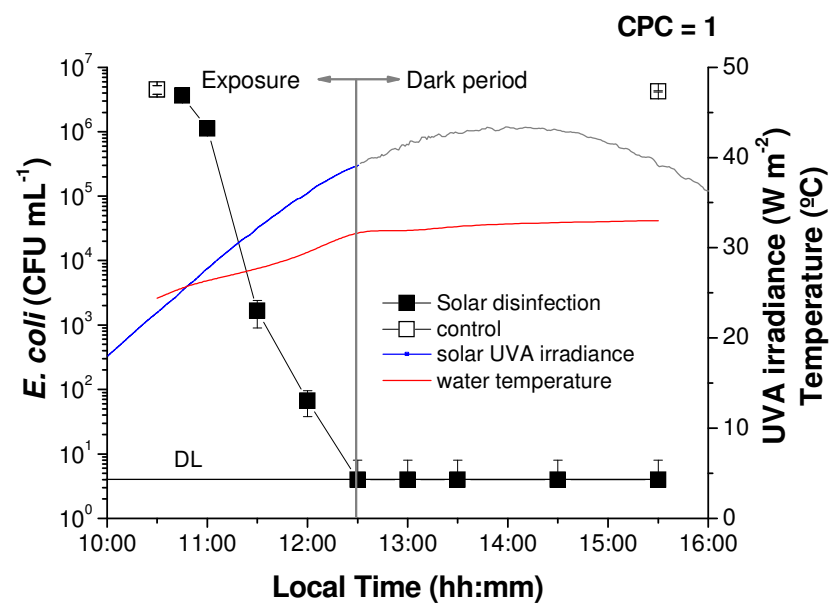
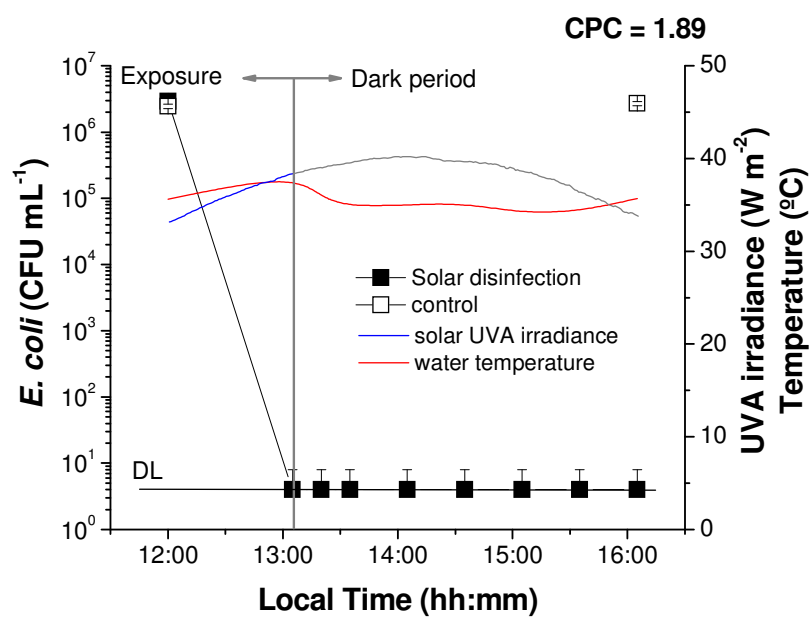


Figure 5.

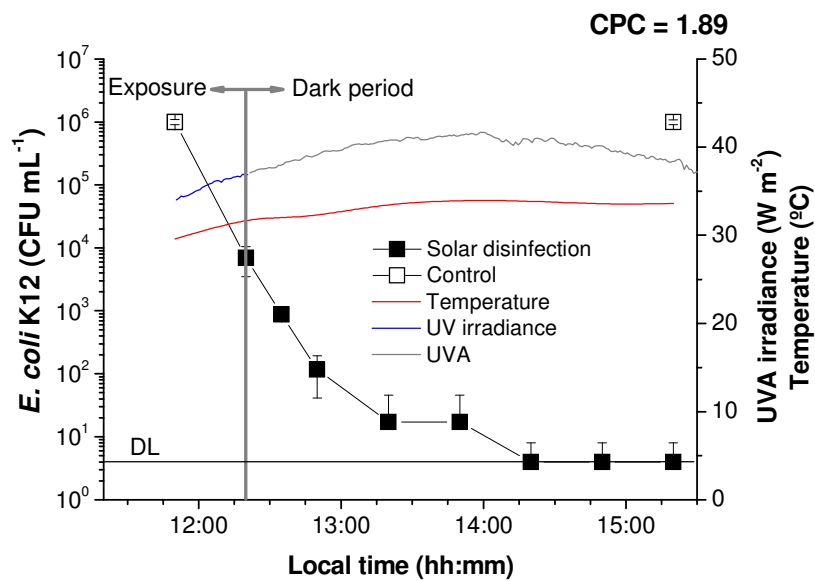


(a)

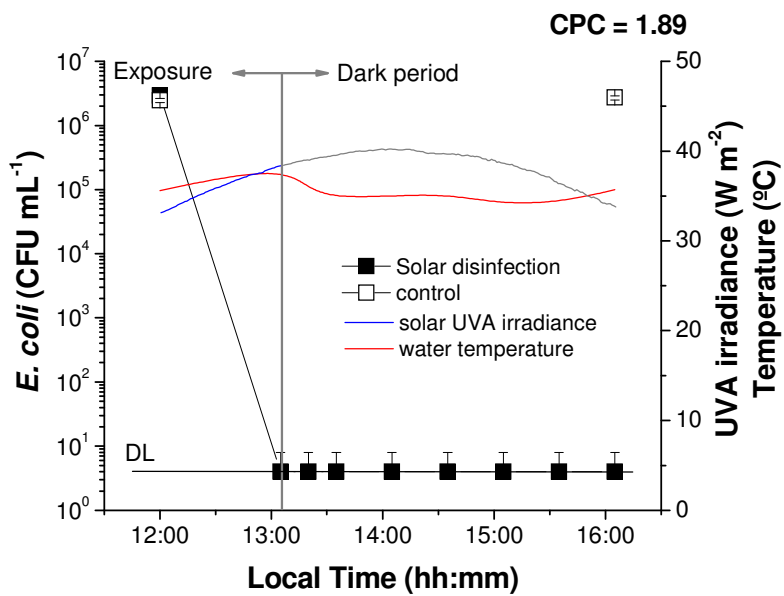


(b)

Figure 6.



(a)



(b)