A Study of the Genotoxic Implications and Enhancement Technologies for Solar Disinfection (SODIS) of Drinking Water

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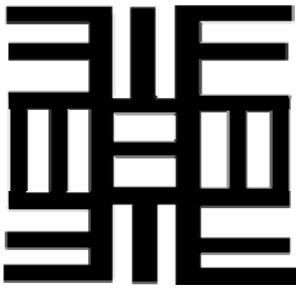

“HE WHO DOES NOT KNOW, CAN KNOW FROM LEARNING”*

*Akan Adinkra symbol of knowledge, life-long education and continued quest for knowledge.

Table of Contents

List of Figures

List of Tables

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To *God,* who makes all things possible.

Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree PhD is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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Summary

Solar disinfection (SODIS) is a household water treatment method that uses sunlight to inactivate pathogens in water. The work presented in this thesis aimed to show that SODIS is an appropriate, effective and acceptable intervention against waterborne disease. This was demonstrated by the inactivation of a highly infectious bacterial pathogen, increasing the volume of SODIS treated water using solar continuous-flow reactors, enhancing SODIS during sunny, cloudy and turbid water conditions by the use of compound parabolic concentrator (CPC) mirrors and finally determining the genotoxicity of SODIS water.

This project identified the inactivation kinetics of *E. coli* O157 under simulated light by following a natural temperature profile which would be comparable to inactivation under real sunlight conditions.

The use of CPC mirrors proved to be enhancement to SODIS in batch reactors under sunny and cloudy conditions and with clear and turbid water (< 300 NTU). Non-homogenous degradation of mirrors occurred in the field, under sunny conditions, there was no significant difference between using an old CPC and no CPC during solar disinfection. However, under cloudy conditions, only the systems with CPC either old or new achieved complete inactivation of bacteria.

An attempt to scale-up SODIS through continuous-flow reactors by treating 14 L and 70 L volumes of water found that increasing flow rate was detrimental to inactivation of *E. coli* K-12, resulting in a residual active population after solar exposure. It was determined that a minimum UV dose, delivered in an interrupted manner was necessary to ensure complete inactivation of bacteria. However, successful inactivation of bacteria in large volumes of water (25 L) occurred in a CPC enhanced batch reactor (EBR) under periods of strong sunlight.

Under standard SODIS conditions, which included daily re-use of plastic bottles over a 6-month period, water contamination by genotoxic compounds was not observed with the *Salmonella* Ames-Fluctuation assay in unconcentrated samples. The Ames-fluctuation test was successful in detecting potential genotoxicity in no-refill samples after 2 months for both dark-control and solar exposed samples. However, genotoxicity was higher for the solar exposed samples.

Abbreviations

ANOVA	One Way Analysis of Variance
ATP	Adenosine 5'-triphosphate
BGR	Borosilicate Glass Reactor
CFU	Colony Forming Units
CO_2	Carbondioxide
CPC	Compound parabolic concentrators
CPD	Cyclobutane Pyrimidine Dimers
DEHA	di(2-ethylhexyl)adipate
DEHP	di(2-ethylhexyl)phthalate
DMT	dimethyl terephthalate
DNA	Deoxyribonucleic Acid
DO_2	Dissolved Oxygen
EAWAG	Swiss Federal Institute of Environmental Science and Technology
EBR	Enhanced Batch Reactor (25 L)
EPEC	Enteropathogenic *E. coli*
EU	European Union
GC	Gas Chromatography
GInaFIT	Geeraerd and Van Impe Inactivation Model Fitting Tool
HIA	Health Impact Assessment
HIV/AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
HPLC	High Performance Liquid Chromatography
HWTS	Household Water Treatment and Safe Storage
LB	Luria-Bertani
log	Logarithm
MS	Mass Spectrometry
MR	Mutagenic Ratio
NTU	Nephlometric Turbidity Unit
$OH^{\bullet}$	Hydroxyl radical
PBS	Phosphate Buffered Saline
PET	Polyethylene Terephthalate
PSA	Plataforma Solar de Almería

RCSI	Royal College of Surgeons in Ireland
RMSE	Root Mean Sum of Squared Errors
ROS	Reactive oxygen species
SODIS	Solar Disinfection
TiO_2	Titanium Dioxide
UNICEF	United Nations Children's Emergency Fund
UV	Ultraviolet
WHO	World Health Organisation

Chapter 1

Introduction

1.1 Overview

The provision of microbially safe water in sufficient quantities still remains an important public health issue. Household water treatment methods and safe storage (HWTS) have been shown to be effective interventions in improving the quality of water [1]. Among these treatment methods is solar disinfection (SODIS). As the sun is a freely available renewable energy source, there is a new interest in its use for various applications. This chapter will give a background to solar disinfection, how it is used in the field and the mechanisms by which it inactivates microbial pathogens. The chapter will also present the aims of this research.

1.2 Solar Disinfection

The use of sunlight to disinfect water has been practised since ancient times; however, the work of solar disinfection as defined presently was pioneered by Acra and co-workers in the late 1970s, when sunlight was used to disinfect oral rehydration solutions [2]. Disinfection of oral rehydration solutions proved successful, and in the early 1980s further work was conducted on using sunlight to disinfect water. Detailed experiments using several organisms in batch reactors of different containers as well as in continuous-flow reactors were conducted at the University of Beirut [3]. Extensive analysis of solar radiation in Lebanon was also carried out to determine the precise effects of different regions of the solar radiation spectrum on the inactivation of organisms. From these results, they were able to determine that the most favourable regions for solar disinfection received 2500-3000 h of full sunshine per year and lay in the latitude

lines of 15° N / S and 35° N / S (Fig. 1.1) [3]. The semi-arid region lies between latitudes 15° N / S and 35° N / S and is characterised by limited cloud coverage and high solar radiation (3000 hours of sunshine per year. The second most favourable region lies between the equator and latitude 15°N/S. In this region scattered radiation is high due to higher levels humidity which results in cloud cover and rainfall. However, 2500 hours of sunshine is still received per year. Coincidentally, areas which are in dire need of drinking water treatment lie within those same latitude lines and hence could benefit from solar disinfection.

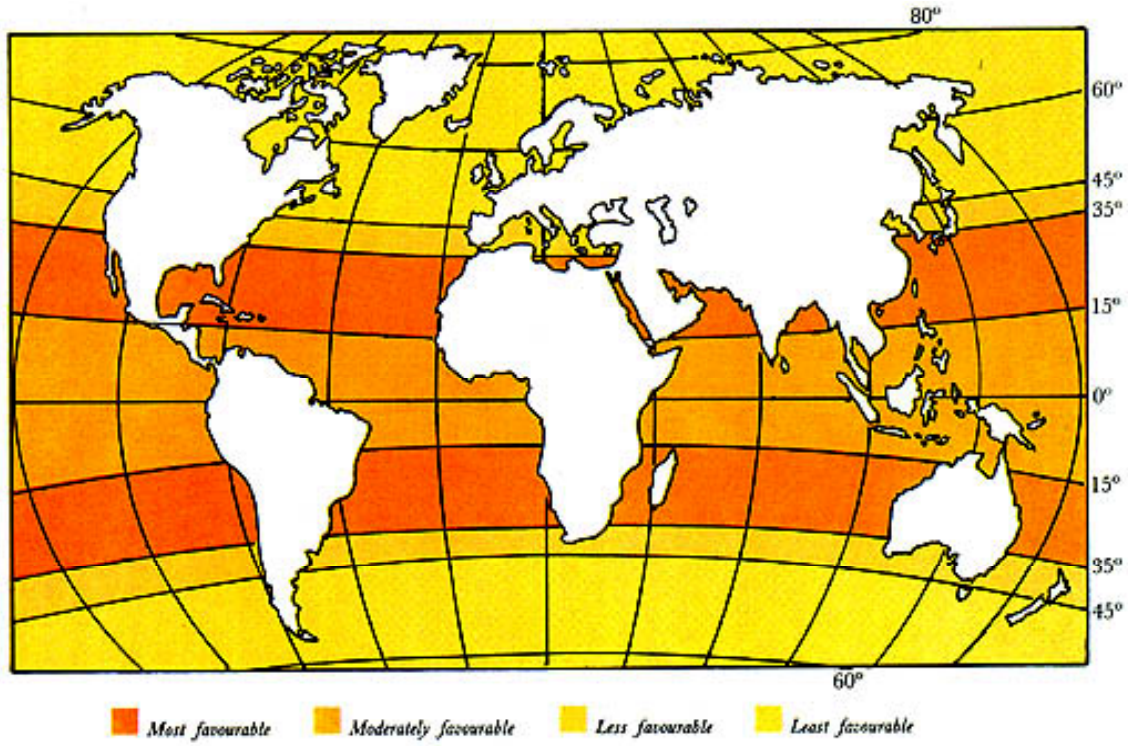


Figure 1.1 *Favourable regions for solar disinfection, determined by number of hours and intensity of sunlight [3].*

Once certain criteria have been met, solar disinfection of water in transparent containers consists of four basic steps, as illustrated in Fig. 1.2. Water to be disinfected should be < 3 L, with a turbidity < 30 NTU [4]. If the turbidity is high, light penetration is reduced and hence water should be filtered before exposure to sunlight. In the rural setting, turbidity can be reduced through the use of cloth which acts as a filter and the seeds of *Moringa oleifera* [5] which act as flocculants [6]. The water to be disinfected is poured into a clean transparent container. The bottle should then be shaken in order for the water to be aerated

[6], and the bottle is placed in direct sunlight for 6 h ensuring that at no time during solar exposure will the bottle be in the shade [4]. The solar disinfected water should be consumed within 48 h post solar exposure. In 2005, SODIS was approved by the World Health Organisation (WHO) as one of recommended household water treatment options [1].

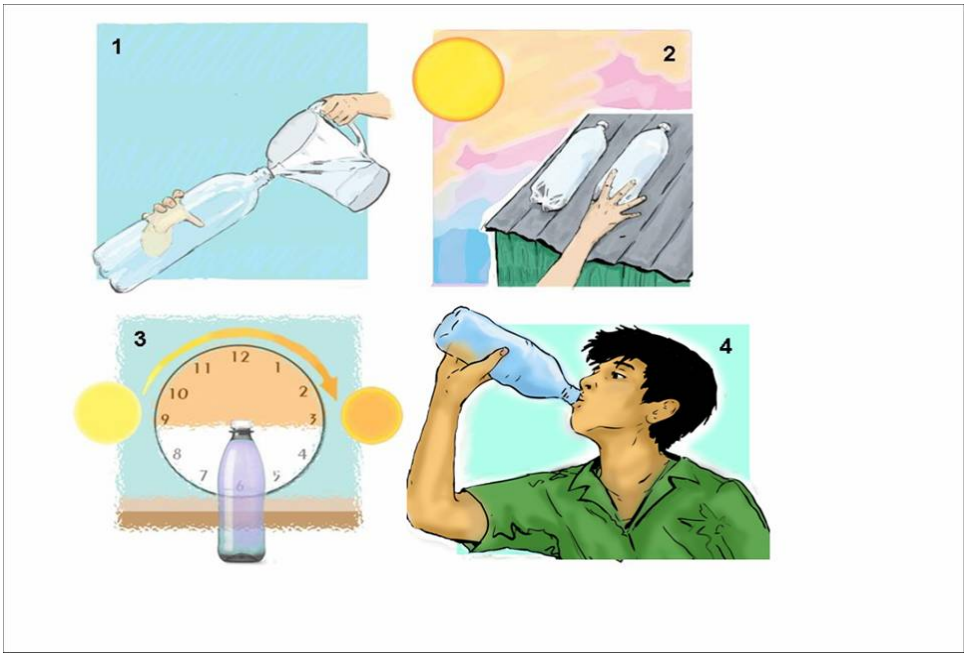


Figure 1.2 *Basic 4-step protocol followed in solar disinfection of water in plastic bottles.*

1.2.1 Optical Inactivation Mechanism of Solar Disinfection

Sunlight inactivates microorganisms through optical and thermal mechanisms. The optical inactivation of sunlight as observed by Acra *et al*. contributes to ~70% of the inactivation observed with sunlight [3]. Optical inactivation of sunlight is governed by the wavelength of light that hits the microorganism. Sunlight reaching the earth's surface is made up of mostly UV-A (320-400 nm) and UV-B (290-300 nm). The DNA molecule strongly absorbs wavelengths below 320 nm, and hence UV-B which ranges from 290-300 nm causes direct damage to the molecule. The most common result of UV-B irradiation is the

formation of cyclobutane pyrimidine dimers [7]. Bacterial response to UV damage includes dark repair mechanisms and photoreactivation [7, 8].

On the other hand, UV-A causes indirect damage to lipids, proteins and DNA through photosensitizers and reactive oxygen species (ROS). Photosensitizers such as humic acids are found in water, while some are found within the microbial cell itself such as flavins and porphyrins [6, 9, 10]. Photosensitizers reach an excited state by absorbing UV-A photons; they then directly react with components of the cell or else react with molecular oxygen forming ROS such as hydroxyl radicals, superoxide and hydrogen peroxide [6]. In order to ensure that a sufficient quantity of ROS is generated, a high level of dissolved oxygen is required. Studies by Reed *et al* [11], and Archer *et al*. [12, 13], found a 4-8 times faster inactivation rate of faecal bacteria in oxygenated water, compared to deoxygenated water [6]. Water is aerated by shaking before exposure to sunlight. However, shaking during exposure to sunlight is not recommended as Kehoe *et al*. reported that at water temperatures > 50˚C, agitation during solar exposure led to the escape of oxygen and hence did not benefit inactivation but rather had a detrimental effect on inactivation [14].

Once ROS molecules are formed they react with DNA, proteins and in particular membrane lipids. ROS affects DNA by causing breaks and base changes in the strands of DNA, resulting in faulty DNA replication or the complete halting of replication. Proteins such as enzymes are destroyed due to changes in amino acids that occur when ROS is present. Most of the water-borne bacteria are gram-negative and have a very thin peptidoglycan layer which makes up only ~ 10% of the cell wall, the rest of the wall is made of outer membrane which consists of lipopolysaccaride, lipoproteins and phospholipids. Hence, mechanisms that result in damage to lipids are very important in the inactivation of bacteria [15]. Once the outer membrane is damaged by excited photosensitizers or by ROS, leaking from the microbial cells occurs leading to loss of function and eventual death [16]. The leaking of membrane was confirmed by experiments showing $^{86}Rb^{+}$ leakage occurred when UV-A was applied to microbial cells [17]. Mechanisms to overcome damage by ROS include antioxidant systems such as catalase and

superoxide dismutase, which act by mopping up ROS; however, these systems are also light sensitive [6].

1.2.2 Thermal Inactivation Mechanism of Solar Disinfection

Although optical inactivation is the major mechanism of sunlight inactivation of microorganisms, the importance of thermal inactivation cannot be underestimated. Heating of water is considered the safest treatment method as it is effective in destroying all waterborne pathogens once pasteurization temperatures (≥ 60˚C) have been reached. Water has to reach a rolling boil for 1 minute at sea level or up to five minutes depending on the altitude to ensure all pathogens are destroyed. Solar infrared radiation causes water temperature to rise when exposed to sunlight. Temperatures ≥ 60˚C are not likely to be reached just by plain exposure to sunlight. Under strong sunlight conditions, temperatures > 50˚C can be reached in small batches of water in polyethylene terephthalate (PET) bottles and have been shown to effective in inactivation of bacteria under field conditions [18]. If temperatures > 50˚C are maintained for 6 minutes this may be sufficient to destroy completely the cysts of *Entamoeba histolytica* (54˚C) and *Giardia* (54˚C) as well as the eggs of *Schistosoma* (55˚C) and *Taenia* (57˚C) [19]. Mild heat leads to the inactivation of microbial cells by breaking of essential bonds and denaturing of enzymes such as catalase which are important to cell function. A synergy between optical and thermal inactivation is not observed for temperatures between 20-40˚C. However, once water temperatures are > 45˚C, a strong synergistic effect is observed where inactivation of microbes occurs faster when the two process are combined than either optical or thermal inactivation on its own [18, 20, 21] . This synergy has been observed in the inactivation of *E. coli* K-12 under solar simulation for temperatures > 45˚C [20] and at temperatures of > 50˚C for *E. coli*, enteroviruses and bacteriophages where the UV dose required for inactivation was reduced by three-fold [21].

1.2.3 Microbial Inactivation Model of Solar Disinfection

The typical inactivation curve for a bacterial population undergoing inactivation by sunlight consists of an initial shoulder followed by an exponential decrease

and in some cases a tailing-off effect. The initial shoulder observed is due to a number of reasons. As mentioned earlier, there are multiple targets involved in the inactivation of microbial cells by sunlight [6, 22]. These sites, which include membranes and enzymes, all have to undergo damage in order for inactivation to take place. Furthermore, a UV dose has to be reached whereby the rate of destruction of these multiple targets overrides the rate of repair. If organisms are formed in clumps or colonies then all the organisms making up that colony have to be destroyed in order to ensure that the colony forming ability of that clump is damaged [23]. Once the threshold UV-dose has been reached, the exponential phase of inactivation begins usually in a single exponential decay pattern. During sunny days with very high solar intensity a double exponential decay might occur due to the inactivation of the more light sensitive population first followed by the more resistant population [6]. In the instances where solar UV-dose is insufficient and solar intensity is not high enough, the more sunlight resistant population may remain viable during the course of solar exposure and generate the tailing effect sometimes observed on microbial inactivation curves.

1.2.4 Solar Disinfection of Waterborne Microbial Pathogens

Most waterborne pathogens are enteric in nature due to the faecal/oral route of contamination of water. These pathogens are able to cause disease by being highly resistant to water treatment methods, surviving for high periods of time outside the body or having a high infectivity rate. Some waterborne pathogens such as *Vibro cholerae* do not have the above mentioned characteristics, but are still important causes of waterborne disease.

Table 1.1 provides a comprehensive list of the waterborne pathogens of concern in developing countries. *V. cholerae*, *Salmonella typhimurium, Shigella dysenteriae* Type 1 and *E. coli* are among the top causes of infectious disease out breaks causing cholera, typhoid fever, shigellosis and acute diarrhoea respectively [24]. Under simulated and real sunlight conditions, concentration of these organisms have been shown to be inactivated by SODIS [18, 20, 25, 26]. *Pseudomonas aeruginosa* was also shown to have a marked reduction in infectivity when SODIS was applied to water containing the organism [27]. Although the organism in drinking water is not a major concern in a general

healthy population [28], for immunocompromised patients, children and infants living under un-satisfactory sanitary conditions, the presence of enterotoxin causing *P. aeurginosa* in water might lead to serious infection [29]. SODIS inactivation of Enteropathogenic *Escherichia coli* (EPEC), an extremely important waterborne pathogen, still remains to be tested. The occurrence of this pathogen in water is a major public health concern, as EPEC is a major cause of diarrhoea in children under 5 years of age in developing countries. As yet, there is no EPEC vaccine; which makes individuals highly susceptible to infection, compared to infection from other waterborne pathogens, where vaccination provides a degree of protection from infection [30] .

When compared with bacterial inactivation by SODIS, only a few studies have been conducted to investigate the effect of SODIS on parasitic (protozoa and helminths), fungal and viral contaminants of water. Of the studies that have been conducted on protozoa, *Giardia muris* cysts and *Cryptosporidium parvum* oocysts were found to be inactivated by SODIS [31, 32].

Giardia sp and *Cryptosporidium sp* are the most prevalent enteric protozoa associated with waterborne disease [34]. A low infectious dose (1- 30 (oo)cysts) and high resistance to water treatments such as chlorination has resulted in these two protozoa not only causing significant persistent (14 days) diarrhoea in individuals in developing countries, but has also been responsible for outbreaks in developed countries such as Ireland, the UK and the USA [35].

Though *Acanthamoeba* trophozites were susceptible to SODIS, *Acanthamoeba* cysts (infective stage) were shown to be resistant to SODIS disinfection [27, 36]. UV radiation studies also confirm the highly resistant nature of *Acanthamoeba* as compared to bacteria and protozoa such as *Cryptosporidium* and *Giardia* [37].

The effectiveness of SODIS against helminths is yet to be tested. *Strongyloides*, *Trichuris trichiura* and *Ascaris lumbricoides* are important water borne enteric helminths in developing regions [33]. Approximately 60,000 deaths per year, mainly in children are due to *Ascaris* infections [24, 33]. The eggs of helminths

are quite big (> 40 μm) and complex in structure [33] and are likely to need a long exposure time to SODIS treatment in order to be inactivated.

Table 1.1 *Microbial pathogens causing significant waterborne disease in developing countries [33].*

Name of microorganisms	Major diseases	Major reservoirs and primary sources
Bacteria		
Salmonella typhi	Typhoid fever	Human faeces
Salmonella paratyphi	Paratyphoid fever	Human faeces
Other *Salmonella*	Salmonellosis	Human and animal faeces
Shigella spp.	Bacillary dysentery	Human faeces
Vibro cholerae	Cholera	Human faeces and fresh water zooplankton
Enteropathogenic *E. coli*	Gastroenteritis	Human faeces
Yersinia enterocolitica	Gastroenteritis	Human and animal faeces
Campylobacter jejuni	Gastroenteritis	Human and animal faeces
Legionella pneumophilia and related bacteria	Acute respiratory illness (legionellosis)	Thermal enriched water
Leptospiria spp.	Leptospriosis	Animal and human urine
Various mycobacteria	Pulmonary illness	Soil and water
Opportunistic bacteria	Variable	Natural waters
Enteric viruses		
Polio viruses	Poliomyelitis	Human faeces
Coxsackie viruses A	Aseptic meningitis	Human faeces
Coxsackie viruses B	Aseptic meningitis	Human faeces
Echo viruses	Aseptic meningitis	Human faeces
Other enteroviruses	Encephalitis	Human faeces
Rotaviruses	Gastroenteritis	Human faeces
Adenoviruses	Upper respiratory and gastrointestinal illness	Human faeces
Hepatitis A virus	Infectious hepatitis	Human faeces
Hepatitis E virus	Infectious hepatitis, miscarriage and death	Human faeces
Norovirus	Gastroenteritis	Formites and water
Protozoa		
Acanthamoeba castellani	Amoebic meningoencephalitis	Human faeces
Blantidium coli	Blantidosis (dysentery)	Human and animal faeces
Cryptosporidium homonis and C. parvum	Cryptosporodisis (gastroenteritis)	Water, human and other mammal faeces
Entamoeba histolytica	Amoebic dysentery	Human and animal faeces
Giardia lamblia	Giardiasis (gastroenteritis)	Water and animal faeces
Naegleria fowleri	Primary amoebic meningoencephalitis	Warm water
Helminths		
Ascaris lumbricoides	Ascariosis	Human and animal faeces

Even though viruses cannot multiply in water, viral contaminants in drinking water are of major concern due to their low infectious dose (1 to 10 infectious units) and their long survival times in water [38]. Viral diarrhoea due to *Rotavirus* and *Adenovirus* are a significant contributor to deaths especially in children [38]. Other enteric waterborne viruses such as *Hepatitis* A and E and *Polio* that do not cause diarrhoea are also of equal concern. Immunisation and vaccination programmes exist in order to help eradicate these viruses. However, outbreaks still occur in areas with inadequate sanitary conditions [39]. SODIS was shown to be an effective means of disinfecting water containing *Poliovirus* [36]. However, studies have shown that of all microbial pathogens, viruses are generally the most resistance to UV radiation - in particular double stranded DNA viruses [37]. A number of waterborne enteric viruses are double stranded [40]. Hence there is an urgent need for more viruses (*Hepatitis* A and E, *Coxsackie* A and B) to be tested in order to ensure that SODIS is an effective method of disinfecting water contaminated with viruses.

Although fungi and yeasts are not listed in Table 1.1, a number of them are potential waterborne infectious agents and some have the ability to produce toxic metabolites [27, 41]. SODIS has been shown to be effective against the yeast *Candida albicans* and the fungus *Fusarium solani* [27]. With the rise in the number of immunocompromised people due to HIV/AIDS, the significance of fungal and yeast pathogens in water will become more apparent and hence testing of SODIS against other fungal species (*Aspergillus*, *Cladosporium*, *Penicillium,* etc) that are often isolated from water will become a necessity [41].

Table 1.2 provides a summary of waterborne pathogens that have been solar disinfected so far under solar simulation and natural sunlight conditions as discussed above. Solar simulation provides a way to see the effect of different intensities, doses wavelengths and temperatures on the inactivation of microorganisms. Solar simulation however, is not a replacement for natural sunlight and hence continued changes to solar simulation experiments are made

to better reflect and approximate inactivation of microorganisms under natural sunlight conditions.

Table 1.2 *Waterborne pathogens that have been successfully inactivated by solar disinfection.*

Waterborne pathogen	Solar simulation	Natural sunlight
Bacteria	*Enterococcus sp.* [43, 44] *Pseudomonas aeruginosa* [27] *Salmonella typhi* [26] *Salmonella typhimurium* [25] *Shigella dysenteriae* Type I [25] *Shigella flexneri* [21] *Vibrio cholerae* [25, 27]	*Campylobacter jejuni* [45] *Escherichia coli* [3] *Pseudomonas aeruginosa* [3] *Shigella dysenteriae* Type I [3] *Vibrio cholerae* [46] *Yersinia enterolitica* [45]
Parasites (protozoa)	*Acanthamoeba polyphaga* trophozites [36] *Cryptosporidium parvum oocysts* [31, 32] *Giardia muris cyst* [31]	
Viruses	*Poliovirus* [36]	*Bacteriophage* f2 [21] *Encephalomyocarditis* virus [21] *Rotavirus* [21]
Fungi	*Candida albicans* [27] *Fusarium solani* [27]	*Fusarium solani* [47]

1.2.5 Health Impact Assessment (HIA) Studies

It has been successfully proven that inactivation of a wide variety of waterborne pathogens occurs during sunlight exposure. However, the true effectiveness of solar disinfection is measured by the reduction of waterborne disease caused by a pathogen in a given population. The earliest field trials occurred in Kenya within the Maasai people who were drinking water contaminated with faecal coliforms [42].

Half of a group of 206 children ranging from ages 5-16 drank SODIS treated water and a 10% reduction in the incidence of diarrhoea was observed, while severe diarrhoea was reduced by 24% [48]. Following a 12 month duration study

with 349 children under 5 years of age, similar reductions in the rate of diarrhoea were observed [49]. A cholera epidemic in the same Maasai population further illustrated the effectiveness of solar disinfection. Out of 155 children (5 years and under) who drank solar disinfected water, only 3 children were infected with cholera. While out of the 144 children who did not drink SODIS treated water, 20 fell sick [46]. A SODIS intervention study performed in Southern India also revealed a 40% reduction in the risk of diarrhoea for SODIS users [50] and furthermore, women who are usually responsible for fetching water reported that SODIS was a feasible and sustainable method for disinfecting water [50]. Ongoing HIA studies are being conducted in Zimbabwe, South Africa and Cambodia and will provide more information regarding the impact of drinking solar disinfected water on diarrhoea rates

1.2.6 Solar Disinfection Reactors

One of the most important criteria for a SODIS reactor is the ability of the reactor wall to transmit sunlight. In this regard, non-coloured glass is preferred. Extensive work by Acra *et al*. [51] showed that ordinary glass bottles and glass jars could transmit up to 90% of solar radiation particularly in wavelengths in the UV-A region [51]. Borosilicate glass tubes which have been used in prototypes for flow solar reactors transmit 89-90% in the UV-A range as well as 45% in the more germicidal UV-B range [52]. Although glass is a suitable material in the use of flow solar reactors, for everyday batch disinfection of contaminated water, SODIS users will be required to fill water in containers everyday and this could be heavy due to the use of glass and could also be a potential source of injury if glass breaks. Use of glass bottles can also place a financial burden on low income users since there is often a deposit paid on return of the bottle to the point of purchase. Glass SODIS reactor bottles are therefore often stolen for this deposit if the user is unable to expose the bottles in a secure location.

Plastic bottles have proved to be suitable SODIS reactors, specifically PET bottles. Though PET bottles do not transmit UV-B, they do efficiently transmit 85-90% in the UV-A region if the bottles are not old or scratched and have

proved effective in solar disinfection of water [18]. Unlike glass which is inert and does not release photoproducts, plastic bottles have the potential to leach compounds into water after exposure to strong sunlight conditions. High solar intensity conditions are necessary in order to achieve complete inactivation of pathogens. Research involving chemical analysis has been conducted concerning the potential leaching of photoproducts into water. So far, results show that in some cases photoproducts such as terephthalate compounds remain on the surface of the water and do not migrate into the water [53]. Other compounds such as the carbonyls and plasticisers are found in the water but are well below the limits set for drinking water quality [53, 54]. However, doubts continue to linger about the safety of water disinfected in plastic bottles given disclaimers made by manufacturers in the bottling industry who instruct users not to reuse plastic bottles. PET bags [55] have also been used as SODIS reactors by placing the bags on a black surface to enhance solar disinfection. A SODIS pouch [56] which consisted of two materials - a metallized plastic to reflect light and a black plastic to absorb solar radiation was also found effective in inactivating *E. coli*, *S. typhimurium*, *S. sonnei* and *S. aureus* [56].

1.2.7 Enhancement Technologies for Solar Disinfection

Thermal Enhancement

Since the strong synergy between optical and thermal inactivation has been observed at temperatures > 45˚C [18, 19, 21], a number of enhancement methods involve accelerating the rate of inactivation of organisms by increasing the temperature of water through the use of absorptive materials and painting PET bottles black [19] in order to aid in the absorption of solar radiation. The use of reflectors [14, 57, 58] also increases the temperature of water but not to the extent as the use of absorptive materials or blackening of bottles. However, unlike blackened surfaces which are unable to raise the temperature of water sufficiently on cloudy days, depending on the reflector, UV-A will still be available on cloudy days for reflectors to enhance the optical inactivation of solar disinfection.

Chemical Enhancement

Titanium dioxide (TiO_2) is a catalyst that that has been shown to increase the rate of solar disinfection, especially in the suspended form by using UV to form ROS. A wide variety of laboratory grown organisms as well as organisms isolated from waste water and river water have been successfully inactivated with the use of the TiO_2 [47, 59-61]. However, the lack of availability of the catalyst in developing regions and having to remove the catalyst after disinfection to improve the aesthetic quality of the water might affect its use in the field. Research into using tubes coated with TiO_2 in order to treat large volumes of water in continuous flow reactors seem promising [62].

Increasing the Volume Disinfected

A number of prototypes of continuous-flow solar reactors have been designed to enhance solar disinfection by increasing the output of treated water in given solar exposure time [51, 63-66]. Some flow reactors have focused on increasing the optical inactivation component of sunlight inactivation by the use of different solar collectors and reflectors [64, 65], while others have focused on increasing thermal inactivation by using flat plates painted black to absorb solar radiation [63, 64]. All these reactors have had varied results in the inactivation of common faecal indicators, but based on literature, none of these reactors are currently being used in the field.

1.2.8 European Union (EU) SODISWATER Project

Since the introduction of solar disinfection by Acra *et al*. [3] almost 30 years ago, SODIS has been extensively researched and used in areas where other means of water treatment are too costly. Furthermore, in 2005, SODIS was approved by the WHO. However, many relief organisations are still unwilling to use the SODIS method, and this reluctance has hampered the use and dissemination of the method in areas which could greatly benefit from the technique. In 2005 a proposal was funded by the EU (FP6-INCO-CT-2006-031650 SODISWATER), in which the effectiveness of SODIS as intervention against waterborne disease

at the household level and as an emergency relief in the aftermath of a natural or man-made disaster would be demonstrated by addressing the following:

1. Health Impact Assessment studies based in three African countries
2. Microbiological studies of the response of the most important untested waterborne pathogens to SODIS.
3. Enhancement techniques designed to improve the efficiency of inactivation (e.g. continuous-flow systems, compound parabolic concentrators, photocatalytic acceleration and UV dosimeteric indicators.
4. Socio psychological studies about successful diffusion and behavioural strategies for sustainable adoption of solar water disinfection.
5. Dissemination strategies so the new knowledge resulting from this research reaches those most at risk from waterborne disease and benefit as quickly as possible.

1.3 Aims of the Project

The aim of this study will be to address points 2 and 3 in the objectives of the EU SODISWATER proposal, which are to conduct microbiological studies of the response of the most important untested waterborne pathogens to SODIS and test enhancement techniques designed to improve the efficiency of inactivation. This will be accomplished by:

- Determining the inactivation of enteropathogenic *E. coli* under solar simulated light by following a natural temperature profile observed in fixed volumes of water. If successful inactivation of EPEC *E. coli* occurs, the range of pathogens shown to be susceptible to SODIS broadens and also provides an inactivation rate likely to be observed under natural sunlight conditions in the field.

- Determining the limitations of PET bottles as efficient SODIS reactors by conducting SODIS experiments under natural conditions and assessing the safety of drinking solar disinfected water. This will involve the use of

natural sunlight on both sunny and cloudy days, the disinfection of real well-water as well as turbid water (well-water and soil that has not undergone sterilisation). The safety of SODIS treated water will be evaluated by using a biological approach to determine if any leached products from PET bottle exposure to sunlight is a cause of concern to human health.

- Determining the potential of compound parabolic concentrator (CPC) mirrors to enhance solar disinfection in both clear and turbid well-water exposed to natural sunlight (sunny and cloudy conditions).

- Determining the potential of continuous-flow solar reactors to enhance solar disinfection in PET bottles by treating large volumes of water. PET bottles are able to disinfect 1-3 L of water for 6 h under strong sunlight conditions. To prove continuous-flow solar reactors are an enhancement to SODIS in PET, the water should be disinfected in under 6 h and the volume disinfected should be sufficient to provide for a family or small communities.

Chapter 2

Inactivation of *Escherichia coli* O157 by Solar Disinfection (SODIS) using Simulated Sunlight

2.1 Introduction

In this chapter, a previously untested waterborne pathogen *E. coli* O157 was exposed to simulated sunlight conditions in order to determine its susceptibility to solar disinfection. Instead of maintaining a set water temperature for the duration of experiments as had been previously performed in other simulated inactivation studies [26, 27, 36], a natural temperature profile, measured earlier in fixed volumes of water and exposed to African sunlight, was used [43]. This enabled better comparison between results obtained under simulated conditions and those under natural sunlight conditions.

2.1.1 *Escherichia coli* O157

E. coli O157 is the leading cause of infantile (under 5 years of age) diarrhoea in developing countries [67]. The diarrhoea is bloody in nature (haemorrhagic colitis) and can sometimes progress to renal failure (haemolytic ureamic syndrome), which is often fatal in children [68]. In the elderly, thrombotic thrombo-cytopaenic purpura and neurological impairment are severe complications associated with infection [69]. The bacteria are both food-borne (un-pasteurised milk) and waterborne. Faecal contamination of water occurs from humans and animals that have access to water catchment areas. Infection can occur directly through drinking contaminated water or indirectly through the preparation of milk formula using contaminated water. In addition, swimming in aquatic environments where the bacteria is known to survive for long periods, has been associated with infection [70]. The bacteria are highly infectious with

an infectious dose of < 100 organisms [68]. *E. coli* bacteria are gram-negative rod-shaped bacteria which are normal inhabitants of the intestinal flora of both humans and animals. Some strains are capable of producing large amounts of Vero toxin, a toxin that is cytotoxic to Vero fibroblastic green monkey kidney line cells [71]. Bacteriophages infect the bacterial cells and integrate into the bacterial genome thereby conferring on them the toxin producing ability. The letter and number designations refer to the antigenic type O, the somatic (lipopolysaccharide) antigen number 157 and H the flagella antigen number 7. For some bacteria K refers to the capsular antigen. These antigens are used to classify the different serotypes [72]. *E. coli* O157:H7, like most *E. coli*, is inactivated by water disinfection methods such as heat, UV-C and chlorine. Although in nutrient limited situations, *E. coli* O157:H7 is able to develop a chlorine resistant phenotype [73].

2.1.2 *Escherichia coli* K-12

E. coli K-12 was first isolated in 1922 from human stool at the University of Stanford [74, 75]. It was used in the 1940's for prominent work in nitrogen metabolism and the biosynthesis of tryptophan from indole and serine [74, 75]. Since then, *E. coli* K-12 has been cultivated and used several times in the laboratory and this has resulted in the loss of its O antigen and therefore makes it safe to use routinely [74, 75]. With regard to solar disinfection, *E. coli* has been used in many studies due to its widespread use as a faecal indicator. Early solar disinfection studies by Acra *et al*. [3], showed that *E. coli* was more resistant to sunlight inactivation when compared to other bacteria (*Salmonella typhi, Salmonella enteritidis* and *Pseudomonas aeruginosa*). Although, biological reasons are still unclear as to why *E. coli* is more resistant, the bacteria acts as a standard to measure the effectiveness of solar disinfection [3]. Consequently, *E. coli* K-12 has been used as a representative of *E. coli* in a number of solar disinfection studies [16, 76, 77]. Although the loss of its O antigen makes *E. coli* K-12 harmless to work with, this also means that it might not be the most suitable model for studies on pathogenesis, where *E. coli* O157 serves a better representative for pathogens such as *Shigella* [74, 75].

2.1.3 Solar Disinfection (SODIS) under simulated sunlight

To generate a solar spectrum which resembles that of natural sunlight occurring in geographic areas where SODIS is practiced, a 1000 W Xenon arc lamp solar simulator apparatus was used. The light source is a 1000W Xenon arc lamp (Model 9119x; KW Large Area light Source, Oriel Corporation, Stratford, CT, USA). An ellipsoidal reflector surrounds the lamp and collects over 70% of the output. This radiation is focused onto an optical integrator which produces a uniform diverging beam which is deflected 90° by a mirror to a final collimating lens. The output is a uniform collimated beam. The lamp has a short cut-off at 260 nm and is fitted with an air-mass 1 filter (AM 1 Direct filter Set P/N 81074 + 81011, Oriel Corporation, Stratford, CT, USA) which simulates the equatorial solar spectrum at ground level when the sun is directly overhead [18]. Light intensity was measured using a 2 W broadband power/energy meter, 13 PEM 001/J (Melles Griot, NY, USA) measures intensity from 200 nm to 20 μm (Fig. 2.1).

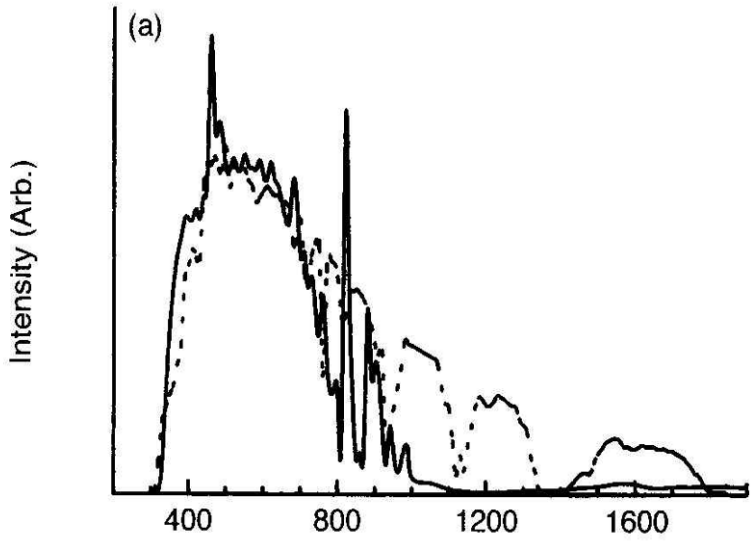


Figure 2.1 *Comparison if irradiance spectra of the solar filtered Xe arc lamp (solid line) and of ground level solar spectrum (dotted line) [18].*

Since the area under the beam is restricted, solar simulated experiments were conducted in transparent polystyrene lidded, 6-well tissue culture plates (Sarstedt AG & Co, Nümbrecht, Germany). This allows for 6 suspensions of bacteria to be tested at a given time as compared to the 2 or less plastic bottles that can fit

under the beam at a given time. Using the culture plates also catered for a more even distribution of light intensity (Fig. 2.2).

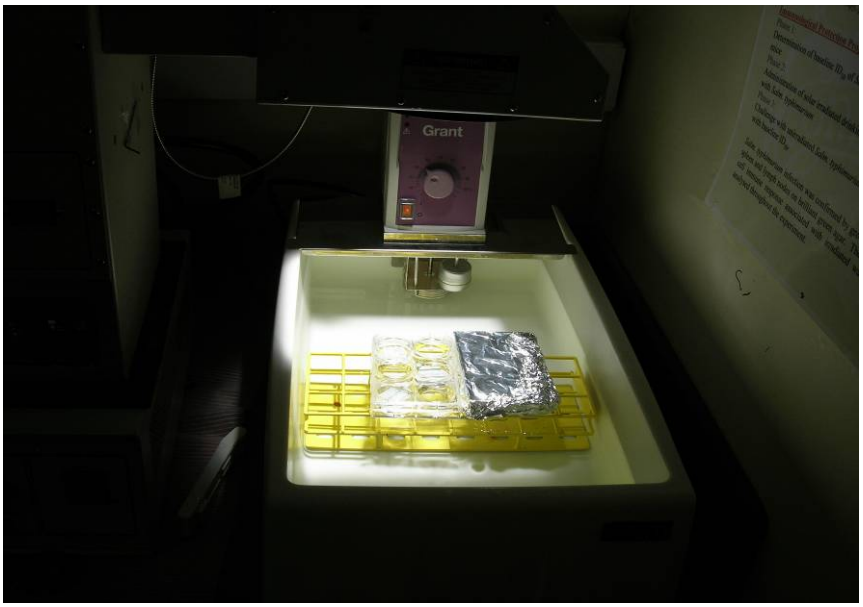


Figure 2.2 *6-well tissue culture plates under light beam during solar simulated experiments.*

Optical transmission properties of polystyrene are comparable to those of PET as both transmit UV-A (320-400 nm) which are the wavelengths responsible for the majority of bacterial inactivation under natural sunlight [31]. Polystyrene also transmits the highly lethal UV-B. However, this is mostly absorbed by the atmosphere and hence is not a major component of sunlight reaching the earth's surface. During the summer months, the solar irradiance of the spectral bands at sea level is UV-B (290-320 nm) = 0.5%, UV-A (290-400 nm) = 6.3%, visible (400-760 nm) = 48.9%, and infrared (720-3000 nm) = 46.3%. UV-A represents roughly 90-92% of the total solar ultraviolet radiation (UVR) [78]. This means that on the earth's surface ultraviolet radiation of sunlight is composed of mostly UV-A radiation with some UV-B.

The solar simulated apparatus has been used to inactivate a variety of pathogens such as bacteria, viruses and parasites [26, 31, 36]. Complete or partial inactivation was dependent on the fixed temperature of the water. Poliovirus and

Acanthamoeba polyphaga cysts, *Cryptosporidium parvum* oocysts and *Giardia muris* cysts showed complete or partial inactivation of a given population at temperatures > 45ºC. The higher the temperature the less exposure time was needed under the simulator [31, 36].

2.1.3 Aims

The aim of this study were to

(i) Determine if solar simulated light can be used to disinfect de-ionised water contaminated with *E. coli* O157.

(ii) Determine inactivation kinetics for *E. coli* O157 by using a realistic temperature profile.

2.2 Materials and Methods

2.2.1 Bacterial Preparation, Cultivation and Enumeration

E. coli K-12 (ATCC 23631) and *E. coli* O157 (non-verotoxin producing clinical isolate provided by the Irish Health Service Executive Public Health Laboratories, Cherry Orchard, Dublin Ireland; laboratory strain identifier 05-E9-25) were obtained from frozen stocks (-80ºC) and streaked onto Luria-Bertani (LB) agar plates (L61746; Sigma, United Kingdom) and incubated at 37ºC. As *E. coli* O157 is a recognized cause of potentially severe diarrhoea, laboratory work with bacteria was conducted in a Level 3 biosafety cabinet using personal protective equipment (gloves and lab coat). Suspensions of both bacteria were then prepared by transferring single colonies from incubated plates and inoculating Luria broth (L3522; Sigma, United Kingdom). Inoculated broths were incubated at 37ºC for 18 h with shaking at 200 rpm to obtain a stationary phase culture. Bacterial cells were harvested by centrifugation at 2000 g for 10 minutes and the pellet obtained was washed three times with phosphate buffered saline (PBS) solution in order to remove any growth medium. The pellet was then resuspended in sterile de-ionised water to obtain a final concentration of 10^6 CFU/ml.

The bacterial population in the water was determined using the Miles and Misra drop count technique [79]. Samples were serially diluted in distilled water and 20 µl of sample dropped, in triplicate, onto LB agar plates. These plates were incubated overnight and counted the following day. The detection limit for this technique was 7 CFU/ml. The log-kill of bacteria for each time point was calculated using the formula (Log N_t / N_0) where N_t is the viable count of bacteria at a given experimental time point and N_0 is the initial bacteria viable count at time zero.

2.2.2 Solar Simulated Experiments

Volumes of 10 ml 10^6 CFU/ml *E. coli* K-12 and *E. coli* O157 preparations were aliquoted into lidded 6-well transparent polystyrene tissue culture plates and placed in position E (Fig. 2.3). Dark controls consisted of suspensions in tissue culture plates but covered with aluminium foil to exclude simulated sunlight. These plates were placed in position F (Fig. 2.3). The temperature was maintained using a stirred thermostatic regulator (GA100, Grant Instruments LTD., Cambridge, UK.) and was calibrated against a thermocouple probe (Checktemp 2; Hanna Instruments**,** Bedfordshire, UK). Temperatures in the wells were taken at the beginning of the experiment at time point 0 and at each time point thereafter to obtain a temperature profile for the duration of the experiment. After exposure to simulated sunlight, tissue culture plates containing inactivated bacteria suspension were placed on the bench at room temperature and plated out on LB agar after 48 h to determine if re-growth of bacteria had occurred.

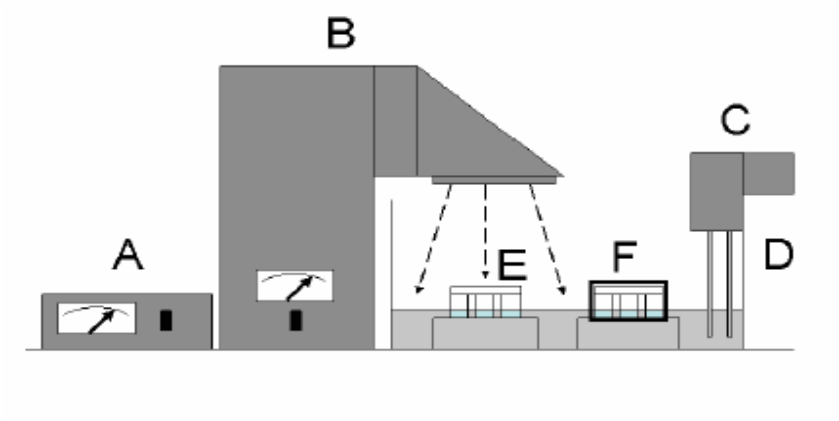


Figure 2.3 *Solar filtered 1000 W xenon arc lamp solar simulator apparatus (A)1000 W arc lamp power supply; (B) lamp intensity controller; (C) water heater; (D) water bath; (E) test microbe suspension sample in tissue culture plate and (F) dark control test microbe suspension sample wrapped in aluminium foil.*

2.3 Results and Discussion

Inactivation of *E. coli* O157 and *E. coli* K-12 using Simulated Sunlight

E. coli O157 was completely inactivated within 4 h exposure to simulated sunlight conditions (885W/m^2) (Fig. 2.4). This demonstrates the susceptibility of the organism to solar disinfection as has been previously reported for other enterobacteriacea such as *Salmonella typhimurium*, *Shigella dysenteria*, *Vibro cholera* and *Pseudomonas aeruginosa* [18, 20, 25, 26]. Differences in the time required for inactivation and pattern of inactivation in relation to dose of sunlight requires that every organism be tested individually to evaluate its response to SODIS under set conditions. Moreover, solar disinfection experiments have been conducted under a wide variety of experimental conditions which makes it difficult to compare results from different studies. Although *E. coli* O157 and *E. coli* K-12 share biological similarities, relying on the inactivation time of *E. coli* K-12 would have resulted in an underestimation of the inactivation time required for *E. coli* O157 and would have significant consequences on infection given the low infectious dose of *E. coli* O157. *E. coli* K-12 only required 3 h to inactivate approximately a 10^6 CFU/ml population of bacteria while *E. coli* O157 required 4 h for the same population of bacteria to be inactivated under similar solar simulated conditions (Fig. 2.4). This difference in two similar organisms within

the same species exemplifies the need for every organism to be tested individually.

Khaengraeng and Reed [80] obtained a T_{90} inactivation time of 38 minutes for their study on solar simulated inactivation of a non-toxigenic strain of *E. coli* O157. In Fig. 2.4, the T_{90} inactivation time obtained also for a non-toxigenic strain of *E. coli* O157 was 75 minutes and a T_{99} inactivation time of 230 minutes. Differences in inactivation times between the two strains are likely due to the different experimental conditions under which the respective SODIS experiments were conducted and the use of different solar simulator lamps. When performing solar disinfection studies under laboratory conditions, experimental factors affect the outcome of solar disinfection. These factors include starting concentration of bacteria, differences in strain, and the osmotic pressure of the liquid that is contaminated with the test bacteria (distilled water, natural water, and turbid water). Growth conditions of bacteria prior to inoculation into water and the way in which bacteria are exposed to sunlight also affect inactivation. These factors could account for the differences in inactivation times observed.

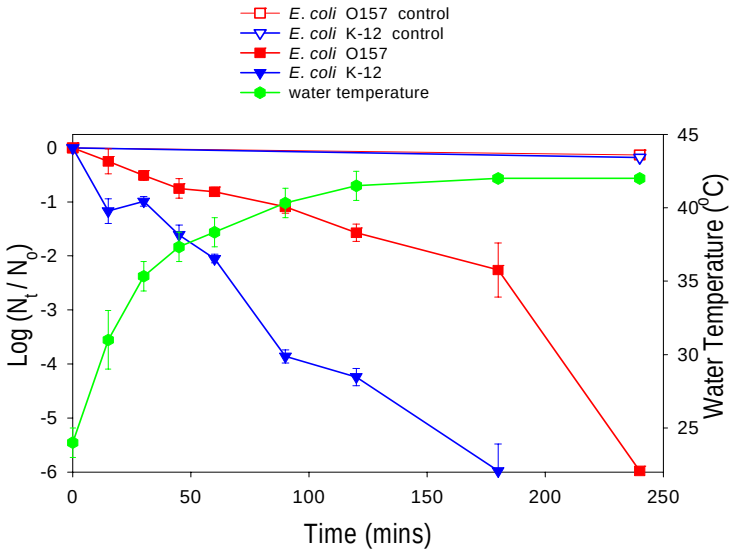


Figure 2.4 *Inactivation curves of E. coli O157 and E. coli K-12 in de-ionised water exposed to simulated sunlight. Each point represents the average of triplicates and error bars show the standard error limits.*

The intermittent exposure to sunlight at the beginning of the experiment as a result of the removal of the tissue culture plate every 15 minutes from simulated

sunlight in order to facilitate sample plating, might have resulted in a slight recovery of some bacterial cells. This was seen at time point 30 minutes during *E. coli* K-12 inactivation [77]. Continuous and intermittent solar exposure are known to have an effect on subsequent inactivation observed [81, 82]. However, optical exposure conditions were the same for *E. coli* K-12 and *E. coli* O157. Differences in inactivation are likely due to the response of the different bacteria to a hypotonic solution such as de-ionised water, with *E. coli* K-12 being more susceptible to osmotic pressure than *E. coli* O157. A study showed a wild type *E. coli* O157: H7 strain having a low permeability after growth at 37ºC allowing it better survival in hypotonic environments [83].

Under natural sunlight conditions water gradually heats up from a minimum temperature to a maximum and then to a minimum. During SODIS under high intensity sunlight and depending on the reactor (i.e. use of reflectors or blackening of the side of the bottles) [6, 14] water temperatures are known to reach levels up to 55ºC which results in pasteurization under field conditions [18, 43]. These high water temperatures do not occur all the time due to low intensity sunlight as a result of seasonal change or cloud cover. Furthermore high temperature resistance has been observed in some faecal coliforms and hence a greater temperature would need to be reached for the thermal process of sunlight alone to be sufficient for inactivation [19].

SODIS works on the basis of two major factors, the lethal action of solar UV-A light, and the synergistic effect which is created when water temperature rises above 45ºC [21]. These temperatures > 45ºC are sometimes reached under real conditions. Berney *et al*. analysed the temperature effect (mild heat) to inactivate *E. coli* and three pathogenic strains, *S. typhimurium*, *S. flexneri* and *V. cholerae* using laboratory tests with temperatures controlled between 41ºC and 52ºC [20]. The increase of temperature yielded an increment of the inactivation rate. They showed that *S. typhimurium, S. flexneri* and *E. coli* were similarly affected by mild heat with *E. coli* being less sensitive at higher temperatures [20]. Nevertheless, temperatures between 20-40ºC do not produce a strong synergy with UV-A to induce a high acceleration on inactivation of *E. coli* by UV-A, however a synergistic effect between thermal and optical processes has been

observed when temperatures > 45ºC are reached. Previous solar experiments have been conducted at a fixed maximum temperature and therefore might have overestimated the amount of time and UV-A dose required for inactivation. As can be seen from Fig. 2.4, where a natural temperature profile from 24ºC to 42ºC was followed, bacterial inactivation for both organisms occurred at low temperatures. However, temperatures > 45ºC were not achieved so a synergistic effect on inactivation was not expected. It should be noted that a cumulative stress effect on bacteria (i.e. exposure to a hypotonic solution followed by solar radiation and an increase in temperature) and increased sensitivity or decreased sensitivity to mild heat, might result in the synergistic effect on bacterial inactivation occurring at a lower temperature or higher temperature [20].

Re-growth of both *E. coli* O157 and *E. coli* K-12 did not occur after 48 h showing the dose of simulated sunlight received by bacterial cells had been enough to cause lethal damage without the possibility of repair.

2.4 Conclusions

1. This study demonstrates the inactivation of *E. coli* O157 under simulated sunlight conditions and confirms the potential of SODIS to be an effective household water treatment method for this highly infectious waterborne pathogen.
2. By following a natural temperature profile during SODIS under simulated conditions, inactivation times obtained for *E. coli* O157 are comparable to those that will be obtained under real sunlight conditions. However, the effects of turbid water which reduces sunlight penetration, as well as chemical constituents of natural water which could exert physiological stress can make organisms more or less sensitive to solar inactivation.

Chapter 3

Solar Disinfection (SODIS) in Continuous-Flow and Batch Systems: The Effect of Temperature, Irradiated Surface Area, Flow Rate, UV Intensity and UV Dose on Inactivation of Bacteria

3.1 Introduction

In this chapter, the limitations of solar disinfection, when it is scaled-up through the use of continuous-flow recirculation reactors, are explored. The effect of temperature, irradiated surface area, flow rate, UV intensity and UV dose on pathogen inactivation under natural sunlight and with larger volumes of water still needs to be investigated, since previous knowledge on the effect of the above-mentioned parameters was generated from small disinfection systems. An understanding of the role these parameters play during solar disinfection in these reactors could eventually lead to the design of a reactor in which large quantities of water are efficiently disinfected under both sunny and cloudy conditions with the shortest residency time possible.

3.1.1 Continuous-Flow Solar Reactors

After demonstrating the process of solar disinfection in batch systems extensively under various solar conditions, Acra *et al*. explored the possibility of solar disinfection in continuous-flow reactors [51]. Two types of solar reactors were constructed: Type I and Type II. Type I solar reactors (Fig. 3.1) consisted of a borosilicate glass tube shaped in a serpentine formation, mounted on a metallic frame inclined at 34º [51]. This inclination maximised the amount of solar radiation reaching the reactor, since the latitude of Beirut, where the solar

disinfection experiments took place is 34º. Solar reactor IA treated a total volume of 4.87 L and solar reactor IIA a volume of 5.20 L [51].

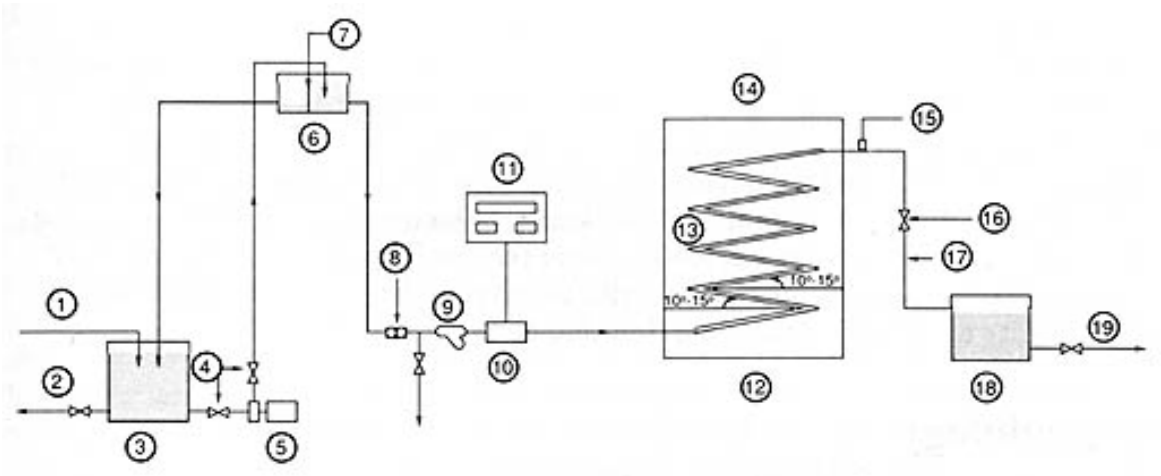


Figure 3.1 *Type IA solar reactor [51].(1)-Water feed; (2)-Drain; (3)-Storage reservoir containing contaminated water; (4)-Gate valve; (5)-Pump; (6)-Constant head-tank; (7)-Overflow; (8)-Flow regulator; (9)-Strainer; (10)-Flow meter; (11)-Digital flow-meter panel and control; (12)-Solar reactor; (13)-Serpentine transparent tube; (14)-Inclined from support(facing south); (15)-Air valve; (16)-Globe-valve; (17)-Effluent; (18)-Storage tank; (19)-Distribution*

Type II solar reactors (Fig. 3.2) were constructed to maximise the amount of sunlight reaching the reactors by using more transparent components. Solar reactor IIA was made of up two Pyrex glass containers to hold a volume of 4.23 L as well as a Pyrex glass helix with a volume of 1.0 L and hence the total volume treated by the reactor was 9.46 L [51]. Solar reactor IIB had four Pyrex glass containers connected to the Pyrex glass helix and treated a total volume of 17.92 L. Both Type I and Type II reactors had a flow rate maintained at 0.07 – 0.62 L/min with a uniform UV-A distribution [51].

Solar collector technology had been found to be effective when used for thermal applications; however, the possible use of collectors for disinfection had yet to be explored. Vidal *et al*. [84] constructed a prototype flow reactor (Fig. 3.3) made up of 12 Pyrex borosilicate glass tubes placed at the linear focus of compound parabolic concentrators (CPCs) which had aluminized reflective surfaces. The

apertures of the CPCs were tilted at 43º, the latitude of Zamudio, Spain, where the solar reactor was located. The reactor treated a total volume of 25 L and could potentially have a daily output of 50 L/m^{2}h [6, 84].

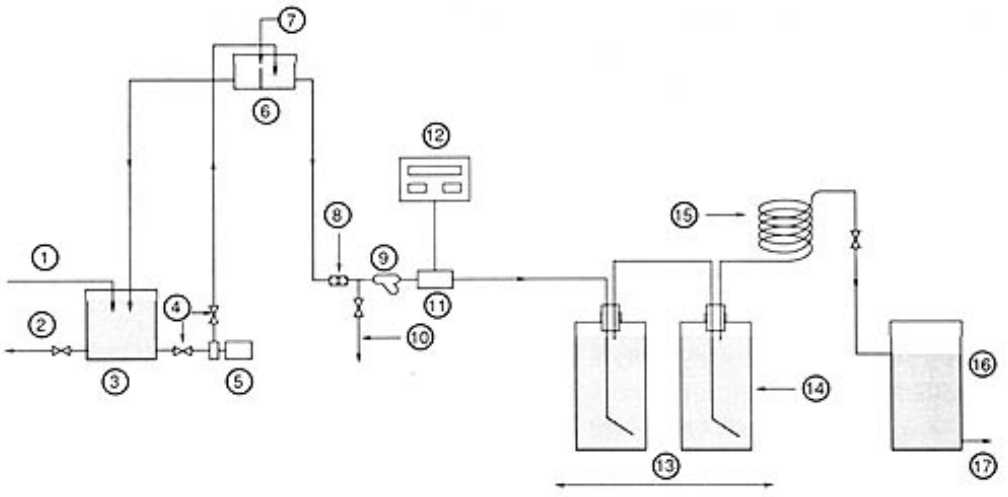


Figure 3.2 *Type IIB solar reactor [51] (1)-Water feed; (2)-Drain; (3)-Storage reservoir containing contaminated water; (4)-Gate valve; (5)-Pump; (6)-Constant head-tank; (7)-Overflow; (8)-Flow regulator; (9)-Strainer; (10)-Drain; (11)-Flow-meter panel; (12)-Digital-meter panel and control; (13)-Solar reactor; (14)-4 L glass bottles; (15)-Transparent spiral tube; (16)-Storage tank; (17)-Distribution.*

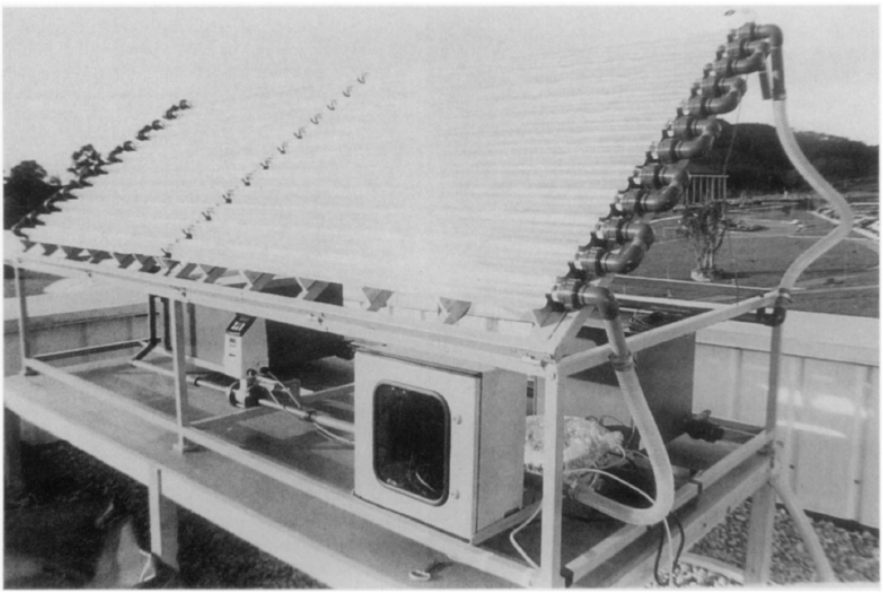


Figure 3.3 *CPC pilot plant [84]*

Three small-scale solar collector reactors were then designed to determine the type of collector that would yield the best microbial inactivation rates [85]. The three different collectors were compound parabolic (CP), parabolic (P) and V-groove (V), all made from polished aluminium (Fig. 3.4) [85]. Each reactor consisted of six tubes mounted at the focal points of their respective collectors. The apertures of the collectors were tilted at 37º, the latitude of Almería, Spain where the three reactors were located. The three reactors treated a total volume of 1 L at a flow rate of 2.8 l/min [85].

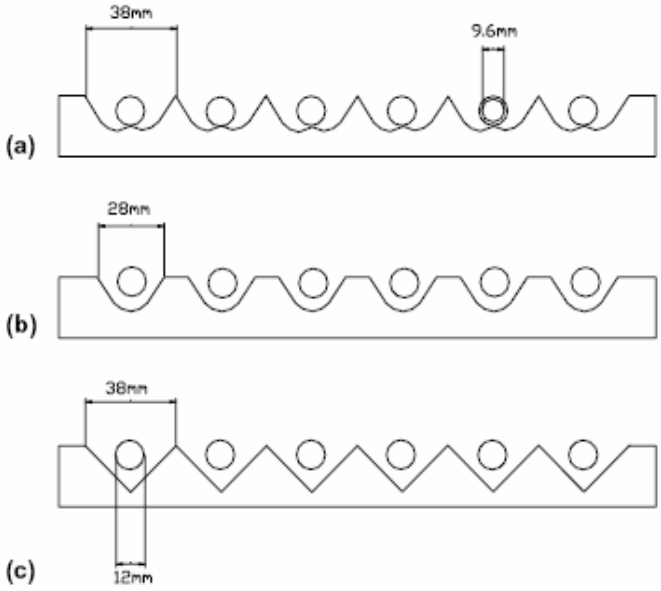


Figure 3.4 *Cross section of reactors: (a) compound parabolic, (b) parabolic and (c) V-groove [85].*

Fjendbo Jørgensen *et al*. [63] evaluated a flow through solar flat-plate reactor (Fig. 3.5) that used solar radiation to pasteurise water. The reactor consisted of a series of copper piping covered by aluminium plates [63]. Both the plates and pipes were painted black to increase absorption of solar radiation. A thermostat valve controlled the flow of water. Once water had reached a desired temperature, the valve opened to allow water to flow. The output of treated water was 50 l/m^{2}d [6, 63].

Saitoh and El-Ghetany [64] tested a flow through reactor (Fig. 3.6) that combined filtration, pasteurisation and UV disinfection. Contaminated water

entered a filter at 10 l/min, after filtration water entered a heat exchanger where it was heated and then finally entered a solar hot box [64]. The inside walls of the solar hot box were covered with aluminium foil, which reflected sunlight on to a transparent Pyrex glass container through which water coming in from the exchanger flowed into. When water reached the desired temperature in the solar hot box, a thermal controlled valve opened to release water into the storage container [64].

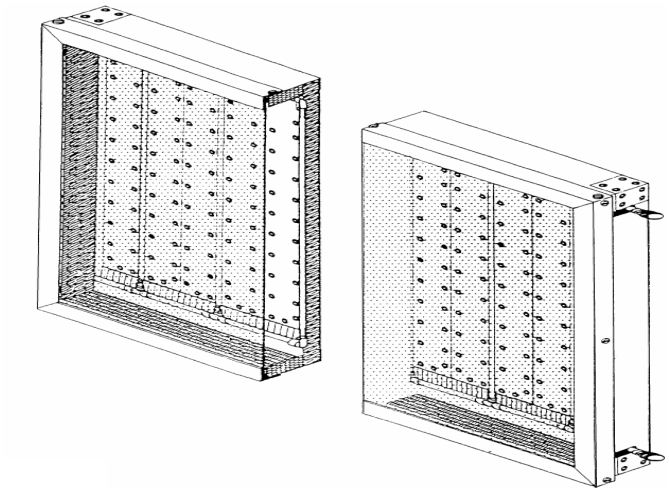


Figure 3.5 *Solar flat-plate collector with copper tubes covered by aluminium plates [63].*

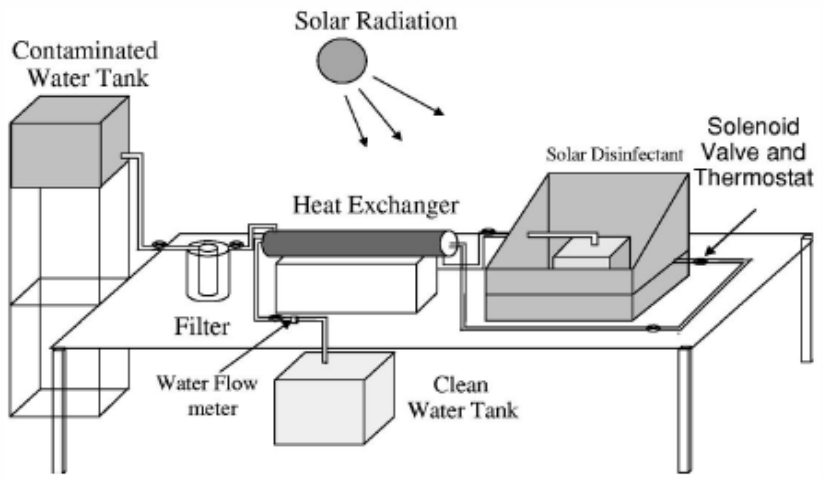


Figure 3.6 *Thermally controlled flow solar water disinfecting system [64].*

3.1.2 Microbial Efficiency in Continuous-Flow Solar Reactors

The reactors set up in Beirut by Acra *et al*. [51] were able to inactivate faecal indicators *E. coli* and *Enterococcus faecalis* in contaminated water. In order to achieve a 99.9 % inactivation of *E. coli*, a UV-A dose of 19.08 Wh/m^2 (68.69 kJ/m^2) was needed and for *E. faecalis*, a UV-A dose of 26.72 Wh/m^2 (96.19 kJ/m^2) [51]. Modelling bacterial inactivation under first order kinetics, the bacterial inactivation rate constants were k = 0.34 m^2Wh^{-1} (1.22 m^2kJ^{-1}) for *E. coli* and k= 0.25 m^2Wh^{-1} (0.90 m^2kJ^{-1}) for *E. faecalis* [51]. Laboratory strain bacteria that were physically seeded into water to be disinfected had faster inactivation rates compared to bacteria that were found naturally in sewage contaminated water [51]. It was concluded that inactivation rates of bacteria in sewage water were more likely to give a better representation of the efficiency of SODIS in flow reactors compared to laboratory strains. Regrowth of inactivated organisms was not observed after inactivation and neither was the growth of microalgae on the surfaces of the reactor, possibly because of the lack of sufficient nutrients for growth in water [51].

The pilot CPC plant set up by Vidal *et al*. [84] was also evaluated by determining the inactivation of an approximately 10^3 CFU/ml concentration of *E. coli* and *E. faecalis*. 99.99% of *E. coli* was inactivated in 30 minutes and 99.99% of *E. faecalis*, in 40 minutes [84]. The inactivation rate constants in the CPC reactor were k = 0.66 m^2Wh^{-1} (2.38 m^2kJ^{-1}) for *E. coli* and k = 0.52 m^2Wh^{-1} (1.88 m^2kJ^{-1}) for *E. faecalis*; both constants were two times greater than those observed by Acra *et al*. in their solar reactor [51, 84]. Both reactors showed that *E. coli* was more sensitive to solar irradiation when compared to *E. faecalis* [51, 84]. This increased resistance of *E. faecalis* has also been observed for other water treatment methods such as chlorine [84].

The inactivation of a 10^6 CFU/ml concentration of *E. coli* K-12 was used to determine which reflector profile provided the best inactivation in flow reactors fitted with different reflectors. There was a 3-4 log reduction bacteria within 60 minutes for all three reactors, however, the highest rate of inactivation, 0.17 was obtained in the reactor with the compound parabolic reflector [85]. The reactor with the CP reflector was 28 % more efficient than the reactors with parabolic

and V-groove reflectors [85]. There was no significant difference in inactivation efficiency between parabolic and V-groove reflectors which had an inactivation rate of 0.13 and 0.12 respectively [85].

The thermally controlled solar flat-plate reactor evaluated by Fjendbo Jørgensen *et al*. [63] reached a minimum temperature of 65°C under strong sunshine conditions (960 W/m^2) and this led to the inactivation of approximately 10^4 CFU/ml concentration of *E. coli*, *E. faecalis* and *S. typhimurium* [63]. Although not tested, it was concluded that other heat resistant protozoa (*G. lamblia*), helminths eggs (*Enterobius vermicularis*) and enteric viruses are likely to be inactivated in the reactor once maximum water temperatures of 85°C have been reached. In order to inactivate *Hepatitis* A, the water has to be maintained at 85°C for at least 4 minutes [63].

3.1.3 Water used in Continuous-Flow Solar Reactors

Microbial inactivation studies in flow reactors have used different types of water, e.g. sewage water, well-water, distilled water and de-ionised water. Using a natural source of water such as well-water, sewage water and river water gives a better prediction of microbial inactivation under real conditions. Using natural water avoids weakening of bacterial cells due to an unfavourable osmotic environment (lack of ions). Bacteria showed better survival rates in flow reactors under dark conditions when suspended in well-water as opposed to distilled water [86]. By using water containing ions, bacterial cells undergo less osmotic pressure as the concentration of water molecules in the well-water is similar to that of the concentration of water in the cell. This prevents water from rushing into the bacterial cell and creating pressure. A greater osmotic pressure does not lead to lyses of the cell with regard to distilled and de-ionised water [25] but it does mechanically weaken the cell membrane by causing the phospholipid bi-layer to flatten, which results in conformational changes in integral membrane proteins [87] . Furthermore, depending on the severity of osmotic pressure, 10-80% of solutes such as K^+ and ATP, which are essential for cell function are also expelled in order to lower the osmotic pressure within the cell [88, 89].

Though the presence of ions may help to retain bacterial integrity, if ions are present in high concentrations they could have a limiting effect on the SODIS process. UV-A mediates its biological effects on bacteria by reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals [90]. If bicarbonates (HCO_3^-) are present in water they react with hydroxyl radicals producing $CO_3^{\bullet -}$ which has a slower reaction with organic molecules when compared to $^{\bullet}OH$ [91]. Furthermore, HCO_3^- induces photo-absorption which limits the amount of light reaching bacteria in water [60]. Other anions such as phosphates, chloride and sulphates are shown to be absorbed by bacteria but do not illicit a direct effect on solar inactivation unless in the presence of a photocatalyst such as titanium dioxide [60].

3.1.4 Aims

The aims of this study were to:

(i) Determine the limitations of SODIS when it is scaled-up through the use of continuous flow recirculation reactors. For this purpose the effects of several parameters on inactivation were studied: temperature (T), the total volume of treated water (V_t), the irradiated area of the solar collector in the photoreactor (A_{irr}) and the flow rate (Q).

(ii) Determine whether in natural sunlight, inactivation of *E. coli* K -12 is driven by: solar UV irradiance, the total solar UV dose received or a combination of both factors.

3.2 Materials and Methods

3.2.1 Bacterial Preparation and Cultivation

For safety reasons and its known susceptibility to solar disinfection, *E. coli* K-12 instead of *E. coli* O157 was used in the subsequent experiments. Cultures of *E. coli* K-12 ATCC 23631 were generated from frozen stocks by streaking onto Luria Bertani (LB) (Sigma-Aldrich, USA) agar and incubated at 37°C for 15-18 h. A single colony was then inoculated into 2.5 ml sterile LB broth (Miller's LB

Broth, Sigma-Aldrich, USA) and was then incubated at 37°C for 18 h on a rotary shaker to obtain a stationary phase culture. Cells were harvested by centrifugation at 800 x g for 10 min. The pellet was resuspended in 2.5 ml PBS to obtain a final concentration of 10^9 CFU/ml. Volumes of 2.5 ml, 14 ml and 70 ml of stock concentration of bacteria were inoculated into the 2.5 L, 14 L and 70 L volume reactors, respectively, in order to obtain a 10^6 CFU/ml starting concentration of bacteria for each experiment. All the experiments were conducted in duplicate, and each sample was plated in triplicate.

3.2.2 Enumeration of Bacterial Regrowth after Solar Exposure

Enumeration of bacteria contained in the borosilicate tubes exposed to sunlight was conducted through the standard plate count method after a series of 10 fold dilutions. 20 µl of diluted sample were spread on LB agar plates in triplicate and incubated 37ºC overnight and counted the following day. To ensure that the starting concentration of bacteria in the tube of water was indeed 10^6 CFU/ml, a sample of water was taken before the tube was exposed to sunlight. This dark control sample was kept on a rotary shaker in a dark 37ºC incubator for the duration of the experiment, and was then plated, incubated overnight and counted the following day. The detection limit for this experimental method was found to be 4 CFU/ml. Re-growth counts of bacteria were determined for all experiments by leaving the last two samples at room temperature for 24 h and 48 h. After 24 h and 48 h, the plate count method as described above was used to determine bacterial counts on both LB agar plates and Endo agar (Sigma-Aldrich, USA) plates.

Endo agar is a selective media, specific for the detection of coliforms and enteric organisms in water and wastewater [92]. Endo agar was also used as a way to suppress and prevent growth of any non-coliforms, non-enteric organisms that might have grown during the 24 h and 48 h post irradiation period and could interfere with accurate counts of *E. coli* K-12 in these samples. Colonies of *E. coli* on Endo agar are red with a permanent greenish intense metallic sheen while other lactose positive bacteria are red but without the sheen [93]. Lactose-negative bacteria are colourless and irregular shaped and gram positive colonies do not grow at all due to inhibition by sodium sulphite and fuchsin [93]. The use

of selective agars might suppress and even prevent the growth of injured and weak organisms [94, 95], however there was no significant ($p<0.05$) difference in growth counts of pure *E. coli* K-12 on LB agar and on Endo agar even after exposure to SODIS. In the case where complete inactivation of *E. coli* K-12 occurred, no colonies were present on both LB and Endo agar. Hence, for this study, Endo agar was deemed as an appropriate media for differentiating between *E. coli* K-12 and other bacteria during solar disinfection of water.

3.2.3 Water Composition

Water was collected from a well situated on the PSA site from a depth of approximately 200 m. The concentration of naturally occurring organisms was determined by the plate count enumeration technique using LB agar and was found to be 100-200 CFU/ml. These organisms were identified as non-coliforms and non-enteric in nature due to their presentation as either irregular, colourless colonies on Endo agar or the complete lack of growth also on Endo agar. So that all the experimental results could be compared, a single batch of 1000 L of well-water was withdrawn at once so the same stock of water could be used in all the experiments, and no variation in the composition of the natural well-water was possible. Table 3.1 provides average values of a list of physical and chemical parameters of the well-water used during the experimental period.

Cation concentrations were determined with a Dionex DX-120 ion chromatograph equipped with a Dionex Ionpac CS12A 4 mm x 250 mm column at a flow rate of 1.2 ml min^{-1}. Anion concentrations were determined with a Dionex DX-600 ion chromatograph using a Dionex Ionpac AS11-HC 4 mm x 250 mm column. The gradient programme for anion determination was pre-run for 5 min with 20 mM NaOH, an 8-min injection of 20 mM of NaOH, and 7 min with 35 mM of NaOH, at a flow rate of 1.5 ml min^{-1}. Turbidity measurements were performed using a turbidimeter (model 2100N, Hach, U.S.A.). For all the experiments, turbidity values between 1 and 2 NTU were obtained. The presence of Fe in the water was not observed using UV-VIS measurements (detection limit 0.05 mg/l).

Table 3.1 *Physical and chemical properties of well-water*

PO_4^{3-}	0.5 mg/l	HCO_3^-	500 mg/l
Cl^-	355 mg/l	Na^+	370 mg/l
pH	7.3	Conductivity	2300 uS/cm
NO_3^-	22 mg/ml	NH_4^+	6 mg/l
Turbidity	1-2 NTU	Bacteria	100 CFU/ml
SO_4^{2-}	329 mg/ml	K^+	11 mg/l
F^-	1 mg/l	Mg^{2+}	64 mg/l
Br^-	2 mg/l	Ca^{2+}	122 mg/l

3.2.4 Solar Reactors

Three solar reactor systems were used (Table 3.2).

Table 3.2 *Physical characteristics of the solar reactors used in the experiments*

Characteristic	Borosilicate glass tubes	14 L solar CPC reactor	70 L solar CPC reactor
Total volume (L)	2.5	14	70
Illuminated volume (L)	2.5	4.7	47
Illuminated surface (m^2)	0.21	0.42	4.2
No. of tubes	1	2	20
Temperature (ºC)	--	--	25, 35, 45, 55
Flow rate (l/min)	0	2, 10	2, 10

(i) *Borosilicate glass tubes (batch systems):* The shape and dimensions of plastic bottles used for SODIS experiments are not standardised. All bottles perform with similar efficiency even though the amount of radiation reaching the water is not necessarily the same. The shape and surface of these bottles are usually irregular and the shape in some parts of the bottle is more hexagonal, than cylindrical. A correct assessment of the radiation entering the system would require detailed optical analysis and calculations for each particular bottle. To avoid this, we used cylindrical borosilicate glass tubes which were closed at both ends (Fig. 3.7a) and positioned axially along the linear focus of a CPC reflector (Fig. 3.7b). The tube is made of glass (Type 3.3, Schott-Duran, Germany), with

an optical cut-off at 280 nm. The tube is 1.3 m in length (excluding sampling valve and closed end), has an outer diameter of 0.05 m, a wall thickness of 1.8 mm, an internal volume of 2.5 L, and an irradiated collector surface area of 0.21 m^2 (Fig. 3.7b). The transmittance of transparent PET bottles is 85-90 % in UV-A (320-400 nm) wavelengths [18] while borosilicate glass tubes have a transmittance of 89-90% in the UV-A range [52].

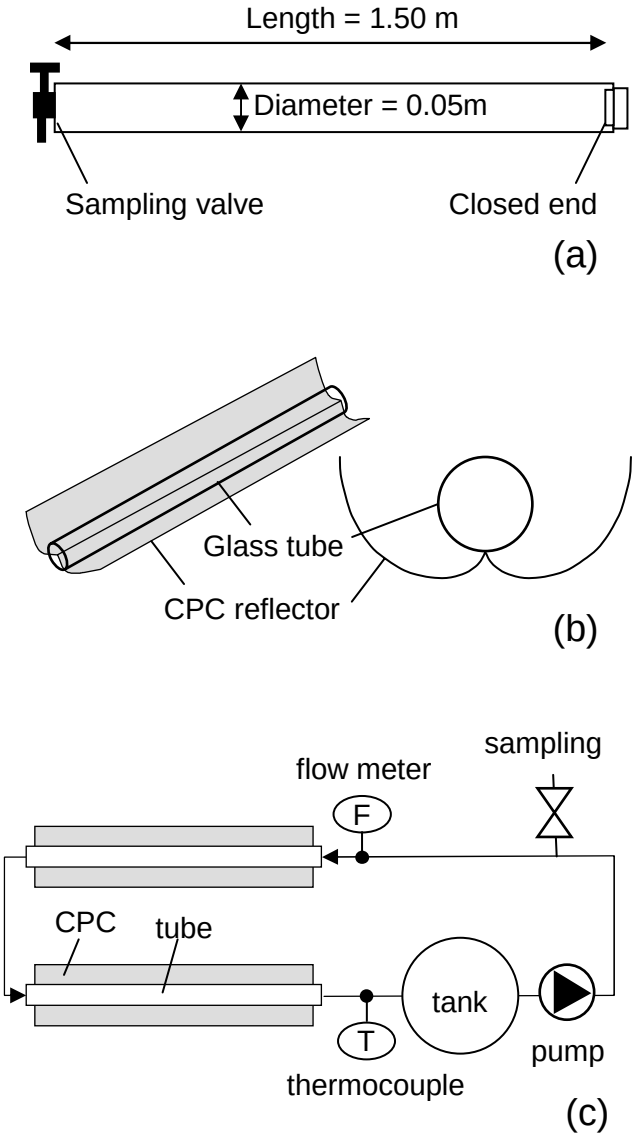


Figure 3.7 *Glass tube configuration (a), Tube + CPC collector configuration (b) and Flow diagram of the 14 L solar CPC reactor (c).*

The temperature of the water was monitored for the duration of each experiment to ensure that temperature of the water did not exceed 40ºC. The same procedure was used for reactor (ii).

(ii) *14 L solar CPC reactor (re-circulation):* The 14 L solar CPC reactor was designed and built specifically for photocatalytic water disinfection using natural solar radiation collected in a CPC [96]. Water is continuously recirculated between an opaque holding tank and a transparent photoreactor unit. This system consists of two concentric borosilicate glass tubes which are coaxially placed in the focus of CPC reflectors (Fig. 3.7b) designed for the best optical performance under varied solar conditions [97].

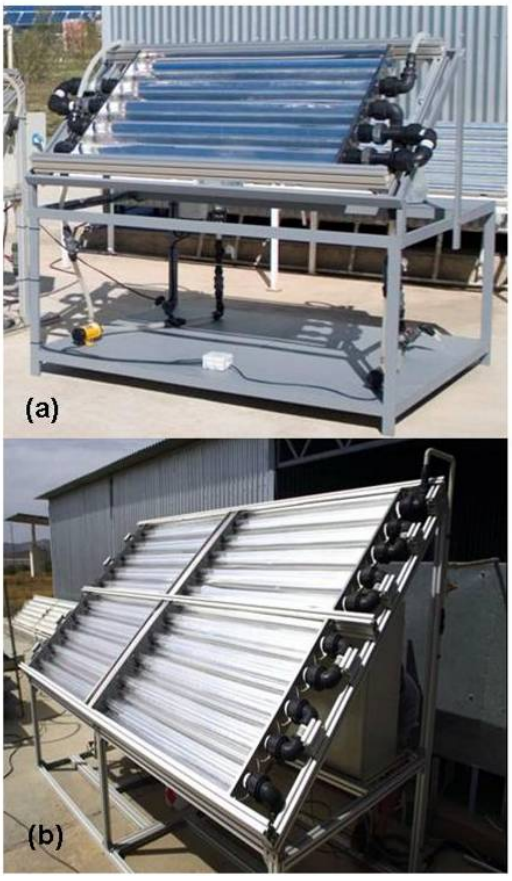


Figure 3.8 *14 L solar CPC reactor (a) and 70 L solar CPC reactor (b) both located at the PSA facilities in Almería, Spain.*

The system (glass tubes + CPC collectors) is held by aluminium frames mounted on platforms tilted at 37° local latitude. The glass tubes are connected so that water flows directly from one to another and finally into a tank (Fig. 3.7c). A

centrifugal pump (20 Watts, Panworld, Spain) then returns the water to the solar collector. The tank has an aperture on top into which contaminated water can be poured. For the disinfection process, this aperture is closed with a plastic lid. The treated water is later recovered by opening the outlet valve. The reactor volume is 14 L, the illuminated volume 4.7 L, and the irradiated collector surface 0.42 m^2. The picture in Fig. 3.8a shows the system of a 14 L solar CPC reactor, installed at PSA facilities. This reactor was used to analyse the behaviour of the solar disinfection in re-circulated systems at two flow rates, 2 and 10 l/min.

(iii) *70 L-solar CPC reactor (re-circulation):* In order to prove the capability of the disinfection process for a higher volume of water, a solar CPC reactor of 70 L was used. This system is similar in design to the 14 L-solar CPC reactor but with larger dimensions. It consists of four panels of solar CPC collectors, each having 5 CPC reflectors and 5 glass tubes of a similar design to those used previously in reactor (ii). The solar collectors are connected in series to a tank and a pump in a whole system as illustrated in Fig 3.7c. The photoreactor volume is 70 L, the illuminated volume 47 L, and the irradiated collector surface 4.2 m^2 (Fig. 3.8b). This solar reactor is equipped with an in-line temperature sensor and also incorporates indirect heating and cooling systems to control water temperatures without injuring the bacteria directly. This reactor was used for large scale experiments with temperatures controlled at 25, 35, 45, and 55 ºC and with different irradiated collector surfaces of 1, 2, 3, and 4 m^2.

3.2.5 Sunlight Exposure and Radiation Measurement

All experiments were performed under natural solar radiation at the Plataforma Solar de Almería (PSA), Almería, Spain, located at 37º84’ N and 2º34’ W. The PSA (facilities and collaborators) was chosen as a site for SODIS experiments due to its long history of extensive work and knowledge in the solar energy field. Furthermore, prototypes of solar reactors had been constructed at the site and were ready for use in preliminary SODIS experiments. Located at a latitude of 37º84’ N, Almería, lies just outside the favourable region of solar disinfection (15° N / S and 35° N / S). Therefore bacterial inactivation rates obtained using

SODIS reactors in Almería will be slow, when compared to faster inactivation rates and a better SODIS reactor performance which is expected to occur in SODIS favourable regions, where SODIS reactors are to be eventually used.

All the systems were inclined at 37º and facing South. This inclination corresponds to the latitude of the Plataforma Solar de Almería, South-East of Spain and maximizes the annual energy collection, enhancing the radiation reaching the system during the winter months. Solar UV irradiance was measured with a global UV radiometer (295-385 nm, Model CUV3, Kipp & Zonen, Netherlands) also inclined at 37º and placed next to the solar reactors. The solar UV energy delivered onto the system or solar UV dose ($Dose_{UV}$, J/m^2) was obtained by integration of solar UV irradiance (I_{UV}, W/m^2) over a given period of time (dt, s) in 1 minute intervals (Eq. 1).

$$Dose_{UV} = \int_{t_1}^{t_2} I_{UV} \cdot dt \qquad \text{(Eq. 1)}$$

Duplicated experiments were performed under similar solar radiation conditions which ensures that results can be reproduced. Clear, sunny days were used for all irradiation experiments to ensure that the amount of irradiance delivered into the systems was as similar as possible. Experiments were usually sampled over a 5 hour period, irrespective of the duration of sunlight exposure. Duration of solar exposure varied depending on the objective of the experiments.

3.2.6 Statistical analysis

Data obtained in the studies were analysed using the one-way ANOVA analysis tool (Origin v7.0300, OriginLab Corp., Northampton, USA). The results of duplicates of each experiment revealed that there are no significant differences ($p<0.05$, Confidence > 95%) in culturable bacterial population of the samples. When statistical analysis of results did not yield the 95% of confidence, triplicates measurements were carried out.

3.2.7 Modelling with Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFIT)

The Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFIT) is used for testing a number of microbial survival models on microbial inactivation curves [98]. The following models are used: log-linear regression , log-linear + tail [99], log-linear + shoulder [99], log-linear + shoulder + tail [99], Weibull model [100], biphasic model [101] and biphasic + shoulder [98]. All models were run for each inactivation curve and the values of the Root Mean Sum of Squared Errors (RMSE) were compared. The RMSE is considered to be the most simple and informative measure of goodness-of-fit for linear and non-linear models [98]. The model with the smallest RMSE was considered the best fit for the respective inactivation curve. If two models had the same or similar RMSE values the simpler model was considered to fit best. *Shoulder length* (h), k_{max} (h^{-1}; rate constant for the log-linear phase), N_{res} (CFU/ml, residual concentration of bacteria after treatment), t_{DL}(h) and Q_{DL} (kJ/l) (exposure time and solar UV-A energy per unit of volume to reduce plate counts to the detection limit (DL). Values were calculated using the best-fit model of GInaFIT (Table 3.3). These fitting results were obtained for results shown in sections 3.3.1, 3.3.2, and 3.3.3.

Table 3.3 *Fitting results of the experimental data derived from the program Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFIT). tDL and QUV-DL values (time and energy per unit of volume received to reach the detection limit (DL) of sunlight exposure for the different solar systems.*

Solar system/ experimental conditions	*Fitting model*	*Parameters*				
(Figure number)		*Shoulder length (h)*	k_{max} *(h^{-1})*	N_{res} *(CFU/ml)*	t_{DL} *(h)*	$Q_{UV\text{-}DL}$ *(kJ/l)*
Reactor (iii), dark, 25ºC (Fig. 3.9)	Log-linear	--	0.10 ± 0.2	--	--	-- (dark)
Reactor (iii), dark, 35ºC (Fig. 3.9)	Log-linear	--	0.0 ± 0.1	--	--	-- (dark)
Reactor (iii), dark, 45ºC (Fig. 3.9)	Biphasic (Log-linear, k_1 and k_2) and shoulder	0.7 ± 0.2	k_1 = 6.1±0.9 k_2 = 1.4±0.5	--	4	-- (dark)
Reactor (iii), dark, 55ºC (Fig. 3.9)	Biphasic (Log-linear, k_1 and k_2) and shoulder	0.9 ± 0.2	k_1 = 9 ± 3 k_2 = 1.6±1.6	--	4	-- (dark)
Reactor (iii), 1 m^2 exposed to sun (Fig. 3.10)	Log-linear, shoulder and tail	1.5 ± 0.2	5 ± 2	7×10^5 ± 10	--	--
Reactor (iii), 2 m^2 exposed to sun (Fig. 3.10)	Log-linear, shoulder and tail	0.69 ± 0.11	5.3 ± 0.5	293 ± 1	--	--
Reactor (iii), 3 m^2 exposed to sun (Fig. 3.10)	Log-linear, shoulder and tail	0.2 ± 0.4	6 ± 2	158 ± 2	--	--
Reactor (iii), 4 m^2 exposed to sun (Fig. 3.10)	Log-linear, shoulder and tail	0.62 ± 0.03	5.7 ± 0.2	503 ± 1	--	--
Reactor (i), 0 l/min (Fig. 3.12)	Log-linear and shoulder	0.41 ± 0.03	9.5 ± 0.2	--	2	18.7
Reactor (ii), 2 l/min (Fig. 3.12)	Log-linear, shoulder and tail	0.7 ± 0.2	7.6 ± 1.5	39.5 ± 1.4	--	--
Reactor (ii), 10 l/min (Fig. 3.12)	Log-linear, shoulder and tail	0.83 ± 0.13	5.1 ± 0.6	436.4 ± 1.3	--	--

3.3 Results and Discussion

3.3.1 Effect of Temperature on Inactivation

In Figure 3.9 the effect of water temperature on inactivation of bacteria in a continuous flow reactor is examined. For this purpose, the 70 L solar CPC reactor (Fig 3.8b) was used. Temperatures were set at 25, 35, 45, and 55 ºC for the duration of 5 h with the aim of monitoring the viability of bacteria in the reactor under operation at 10 l/min flow rate without solar exposure.

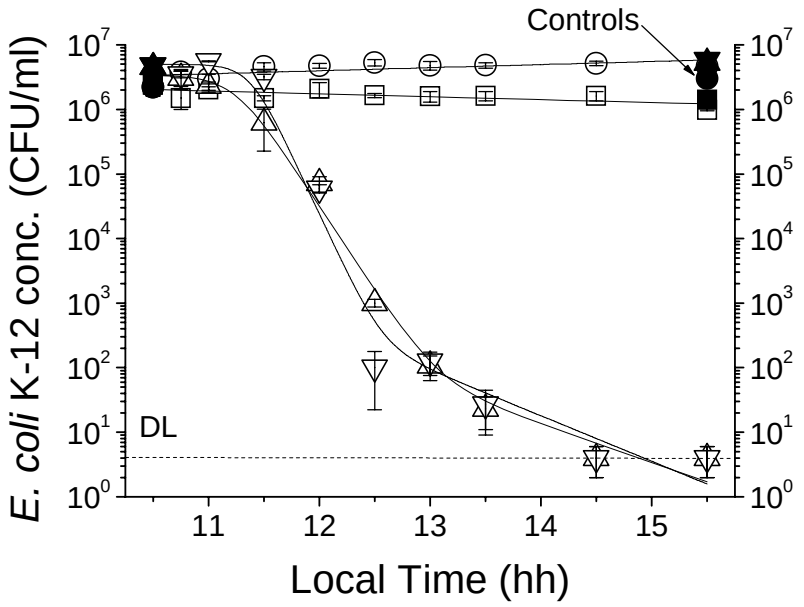


Figure 3.9 *E. coli K-12 behaviour in reactor (iii), under dark conditions, flow rate: 10 l/min, and controlled temperature: 25ºC (-□-), 35ºC (-○-), 45ºC (-Δ-), 55ºC (-▽-). Closed symbols represent control samples. Lines represent modelling results obtained with the software Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFIT) [98]. Dashed line (--) shows the detection limit (DL= 4 CFU/ml). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

At 25ºC there was a slight reduction (< 1 log) in bacterial population which can be attributed to the effect of shear forces caused by pumping the water through the continuous flow reactor. At 35ºC, favourable growth temperature facilitates bacterial growth and stability and thereby compensates any reduction in population due to shear force and results in a small increase in bacterial population (< 1 log). Complete bacterial inactivation occurred at temperatures of

45ºC and 55ºC after 5 h, confirming the well-known phenomenon of thermal inactivation of *E. coli* only occurring at temperatures > 40ºC [18]. In order for heat inactivation to play a role in SODIS, temperatures of at least 45ºC are sufficient to inactivate 10^6 CFU/ml concentration of *E. coli* K-12 even for 70 L of well-water at a constant flow rate of 10 l/min. Samples were stored for 24 h and 48 h at room temperature to evaluate re-growth of bacteria after inactivation at 45ºC and 55ºC. At both temperatures, bacterial re-growth was not observed after 48 h. Bacterial inactivation does not occur for temperatures below 40ºC and, inversely, the bacterial suspensions lose viability for temperatures above 40ºC; thus the reactor offers the typical thermal response of batch solar disinfection systems. Consequently any inactivation observed in solar reactors (ii) and (iii) during solar exposure can be attributed to solar radiation and, at most, only mild solar heating. According to GInaFIT modelling, mild heat inactivation was observed only for 45 and 55 ºC with shoulder length values of (0.7±0.2) h and (0.9±0.2) h respectively and biphasic log-linear decay for both cases. On the other hand, the very slow decrease observed for 25 and 35 ºC is also predicted with GInaFIT.

3.3.2 Effect of Irradiated Surface Collector Area on Inactivation

The effect of varying solar collector surface area on disinfection rates was studied using reactor (iii) (Fig. 3.8b). The objective was to evaluate the effect of increasing the illuminated surface on the disinfection result using 1, 2, 3, and 4 m^2 exposure areas (Fig. 3.10). The experiments were carried out using a flow rate of 10 l/min. The temperature was kept at a constant 25ºC during the duration of the experiments to ensure that bacterial inactivation due to heat was not a factor.

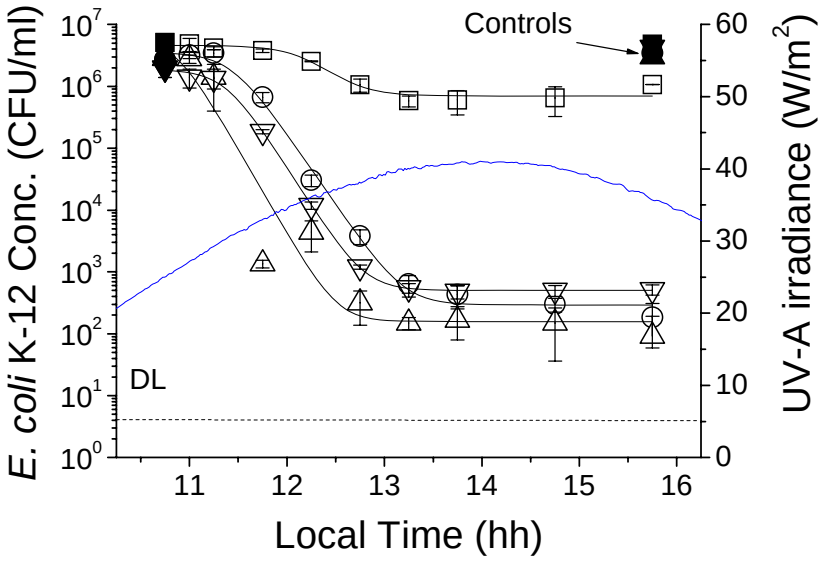


Figure 3.10 *Inactivation curves of E. coli K-12 in reactor (iii) during natural sunlight exposure. Flow rate: 10 l/min; illuminated collector surface: 1 m^2 (-□-), 2 m^2 (-○-), 3 m^2 (-Δ-), 4 m^2 (-▽-). Closed symbols represent control samples. Lines represent modelling results obtained with the software Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFIT) [98]. Dashed line (--) shows the detection limit (DL). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits and UV irradiance (—) (295-385 nm) data representative of one of the days.*

At 1 m^2 illumination, there was approximately a 1 log reduction in bacterial population. This reduction is likely to be a combination of natural inactivation of bacteria due to shear stress induced by the continuous flow process as well as inactivation by solar irradiation. With 2 m^2, an approximate 4 log reduction is observed. Complete inactivation of all bacteria is not achieved. A residual viable concentration of ~10^2 CFU/ml remained after 5 hours. Consequently, two further experiments were added to investigate whether the sequence or order of the way in which water passed through the 1 m^2 panels in the 2 m^2 cumulative exposure configuration, influenced the final inactivation achieved. The covered panels were arranged in diagonal and consecutive exposure configurations (Fig. 3.11).

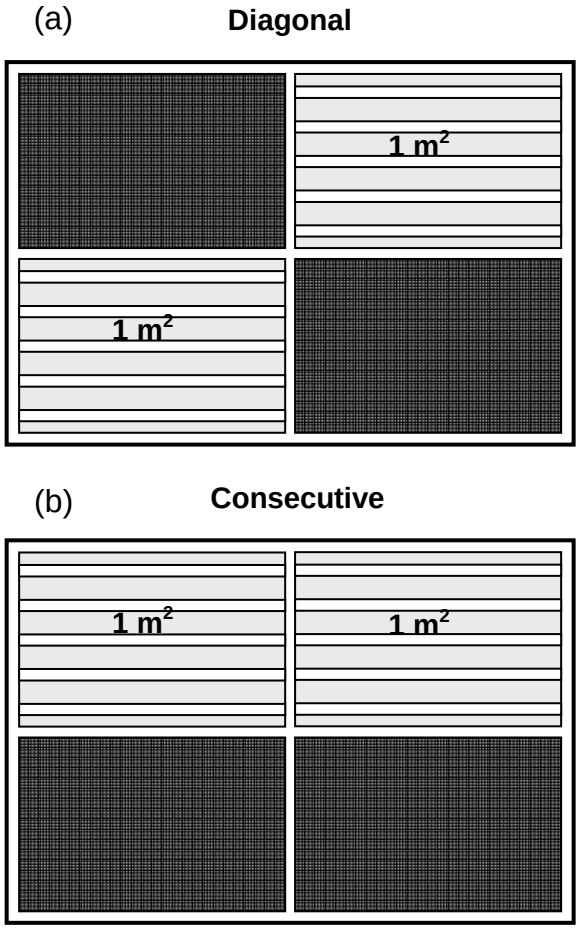


Figure 3.11 *Diagonal (a) and consecutive (b) exposure configurations used during illuminated area tests.*

No significant difference ($p<0.05$, one-way ANOVA analysis) was observed using both configurations - consecutive or diagonal exposure - since both result in similar inactivation rates (data not shown). A surface area $> 2\ m^2$ slightly decreases the time needed for inactivation by approximately an hour (from 2 to $4\ m^2$). However, it has no effect on the residual concentration of organisms remaining. This may be due to some of the organisms becoming UV resistant during the continuous flow process. Irradiation experiments indicate that once the minimum inactivation dose is reached, a higher dose does not necessarily produce a greater reduction in bacteria. Hence the manner in which the bacteria are suspended in the water and receive their irradiation dose influences the ultimate inactivation. For the described experiments, no significant enhancement

in efficiency was achieved when the ratio of illuminated volume to total volume was greater than 0.312.

These results were fitted with the GInaFIT Model Fitting tool and the parameters obtained (Table 3.3) predicted a behaviour described by a shoulder followed by a log-linear decay and a tail. This is quite common in disinfection processes using SODIS. It is usually found an initial phase of bacterial resistance to the treatment (shoulder), then bacteria start to loose their culturability (log-linear), and finally they can be totally inactivated or not depending on the bacteria and the process. In this case we observe a final residual concentration of bacteria which remain persistent to the treatment due probably to the presence of a small fraction of resistant bacteria or of a part of bacteria which overcome the process and develop defence mechanisms after being exposed to the treatment (tail). The parameters (Table 3.3) show a very similar behaviour in the four cases evaluated. All cases can be described with a shoulder + log-linear equation. The higher the irradiation surface, the lower shoulder length and concentration of residual bacteria, while inactivation rate constant is similar in all cases without reaching the detection limit.

3.3.3 Effect of Flow Rate on Inactivation

The flow rates used to evaluate the performance of solar reactors in previous experiments had been less than < 2 l/min. In these experiments, 2 l/min and 10 l/min were tested. A high flow rate is not only preferable in terms of treating a large volume of water in a shorter time, but also prevents biofilm formation, which has been known to occur with low flow rates in nutrient rich water [102]. However, experiments revealed that increasing flow rate (Fig. 3.12) had a negative effect on inactivation of bacteria, irrespective of the long exposure time of 5 h.

The results of the GInaFIT modelling tool (Table 3.3) showed again a behaviour described by a shoulder followed by a log-linear decay and a tail in the cases of flow rate, and without tail for the static system. At a higher flow rate there is a

higher shoulder length and concentration of residual bacteria with a lower inactivation rate constant.

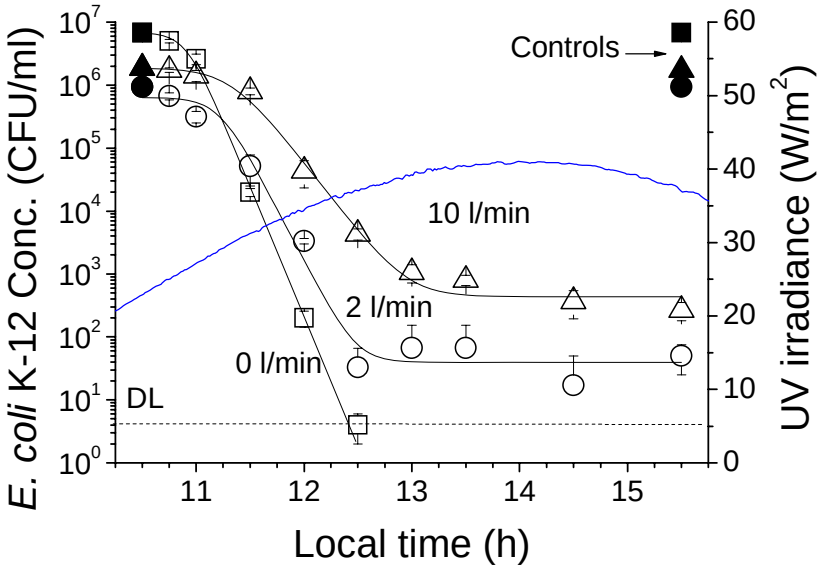


Figure 3.12 *Inactivation curve of E. coli K-12 in reactors (i), (ii) and (iii) during natural sunlight exposure. Open symbols represent control samples. Flow rate: 0 l/min (-□-), 2 l/min (-○-), 10 l/min (-Δ-). Lines represent modelling results obtained with the software Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFIT) [98]. Dashed line (--) shows the detection limit (DL). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits and UV irradiance (—) (295-385) nm.*

The negative effect of flow rate on bacterial inactivation suggests the need for the maximum exposure of bacteria to high UV doses in a short period of time to ensure inactivation; compared to having bacteria exposed to sub-lethal doses over a long period of time. During a 5 h illumination period, the batch reactor, made up of the borosilicate glass tube and filled with 2.5 L of water, received a total solar UV dose > 108 kJ/m^2. The batch system was continuously illuminated because it has no dark spaces and the bacteria were inactivated to below the detection level within 2 h. With the other two solar CPC reactors (Fig. 3.8 a and b), exposure of bacteria to intermittent doses was due to the recirculation rate of the water being 2 and 10 l/min, and even after a 5 h exposure to sunlight (a 2.5 times longer solar exposure time than the batch reactor), a residual concentration of 2 log bacteria still remained inactivated. Since the three systems have different irradiated surface areas and total treatment volumes, their efficacy during

treatment can be compared using the "*total solar-UV energy accumulated during the exposure per unit of treated volume"* (Eq. 2: Q_{UV}, in terms of kJ/l).

$$Q_{UV} = \int_{t_1}^{t_2} I_{UV} \cdot \frac{A_r}{V_t} dt \qquad \text{(Eq. 2)}$$

The system batch reactor system (i), received 18.7 kJ/l after the 2 h exposure (when total inactivation was observed); the 14 L solar CPC system (ii) accumulated a similar UV-A energy per unit volume of 19.5 kJ/l during the 5 h exposure; while the 70 L solar CPC system (iii) received 39.0 kJ/l also after a solar exposure of 5 h. Hence, the disinfection efficiency seems to be more dependent upon the uninterrupted received dose rather than on the accumulated dose over time.

3.3.4 Effect of UV Intensity and UV Dose on Inactivation

Based on the observations of bacterial inactivation under flow rate, the following experiments were carried out to confirm that once a certain uninterrupted dose is received by the system, total disinfection occurs. The borosilicate glass tube reactor was used with different exposure times and under different solar intensity conditions by varying the time of day at which the exposure started. All the experiments presented in this section were conducted over 5 h and once the required solar UV dose was received (calculated using Eq. 2), the tubes were covered with an opaque black plastic cover to prevent further illumination.

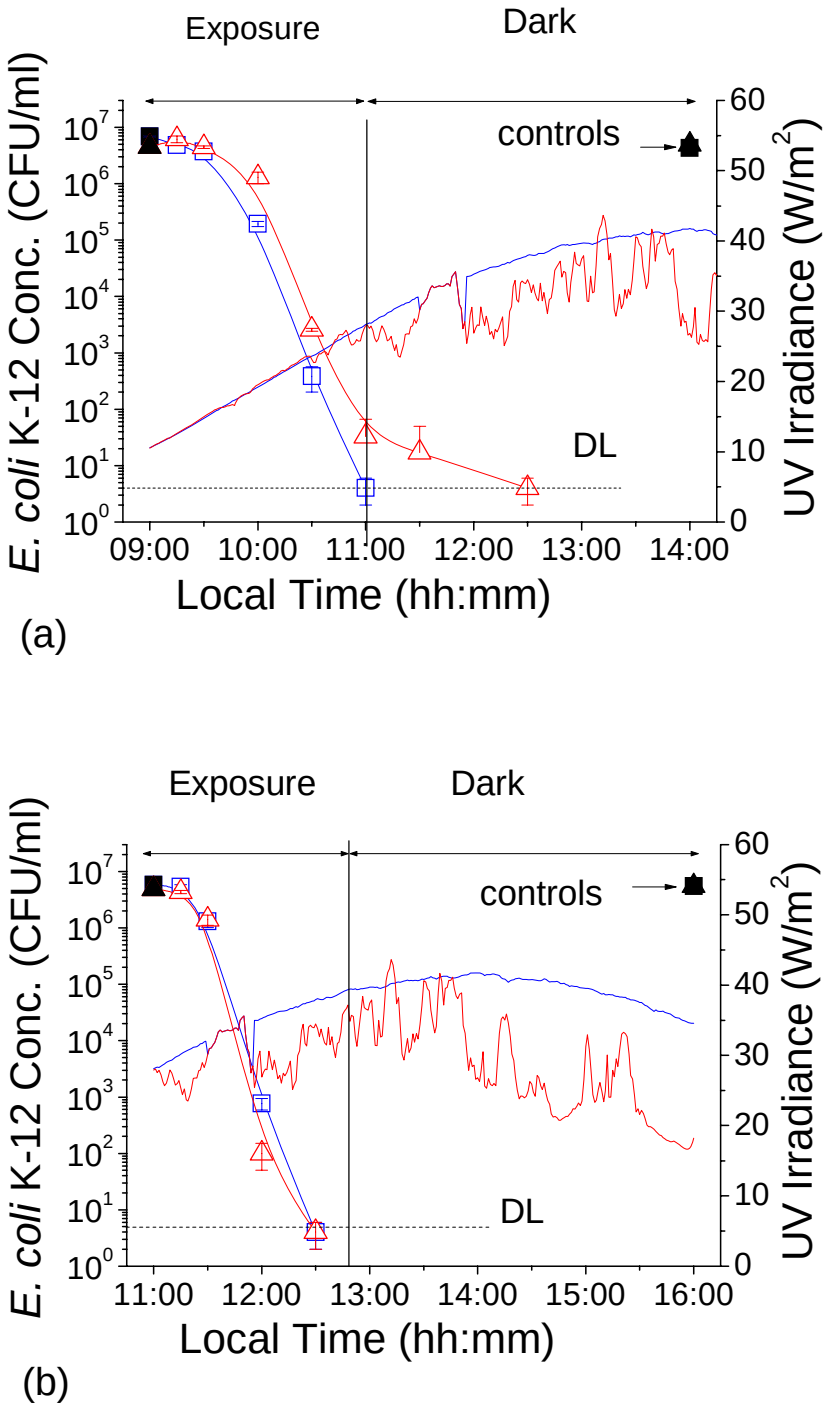


Figure 3.13 *Inactivation curves of E. coli K-12 during 2 h exposure to sunlight in the borosilicate glass tubes for 4 experiments carried out over two consecutive days, 30-05-2007 (-□-) and 31-05-2007 (-Δ-). The corresponding coloured, solid lines (—) represent solar UV irradiance (295-385 nm) measured on the day of each experiment. Starting local times were 09:00 (a), and 11:00 (b). The vertical solid line denotes the end of the illumination period. Dashed line (--) shows the detection limit (DL). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

(i) *Two hour solar exposure:* Previously, inactivation kinetics of *E. coli* K-12 showed that a 10^6 CFU/ml bacterial population was inactivated to below the detection limit within the first 2 h of a 5 h exposure to natural sunlight (Fig. 3.12). Consequently, the first series of experiments involved 2 h solar exposures of bacteria, starting at different times of the day and at different intensities. Fig. 3.13a and 3.13b show experiments that took place from 09:00-11:00 h and from 11:00-13:00 h, respectively. In both experiments, bacteria were inactivated to below the detection level during the first hour of dark cover and remained below the detection level throughout the remainder of the experiment. However, bacterial inactivation experiments that were conducted at 11:00 h with an average UV irradiance of 32.55 W/m^2 and an accumulated UV dose of 234.36 kJ/m^2 occurred at a faster rate during the exposure period to sunlight than experiments that started at 09:00 h with an average UV irradiance of only 19.2 W/m^2 and an accumulated UV dose of 138.24 kJ/m^2.

(ii) *One hour solar exposure:* Duration of sunlight exposure was then reduced to one hour (from 09:00h-10:00 h and 11:00-12:00 h); like the first series of experiments, different starting times (and consequently intensities) were used. In Fig. 3.14a, experiments started at 09:00 h with an average UV irradiance of 14 W/m^2 and an accumulated UV dose of 50.4 kJ/m^2 and did not result in total inactivation; instead a 2 log concentration of bacteria remained for the duration of the experiment. In Fig. 3.14b, as with Fig 3.13a, total inactivation is achieved within one hour after the tube was covered, this occurred with the experiment starting at 11:00h and an average irradiance of 30 W/m^2 and an UV dose of 108 kJ/m^2.

(iii) *Fifty minute solar exposure:* To complete this study, experiments of 50 min exposure duration were also performed (Table 3.4). Although the final concentration was very near the detection limit, total inactivation is not observed after more than 4 hours after the solar exposure has completed. This occurred with the experiment starting at 11:00 h and an average solar UV irradiance of 30.9 W/m^2 and an accumulated UV dose of 92.16 kJ/m^2. With this result, it can be said that total inactivation (to below the detection limit) occurs only for UV doses higher than 108 kJ/m^2.

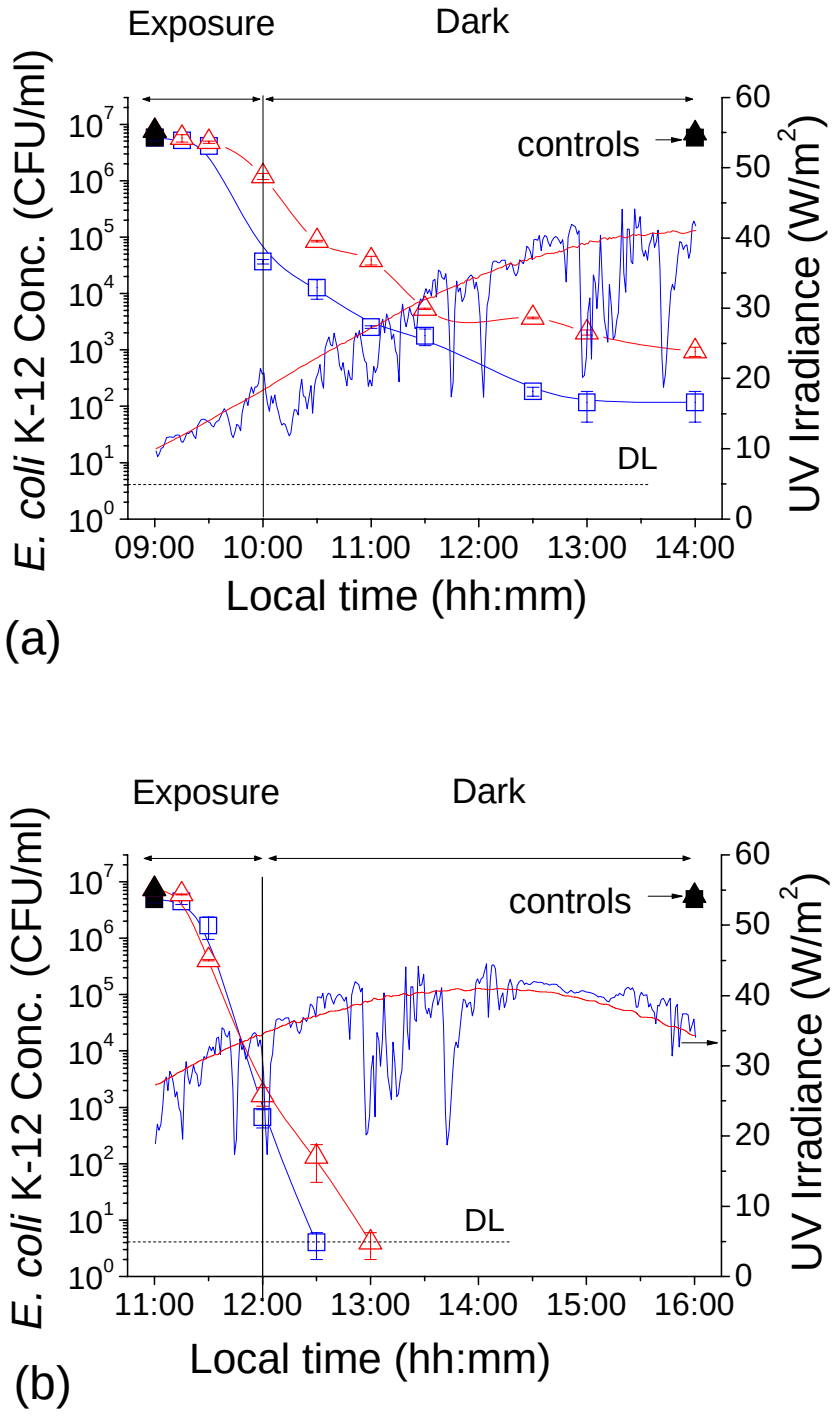


Figure 3.14 *Inactivation curves of E. coli K-12 during 1 h exposure to sunlight in the borosilicate glass tubes during 4 experiments performed under similar conditions and over relatively adjacent days, 01-06-2007 (-□-) and 06-06-2007 (-Δ-). The corresponding coloured, solid lines (—) represent solar UV irradiance measured on the day of each experiment. Starting local times were 09:00 (a), and 11:00 (b). The vertical solid line denotes the end of the illumination period. Dashed line (--) shows the detection limit (DL). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

(iv) *Thirty minute solar exposure:* To demonstrate that a dose threshold, unrelated to irradiance, is necessary to reach total inactivation near 108 kJ/m^2, a new series of 30 min exposure experiments was performed. Three experiments starting at 10:00, 10:30, and 13:15 h local time were carried out to observe inactivation kinetics for *E. coli* K-12 for UV doses < 108 kJ/m^2 but with high and low irradiances. In these experiments, complete inactivation was not achieved even with a starting UV irradiance as high as 39.2 W/m^2 (Table 3.4), regardless of irradiance values. Table 3.4 and Fig. 3.15 which give a summary of final concentrations versus the UV dose and UV irradiance, clearly shows that the effective UV dose that produces complete inactivation must be approximately 108 ± 4 kJ/m^2 in the 295-385 nm wavelength region. The described dose is roughly equivalent to 2160-2520 kJ/m^2 in the global solar spectrum (300-3000 nm) [103] and to 126-137 kJ/m^2 in the UV-A spectrum (315-400 nm) [104]. This lethal uninterrupted UV dose of 108 kJ/m^2 has been experimentally determined to disinfect 2.5 L suspensions of 10^6 CFU/ml *E. coli* K-12 in real well-water using a CPC of concentration factor 1 (irradiated surface: 0.21 m^2), which is equivalent to delivering 9 kJ/l into the treated water.

It is widely accepted that inactivation of microbial cells occurs through a variety of mechanisms depending on the type of UV used for inactivation. Solar UV used during the SODIS process consists mainly of UV-A and hence the main inactivation mechanism is a photooxidative process as well as the generation of reactive oxygen species (ROS) [105, 106] . However many aspects of solar UV-A inactivation are still unclear. Some studies have shown that UV-A damage depends on irradiance and dose [90]; defining the first as the power incident per unit area (W/m^2) and the second as the energy reaching a unit area (J/m^2) of the target system during a given exposure time. Other studies indicate that the manner in which the dose is delivered (continuously or intermittently) has an effect on the subsequent inactivation observed [81, 82]. Due to a variety of experimental conditions, (the use or non-use of simulation lamps, natural sunlight, volumes and types of water as well as different organisms) it is very difficult to compare these inactivation results and draw concrete conclusions as to what drives UV-A inactivation.

Table 3.4 *Summary of parameters and results for the experiments performed*

Date (2007)	Start-End (Local time)	*Solar UV-Irradiance (W/m^2)	*Solar UV-dose (kJ/m^2)	C_0** (CFU/ml) $x10^6$	C_f*** (CFU/ml)	Complete Inactivation
2 h-exposure						
30-05	09:00-11:00	19.2	138.24	6.9±0.2	DL	YES
30-05	11:00-13:00	34.6	235.44	5.8±0.1	DL	YES
31-05	09:00-11:00	19.2	138.24	4.5±0.1	DL	YES
31-05	11:00-13:00	30.5	219.60	4.9±0.5	DL	YES
1 h-exposure						
01-06	09:00-10:00	13.8	49.68	5.8±0.7	120±40	NO
01-06	11:00-12:00	29.4	105.84	4.9±0.7	DL	YES
06-06	09:00-10:00	14.1	50.76	7.6±0.5	930±110	NO
06-06	11:00-12:00	31.1	111.96	7.5±0.6	DL	YES
50 min-exposure						
13-06	11:00-11:50	30.9	92.16	6.4±0.8	10±4	NO
13-06	11:00-11:50	30.9	92.16	7.0±0.6	110±110	NO
30 min-exposure						
07-06	10:00-10:30	20.4	36.72	6.4±0.5	5300±1100	NO
08-06	10:00-10:30	18.2	32.76	5.9±0.2	$(50±5)\cdot10^3$	NO
07-06	10:30-11:00	24.7	44.46	4.7±0.1	7300±400	NO
08-06	10:30-11:00	23.2	41.76	5.5±0.1	330±70	NO
12-06	13:15-13:45	39.2	70.56	8.4±0.1	4200±200	NO
12-06	13:15-13:45	39.2	70.56	6.6±0.7	2700±500	NO

* Average solar UV-irradiance in the solar spectral range of 295-385 nm during exposure.
** C_0: Initial bacteria concentration.
***C_f: Final bacteria concentration. Experiments were conducted during 5 hours, regardless of the exposure time. DL= 4 CFU/ml

Table 3.4 summarises inactivation patterns of *E. coli* K-12 during exposure to sunlight but under varied UV irradiances and accumulated doses. Complete inactivation of *E. coli* K-12 to below the detection level occurs at both low and high UV intensities as long as an uninterrupted solar-UV dose > 108 kJ/m^2 is reached and indicates that inactivation of bacteria is dependent on the UV dose rather than the UV irradiance. This result correlated well with previous experiments exploring the effect of flow rate and irradiated collector surface area, which also indicated that UV dose must be sufficient and is the main driving factor compared to exposure time or a minimum UV irradiance [107]. However, the threshold uninterrupted UV dose that is required by a given organism to ensure inactivation has not been characterised before. From the experiments conducted, a ~10^6 CFU/ml of *E. coli* K-12 in clear well-water (turbidity < 5 NTU) requires an uninterrupted dose > 108 kJ/m^2 for complete

inactivation and to prevent re-growth of organisms. Rincon and Pulgarin reported similar UV doses of 134.2, 136.2 and 157.5 kJ/m^2, which were sufficient to induce a 4-log reduction in concentration of *E. coli* K-12 in natural lake waters [81].

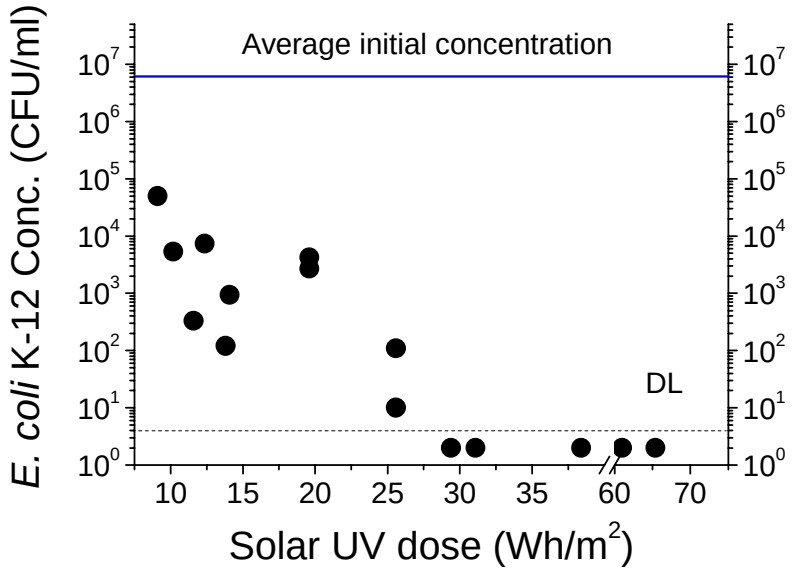


Figure 3.15 *Final bacteria concentration versus UV dose received during 16 experiments of Table 3.3. Dashed line (--) shows the detection limit (DL). Solid line (—) represents the average initial bacteria concentration ~ 6·10^6 CFU/ml.*

Flow cytometry studies of *E. coli* by Berney *et al.* [16], have shown that inactivation by solar disinfection is caused by a sequence of disruptions to normal cellular functions. Shortly after the start of solar exposure, ATP synthesis and efflux pump activity both cease. These are followed by a gradual loss of membrane potential and a reduction in glucose uptake. Finally, the cytoplasmic membrane of the bacterial cells becomes permeable and there is a corresponding loss of cultivability. Membrane permeability was shown to indicate cell death. The loss of cultivability on sodium pyruvate-supplemented tryptic soy agar (a procedure for the recovery of injured cells) was also shown by Berney *et al*. to have a close correlation to the loss of membrane potential [16]. Cells were no longer able to repair the damage and recover after exposure to >1500 kJ/m^2 of solar UV-A radiation. Furthermore, the loss of pathogen infectivity, an important issue for the SODIS user, was shown to occur after *S. typhimurium* was subjected to simulated solar disinfection and found to be non-infective in BALB/c mice

[26]. Since loss of bacterial cultivability is one of the last symptoms of SODIS-induced cell damage, Berney *et al.* suggest that bacteria may retain cultivability for a short period of time even after they have received an ultimately lethal dose of solar radiation [16]. This explains experimental results on *E. coli* K-12 inactivation observed in Figures 3.13a and 3.14b, where inactivation to below the detection limit continues in the dark periods after solar exposure.

The use of a higher UV irradiance over a short time is more advantageous than lower intensity over longer periods in terms of the capacity of the bacteria to respond and repair the damage caused by the incident UV. A faster inactivation rate was seen in Fig. 3.13b compared to Fig. 3.13a, since a high starting irradiance of 28 W/m^2 was used in Figure 3.13b compared to 11 W/m^2 in Fig. 3.13a. A high UV irradiance has been postulated to adversely affect the efficiency of cellular repair enzymes [107] as well as attacking defence mechanisms and preventing photoreactivation. These all serve to enhance the rate of inactivation of bacteria [81].

As illustrated in Fig. 3.12, bacterial inactivation is not only dependent on the dose received, but on the way in which the dose is delivered. The three systems described in section 3.2.3 were all exposed for 5 h to illumination but at different flow rates. However, complete inactivation of bacteria was only seen in the batch system with the borosilicate glass tube with no flow. In this configuration, water with bacteria was constantly illuminated and hence the needed uninterrupted UV dose was achieved and complete inactivation to the detection level took place. With the continuous flow systems at 2 l/min and 10 l/min, an accumulated UV dose of > 108 kJ/m^2 was also deposited to the bacteria but in an intermittent manner, resulting in a 2 log concentration of residual viable bacteria remaining after the 5 h period. This also explains the results obtained in the studies of irradiated collector surface. In this case, results showed that intermittent exposure is detrimental to bacterial inactivation as the bacteria are given a chance to switch on self – defence mechanisms during the dark period and hence are more resistant when re-illuminated [81]. For all experiments summarised in Table 3.3, water temperatures were less than < 40ºC, which once again illustrates that temperature is not the predominant factor in sunlight inactivation of bacteria.

Instead once the appropriate dose of UV is obtained, bacteria are eliminated - a similar finding was also noted by Martin-Dominguez *et al.* [108].

3.4 Conclusion

1. This study demonstrates an attempt to scale-up SODIS through the use of pumped, re-circulatory, continuous flow reactors. If the operational parameters such as flow rate and irradiated surface area are set in such a way that the microbial pathogens are repeatedly exposed to sub-lethal doses of solar radiation followed by a period within which the cells have an opportunity to recover or repair, complete inactivation may not be achieved.

2. This study shows that characterising the UV dose needed for inactivation of an organism and then obtaining that dose during a high UV irradiance period is an efficient way to disinfect water.

Chapter 4

Solar Disinfection (SODIS) in Batch Reactors: The Effect of Compound Parabolic Concentrators (CPCs) and Turbidity on Microbial Inactivation

4.1 Introduction

In this chapter, the effectiveness of compound parabolic concentrators (CPCs) to enhance solar disinfection under real sunlight and varying cloud conditions is explored. The effect of CPC degradation on enhancement of SODIS was evaluated by testing a CPC that had been under field use for 3 years. The effectiveness of PET bottles as SODIS reactors to disinfect turbid water was also evaluated. Turbid water was also disinfected in reactors with CPCs to determine if CPCs provided an enhancement to the disinfection of turbid water.

4.1.1 Compound Parabolic Concentrators (CPCs)

Concentrating systems can be classified into imaging systems and non-imaging systems, depending on their shape and geometry. Image forming systems such as parabolic mirrors, focus an image of the sun at a point, which is where the absorber is positioned. These systems only work with rays parallel to the axis of the parabola which means that they can only use direct solar radiation and only on clear days. Non-imaging systems have a diffuse focus, and no image is formed. The CPC is a non-imaging system which was invented by scientists in the former Soviet Union [109] and United States [110]. With the CPC, concentrated rays are homogeneously distributed in the absorber. Their main advantage is that they concentrate diffuse radiation. Hence they do not rely solely on direct solar radiation and should be effective even on cloudy days. In addition, they concentrate radiation independently of the direction of sunlight and do not require sun tracking, in contrast to direction dependant image forming systems. Other reflecting systems tested in previous studies have a varying concentration

during the day because they are essentially image forming systems and depend on the angle of incidence of the sun on the reflector [57, 58, 111]. A major advantage of CPC systems is that the concentration factor remains constant for all values of the sun's zenith angle within the acceptance angle limit. CPC mirrors have been widely used and tested in the field of photocatalysis to enhance the UV radiation reaching the photocatalyst and it has been proven that the CPC is the best collector to use in order to concentrate the solar UV spectrum [96].

4.1.2 Effect of Turbidity on Solar Disinfection

The presence of suspended organic and inorganic matter such as microscopic organisms, clay, silt and plankton are responsible for turbidity in water [112, 113]. Turbidity not only has a negative effect on the aesthetic quality of the water by generating disagreeable odours and tastes but also on disinfection efficiency. The effectiveness of chlorination is known to be hampered by turbidity [112]. Based on the fact that turbidity is the measure of the degree of light-scatter by particulates, it follows that solar disinfection should be less efficient in turbid water. The microbicidal wavelengths of sunlight (mostly UV-A) may be scattered or absorbed by turbid agents thereby causing incomplete inactivation of pathogens. Particles can also act as a physical shield and block UV-A from coming into contact with microorganisms [113]. Inactivation of organisms by UV-A occurs through an indirect process and involves the formation of oxidative species such as superoxide and hydroxyl radicals as well as hydrogen peroxide which destroy vital proteins, membranes and cause damage to DNA [90, 106]. Natural organic matter which is likely to be found in turbid water is a scavenger of these essential radicals needed for inactivation of pathogens. Furthermore, high concentrations of carbonates or bicarbonates, which again are to be found in turbid water, react with hydroxyl radicals forming less reactive radicals and shielding bacteria from light by photo-absorption [60].

Although solar disinfection is expected to be less efficient when water is turbid, there have been very few studies that illustrate the extent to which SODIS is compromised at a given turbid level and under specific weather conditions [14, 43]. However, these studies show that even in samples that have turbidity levels

> 30 NTU solar disinfection is still feasible even though it might take longer for pathogens to be inactivated than the recommended 6 h of strong sunlight. Local communities in extremely remote areas and with very little resources might solely depend on SODIS as a means to disinfect water. In Kenya, turbidity values ranged from 5 to 2000 NTU depending on the time of the day, year and weather conditions. However, a later study showed a reduction in diarrhoea for children also in Kenya drinking solar disinfected water that had turbidity levels greater than 200 NTU and water samples had reached temperatures > 55°C [43, 46]. Without the ability to filter the water prior to solar exposure, it is essential to determine the extent to which solar disinfection is affected when turbid water is used and under different weather conditions (low ambient temperature and during months when there is less illumination). Pathogens must be fully inactivated without the potential for re-growth since the turbid agent could be a potential source of nutrients for microorganisms [112] that are able to recover after illumination.

4.1.3 Aims

The aim of this study were to

(i) Evaluate the use of CPCs to enhance solar disinfection under real solar radiation conditions on clear and cloudy days.

(ii) Determine the efficiency of solar disinfection in inactivation of *E. coli* K-12 contained in real turbid water and exposed to sunlight in batch reactors (PET bottles and CPC enhanced borosilicate glass tubes).

4.2 Materials and Methods

4.2.1 Bacterial Preparation, Cultivation and Enumeration

E. coli K-12 was prepared according to the methods in section 3.2.1 in order to obtain a 10^6 CFU/ml concentration of bacteria in 2 L of well-water for PET bottles and 2.5 L of well-water for borosilicate glass tubes.

4.2.2 Enumeration of Bacterial Regrowth after Solar Exposure

The membrane filtration method was used to assess the possible presence of viable bacteria and assess regrowth. A total volume of 750 ml of solution was collected at the end of the experiment. 250 ml of this solution was filtered through 47 mm diameter 0.45μm pore size cellulose nitrate filters (Sartorius AG, Germany). The filter was then aseptically placed on LB media and then on Endo agar to differentiate between *E. coli* and naturally occurring soil bacteria. The remaining 500 ml was left on the bench at room temperature. Further 250 ml volumes were taken from the test samples after 24 h and 48 h and filtered as described previously to determine bacterial regrowth. Since the soil used to prepare turbid solutions was not sterilised, gram staining was used to identify non-coliforms and presumptive bacilli were streaked onto LB agar in order to isolate different colonies. The colonies were then transferred into API 50 CHB/E medium (bioMérieux Inc, Spain) and carbohydrate metabolism was assessed by API 50 CH strip (bioMérieux Inc, Spain) according to the manufacture's instructions and colonies identified by patterns generated from carbohydrate usage.

4.2.3 Preparation and Measurement of Turbidity Solution

Natural well-water was collected from a well with an approximate depth of 200 m as described in section 3.2.3.

Table 4.1 *Physical and chemical properties of well-water*

Cl^-	332 mg/l	Na^+	434 mg/l
NO_3^-	12 mg/l	NH_4^+	6 mg/l
SO_4^{2-}	294 mg/l	Mg^{2+}	41 mg/l
F^-	0.7 mg/l	Ca^{2+}	50 mg/l
Br-	2 mg/l	HCO_3^-	170 mg/l
PO_4^{3-}	0.5 mg/l	TOC	6 mg/l
pH	7.7	Conductivity	2.710 uS/cm
Turbidity	1.5 NTU	Bacteria	0 CFU/ml

Table 4.1 provides average values of a list of physical and chemical parameters of the well-water used during the experimental period. Cations and anions were determined according to methods described in section 3.2.3.

Turbid solutions (5, 100 and 300 NTU) were prepared in 1000 ml erlenmeyer flasks. Edaphologically classified Red soil was obtained from the Michelin test field, Almería, Spain. 0.3, 7 and 13 g of soil were weighed out and added to 500 ml of well-water for 5, 100 and 300 NTU turbid solutions respectively. The mixture for 100 NTU and 300 NTU was agitated every 2 minutes over a 30 minute period and left to stand for an hour. The mixture for 5 NTU was agitated every 10 minutes over a 30 minute period and also left to stand for an hour. After sedimentation, the solution was then pipetted off, and measured to ensure correct turbidity using a turbidimeter (Model 2100, Hach, USA). Tables 4.2a and 4.2b lists the constituents and chemical properties of soil used. Soil analysis was conducted by the Department of Edaphology at the University of Santiago de Compostela, Spain and well-water analysis, by the Chemistry Laboratory at the PSA also in Spain.

Table 4.2a *Physical and chemical properties of Red soil*

pH (water)	8.61
pH (KCl)	7.58
Total Organic Carbon	0.935%
Total Nitrogen	0.048%
Relation C/N	8.2
Fe_2O_3	0.060%
Al_2O_3	0.162%
Carbonates	8.1%
P-ClH	50.93 mg/kg
$P\text{-}CO_3H^-$	14.93 mg/kg
$P_{inorganic}\text{-}CO_3H^-$	10.62 mg/kg
$P_{organic}\text{-}CO_3H^-$	4.31 mg/kg
Granulometric analysis (without destruction of carbonates)	
% Sand	33
% Fine silt	9
% Thick silt	13
% Clay	44
Granulometric analysis (destruction of carbonates)	
% Sand	37
% Fine silt	7
% Thick silt	20
% Clay	36
Mineralogy of the fraction of clay	
Main minerals	Illite, Halloysite
Minor minerals	Calcite

Table 4.2b *Analysis of Turbidity samples prepared with well-water and Red soil*

Turbidity	pH*	$C_{soluble}$ (mg/l)	P_{total}* (mg/l)	$P_{inorganic}$* (mg/l)	$P_{organic}$* (mg/l)
5 NTU	7.01	0.7	0.004	0.000	0.0004
100 NTU	7.41	0.6	0.055	0.013	0.042
300 NTU	7.47	12.2	0.254	0.021	0.232

* Measured in filtered samples.

4.2.4 PET Bottles and Glass Tubes

The shape and dimensions of purchased plastic bottles used for SODIS experiments are not standardised. The correct assessment of the radiation entering the system would require detailed optical analysis and calculations for each particular bottle. However, most bottles perform with similar efficiency even though the amount of radiation reaching the water is not necessary the same. The surfaces of the bottles are usually irregular and the shape is, in some parts of the bottles, not cylindrical but rather hexagonal. Transparent PET bottles are opaque below wavelengths of 320 nm and transmittance might be as high as 85%-90% in UV-A (320-400 nm) wavelengths [18]. The plastic bottles (Fig. 4.1) used for this experiment were 2 L bottles with hexagonal cross sectional shape, 9 cm equivalent diameter and 30 cm height.

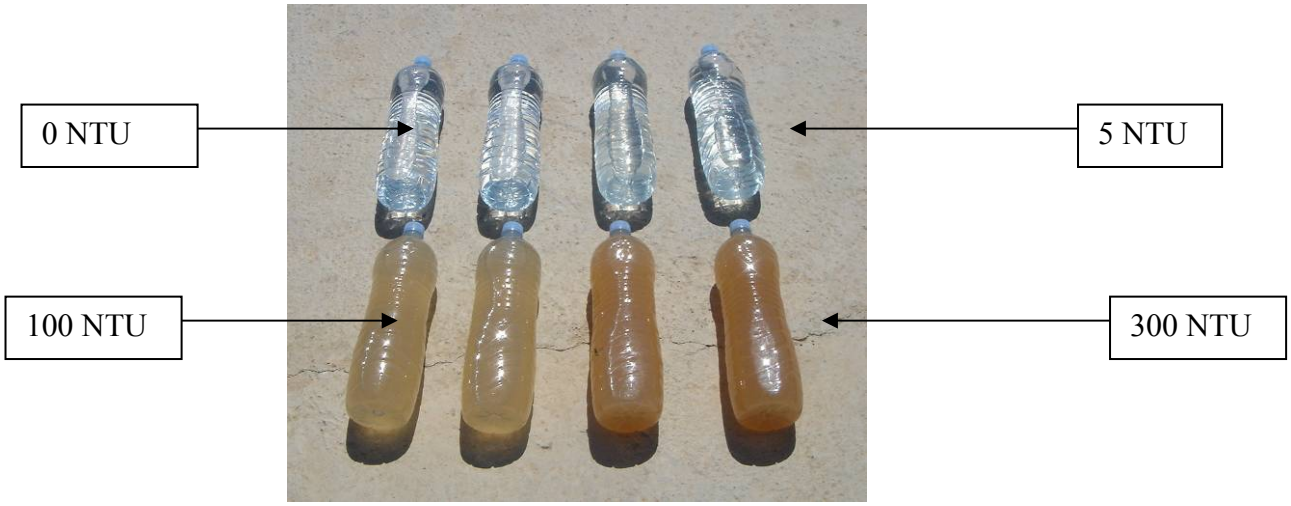


Figure 4.1 *2 L PET bottles filled with turbid water (0, 5, 100, and 300 NTU) during solar exposure.*

With borosilicate glass tubes, calculating the amount of radiation reaching the system is possible due to the uniformity of the glass. Glass tubes which were closed at both ends were used as batch reactors. One end was completely closed and the other had a sampling valve fitted, as can be seen in Figure 4.2a. Each tube had dimensions of 1.50 m length, 0.05 m outer diameter, 1.8 mm wall thickness and 2.5 L internal volume. Borosilicate glass tubes were then placed on

the linear focus of a CPC to assess the enhancement of the use of a CPC (Fig. 4.2c). Glass tubes were filled with 2.5 L of turbid water to asses the efficiency of solar disinfection in inactivation of turbid water containing bacteria (Fig. 4.3). In order to assess any efficiency difference arising from the transmittance, of PET and borosilicate glass tubes, comparison experiments were performed. Both the bottle and the tube were exposed to sunlight under the same atmospheric, radiometric and meteorological conditions in duplicated experiments.

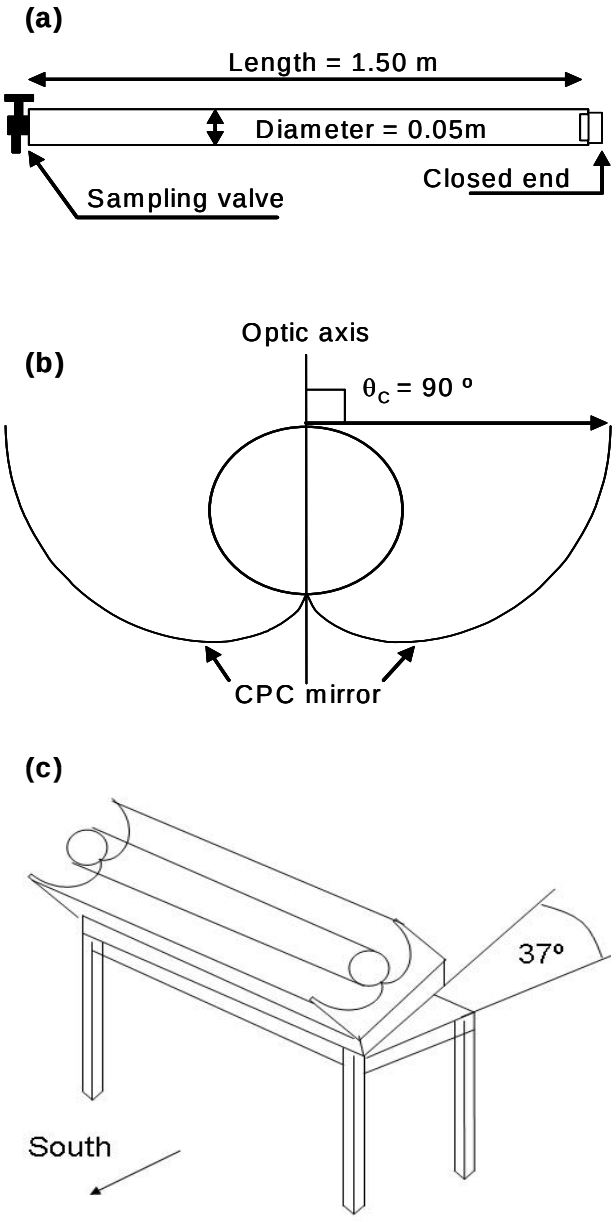


Figure 4.2 *(a) Glass tube configuration. (b) Design of CPC for the glass tube experiments. (c) Experimental tube fitted in CPC mirror inclined at 37° with respect to the horizontal and facing south.*

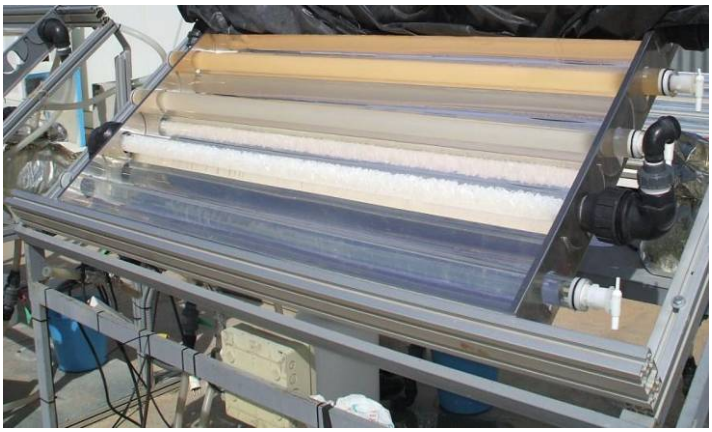


Figure 4.3 *Configuration of CPC enhanced borosilicate glass tubes (2.5 L) filled with turbid water (0, 5, 100, 300 NTU) exposed to sunlight.*

4.2.5 Compound Parabolic Concentrator Mirrors

In construction of the CPC, the following terms are defined as: Aperture area = *A;* Absorber area = A_{abs}; Sun zenith angle on the concentrator = θ; Acceptance angle of the CPC = θc; Concentration factor = $C = A/A_{abs}$. The main issue during concentration of radiation is to ensure that radiation which is incident on *A* and uniformly distributed over a range of angles ($\theta \leq \theta c$), can be concentrated onto the smaller absorber area A_{abs} without the need for repositioning the system as the value of θ varies [114]. For a cylindrically shaped SODIS reactor the concentrating system is two dimensional. In this case, the second law of thermodynamics states that the maximum possible concentration or ideal concentration is $C_{ideal} = 1 / \sin \theta c$. In the case of SODIS and given the diffuse nature of the UV-A spectrum [57], only a homogeneous distribution of light on the absorber tube is required, not a high concentration. Hence, a system with a concentration factor of 1 was designed. Then, θ_C = 90º and the shape of the mirror is defined by an involute to the absorber as can be seen in Fig. 4.2b. The aperture of the system is 15.7 cm and is numerically equal to the perimeter of the absorber. Due to the non-imaging nature of the reflectors, the entire absorber is homogeneously illuminated at all times, even on cloudy days. The CPC mirrors were built with 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. The reflectivity of the mirrors was assessed with a reflectometer from Devices and Services (U.S.A.), model ISR, measurement spectra of 635-685 nm centred at 660 nm and a precision in measurement of ±0.2%. In order to assess the influence of mirror degradation upon the SODIS process, the tubes were tested with old and new CPC mirrors of the same material. The older CPCs had been exposed to field conditions for 3 years with relatively low maintenance.

4.2.6 Sunlight Exposure and Radiation Measurement

All experiments were performed under natural solar radiation at the Plataforma Solar de Almería, Spain, located at 37º84' N and 2º34' W as described in section 3.2.5.

4.2.7 Geeraerd and Van Impe Inactivation Fitting Tool (GInaFIT)

The Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFIT) is used for testing a number of microbial survival models on microbial inactivation curves [98] as described in section 3.2.7.

4.3 Results and Discussion

4.3.1 CPC Influence on Sunny and Cloudy Days

Figure 4.4 shows the results of inactivation of bacteria in PET bottles and in glass tubes with and without CPC. These experiments were conducted to assess any difference in SODIS efficiency due to material transmittance and also to the use of a CPC. Tube inactivation reaches the detection limit one hour before the bottle. This can be attributed to the fact that the borosilicate glass tube has a 45% UV-B transmittance (compared to 0% for PET) and a 5% advantage over PET in the UV-A. Larger differences in transmittance can be seen beyond UV-A but since very little UV-B and no UV-C are present in terrestrial natural sunlight, the effect of the different diameter of the glass tube and the bottle [18] can be considered negligible. Both glass tubes, with and without CPC, reached the

detection limit (4 CFU/ml). The glass tube with the CPC reached the detection limit one hour before the tube with no CPC. In terms of UV-A dose, the tube with CPC reached the detection limit after receiving 150 kJ/m^2 (Table 4.3), while the glass tube with no CPC needed 210 kJ/m^2. This represents 40% more UV-A required to achieve the same result on clear days with the non-CPC system. Hence, the system with CPC is more efficient during solar disinfection.

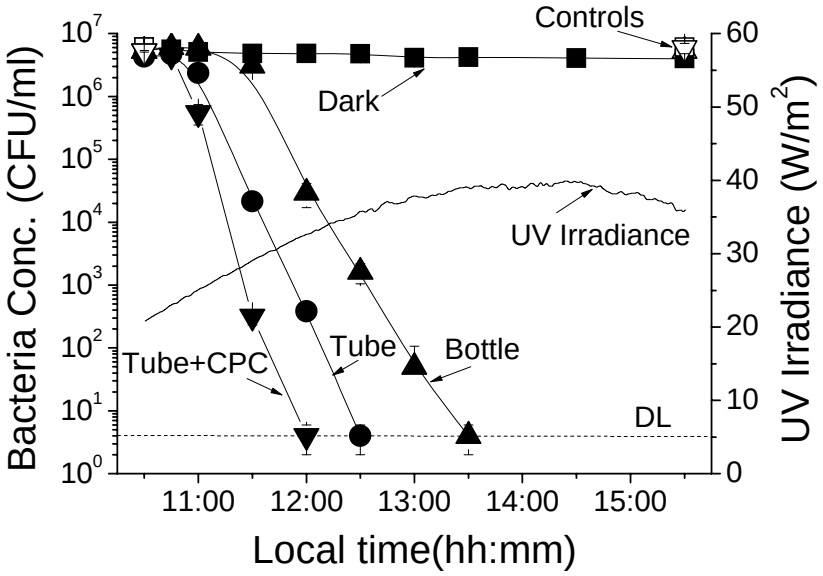


Figure 4.4 *E. coli K-12 inactivation during real sunlight exposure on sunny days with real sunlight exposure in a glass tube with CPC (-▼-), without CPC (-●-), PET bottle (-▲-) and dark control (-■-). The solid line (—) is the solar UV irradiance. Dashed line (- -) shows the detection limit (DL= 4 CFU/ml). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

Figure 4.5 shows the results of similar experiments performed on cloudy days. The inactivation of bacteria in PET bottles under cloudy conditions was not tested since in Fig. 4.4, even in full sunshine PET bottles had a lower inactivation efficiency. In Fig. 4.5 only the system with the CPC reflectors reached the detection limit. When turbidity of the water is very low (1-5 NTU), the SODIS process is dominated by the amount of sunlight reaching the absorber [14]. On clear days, the direct component of sunlight predominates. Approximate contributions of direct and diffuse sunlight for the UV-A spectrum are of 60% and 40 % respectively [115]. This means that the UV-A dose needed for SODIS

to reach detection limit will eventually be reached in both systems as seen in Fig. 4.4. Nevertheless, the reflectors contribute by delivering more energy in less time, producing a faster inactivation rate and reaching the detection limit one hour before. As stated earlier, the system without the CPC is only exposed to the sun on the front side whereas that with the CPC has the entire tube illuminated. On cloudy days, most of the available UV-A is in the diffuse form with a negligible value for the direct component. In this case, the reflectors distribute all of the diffuse radiation reaching the aperture onto the absorber. The system without reflectors mainly receives the circumsolar diffuse radiation that comes directly from the direction of the sun and not from the entire sky hemisphere. This is known as *forward scattered radiation* and is very low on cloudy days. This explains the fact that the system fitted with the CPC reaches detection limit while the system with no CPC does not. In terms of optical path length, only the forward scattered radiation reaches the system without reflectors and it must pass through 5 cm of water optical path length while the system with CPC receives radiation from the entire sky hemisphere and must only go through 2.5 cm because the whole tube is illuminated.

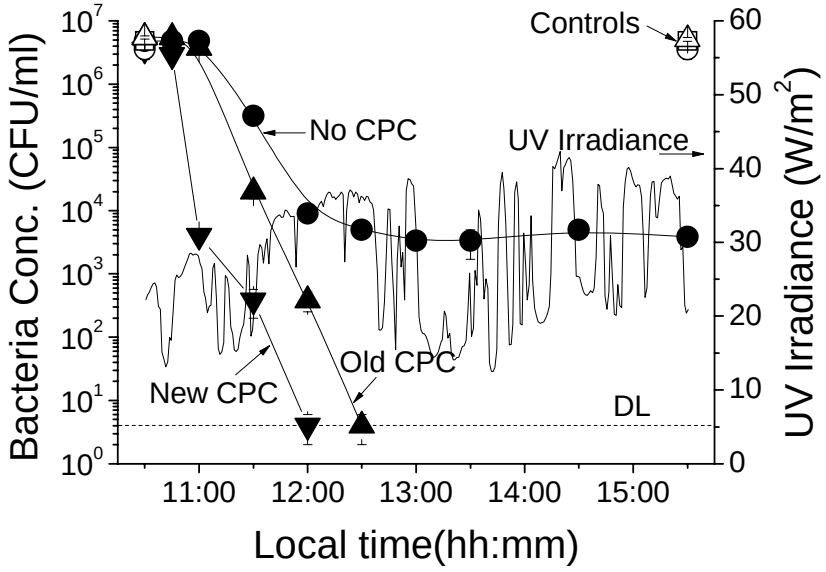


Figure 4.5 *E. coli K-12 inactivation during real sunlight exposure on cloudy days in a glass tube with new CPC (-▼-), old CPC (-▲-), without CPC (-●-).The solid line (—) is the solar UV irradiance. Dashed line (--) shows the detection limit (DL= 4 CFU/ml). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

Table 4.3 *Summary of parameters and results for the experiments performed*

Date [(1)], solar conditions & Figure	Reactor system	Av.[(2)] solar UV-A irradiance (W/m^2)	C_i [(3)] (CFU/ml)	C_f [(4)] (CFU/ml)	Exposure to reach DL (h) / UV-A dose[(5)] (kJ/m^2)	Min. - Max. temperature (ºC)
29/5/07 Dark Fig. 4.5	Glass Tube	----	$(6.1\pm0.6)x10^6$	$(7.0\pm3.0)x10^5$	---	21.3-27.5
29/5/07 Sunny Fig. 4.5	Bottle /No CPC	35±5	$(5.3\pm0.4)x10^6$	DL	3 / 340±60	21.3-29.5
	Tube / No CPC	35±5	$(4.3\pm0.4)x10^6$	DL	2 / 210±30	21.5-30.4
	Tube / New CPC	35±5	$(5.23\pm0.14)x10^6$	DL	1.5 / 150±20	21.0-33.0
24/5/07 Cloudy Fig. 4.6	Tube / No CPC	28±8	$(4.8\pm0.5)x10^6$	$(3.8\pm1.1)x10^3$	---	20.7-28.7
	Tube / old CPC	28±8	$(5.5\pm0.3)x10^6$	DL	2 / 200±50	20.3-31.2
	Tube / New CPC	28±8	$(3.5\pm1.1)x10^6$	DL	1.5 / 140±40	20.2-33.0

(1) All the experiments were performed in duplicate in twin systems under the same climatic conditions from 10:30 h to 15:30 h local time.
(2) The average for the 5 h duration of the experiment.
(3) Ci: Initial bacterial concentration (CFU/ml)
(4) C_f : Final bacterial concentration (CFU/ml). DL: when zero CFU was detected, concentration was the detection limit (DL: 4 CFU/ml). Each value of bacterial concentration is the average measurement and errors are standard deviation from triplicate measurements taken in duplicated experiments.
(5) Accumulated UV-A dose calculated as the integral of the solar UV-A irradiance (W/m^2) on the time of exposure until DL was reached.

4.3.2 Use of CPC on Sunny and Cloudy Days – Modelling with GInaFIT

The Gearaerd and Van Impe Inactivation Model Fitting Tool (GInaFIT) was used for analysing different configurations of the solar systems under sunny and cloudy conditions. The following models were used: log-linear regression, log-linear + tail, log-linear + shoulder, log-linear + shoulder + tail, Weibull model,

biphasic model, biphasic + shoulder [98]. T_{DL} and F_{DL} values (exposure time and solar UV-A dose required to reduce plate counts to the detection limit (DL)) were calculated using the best-fit model of GInaFIT (Table 4.4). These fits show that the experiments carried out in the glass tube using the new CPC yields the fastest inactivation rate (k_{max}) compared with the other systems evaluated. All kinetics results obey the Geeraerd Shoulder model as expected for the SODIS process [20], except for the case of the tube without CPC mirror under cloudy conditions which obeys the Geeraerd Shoulder and Tail model (Fig. 4.6).

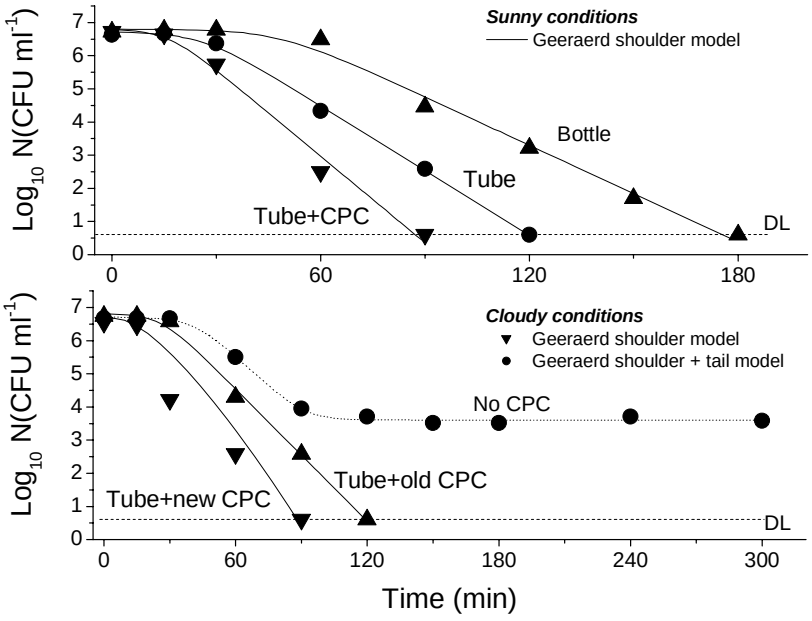


Figure 4.6 *Inactivation curves of E. coli K-12 during real sunlight exposure on clear days (top) in a glass tube with CPC (-▼-), without CPC (-●-), PET bottle (-▲-), and on cloudy days (bottom) in a glass tube with new CPC (-▼-), old CPC (-▲-), without CPC (-●-). Lines represent theoretical fits using GInaFIT.*

A number of studies have tried to enhance disinfection using some kind of solar thermal system. The most economic solar collectors have an average conversion efficiency of around 30% [116]. Painted bottles or tubes have a somewhat lower efficiency than these. According to the principles of heat transfer [117, 118], it takes approximately 1-2 hours to heat 1 L of water inside a painted bottle from 20ºC to 45ºC, assuming summer weather conditions, global irradiances of around 800 W/m^2 and a 30 % conversion efficiency. In winter, depending on ambient temperature, the system would not reach the desired temperature mainly due to

heat losses to the environment. Previous works have tried to couple a solar thermal collector to a radiation collector device [64] to address this problem. More efficient solar thermal collectors are available but their cost is prohibitively high to be considered as a part of a SODIS system.

Table 4.4 *Fitting results of experimental data using GInaFIT obtained from CPC/No CPC systems exposed to real sunlight.*

Reactor system	Geeraerd Fitting model	Parameters		
		Shoulder length (min) / Inactivation rate k_{max} (min^{-1})	T_{DL} (min)	F_{DL} (kJ/m^2)
Sunny conditions				
Bottle	Shoulder	48±6 / 0.11±0.01	174.6±0.8	330±60
Glass tube	Shoulder	26±3 / 0.15±0.01	120.0±0.3	210±30
Glass tube + new CPC	Shoulder	16±8 / 0.20±0.02	87.3±0.3	140±20
Cloudy conditions				
Glass tube	Shoulder & Tail	41±3 / 0.14±0.01 $Log(N_{res})$=3.60±0.03	---	---
Glass tube + old CPC	Shoulder	26±5 / 0.15±0.01	120.0±0.5	200±50
Glass tube + new CPC	Shoulder	18±9 / 0.16±0.04	88.0±0.4	130±30

T_{DL} – time required to reach detection limit (DL)

F_{DL} – UV-A dose required to reach detection limit (DL)

$Log(N_{res})$ - Residual concentration of bacteria remaining after solar exposure

By enhancing solar disinfection with the use of a CPC (optical system), the solar UV-A radiation reaching the system is limited by the transmittance of the tube or bottle and the reflectivity of the mirror. The transmittance of most PET bottles and borosilicate glass tube is around 85-90%. The reflectivity of the mirror can vary according to the material used to build the reflectors. In most cases it is above 80% [119]. The major advantage of UV-A light is that it still reaches the Earth's surface on cloudy days; so solar radiation eventually gets into the water and reaches the bacteria. It therefore seems more profitable to invest efforts to enhance the optics of radiation collection in SODIS systems than to enhance the thermal component.

4.3.3 Mirror Degradation

The reflectivity measurements performed on new mirrors reported homogeneous values of 82% for the entire surface. Those performed on 3 year old mirrors reported non-homogeneous values between (27.0±0.5)% and (72.0±0.5)% of surface reflectivity over the length of the material. On clear sunny days, use of the CPC only allows for a faster inactivation, so the degradation of the mirror retards this effect and inactivation times are the same in the case of none and old CPC. On cloudy days, the advantage of even an old degraded CPC can still be observed (Figure 4.5). Both CPC (old and new) systems reached the detection limit on cloudy days. The system without CPC did not. Given that SODIS is usually used under rough field conditions, degradation of reflectivity is likely to be a problem for which ever reflective material is used. The older CPCs were exposed to only moderate field conditions and yet suffered an important decrease in the reflectivity after 3 years of exposure. Therefore, material degradation should not be overlooked for any reflector used together with SODIS systems. The cost of the aluminium mirror itself is similar to that of the bottle or even higher depending on the quality, so the use of reflectors is justified in larger SODIS systems that provide drinking water for several households.

4.3.4 Survival of Bacteria in Turbid Water without Solar Exposure

Concentrations of *E. coli* K-12 were monitored in turbid water under dark conditions for a period of 6 hours to determine if a decrease in bacterial population occurred and ensure that any subsequent decrease in concentration observed during solar exposure was due to the bacterial inactivation properties of sunlight. Turbid water samples that are not autoclaved serve to reflect turbid water samples in the natural environment. By using these natural samples the monitoring of initial bacterial concentration in the dark is extremely important, as bacteria might attach to suspended particulates causing an apparent decrease in concentration especially for heavily turbid levels. Secondly, the use of turbid water samples that are not autoclaved results in the presence of bacteria that are indigenous to the soil used, and these organisms might prove to be antagonists. *Pseudomanas*, *Actinomyces*, *Micrococcus* and *Flavobacterium* are examples of antagonists that have been shown to reduce the number of *E .coli* in suspension [120]. Dark conditions also allow for the observation of inactivation of organisms due to thermal effect only. The highest temperature, 45.7°C which was recorded after 6 h was for the 300 NTU sample in the 2 L PET bottle during the warm summer months. The lowest recorded also after a 6 h exposure was 25.8°C for the 5 NTU sample in the CPC reactor during cloudy conditions in the winter months. Irrespective of the temperature reached during dark conditions or the type of SODIS reactor used for all levels of turbidity (0, 5, 100, 300 NTU), the concentration of both types of bacteria remained ~ 10^6 CFU/ml, which was the concentration of the initial seeded bacterial population. Subsequent inactivation of bacteria observed on solar exposure can then be attributed to the synergistic effect between thermal and optical process for temperatures 45-50°C [18, 21] or else solely due to optical properties of solar radiation for lower temperatures.

4.3.5 Inactivation of Bacteria in Turbid Water contained in PET Bottles

PET bottles are most frequently used as the SODIS reactor of choice for SODIS users, due to their widespread availability, their efficient transmittance of UV-A, their robustness, as well as being light-weight [121]. Bottles were filled with

contaminated water (2 L) and exposed to high intensity UV-A (34.2 ±4.0 W/m^2) sunlight for 6 h to determine the turbidity level that would result in complete inactivation of 2 L of turbid water. Figure 4.7 shows the results of inactivation of *E. coli* K-12 in PET bottles for, 0, 5, 100, 300 NTU water samples under sunny conditions. As expected, inactivation rates are reduced with increasing turbidity. Within 5 hours, both the 0 NTU and 5 NTU water samples were both completely inactivated, their inactivation curves followed the standard microorganism inactivation curve: an initial delay, followed by a log-linear inactivation region and then a "tail" where the inactivation processes becomes slower [122]. For the 100 NTU sample, complete inactivation only occurred after 6 hours with a longer initial delay "shoulder" when compared to that of 5 NTU. Complete inactivation did not occur with the 300 NTU water samples, after 6 h of solar exposure. A 2.5 log concentration population of *E. coli* K-12 remained despite the synergistic effects of SODIS [18, 21] which should have occurred due to a maximum temperature of 45.7°C being reached.

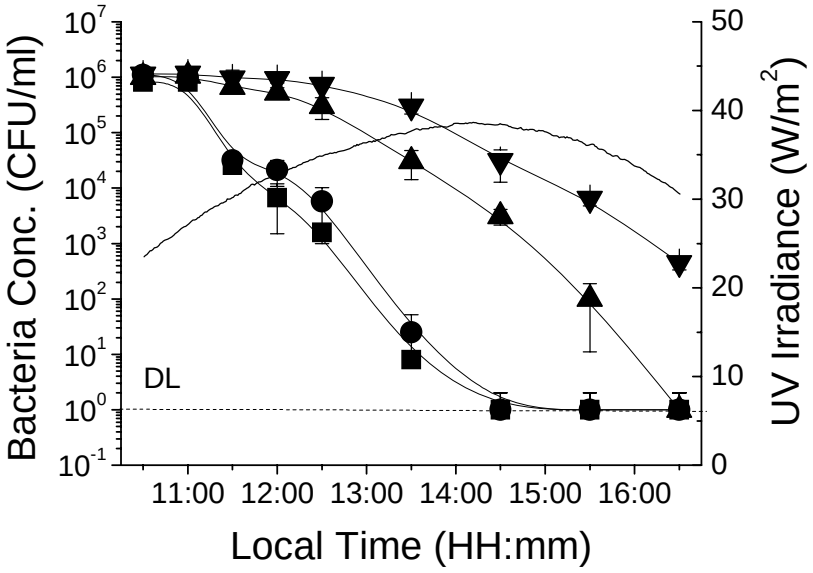


Figure 4.7 *Inactivation of E. coli K-12 in turbid water contained in PET bottles during real sunlight exposure on sunny days, 0 NTU (-■-), 5 NTU (-●-), 100 NTU (-▲-) and 300 NTU (-▼-). The solid line (—) is the solar UV irradiance. Dashed line (--) shows the detection limit (DL= <1 CFU/ml). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

However, on observation of the inactivation curve for 300 NTU, solar exposure was stopped at the log linear inactivation region of the curve and no tail was present. The curve indicated that a longer time exposure was needed in order to further inactivate the bacterial population left, rather than the remaining inactivated population being resistant to solar disinfection.

Under cloudy conditions solar disinfection of turbid water in PET bottles was severely compromised. After 5 h exposure, there was approximately a 4 log reduction of bacteria in both the 0 and 5 NTU samples (Fig. 4.8). For the 100 NTU sample, there was an approximately 3 log reduction of bacteria and for the 300 NTU sample less than a 1 log reduction (Fig. 4.8). Maximum water temperatures occurred in the 300 NTU sample and were less than 30°C, hence bacterial inactivation observed is due to optical properties of sunlight and not thermal properties.

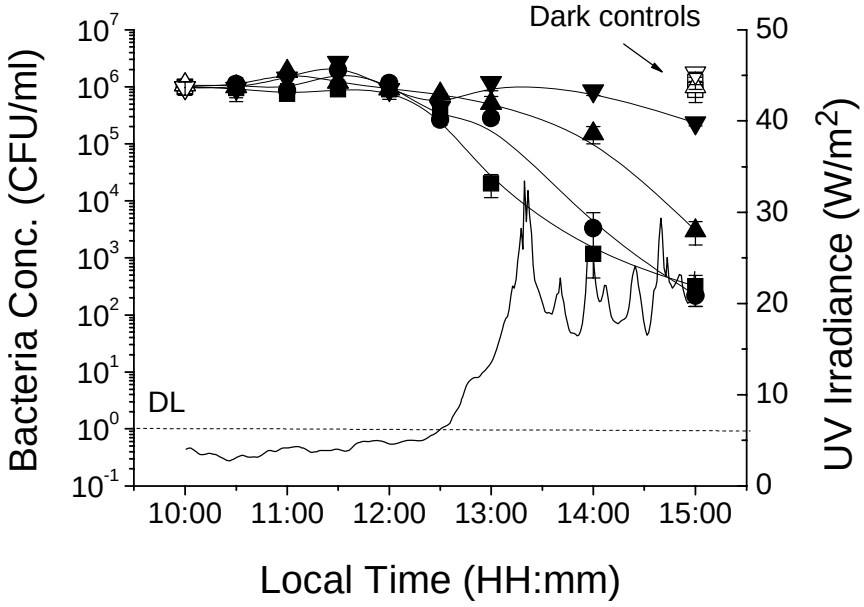


Figure 4.8 *Inactivation of E. coli K-12 in turbid water contained in PET bottles during real sunlight exposure on cloudy days, 0 NTU (-■-), 5 NTU (-●-), 100 NTU (-▲-) and 300 NTU (-▼-). The solid line (—) is the solar UV irradiance. Dashed line (--) shows the detection limit (DL= <1 CFU/ml). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

4.3.6 CPC Influence of Bacterial Inactivation in Turbid Water

The water used in the experiments described in section 4.3.1 was transparent with turbidity of less than 5 NTU. In field conditions, water turbidity can go up to 200 NTU. Under such conditions an important attenuation of sunlight is expected as mentioned by Kehoe *et al.* [14]. In these cases, the difference in inactivation efficiency between systems with and without CPC reflectors is expected to be larger on both clear and cloudy days. The path length of light inside the water when a CPC is used reduces to half of that with no reflectors. Given that the attenuation of sunlight is exponential according to the Beer-Lambert law, an important enhancement of the process in turbid water systems with CPC is expected.

Figure 4.9 shows inactivation of *E. coli* K-12 during clear sunny conditions in winter (February 2008). Within 2 h complete inactivation had occurred in all turbid samples (5, 100 and 300 NTU). With inactivation in the 5 NTU sample occurring half an hour faster than that of the 100 NTU and 300 NTU. The average solar UV-A radiation was 29.1±4.1 W/m^2 and the highest temperature reached was 38.5°C.

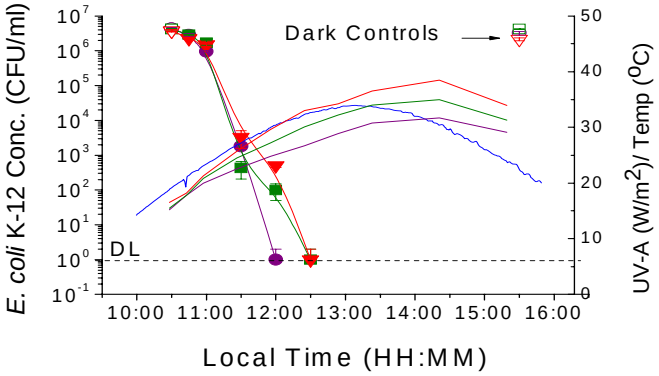


Figure 4.9 *Inactivation of E. coli K-12 in turbid water contained in CPC enhanced glass tubes, during real sunlight exposure on clear sunny days, 5 NTU (-●-), 100 NTU (-■-) and 300 NTU (-▼-). The solid line (—) is the solar UV irradiance. Water temperature in 5 NTU (—), 100 NTU (—) and 300 NTU (—) Dashed line (--) shows the detection limit (DL= <1 CFU/ml). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

Figure 4.10 shows inactivation curves obtained in turbid water under sunny day with cloudy intervals. Inactivation took longer than under clear sunny conditions

(Fig. 4.9). *E. coli* K-12 was completely inactivated in the 5 NTU sample after 2.5 h of solar exposure, 100 NTU after 3 h exposure and 300 NTU after 4 h. Inactivation curves under sunny days with cloudy intervals showed an initial delay (shoulder) which was not observed for sunny conditions (Fig. 4.9). This shows that the system under cloudy conditions required more time for enough dose to be acquired in order for inactivation to begin. Average UV-A irradiance under cloudy conditions was 23.8±5.8 W/m^2 and the maximum temperature which was recorded in the 300 NTU sample was 33.2°C. Water temperature increased with increasing turbidity.

When compared to inactivation observed in turbid water using PET bottles, there is a clear enhancement of the SODIS process with the use of CPC enhanced glass tubes whereby even under cloudy conditions, bacteria in a 300 NTU sample is inactivated under 6 h. Kehoe *et al*, showed inactivation of bacteria in a 200 NTU sample in PET bottles within an 8.5 h period under sunny conditions (global irradiation of 956 W/m^2) and under a sunny day with cloudy conditions (global irradiation of 190 W/m^2) only a 3.5 log reduction was observed [14]. Even though Kehoe *et al* observed complete inactivation for the 100 NTU sample under cloudy conditions, re-growth was observed 24 h later [14].

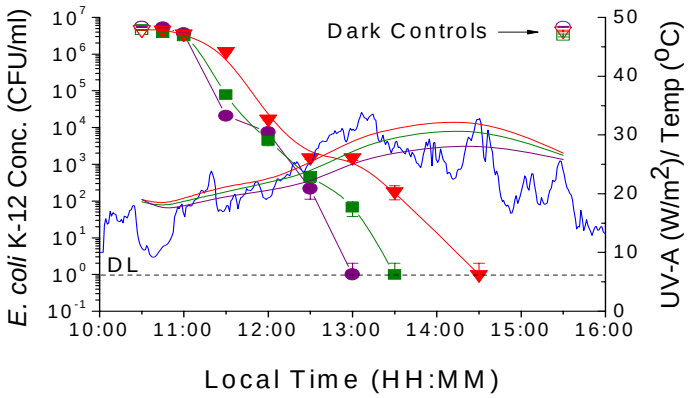
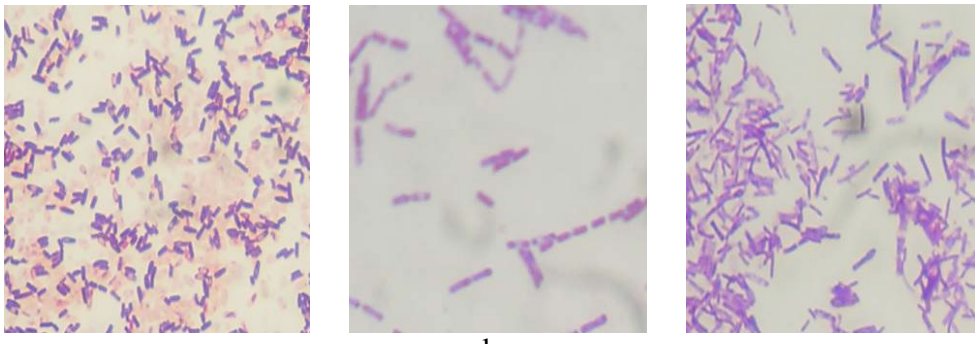


Figure 4.10 *Inactivation of E. coli K-12 in turbid water contained in CPC enhanced glass tubes, during real sunlight exposure on a sunny day with cloudy intervals, 5 NTU (-●-), 100 NTU (-■-) and 300 NTU (-▼-). The solid line (—) is the solar UV irradiance. Water temperature in 5 NTU (—), 100 NTU (—) and 300 NTU (—) Dashed line (--) shows the detection limit (DL= <1 CFU/ml). Each point represents the average of triplicate measurements of duplicate experiments and error bars show standard error limits.*

4.3.7 Bacterial Re-growth after Solar Exposure of Turbid Water

Studies have shown that bacterial cells may retain their cultivability even after receiving a lethal dose of solar radiation. But ultimately if the dose is high enough, cells are unable to repair damage and do not recover afterwards [16]. The inability to repair is even more certain when high UV irradiance is used over a short period of time, thereby attacking cellular repair enzymes and defence mechanisms [107, 123]. During solar exposure of turbid water especially under cloudy conditions in the winter months, solar intensity is reduced creating an opportunity for bacteria to detect damage and respond with repair mechanisms. Surviving microorganisms might not only be resistant to UV but may have their growth facilitated by a readily available carbon source [60] which could be present in turbid water. Due to the use of unautoclaved water soil, background microflora was expected. A 10^2 CFU/ml concentration of background flora was only observed in samples taken at the end of 5 h solar exposure period probably due to the inactivation of *E. coli* K-12 which could have served as a competition when present. This phenomenon of naturally occurring flora only being detected after seeded bacteria has being inactivated was also observed when testing the effectiveness of a SODIS pouch, which was conducted with natural river water [56]. When API 50 CH strip testing was performed, the gram positive rods were most likely *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus lentus* (Fig. 4.11). Though these organisms remained after solar exposure they are unlikely to be important causes of waterborne disease.



a b c

Figure 4.11 *Gram stain of Bacillus subtilis (a), Bacillus cereus (b) and Bacillus lentus (c).*

4.4 Conclusion

1. The use of compound parabolic concentrators (CPCs) provides an enhancement to solar disinfection for both turbid (≤ 300 NTU) and clear water on sunny and cloudy days, with a more pronounced enhancement under cloudy conditions.
2. Under field conditions, non-homogenous degradation of CPC mirrors occurs. Under sunny conditions, there is no significant difference between using an old CPC and no CPC during solar disinfection. However, under cloudy conditions, only the systems with CPC either old or new achieved complete inactivation of bacteria.
3. Solar disinfection of turbid water in PET bottles is limited to turbidity levels less than 100 NTU if conducted on days with at least 6 h exposure to high intensity solar radiation.

Chapter 5

Solar Disinfection (SODIS) as a Household Water Treatment Method: Design and Evaluation of a 25 Litre Batch Solar Disinfection (SODIS) Reactor Enhanced with a Compound Parabolic Collector (CPC)

5.1 Introduction

This chapter will present the design of a simple point-of-use SODIS reactor to treat 25 L of contaminated water**,** constructed from a methacrylate tube placed at the linear focus of a compound parabolic concentrator (CPC) and mounted at a 37º inclination. The microbial inactivation efficiency of the reactor in disinfecting well-water and turbid water is evaluated by experiments carried out under high and low solar intensity conditions, over a seven month period to mimic field conditions. The ease-of-use and cost analysis of the reactor was also determined.

5.1.1 Household Water Treatment Methods

Due to breakdown in water infrastructure and unreliable supply of treated water, the responsibility of ensuring safe drinking water often ultimately rests with the consumer. In cases where consumers are able to obtain water from an improved source (public taps, protected dug wells, boreholes and rainwater harvesting) [124], approximately 83% of users will end up drinking water of poor microbial quality. This is as a result of contamination occurring when transporting water home and unhygienic handing practices [125]. A number of household water treatment methods which include filtration, flocculation, chlorination, thermal and ultraviolet disinfection have been implemented and found to be effective in

improving the microbiological quality of water in the home or at the point-of-use. These methods have been approved by the World Health Organization (WHO) [126, 127]. Household water treatment methods have been shown to be sustainable and cost-effective and are an added benefit in intervention programmes such as HIV/AIDS, nutrition and water supply programmes. In field settings where household water treatment methods are being used, reductions in diarrhoea usually range from 30-50%.

5.1.2 SODIS as a Household Water Treatment Method

In geographic areas where prolonged sunlight is available, solar disinfection (SODIS) is an economically viable household water treatment. Health impact assessment studies showed a 26–37% reduction in diarrhoea for SODIS users [46, 48-50]. The term SODIS often refers to exposure of small volumes of water (< 3 L) contained in transparent bottles (usually polyethylene terephthalate (PET)) to sunlight [21]. However, even solar reactors which are blackened and rely on solar radiation in order to reach pasteurization temperatures, are part of the household water treatment methods that are classified under solar disinfection. The ability of a household water treatment method to treat a variety of microorganisms ensures that it can be disseminated and widely used in areas which might have a mixture of different pathogens present in water. Solar exposure of contaminated water, if a transparent container is used, results in inactivation of pathogens by two processes, UV irradiation and thermal effects. Most other water treatment methods utilise one process to inactivate organisms. Studies conducted under laboratory and field conditions have shown a wide range of pathogens to be inactivated by SODIS [6, 11, 25, 27, 31, 32, 45]. Not all household water treatment methods can inactivate every pathogen; enteric viruses may remain after filtration and chlorination has been shown to be ineffective against *C. parvum* [128]. Hence if these methods are to be used in a certain area it would be essential to determine what the microbial target is.

Although SODIS in PET bottles is effective, there are a number of limitations that might result in low compliance rates after the disinfection method has been introduced into a given community. Periods of cloudy weather will require

SODIS users to expose bottles for 2 consecutive days in order to inactivate pathogens. During rainy seasons, an alternative disinfection method has to be used. The use of filtration before solar exposure is often recommended for water that has a turbidity ≥ 30 NTU [129].

The volume of water disinfected at a given time during SODIS is restricted to < 3 L, which requires users to have sufficient bottles as well as labour provide an adequate volume of disinfected water for an average household [50, 130]. Average daily drinking water requirements recommended by the WHO for an individual are 2 L, but depending on climatic conditions, pregnancy and sickness more water might be required. The small volume of water disinfected in PET bottles is used up daily and ensures that recontamination of water does not occur after disinfection. The PET bottle also serves as a safe water storage vessel due to its narrow opening. Using a container that prevents recontamination after treatment of water is essential, as SODIS does not have residual disinfection. However, not only the quality but the quantity of water is important in order to reduce waterborne disease [131].

5.1.3 Aims

The aims of this study were to:

(i) Design and construct an enhanced SODIS batch reactor (EBR) which is easy to operate and treats large volumes of water (25 L).
(ii) Assess the microbial inactivation performance of the EBR by comparing inactivation kinetics of *E. coli* K-12 in the EBR to inactivation in a borosilicate glass tube reactor (BGR) under varying solar conditions.
(iii) Examine the effect of turbidity on the inactivation of *E. coli* K-12 in 25 L of water.
(iv) Determine the cost of building and operating the EBR.

5.2 Materials and Methods

5.2.1 Enhanced SODIS Batch Reactor (EBR)

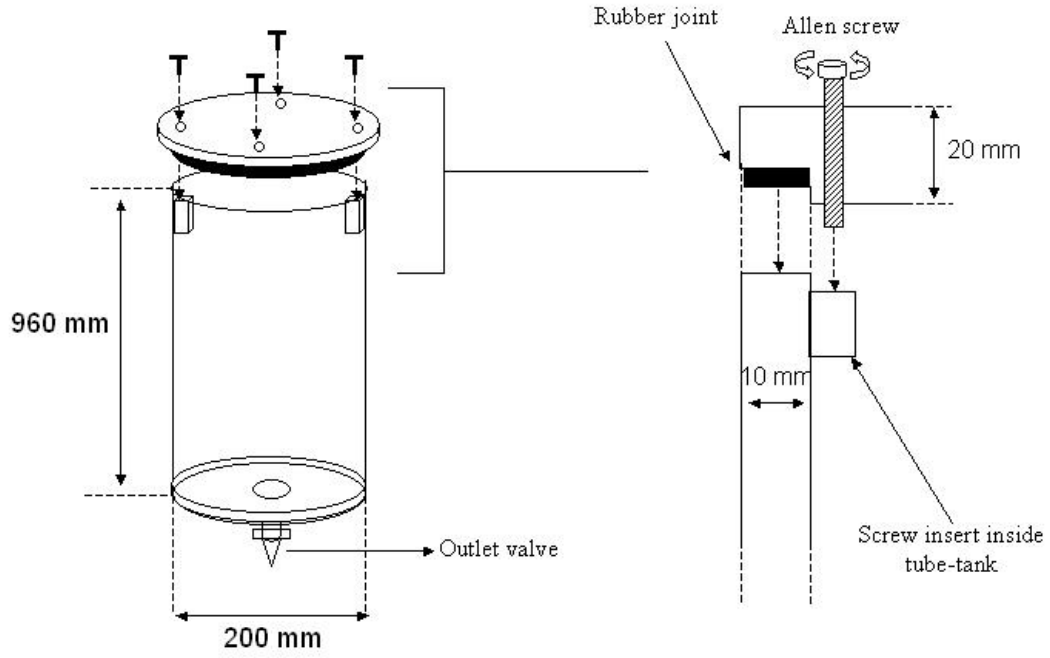
The SODIS reactor (Fig. 5.1) was constructed by placing a methacrylate plastic tube along the linear focus of a CPC mirror with a N-S orientation; which was fixed to a metal frame inclined at 37º (equal to the local latitude to recover maximum UV-A radiation during one year)**.** The methacrylate tube was made with an outlet valve at the bottom and a removable top (Fig. 5.2a and 5.2b), which was built with the same methacrylate material (Fig. 5.2a and 5.2b).

Figure 5.1 *Enhanced SODIS batch reactor (EBR) filled with E. coli K-12 contaminated turbid water.*

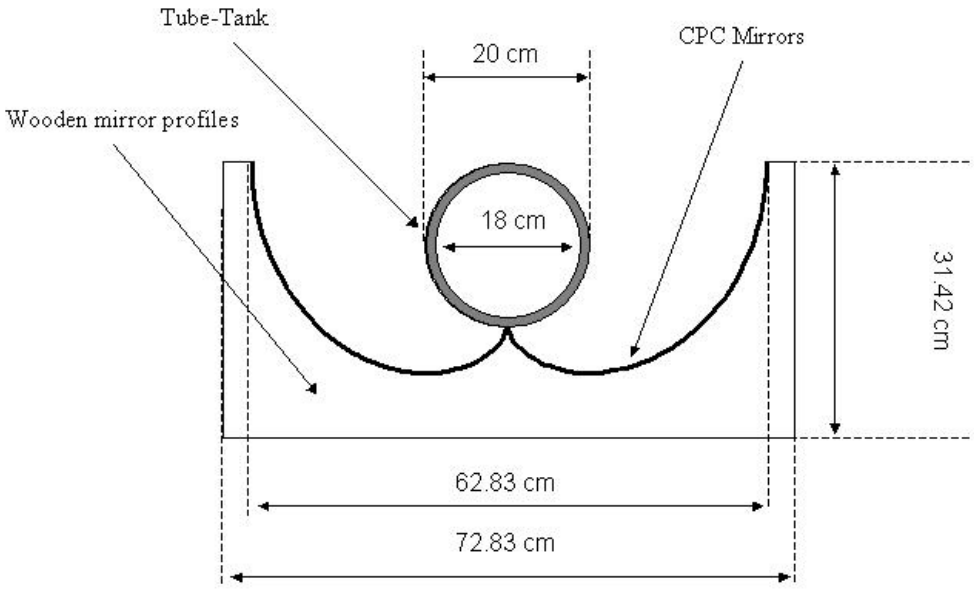
The tube was positioned at the linear focus of a CPC reflector made of highly reflective anodized aluminium sheet (MicroSun® Aluminium, Alanod GmbH, Ennepetal, Germany) with a concentration factor of 1 (CF=1) (Fig. 5.2b). The reflectivity of the aluminium sheets was 87% for UV and 95% for the visible and infra-red portions of the solar spectrum.

PET and borosilicate glass transmit more UV-A then methacrylate (Fig. 5.3). However, methacrylate is much more robust and less expensive when compared to a system constructed from borosilicate glass tube.

The dimensions of the SODIS reactor were determined by UV-A transmittance and by market constraints. The length of the tube was 1 m. Even though 2 m tubes were commercially available, a 2 m tube batch system would be highly impractical for transportation and filling the reactor. Sommer *et al*. [19] showed the maximum diameter of the tank is related to the UV-A transmittance and established that at a depth of 10 cm in 1 NTU turbid water, the UV-A transmittance is 75%.



(a)



(b)

Figure 5.2 *Scheme of the EBR (a) and the CPC mirror dimensions for EBR (b)*

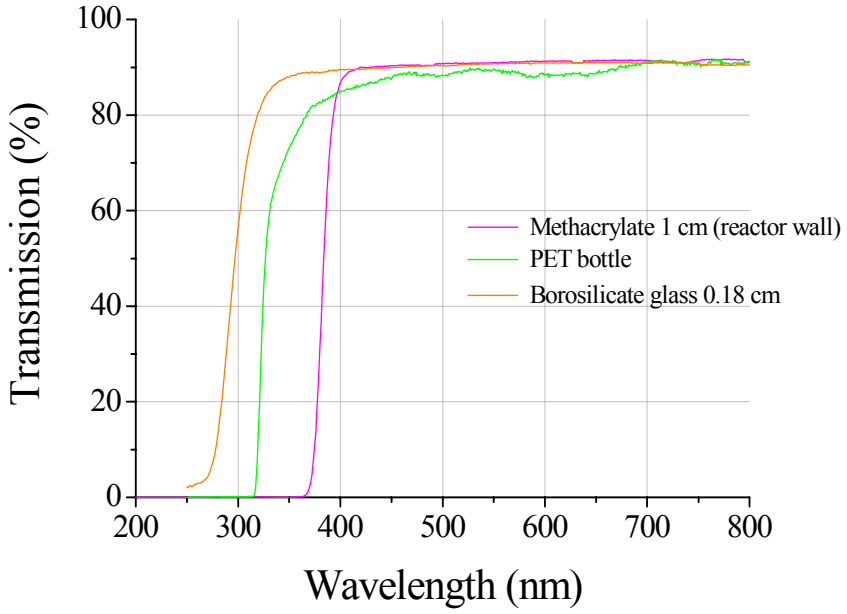


Figure 5.3 *Transmittance of different transparent materials*

However, the use of a tube with a 10 cm diameter, 100 cm in length and with a thickness of 5 mm, would yield an approximate volume of 6 L of treated water, which would be insufficient for the daily drinking water demands of a household. In construction of the EBR, a tube with a diameter of 20 cm and a thickness of (10 mm was used with a CPC with CF=1 and ensuring a treated volume of approximately 25 L of water per batch (Table 5.1). By using a CPC reflector, the tube is homogeneously illuminated even on cloudy days. Sunlight passes through a distance of one tube radius to reach the centre of the tank and assuming 75% UV-A transmittance as the maximum radiation loss tolerance, the maximum tube radius is 10 cm.

The top of the system is secured to the EBR using four Allen screws and a rubber seal. Source water is poured in the unit through the top of the tank (Fig 5.2a). Once the tank is filled with water, the top is closed with the screws and the rubber seal avoids any loss of water due to evaporation or further contamination of water from the environment. After the required exposure time to sunlight, treated water is then taken out at the exit valve on the other end. During the course of the study, filling the reactor was slightly inconvenient due to the use of the Allen screws. A follow up prototype will be designed using wing nuts which would make it easier to refill.

To compare results of the new EBR, the CPC enhanced borosilicate glass tube (BGR) was also used. This system was described in section 3.2.4 (Fig. 5.4, Table 5.1).

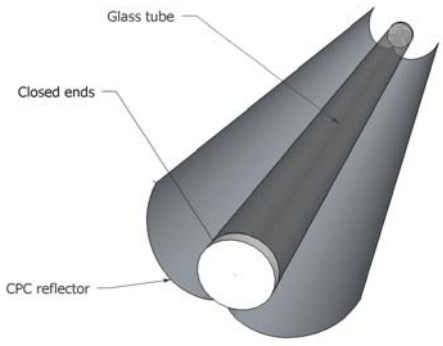


Figure 5.4 *CPC enhanced borosilicate glass tube (BGR)*

Table 5.1 *Characteristics of Enhanced SODIS Batch Reactor (EBR) and CPC enhanced borosilicate glass tube (BGR)*

	EBR	**BGR**
TUBE		
Total volume	25 L	2.5 L
Treated volume	22.5 L	2.5 L
Material	Methacrylate	Borosilicate glass
External diameter	20 cm	5 cm
Thickness	1 cm	1.8 mm
CPC mirror		
Irradiated length	92.5 cm	148 cm
Irradiated width	62.5 cm	14.2 cm
Aperture area	0.58 m^2	0.21 m^2
Concentration factor	1	1
Mirror surface	Highly reflective anodized aluminum	

5.2.2 Bacterial Preparation, Cultivation and Enumeration

E. coli K-12 was prepared according to the methods in section 3.2.1 in order to obtain a 10^6 CFU/ml concentration of bacteria in 25 L of water.

5.2.3 Enumeration of Bacterial Regrowth after Solar Exposure

Bacterial regrowth was assessed as described in section 3.2.2.

5.2.4 Preparation and Measurement of Turbidity Solution

Natural well-water was collected from a well with an approximate depth of 200 m as described in section 3.2.3. Table 5.2 provides average values of a list of physical and chemical parameters of the well-water used during the experimental period. Cations and anions were determined according to methods described in section 3.2.3. Turbidity solutions (100 NTU) were prepared as described in section 4.2 with edaphologically classified Red soil which was obtained from the Michelin test field, Almería, Spain and natural well-water.

Table 5.2 *Physical and chemical properties of well-water*

Cl^-	332 mg/l	Na^+	434 mg/l
NO_3^-	12 mg/l	NH_4^+	6 mg/l
SO_4^{2-}	294 mg/l	Mg^{2+}	41 mg/l
F^-	0.7 mg/l	Ca^{2+}	50 mg/l
Br-	2 mg/l	HCO_3^-	170 mg/l
PO_4^{3-}	0.5 mg/l	TOC	6 mg/l
pH	7.7	Conductivity	2.710 uS/cm
Turbidity	1.5 NTU	Bacteria	0 CFU/ml

5.2.5 Sunlight Exposure and Radiation Measurement

All experiments were performed under natural solar radiation at the Plataforma Solar de Almería, Spain, located at 37º84' N and 2º34' W as described in section 3.2.5.

5.2.6 UV Measurement of Different Transparent Material

For each material, 2 cm x 3 cm sections were cut in triplicate and measured using a Unicam spectrometer (Unicam Limited, Cambridge, UK).

5.2.7 Temperature Measurement of Water Samples

Temperature of water samples was measured each time that samples were taken from solar units with a thermometer (model HI 98509-1, Hanna Instruments, S.L., Eibar, Spain).

5.3 Results

Table 5.3 provides a summary of all experiments conducted in the EBR and the BGR using distilled water and well-water during the winter, spring and summer seasons. From Table 5.3, its clear that inactivation of *E. coli* K-12 in distilled water requires a shorter exposure time to sunlight for both the EBR and BGR than in well-water.

Table 5.3 *Summary of results from all experiments conducted in the EBR (22.5 L) and the BGR (2.5 L) using distilled and well-water*

#	DATE	Water	Volume (L)	Treatment time (h)	$UV_{ave.}$ (W/m^2)	Dose (kJ/m^2)	Q_{UV} (kJ/l)	$T_{máx}$ (ºC)	T_{min} (ºC)	Initial conc. (CFU/ml)	Final conc. (CFU/ml)
WINTER											
1	31-10-07	WW	22.5	5	26.9	486	12.5	25.4	15.9	$(2.7\pm0.5)\times10^6$	2100±200
2	23-01-08	WW	22.5	5	25.4	457	11.8	23.4	13.1	$(1.2\pm0.1)\times10^6$	900±100
3	24-01-08	WW	22.5	5	25.3	454	11.7	22.9	9.7	$(3.9\pm0.3)\times10^6$	930±130
4	29-01-08	WW	22.5	5	24.8	446	11.4	23.5	10.9	$(1.9\pm0.2)\times10^6$	1100±200
5	30-01-08	WW	22.5	5	26.9	482	12.5	25.0	8.6	$(1.6\pm0.5)\times10^6$	670±30
6	01-02-08	WW	22.5	5	26.7	482	12.4	23.1	11.0	$(2.8\pm0.7)\times10^6$	600±200
7	05-02-08	WW	22.5	5	28.6	515	13.3	26.5	12.3	$(1.0\pm0.2)\times10^6$	600±200
8	06-02-08	WW	22.5	5	28.9	522	13.4	27.5	12.9	$(1.3\pm0.2)\times10^6$	600±50
9	08-02-08	WW	22.5	5	20.6	371	9.6	23.4	9.6	$(2.6\pm0.1)\times10^6$	1100±300
10	13-02-08	WW	22.5	5	8.9	162	4.1	12.6	10.9	$(2.3\pm0.4)\times10^6$	820±120
11	13-02-08	WW	2.5	5	8.9	162	13.5	13.3	11.3	$(6.8\pm0.7)\times10^6$	DL
12	07-03-08	WW	22.5	5	35.4	637	16.4	31.9	21.3	$(3.4\pm0.1)\times10^6$	DL
13	14-03-08	WW	22.5	2	30.1	216	5.6	27.2	19.1	$(4.1\pm0.2)\times10^6$	$(8\pm3)\times10^3$
14	14-03-08	WW	2.5	1.5	30.3	166	13.8	31.2	24.3	$(2.3\pm0.1)\times10^6$	DL
SPRING											
15	25-03-08	WW	22.5	4	38.1	547	14.2	30	14.9	$(2.8\pm0.5)\times10^6$	DL
16	25-03-08	WW	2.5	2.5	37	335	28.0	31.7	21.9	$(4.3\pm0.2)\times10^6$	DL
17	28-03-08	WW	22.5	5	38.2	191	17.7	34.7	20.0	$(2.6\pm0.4)\times10^6$	DL
18	28-03-08	WW	2.5	2	36.9	74	22.3	33.5	25.1	$(4.1\pm0.2)\times10^6$	DL
19	01-04-08	WW	22.5	5	36.4	182	16.9	33.1	17.3	$(3.8\pm0.1)\times10^6$	180±60
20	01-04-08	WW	2.5	2	29.8	60	18.0	28.2	23.0	$(4.5\pm0.6)\times10^6$	DL
21	02-04-08	WW	22.5	4	35.6	142	13.2	32.5	16.0	$(2.5\pm0.3)\times10^6$	DL
22	02-04-08	WW	2.5	2	29.7	59	18.0	31.7	22.4	$(5.0\pm0.2)\times10^6$	DL
23	03-04-08	WW	22.5	4	35.9	144	13.3	34.7	17.2	$(3.1\pm0.1)\times10^6$	DL

#	DATE	Water	Volume (L)	Treatment time (h)	$UV_{ave.}$ (W/m^2)	Dose (kJ/m^2)	Q_{UV} (kJ/l)	$T_{máx}$ (ºC)	T_{min} (ºC)	Initial conc. (CFU/ml)	Final conc. (CFU/ml)
24	03-04-08	WW	2.5	2	30.1	60	18.2	30.2	20.7	$(4.9\pm0.2)\times10^6$	DL
25	04-04-08	WW	22.5	5	36.4	182	16.9	33.1	16.9	$(3.8\pm0.5)\times10^6$	130±60
26	04-04-08	WW	2.5	2	29.8	60	18.0	26.2	20.1	$(5.2\pm0.2)\times10^6$	DL
27	21-05-08	WW	22.5	7	33.1	232	21.5	37.8	18.4	$(2.4\pm0.3)\times10^6$	DL
28	21-05-08	WW	2.5	3	23.9	72	21.7	31.3	19.6	$(4.4\pm0.2)\times10^6$	DL
29	11-06-08	DW	22.5	4	27.2	109	10.1	36.9	22.7	$(4.0\pm1.0)\times10^6$	DL
30	11-06-08	DW	2.5	1	25.5	26	7.7	31.7	22.4	$(4.3\pm0.6)\times10^6$	DL
31	12-06-08	DW	22.5	3	30.4	91	8.5	33.3	23.9	$(2.7\pm0.3)\times10^6$	DL
32	12-06-08	DW	2.5	1.5	27.9	42	12.7	34.6	23.5	$(1.9\pm0.5)\times10^6$	DL
33	13-06-08	DW-100 NTU	22.5	5	35.7	179	16.6	40.6	23.6	$(5.1\pm0.6)\times10^6$	DL
34	13-06-08	DW-100 NTU	2.5	3	32.3	97	29.3	35.2	24.6	$(4.8\pm0.2)\times10^6$	DL
35	13-06-08	DW-0 NTU	2.5	2	29.2	58	17.7	31.8	26.4	$(5.1\pm0.3)\times10^6$	DL
36	17-06-08	DW-100 NTU	22.5	5	36.1	181	16.7	45.8	25.2	$(1.3\pm0.3)\times10^6$	DL
37	17-06-08	DW-100 NTU	2.5	3	32.9	99	29.8	38.4	24.3	$(3.9\pm0.6)\times10^6$	DL
38	17-06-08	DW-0 NTU	2.5	2	29.8	60	18.0	35.1	26.0	$(3.6\pm0.1)\times10^6$	DL
39	18-06-08	WW	22.5	7	35.2	246	22.9	40.7	23.6	$(1.2\pm0.1)\times10^6$	DL
40	18-06-08	WW	2.5	3	32.7	98	29.7	36.9	24.4	$(4.1\pm0.2)\times10^6$	DL
41	18-06-08	DW	2.5	1.5	27.8	42	12.6	37.0	26.7	$(2.5\pm0.5)\times10^6$	DL
SUMMER											
42	08-07-08	WW-100 NTU	22.5	7	33.1	232	21.5	50.8	19.4	$(1.7\pm0.2)\times10^6$	DL
43	08-07-08	WW-100 NTU	2.5	3	30.7	77	23.2	36.8	24.0	$(2.8\pm0.2)\times10^6$	DL
44	08-07-08	WW-0 NTU	2.5	2	29.3	59	17.7	32.4	27.3	$(4.2\pm0.5)\times10^6$	DL
45	11-07-08	WW-100 NTU	22.5	6	36.3	218	20.2	50.3	23.9	$(7.8\pm0.4)\times10^6$	DL
46	11-07-08	WW-100 NTU	2.5	3	33.4	100	30.3	43.2	27.5	$(4.0\pm0.5)\times10^6$	DL
47	11-07-08	WW-0 NTU	2.5	2	30.4	61	18.4	39.2	30.2	$(8.3\pm0.5)\times10^5$	DL
48	23-07-08	DW	22.5	4	34.0	136	12.6	45.2	26.6	$(2.4\pm0.6)\times10^6$	DL
49	23-07-08	DW	2.5	2	29.0	58	17.5	42.9	28.0	$(1.9\pm0.3)\times10^6$	DL

*

- $UV_{ave.}$: average solar UV-A irradiance during the whole exposure (when the detection limit (DL) was not reached) or during exposure to reach DL.
- Dose: solar UV-A dose during the whole exposure (when DL was not reached) or during exposure to reach DL.
- Q_{UV}: accumulated solar UVA energy per unit of volume during the whole exposure (when DL was not reached) or during exposure to reach DL.
- $T_{max.}$: maximum temperature reached during the whole exposure (when DL was not reached) or during exposure to reach DL.
- $T_{min.}$: minimum temperature reached during the whole exposure (when DL was not reached) or during exposure to reach DL.
- Treatment time: the whole exposure (when DL was not reached) or exposure time to reach DL.
- WW: well-water
- DW: distilled water

5.3.1 Inactivation Kinetics in CPC Enhanced Batch Reactor (EBR) and CPC Enhanced Borosilicate Glass Tube Reactor (BGR)

Previous experiments have shown that the use of a borosilicate glass tube (BGR) (Fig. 5.4) surrounded by a CPC was the most optimum batch reactor for inactivation of *E. coli* K-12 in small volumes of water (< 3 L) [132]. Borosilicate glass transmits 89-90% in the UV-A region of 320-400 nm as well as 45% in the lethal UV-B region of 280-300 nm. The 25 L enhanced batch reactor (EBR) was constructed from methacrylate which is opaque to UV-B and transmits a significantly lower percentage of UV-A when compared to borosilicate glass. Furthermore, the reactor was designed to treat 10 times the volume of water (25 L) that was inactivated in the 2.5 L borosilicate glass tube reactor (BGR) under the same solar exposure conditions. The diameter of the SODIS reactor tube is 20 cm and so light has to travel a longer distance compared to that in the glass tube with a smaller diameter. Dark control experiments were carried out to ensure that bacteria were not inactivated before exposure to sunlight. For both the EBR and BGR, after sampling at given intervals for 5 h, bacterial concentration remained 10^6 CFU/ml, the same concentration as the initial seeded sample. Figure 5.5 shows inactivation kinetics of bacteria on three different days in the two reactors. In Fig 5.5a, under clear sunny conditions with a high level of solar UV-A irradiance at the start of the exposure (29.6 W/m^2), bacteria in both reactors were inactivated to the detection limit (< 1 CFU/ml) in less than 6 h after a total

received UV-A dose of 688 kJ/m^2 (191 Wh/m^2). Bacteria in the BGR were inactivated 3 h faster than those in the EBR. In Fig 5.5b, the day was clear but solar irradiance at the start of the experiment was low (20.2 W/m^2) followed by an intermittent period of cloudy weather towards the end of the exposure period. Bacteria were still inactivated in the BGR within 2 h as it was for Fig. 5.5a. However in the EBR, even after 5 h exposure to sunlight with a UV- A dose of 655 kJ/m^2 (182 Wh/m^2), a 2 log concentration of bacteria still remained. In Fig 5.5c, the starting irradiance was 27.4 W/m^2, just 2 W/m^2 less than that of Fig. 5.5a; however, there were several cloudy intervals resulting in a total UV-A dose 378 kJ/m^2 (105 Wh/m^2).

In both reactors, complete inactivation did not occur, with a residual ~ 2 log concentration of bacteria remaining.

In Fig. 5.5a, 5.5b and 5.5c, maximum water temperatures, 34.7ºC, 33.1ºC, and 26.9ºC respectively, occurred in the 25 L EBR and were 5ºC higher than maximum temperatures in the BGR. In the smaller volume BGR, variations in water temperature closely followed variations in solar irradiance. However as a result of the increased thermal inertia associated with the larger 25 L volume of water in the EBR, water temperatures were slower to respond to changes in irradiance and continued to increase even when solar irradiance has started to decrease.

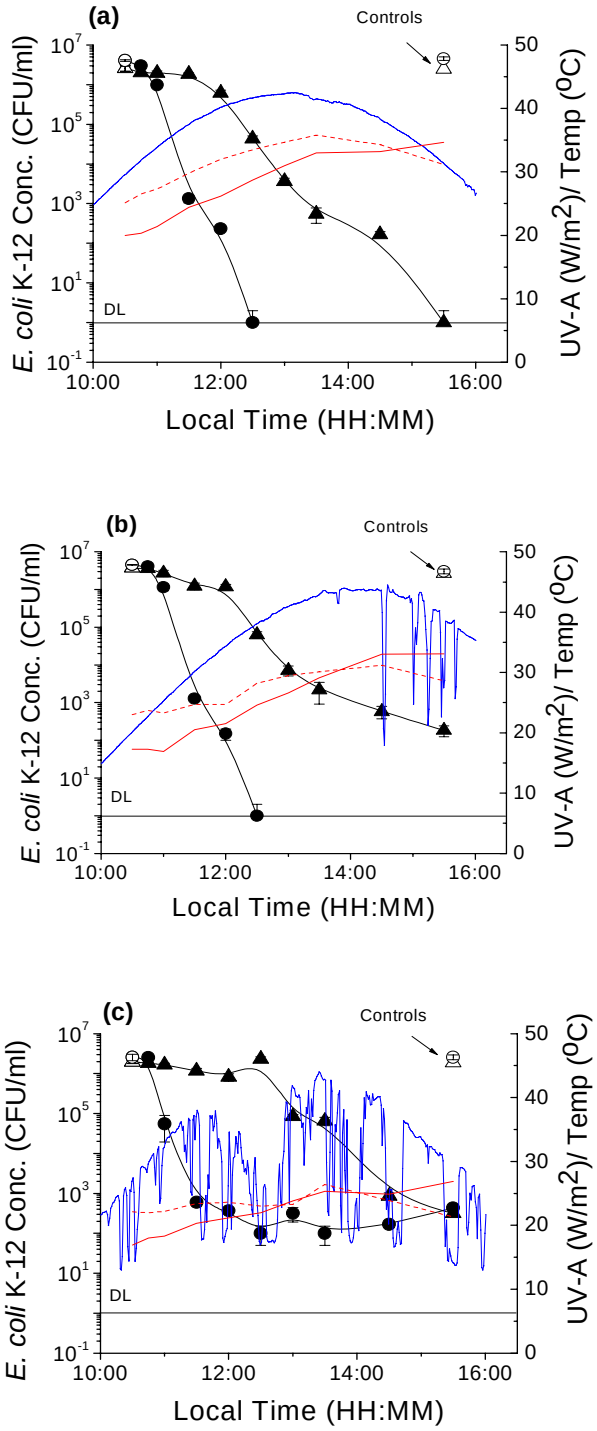


Figure 5.5 *Inactivation curves of E. coli K-12 under sunny (a), partially sunny (b) and cloudy conditions (c). 2.5 L BGR (-●-), 25 L EBR (-▲-),solar irradiance on day of experiment (—), water temperature in 2.5 L (---) , water temperature in 25 L (—) and detection limit (DL) (—) of < 1 CFU/ml. Each point represents the average of triplicates, and bars show the standard errors.*

5.3.2 Effect of Turbidity

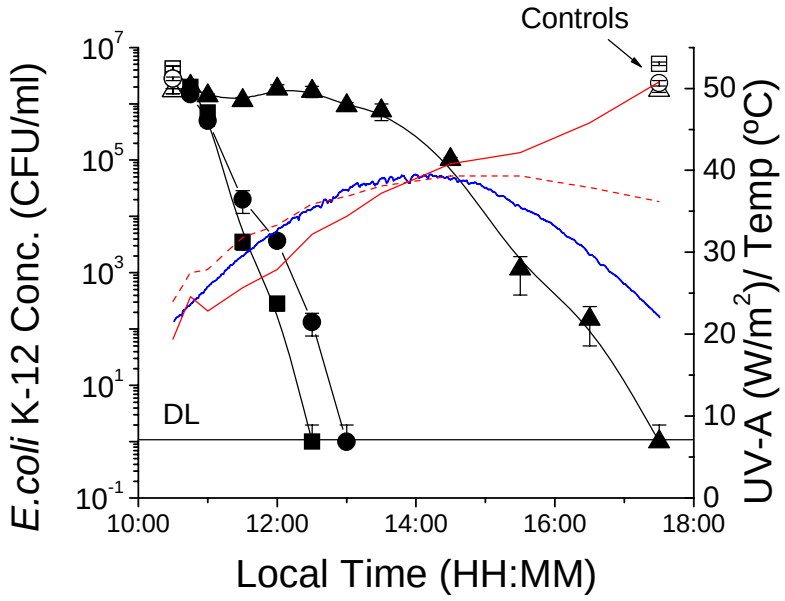


Figure 5.6 *Inactivation curves of E. coli K-12 in turbid water exposed to sunlight. 2.5 L BGR – 0 NTU (-■-), 2.5 L BGR – 100 NTU (-●-), 25 L EBR – 100 NTU (-▲-), solar irradiance on day of experiment (–), water temperature in 2.5 L (100 NTU) (---), water temperature in 25 L (–) and the detection limit (DL) (–) of < 1 CFU/ml. Each point represents the average of triplicates, and bars show the standard errors.*

The effect of turbidity on the inactivation of bacteria in large volumes of water was determined in the 25 L EBR and compared to that of inactivation of smaller volumes of turbid water in the BGR. In Fig. 5.6, inactivation of bacteria in the 100 NTU 25 L EBR sample took 7 h for complete inactivation after receiving a total UV-A dose of 868 kJ/m^2 (241 Wh/m^2). Unlike bacterial inactivation in the 0 and 100 NTU 2.5 L BRG water samples; there was a long initial delay (shoulder) before inactivation started for the 25 L 100 NTU sample (Fig. 5.6). The water temperature (50.8ºC) in the 100 NTU 25 L EBR was 14.6ºC higher than that of the 100 NTU 2.5 L BGR sample (36.2ºC) at the end of the exposure.

5.3.3 Seasonal Variation in Inactivation

As seen in Fig. 5.7, during the winter months of January and February, solar irradiation levels were low and as a result solar exposure within a 5 h period only resulted in a 3.5 log inactivation of bacteria in the 25 L EBR. In March and

April, there were days where solar irradiance levels were high enough to result in complete inactivation of bacteria. However, on some days, solar irradiance levels were low possibly due to hazy conditions.

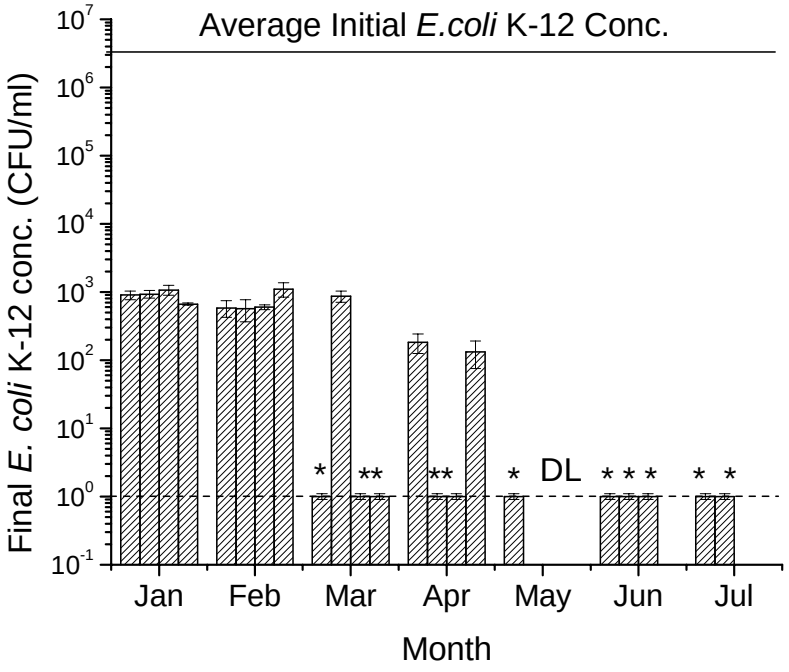


Figure 5.7 *Monthly inactivation of E. coli K-12 in 2008 for the winter - summer seasons in Almería, Spain and the dashed line represents the (DL) detection limit of < 1 CFU/ml. No viable colonies were detected (*).*

This resulted in incomplete inactivation of bacteria since the total UV dose received during the exposure period was insufficient. During the summer months, from May onwards, complete inactivation of bacteria was obtained within a 5 h exposure period on all experimental days, as solar irradiance levels had increased. Complete inactivation occurred after a total UV-A dose of ≥ 659 kJ/m^2 (183 Wh/m^2) (Fig. 5.8) had been received irrespective of the time of year but generally occurring during the summer months.

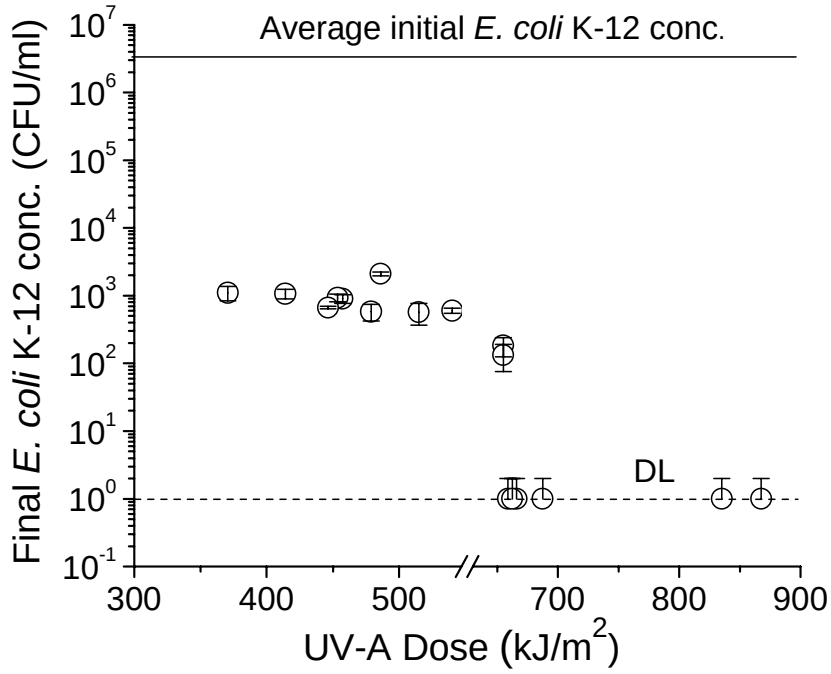
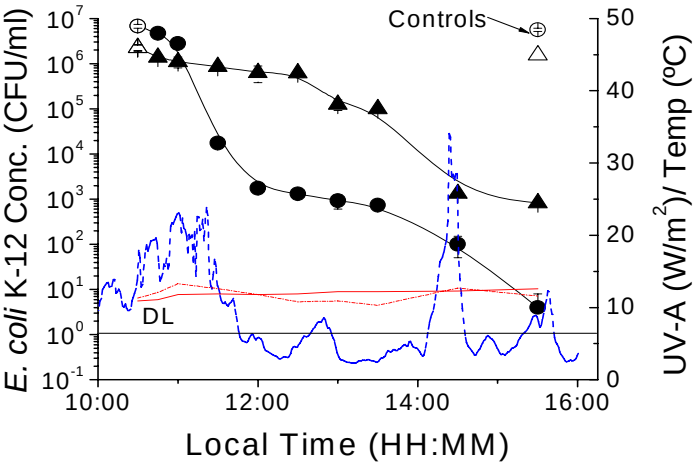


Figure 5.8 *Final bacterial concentration versus UV-A dose received during 5 h solar exposure. The solid line represents the average initial bacterial concentration (~ 2.5 x 10^6 CFU/ml) and the dashed line represents the (DL) detection limit of < 1 CFU/ml.*

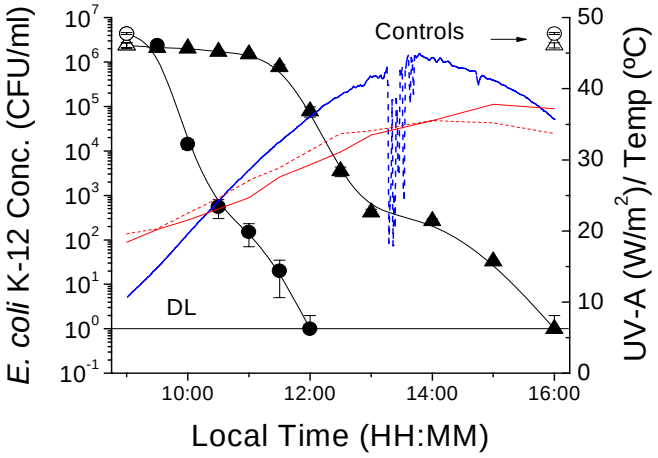
5.3.4 Effect of Cloudy Periods

Fig. 5.9 shows a test on a cloudy day with sufficient UV-A irradiance to initiate the disinfection process during the first 2 hours of exposure, after which the process slows due to the presence of more clouds and a decrease of UVA irradiance.

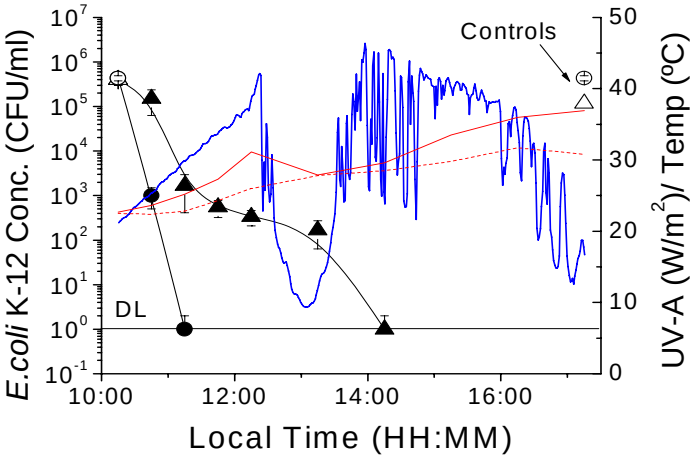
As seen in 5.9b and 5.9c, during days of full sunshine a short cloudy period also decelerates the kinetics. These tests done on May 25th 2008 and on June 11th 2008 show a clear decrease on the *E. coli* K-12 inactivation process when just a few clouds appeared. The presence of clouds decreases the accumulated UV-A dose, since UV-A irradiance also diminishes.



(a)



(b)



(c)

Figure 5.9 *Inactivation curves of E. coli K-12 in well water exposed to sunlight. 2.5 L BGR (-●-), 25 EBR (-▲-), solar irradiance on day of experiment (—), water temperature in 2.5 L (100 NTU) (---), water temperature in 25 L (—) and the detection limit (DL) (—) of < 1 CFU/ml. Each point represents the average of triplicates, and bars show the standard errors. 13th February 2008 (a), 25th May 2008 (b), 11th June 2008 (c).*

5.3.5 Effect of Water Temperature

All experiments were conducted between January and July 2008. Temperature of water samples was measured during exposure to sunlight. Figure 5.10 shows the average, minimum and maximum values measured in all tests done with well water in the EBR system. As seen in this figure the lowest temperatures were found in experiments done in January and February. The highest temperatures were measured in disinfection experiments done in July. Experiments in July reached 45 ºC, the temperature required for synergy of solar irradiation and temperature [18]. Nevertheless we observed that total inactivation was achieved in most experiments done in March, April, May and June.

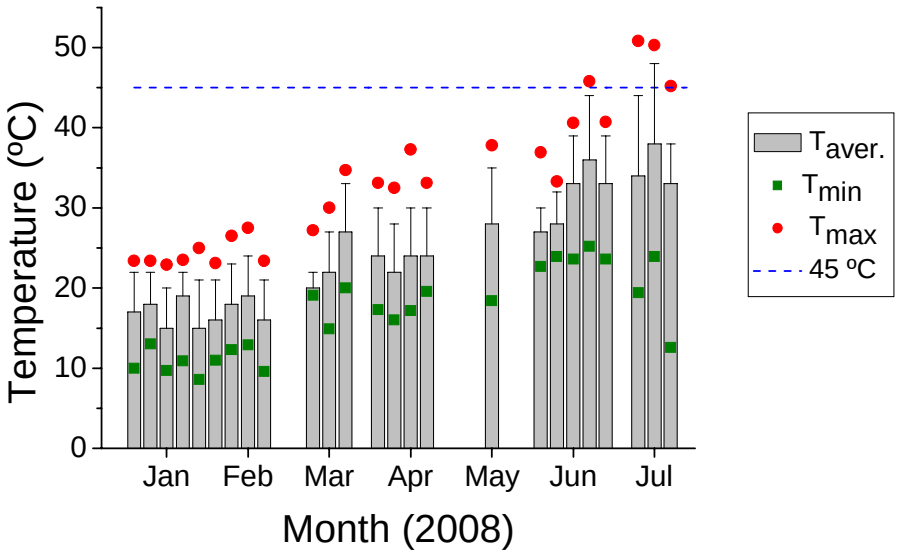


Figure 5.10 *Maximum (●), minimum (■), and average (bars) water temperatures measured while conducting solar tests in the EBR with well-water and E. coli K-12 during exposure (when DL was not reached) or during period needed to reach DL.*

5.4 Discussion

In this study a SODIS enhanced batch reactor (EBR) fitted with a Compound Parabolic Collector (CPC) was constructed for the purpose of treating 25 L of water in ≥ 6 h of strong sunlight, thereby removing the need for a constant supply of PET bottles. As SODIS in PET bottles is a virtually zero cost technology, the EBR needed to: (i) be constructed from materials of minimum cost; (ii)) be

robust in mature so that it can withstand adverse environmental conditions and (iii) require very little maintenance. The reactor consists of a cylindrical methacrylate tube with one inlet and one outlet valve, placed along the linear focus of the CPC. The use of flow was avoided in the reactor, as previous experiments had shown increasing flow rate had a negative effect on bacterial inactivation [133]. This was due to the fact that inactivation of bacteria was more effective when bacteria were exposed to maximum UV-A doses over a short period of time, rather than receive repeated sub-lethal doses over a longer period of time, which is more likely to occur using re-circulated flow [133]. Furthermore, flow-through systems might require a high maintenance cost and a constant supply of power to operate the system. The use of the CPC provides an enhancement to the disinfection process by concentrating available solar radiation and therefore reducing the amount of exposure time required for activation under cloudy conditions [132]. In previous studies, a three year old CPC with reduced reflectivity from a homogeneous 82% along the concentrator to a non-homogeneous value between 27 and 72%, still ensured that complete bacterial inactivation was achieved on cloudy days [132]. CPC's utilise non-imaging optics which allows the system not only to concentrate direct radiation but also diffuse radiation into the absorbing reactor without the need to reorient the system as the sun tracks across the sky [96, 132]. Since UV-A is made up of both direct (~ 60%) and diffuse (~ 40%) solar radiation [115], the use of a CPC ensures that all UV-A is concentrated and available for microbial inactivation without the need to reposition the mirror. Furthermore, the concentration factor (CF) of the CPC remains constant throughout the day unlike non-imaging mirrors where the CF is based on the angle of incidence of the sun on the reflector, which changes through the day [96, 132].

On the earth's surface, ultraviolet radiation of sunlight is composed of mostly UV-A (320 -400 nm) radiation (90-92%) with some UV-B (290-320 nm). UV-B is known to be highly lethal to microbial cells. However, since these UV-B wavelengths only make up a small component of solar radiation, most inactivation of bacteria under sunlight is attributed to UV-A wavelengths. Filtering out most of the UV-B by the use of PET, shows that even without UV-B, inactivation of bacteria still occurs with wavelengths > 320 nm [18].

Compared to PET and borosilicate glass, methacrylate filters out solar UV-B and a significant portion of the UV-A radiation. From Fig. 5.4, methacrylate has a high-pass transmission cut-off at about 370 nm, whereas for PET and borosilicate glass transmission starts at 320 nm and 290 nm, respectively. Despite this significant reduction in UV-A transparency, the final concentrations of bacteria in the 25 L EBR compared well with those in the BGR under solar exposure. Under sunny conditions, complete inactivation of bacteria occurred in both reactors and under cloudy conditions both reactors achieved similar final inactivation levels of ~ 3-log units below the starting concentration (Fig. 5.5a and 5.5c). These results indicate that useful inactivation can still occur not only in the absence of UV-B, but also when there is severe reduction in the amount of UV-A. Results also add to evidence by other studies that visible light wavelengths are an important component in sunlight inactivation of microorganisms and was shown by Acra *et al*. to account for 30% of bacterial inactivation [122, 134].

By building the EBR out of transparent plastic and using a CPC mirror, the emphasis while constructing the reactor was on maximising the optical inactivation properties of sunlight. Reaching pasteurisation temperatures in a reactor on cloudy days is difficult especially under low ambient temperatures and high wind speed conditions [135]; but on cloudy days, diffuse UV-A is still available for inactivation. Saitoh *et al*. [135] showed that after 1.5 h, all coliform bacteria had been eliminated from a CPC reactor while it took double the time for the same concentration of bacteria to be eliminated in the solar hot box once pasteurisation temperatures had been reached [135]. Mani *et al*. [57] also studied a comparison between reflective and absorptive surfaces in small scale reactors and found that under low sunlight conditions, only the reactor with the reflective surface continued to show an enhancement [57]. During experiments conducted in the EBR under sunny conditions (Fig. 5.5a), maximum water temperatures for < 5 NTU samples reached only 34.7ºC and still inactivation of bacteria occurred under 5 h. Nevertheless, there are advantages in increasing the temperature of water especially for temperatures > 45ºC where a synergy between optical and thermal mechanisms were noted in the inactivation of *S. typhimurium* and *E. coli* [18, 20]. Even if > 45ºC temperatures are not achieved, elevated temperatures have been shown to increase inactivation rates [36]. Using a CPC mirror and

aluminium foil reflectors may not enhance water temperatures as much as blackened or absorptive surfaces; however, small increases in temperature do occur. Kehoe *et al*. [14] showed that water temperatures in bottles with foil backing were 0.9ºC higher than bottles without and Navntoft *et al*. [132] showed that on sunny days the use of a CPC increased water temperatures by 2.6ºC and on cloudy days by 4.3ºC.

The relationship between turbidity and inactivation is not straightforward. Penetration of optical radiation through the water is hampered by increasing turbidity. The turbid agent scatters and absorbs the light [14, 43]. However as the radiative emissivity of the water increases with turbidity we see that water temperature is raised to values higher than that achieved in the 0 NTU sample. The heat is trapped within the reactor via a green-house mechanism. Higher temperatures produce convective currents which facilitate mixing thereby ensuring that the entire bulk of the liquid is periodically subjected to higher photon fluxes while closer to the sides of the reactor. Bacteria in turbid water (100 NTU) were disinfected within 7 h with water temperatures reaching a maximum of 51ºC even after solar irradiance levels had begun to decrease (Fig. 5.6). Furthermore, Meera and Ahammed [136] show that during solar disinfection, moderate turbidity (38 NTU), was an enhancement to microbial inactivation when compared to inactivation in low turbidity samples (< 5 NTU) [136].

All experiments were conducted in Almería, Spain which has four distinct seasons. Even though experiments were conducted during the first seven months the year (January to July), it is reasonable to expect that inactivation results for the rest of the year mirror those obtained from January to June. Acra *et al*. [51] show variations in the solar tilt and solar altitude to be symmetrical during the course of the year (Fig. 5.9), with these two factors affecting the amount of solar UV-A at 340 nm reaching the earth's surface [51].

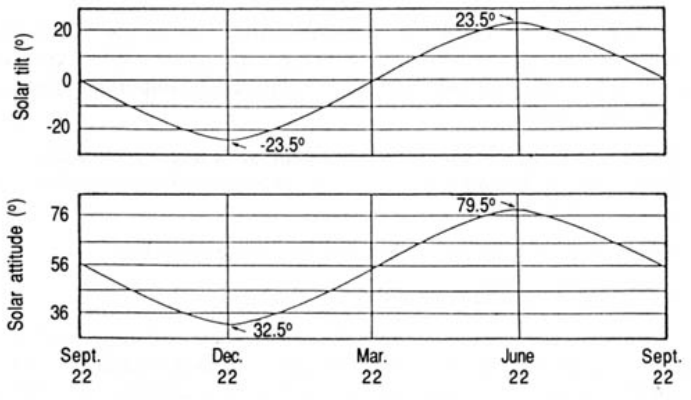


Figure 5.9 *Variations in angles of solar tilt and altitude during the year in Beirut [51].*

In the warmer months where solar UV irradiance was on average 36.9±6.5 W/m^2, complete inactivation of bacteria in the 25 L enhanced batch reactor occurred within 5 h. During the winter months with lower solar irradiance (average = 26.9±4.0 W/m^2), more than 5 h were required to achieve complete inactivation. In areas where solar disinfection is used, full sunshine days and partly sunny days are approximately 200-300 days or 2500-3000 h in a year depending on how far the location is from the equator [126, 134]. Strong sunlight for 3-5 h at an intensity of 500 W/m^2 [137], is the requirement necessary for effective solar disinfection, and is readily achieved in these areas. Hence, the 25 L enhanced batch reactor will function efficiently for most days in the year because sunny days will ensure that solar irradiance levels are high enough to achieve the UV-A dose of 183 Wh/m^2 needed for disinfection in 5 h.

The average monthly income in Kenya is approximately US $ 150.00 and in Maasai populations, can be as low as US $ 30.00 (*personal communication*, Dr Kevin McGuigan).The estimated construction cost for the prototype reactor is US $ 200.00, and assuming an operational life time of 10 years the cost of providing 1 L of treated drinking water from the EBR is ~US $ 0.002. Once the reactor has been purchased there are no operational costs involved and the price to treat a litre of water over 10 years time is expected to remain the same. When compared to the cost based analysis provided by Sobsey *et al*. [138] of different point-of-use water treatment technologies, water treatment in the EBR is more expensive than SODIS in PET bottles (< US $ 0.001/L) and the use of ceramic filters (< US $ 0.001/L) [138]. However, solar disinfection of 25 L of water in

PET bottles will require exposing approximately 13 PET bottles (2 L/bottle) at a given time. The ceramic filter could take up to 10 h at optimal flow rates to produce 20 L of filtered water. The EBR has the same cost per liter as the biosand filter system and is cheaper than the coagulant/chlorine system (PuR sachet) which costs > U$ 0.01/L [138]. In any case, the EBR has the potential to add to the number of household water treatment technologies available, thereby increasing the likelihood that a user will find a suitable water treatment option for their particular socio-economic level.

5.5 Conclusions

1. This study confirms that in a simple low cost SODIS reactor, significant disinfection can be achieved for 25 L of natural well-water seeded with a 10^6 CFU/ml concentration of *E. coli* K-12. Throughout the study period there was at least a 3 log reduction in bacterial concentration. A 40% reduction in diarrhoea has been noted for water contamination that is reduced by 2 magnitudes in conjunction with improved sanitation [139, 140].
2. During sunny conditions, bacteria were completely eliminated within 5 h, without water temperature reaching > 40ºC. When water temperatures were > 45ºC, disinfection occurred in highly turbid water (100 NTU) within 7 h. On cloudy days, 5 h solar exposure was sufficient to decrease bacterial population by 3 logs although not completely. It is therefore recommended that water be exposed for 2 days under cloudy conditions, as is also indicated for disinfection of < 3 L of water in PET bottles under cloudy conditions [129].
3. Inactivation studies of other microbial organisms are still to be conducted and may require a longer exposure time.

Chapter 6

Solar disinfection (SODIS) of Water in Polyethylene terephthalate (PET): Use of the *Salmonella* Ames-Fluctuation Assay as a Preliminary Assessment of Genotoxicity

6.1 Introduction

This chapter investigates the possibility of the release of potentially dangerous compounds from PET bottles into water under both solar disinfection and long term exposure conditions. A biological approach was used in the form of the *Salmonella* Ames-Fluctuation assay to detect genotoxins in water samples stored in PET bottles exposed to SODIS conditions. Genotoxic results from this study in combination with previous studies carried out on migration of chemical compounds from PET, would give a better understanding off the overall health risk of drinking SODIS treated water.

6.1.1 Polyethylene Terephthalate (PET)

PET is a polymer formed from the condensation reaction between terephthalic acid and ethylene glycol with water as a by-product and can also be formed from dimethyl terephthalate and ethylene glycol with methanol as a by-product (Fig. 6.1).

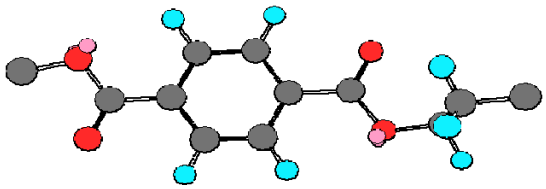


Figure 6.1 *Chemical structure of PET*

Over the years, the use of PET as a packaging and bottling material for water, beverages and food has increased, particularly in the carbonated beverage industry where the use of PET compared to other plastics is more effective in preventing carbon dioxide losses due to permeability [141] .This widespread use of PET bottles in the beverage industry ensures that PET bottles are usually locally available and since PET has a high transmittance of UV-A, these bottles are suitable SODIS reactors [18, 142]. Furthermore, PET bottles were shown to be relatively inert when compared to polyvinyl chloride (PVC) due to the use of fewer additives. Residual vinyl chloride monomer (VCM) a known mutagenic substance was shown by Benfenati *et al*. to be present in bottled water contained in PVC [143]. The concentration of VCM in samples was directly related to its level in the packaging material as well as related to temperature and storage time [143].

Despite being chemically inactive when compared to other plastics, a number of studies have shown that migration of compounds does occur from PET into water and food depending on the effect of consumer use, long term storage and storage conditions [144-148]. Analytical methods such as high performance liquid chromatography (HPLC), gas chromatography (GC) and mass spectrometry (MS) have proved invaluable in identifying and quantifying these compounds. The thermal degradation products of PET identified include the carbonyls: formaldehyde, acetaldehyde, acetone, CO_2 and water [146, 149, 150]. Sunlight and higher temperatures generally enhanced the migration of carbonyls from PET bottles [142, 146].

Plasticisers are added to many plastics to aid in their flexibility. The following plasticisers have been identified from PET during thermal degradation: di(2-ethylhexyl)phthalate (DEHP), di(2-ethylhexyl)adipate (DEHA), phthalic acid, dimethyl terephthalate (DMT), disobutyl phthalate and dibutyl phthalate [144]. DMT was found to be non-genotoxic by short term genotoxic assays, however both DMT and DEHP were shown to induce changes in the genes of mice and rats as well as the development of the central nervous system of the foetus [151, 152]. Studies on PET bottle re-use among students showed DHEA exceeded the acceptable carcinogenic risk set for drinking water [153]. Antimony which is

used as a catalyst during the production of PET was also found to leach from PET into water but only under very high temperatures of 80°C [148].

6.1.2 Migration of Compounds from PET under SODIS conditions

Due to extensive research conducted by the food packing and bottling industry which proved that migration of compounds does occur from PET, a number of chemical studies were conducted to determine if under SODIS conditions compounds leach from PET into water. In 2001, Wegelin *et al*. [142] exposed bottles in Switzerland and Malaysia to dark and sunlight conditions for 15, 31, 63 and 128 days. Not all compounds were identified using the SPME-GC-MS analysis. However, for those that were identified, there was no difference in the chemical composition of water with or without sunlight exposure [142]. Terephthalate compounds were formed on the outer surface of the bottles, while acetaldehyde and formaldehyde were found in the water itself, with higher concentrations of these carbonyls in samples that had been stored for a longer period of time. All concentrations of formaldehyde were below 15 mg/l, the limit set for safe drinking water by Swiss legislation at the time the study was conducted [142].

A more in-depth study was performed in 2003 by the Swiss Federal Laboratories for Material Testing and Research with the aim of quantifying the plasticisers DEHA and DEHP and identifying several unknown compounds that have been found to be present in the water from PET bottles both under SODIS use and non-SODIS use [54]. PET bottles from Honduras, Nepal and Switzerland were used in the study and exposed to sunlight for 17 h during two consecutive days. Controls were placed in the shade. The highest concentration of DEHA was found to be 0.046 µg/l and was from water contained in a reused bottle from Honduras, while the highest concentration of DEHP was 0.71 µg/l and was found in water from a bottle from Nepal. Both bottles had been exposed to sunlight and reached temperatures of 60°C, which are conditions that might occur under SODIS [54]. However, only the concentration of DEHP was significantly different from the blank of pure distilled water. Furthermore, both plasticisers were well below the limits set by the WHO in the Guidelines for Drinking-water Quality which are 80 µg/l and 8 µg/l for DEHA and DEHP respectively [128].

Some of the unknown compounds were identified as flavouring components of drinks that had been previously stored in the bottles, and other compounds remained unidentified as their concentrations were below the detection limit of the GC/MS method used [54].

In a third study, PET bottles containing drinking water were purchased and exposed to sunlight for 12 months at the Plataforma Solar de Almería (PSA), control bottles were kept in the dark also for the same period of time [154]. GC/MS was used to evaluate and possibly identify photodegradation products from the PET bottles. Analysis of samples showed the presence of a number of organic compounds. Under sunlight conditions the plasticiser, DEHP had the highest sample peak, while under dark conditions an alkene compound, nonadecene had the highest peak. An unknown peak was also found in both the dark and sunlight exposed samples [154].

Although all three analytical studies described above were able to detect and quantify a number of important compounds, these studies also illustrate the difficulty in predicting and the almost impossible task of successfully identifying all compounds that are likely to migrate from a PET bottle under certain conditions (heat, sunlight, long term storage). The toxicological significance therefore of all leached products still remains uncertain. Genotoxicity testing which assess the biological effects of compounds was not performed on samples in any of these studies and would have served to give a better evaluation of the overall risk of both known and unknown substances. In genotoxicity testing, substances do not have to be chemically identified in order to assess their genotoxicity [155].

6.1.3 The Ames Assay

The importance of genotoxic testing of drinking water is justified by epidemiological studies that have shown a link between increased cancer risk and genotoxicity in chlorinated, UV-C irradiated and ozone treated drinking water, particularly during repeated and extended use [156-158]. The potential presence of genotoxins in water results not only from anthropogenic activities such as

pharmaceutical, biocidal and industrial chemical contamination, but also arise from water treatment methods [159]. Disinfection of drinking water to remove and inactivate pathogens by chlorination, ozone and UV-irradiation has been shown to release disinfection-by-products that were found to be potentially genotoxic on testing with short-term mutagenicity tests [158, 160].

Mutagenicity tests involving bacteria are the most commonly used and are highly standardised and cheap when compared to other mutagenicity tests. These tests involve the use of bacterial strains which have mutations and as a result have increased sensitivity to the presence of genotoxins. Of the bacterial tests, the Ames test has been used in testing for a wide range of chemicals and water samples including waster water [161, 162]. The Ames test was developed by Ames *et al*. in 1973 [163], and uses mutant *Salmonella typhimurium* strains which cannot grow on their own without histidine supplementation. When these strains are exposed to genotoxins they revert to the ability to synthesise histidine and can grow in the absence of the histidine. The number of revertant colonies that grow after exposure to potential genotoxic substances is compared to that of the negative control (spontaneous revertants) in order to determine the magnitude of mutagenicity and is calculated as the mutagenic ratio (MR) [163-165].

MR = number of revertant colonies (samples)/ number of spontaneous revertants (negative control)

The *Salmonella* Ames-fluctuation test developed by Green *et al*. [166] is a more sensitive, liquid based version of the *Salmonella* Ames test developed by Ames *et al*. [163, 165]. The Ames-fluctuation test is well validated, widely used and allows for comparison with the results of researchers who commonly use the Ames test as the sole assay for testing genotoxicity in water [157]. In the fluctuation assay a greater amount of sample volume can be tested without the need for concentration, thereby avoiding concentration methods that might change the original genotoxicity of the water sample [162, 167, 168].

6.1.4 Genotoxicity of water contained in PET bottles

Using the prokaryotic Ames test (in solid agar medium) with *Salmonella typhimurium* TA98 and TA100 strains), De Fusco *et al*. found that slight mutagenic activity occurred only in mineral water stored for 1 month [141]. Higher mutagenic activity was observed for mineral water (4000 ml) that was stored in bottles exposed to sunlight (MR = 3.6) compared to those in the dark (MR = 2.1) [141]. A similar study conducted by Monarca *et al*. revealed no mutagenic activity in the mineral water after 1 month of storage or at any other month during the 6 month exposure period to sunlight and dark conditions [169]. Evandri *et al*. and Biscardi *et al*. used two plant based genotoxic assays, *Allium cepa* and *Tradescantia*/micronuclei, respectively, to evaluate migration of mutagens from PET bottles [170, 171]. Evandri *et al*. showed that genotoxic activity was present in water samples after 8 weeks regardless of light exposure [171]. Biscardi *et al*. not only observed mutagenic behaviour in water samples without light exposure during mineral bottle storage but also from pipes supplying water for the bottling process [170]. None of these exposure conditions (temperature and sunlight) resembled those experienced by PET bottles during SODIS use.

6.1.5 Aims

The aims of this study were to:

(i) Determine if there is an observable genotoxic activity in water samples associated with prolonged use of SODIS when using PET containers and adhering to standard SODIS protocols (daily refill of PET containers, minimum of 6 h exposure to natural sunlight and water consumed within 24 h).

(ii) Determine if genotoxicity is observed in water samples where PET containers are not refilled but subjected to prolonged and continuous exposure to sunlight for extended periods of time up to 6 months. This was necessary in order to determine sensitivity of the method, where concentrations of leached compounds were expected to be higher in samples without refill. Secondly, to aid in comparison of other previous studies on genotoxicity testing from

PET which had been conducted on samples which had undergone long term exposure without refill.

6.2 Materials and Methods

6.2.1 Samples

PET-bottled (2 L) mineral water samples purchased in Almería, Spain in May 2007 were used for the duration of the experiment. The main physico-chemical parameters of the water were listed on the labels of the bottles. Concentrations of the given parameters were verified using ion chromatograph methods, by running water samples from two separate bottles in duplicate. Cation concentrations were determined with a Dionex DX-120 ion chromatograph (DIONEX, USA) equipped with a Dionex Ionpac CS12A 4 mm x 250 mm column at a flow rate of 1.2 ml min^{-1}. Anion concentrations were determined with a Dionex DX-600 ion chromatograph (DIONEX, USA) using a Dionex Ionpac AS11-HC 4 mm x 250 mm column. The gradient programme for anion determination was pre-run for 5 min with 20 mM NaOH, an 8-min injection of 20 mM of NaOH, and 7-min with 35 mM of NaOH, at a flow rate of 1.5 ml min^{-1}. Table 6.1 provides a comparison between concentrations listed on the labels and those obtained by our methods.

Table 6.1 *Chemical parameters of water as given on bottle labels compared to concentrations obtained by ion chromatograph methods conducted at the Plataforma Solar de Almería (PSA)*

Ion	Concentration (mg/l)	
	Manufacturer	Laboratory
Bicarbonate	314	309 ± 1
Sulphate	26.6	28.6 ± 0.7
Chloride	10.8	15.6 ± 0.8
Calcium	82.8	91.6 ± 0.4
Magnesium	24.2	26.3 ± 0.4
Sodium	4.5	6.8 ± 0.6

6.2.2 Sunlight exposure and storage conditions of water

During the months of June to December 2007, bottles containing mineral water were exposed in triplicate to sunlight for 1, 2, 3, 4, 5, and 6 months. The bottles were placed horizontally on the roof of the chemistry laboratory at the Plataforma Solar de Almería (PSA) (Latitude 37^{o} 05' N, Longitude 2^{o} 21' W, altitude 500m) (Fig. 6.2).

Figure 6.2 *PET bottles exposed to sunlight on the roof at the PSA*

(i) *SODIS protocol (daily refill) samples:* In order to simulate the way in which PET bottles are used during SODIS, three 2 L bottles with distilled water were exposed to sunlight for 6 hours and then stored in the dark. The following day (approximately 24 hours after initial exposure to sunlight), the bottles were emptied, refilled and then exposed to the sun again. This was done for 5 consecutive days of each week. During the weekend, bottles were emptied and kept in the dark until the following Monday. The water was collected after each month and tested. Controls were prepared and maintained in a similar manner but were kept in the dark rather than exposed.

(ii) *Continuous exposure (no refill) samples:* Twenty-one sealed 2 L mineral water bottles were placed outside for exposure in the manner described previously. At each time point (months 0, 1, 2, 3, 4, 5 and 6) 3 bottles were retrieved and tested. Control bottles also containing mineral water were stored in the dark at room temperature (23 - 25°C) for the duration of the experiments.

Solar UV irradiance was measured with a global UV radiometer (295-385 nm), Model CUV3, Kipp & Zonen, Netherlands) inclined at 37º. The solar UV dose ($Dose_{UV}$, J/m^2) delivered onto the bottles was obtained by integration of solar UV irradiance (I_{UV}, W/m^2) over a given period of time (dt, s) in 1 minute intervals (Eq. 1).

$$Dose_{UV} = \int_{t_1}^{t_2} I_{UV} \cdot dt \qquad \text{(Eq. 1)}$$

The typical variation in UV-A irradiance during the exposure period is illustrated in Fig. 6.3. The end of daylight saving time in October 2007, accounts for the left shift in irradiance curve observed in December 2007.

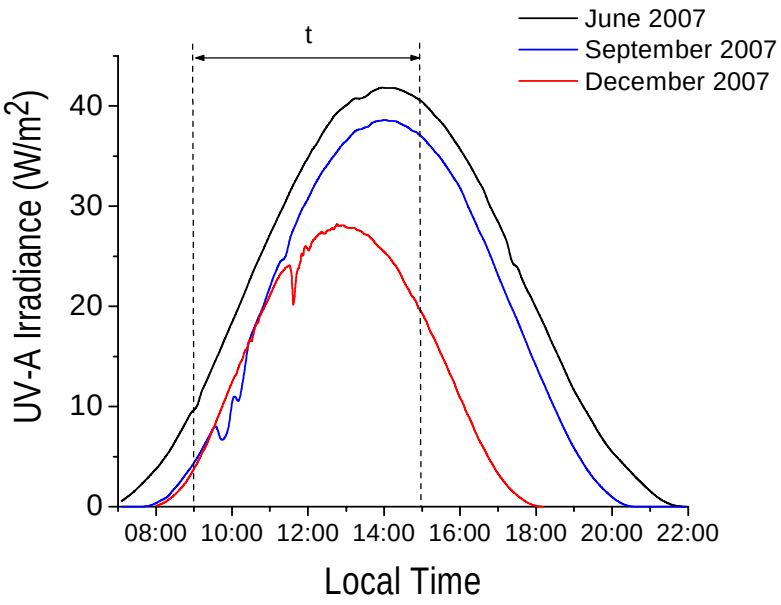


Figure 6.3 *UV-A irradiance curves for the experimental period of June 2007-December 2007. t represents the exposure period for SODIS daily refill samples.*

6.2.3 Ames fluctuation assay

The Ames fluctuation test was performed using reagents from the commercially available Muta-ChromoPlate™ Ames test kit (EBPI Inc., Mississauga, Ontario,

Canada) [172]. The tester strain *S. typhimurium* TA100 without S9 mix was used. Lyophilised bacteria was transferred into nutrient broth and grown overnight for 16 to 18 h. The liquid reaction medium consisted of Davis-Mingioli salts, D-glucose, D-biotin, L-histidine and bromocresol purple, sterile distilled water and *S. typhimurium* TA100. Unconcentrated water samples were added to the reaction medium and the suspension was then distributed into each well of a 96-well microplate (200µl/well). Plates were incubated at 37°C for 5 days in sterile Ziploc bags to avoid evaporation. All yellow, partially yellow or turbid wells were considered positive, and all purple wells were recorded as negative. For each experiment a blank and two controls were run. The blank (did not contain bacteria) was performed to ensure sterility of the experiment; all wells in the blank were expected to be purple. The positive control was conducted using the standard mutagen Sodium azide (0.5µg/100µl); all wells were expected to be yellow. DMSO (dimethyl sulfoxide) was used as a negative control to estimate the number of spontaneous reversions that would occur in the bacterial population.

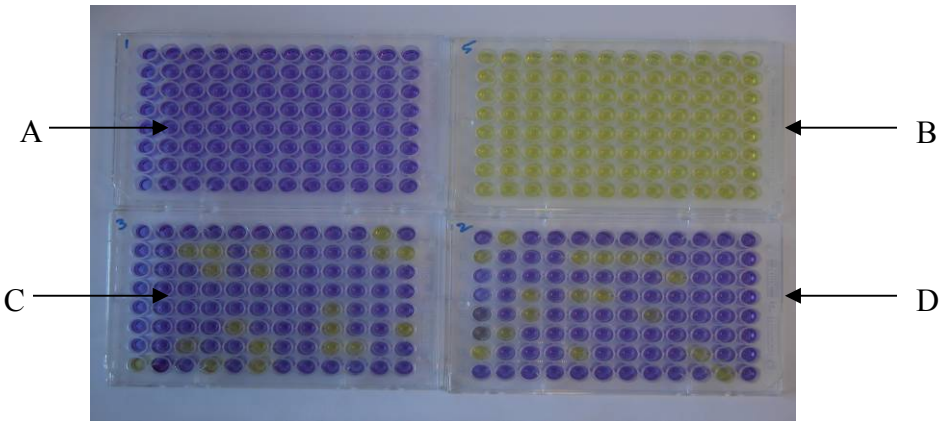


Figure 6.4 *Ames-fluctuation assay in 96-well microplate: (A)-Blank sterility check, (B)-Positive control and (C & D)-Test samples.*

6.2.4 Data Analysis

The number of positive (yellow) wells out of 96 wells per replicate was compared with the number of spontaneous revertant wells obtained with the negative control. The results were an average of three experiments (±S.D.) and were expressed as a mutagenicity ratio (MR = number of positive wells in

samples/number of positive wells in the negative control). A sample was considered genotoxic when a statistically significant increase occurred in the number of positive wells compared to spontaneous revertant wells. Statistical significance was determined using the chi-square (χ^2) analysis illustrated by Gilbert [172, 173].

6.3 Results

6.3.1 Ames Fluctuation assay on samples exposed to SODIS conditions

The effect of exposure to sunlight on the genotoxic content of water in PET bottles was examined. Genotoxicity was not observed in any of the daily-refill samples that were exposed to SODIS conditions or their corresponding control samples regardless of storage time and UV- A dose received (Fig. 6.5a and 6.6a).

In the prolonged exposure (no-refill) samples (Fig. 6.5b) significant ($p<0.05$) genotoxic activity was observed after 2 months for both control (in the dark) and test (exposed to sunlight) samples. Sunlight increased genotoxic activity. Genotoxic samples received a cumulative UV-A dose of 64 MJ/m^2 (Fig. 6.6b). No other significant genotoxicity activity was observed at any other time.

6.4 Discussion

During solar disinfection, PET bottles are subjected to two physical stresses; exposure to sunlight and an increase in water temperature. As the PET bottles age and are re–used, these factors could lead to a change in structure and composition not only of the PET bottle itself, but also of any photodegradation products that might migrate from the plastic bottle into the water. The expected decrease in UV transmittance was observed with sunlight exposure time as was reported by Wegelin *et al*. [142].

The *S. typhimurium* strain used for the mutagenicity testing was TA100. The TA100 strain detects a point mutation which involves the substitution of base pairs and is specific to G → A transition in the *hisG46* gene. It is also capable of

detecting G → T and G → C transitions. Acetaldehyde and formaldehyde, which have previously been shown to migrate from PET bottles, and are the most important degradation products present in PET [146, 149], give a mutagenic response in TA100 without the need for metabolic inactivation with S9 [174]. Nawrocki *et al*. [146] report that at room temperature, carbonyls (formaldehyde, acetaldehyde, and acetone) migrated within a 2.5 h period from PET into water. Acetaldehyde, concentrations were higher in newer bottles compared to bottles that were 1 month old.

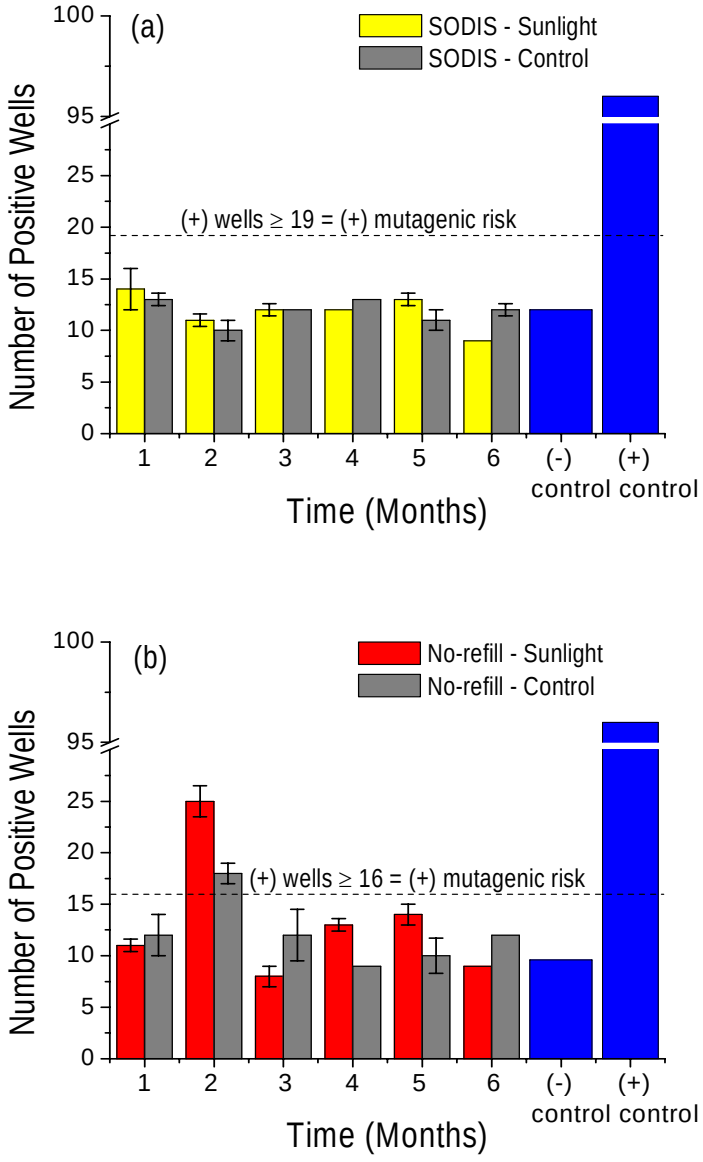


Figure 6.5 *Number of positive wells obtained for SODIS daily refill samples (a) and no-refill samples (b) exposed to sunlight and under dark conditions. Each column represents the average of triplicates, and error bars show the standard error limits.*

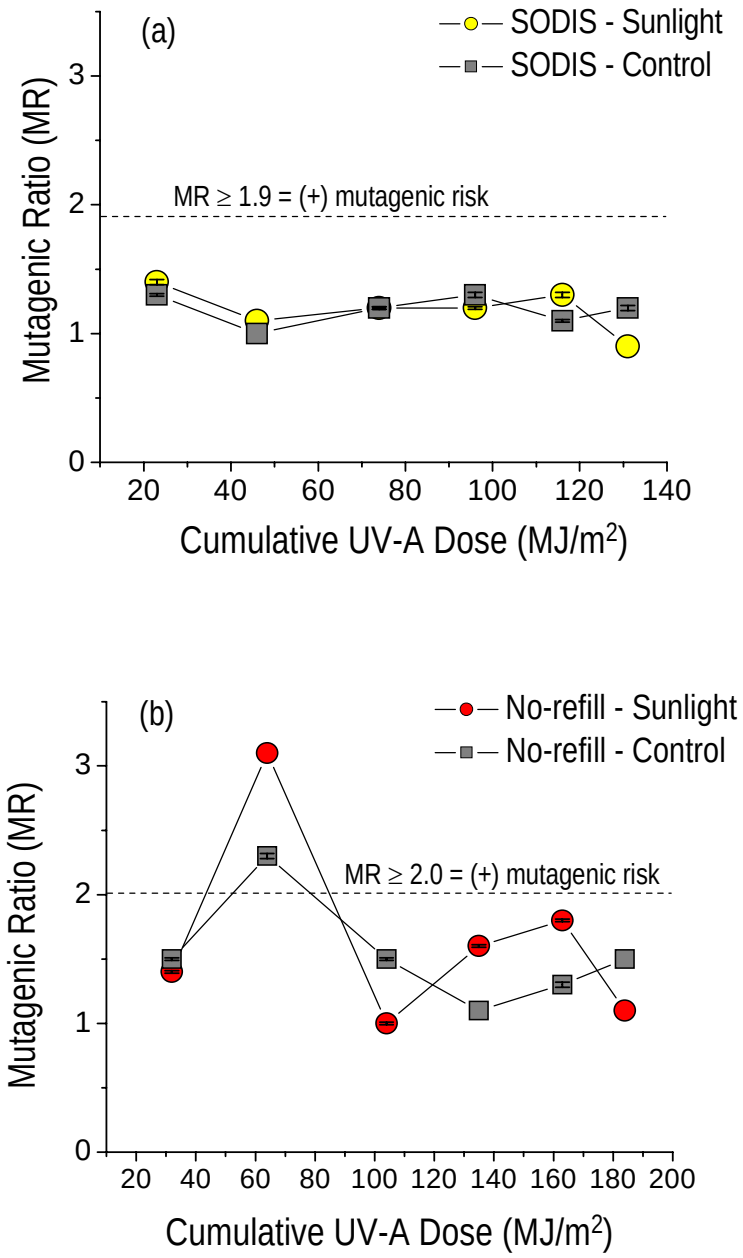


Figure 6.6 *Mutagenic ratios obtained for SODIS daily refill samples (a) and no-refill samples (b) exposed to sunlight and under dark conditions. Each point represents the average of triplicates, and error bars show the standard error limits.*

However, genotoxicity was not detected in any SODIS protocol (daily-refill) water samples at any stage during this study (Fig. 6.5a). This may be due to the escape of volatile compounds into the atmosphere when PET bottles are opened [141, 169, 171]. The water in SODIS bottles reached a maximum temperature of 43°C and was subsequently stored at room temperature (23-25°C). Potentially

genotoxic compounds such as acetaldehyde may be highly volatile and could have been released into the air. However, if the supposed volatility of photoproducts can be invoked to explain their absence from observed results then the escape of volatile compounds is expected to occur during normal SODIS use and thus should not present a risk to the SODIS user.

It should also be noted that usually a SODIS user consumes water that has been treated on the previous day while tomorrow's water is treated today. Thus, under normal conditions of use, SODIS containers are only exposed to sunlight on every second day. Daily-refill samples were exposed daily from Monday to Friday but not over weekends. For a typical 28-day period one would then expect daily-refill SODIS containers to be exposed on 20 days, which is ~17% more frequently than the 14 days exposure experienced by containers under normal SODIS usage conditions. Since genotoxicity was not detected in any of the daily-refill samples over a 6 month time frame, it is reasonable to suggest that under realistic conditions no toxicity would have been detected after at least 7 months (7.01 months = 6 months x 1.17).

De Fusco *et al*. [141] concentrated their samples and using the solid agar Ames Test, only observed significant mutagenicity in water samples in PET bottles stored for 1 month in the dark and increased mutagenicity on exposure to sunlight. Subsequent experiments also performed by the same research group did not detect any further mutagenicity. The difference in mutagenicity was attributed to the use of different PET bottles and different types of mineral water [169]. Evandri *et al*. showed genotoxic activity in water samples after 8 weeks of storage both in the dark and in the light, using a plant based genotoxic assay [171].

In order to have a more accurate estimate of the genotoxic risk to which a SODIS user will be exposed, water samples in this study were not concentrated since SODIS users do not concentrate their water before drinking. Despite the fact that samples were not concentrated, significant genotoxicity occurred in both the dark and sunlight no-refill samples after 2 months (Fig. 6.5b). However the mutagenic ratio was higher for the solar exposed samples (3.1±0.2) compared with the dark

controls (2.3±0.2) (Fig. 6.6b). As no-refill bottles were not opened, the observed genotoxicity might be due to not only non-volatile compounds but volatile compounds as well [171]. Furthermore, genotoxicity was not observed at any time point after the 2 month period; it is therefore likely that genotoxic compounds detected after 2 months have undergone further degradation into non-genotoxic forms. High temperature and sunlight might increase the leaching of these products from PET and increase the rate of degradation of photoproducts. This results in the increased genotoxicity which is observed when bottles are exposed to sunlight. Most importantly, under standard SODIS conditions, which included daily re-use of plastic bottles over a 6-month period, water contamination by genotoxic compounds was not observed. Genotoxic results obtained could be combined with previous studies carried out on migration of chemical compounds under SODIS conditions [54, 142] to give a better understanding of the overall health risk of drinking SODIS treated water.

No indicator organisms were added to the sample bottles in this study to help monitor levels of disinfection. There is a possibility that organic compounds originating from the destruction of microbial cell membranes and organelles may contribute in some way to the proposed genotoxicity. All of the experiments reported here used sterile distilled water (for the daily refill samples) or the original commercially available mineral water (for the no-refill samples) rather than natural waters, in order to eliminate the possibility that the presence of extraneous compounds might interfere with experimental studies.

Although this preliminary investigation has not identified any genotoxic risk associated with unconcentrated SODIS water, further study is required. In particular, an evaluation of the genotoxicity of SODIS water over a range of sample concentrations and for a variety of different PET containers. Studies highlighted in section 6.1.2 demonstrate that the country of origin of the bottles is a determining factor in terms of what compounds might leach into water and hence it would be useful to examine bottles originating from areas where SODIS is used. A more realistic microbiological profile of water to be disinfected would also be beneficial in assessing whether the presence of organisms increases the genotoxicity of water or whether leached products might have a role in the

inactivation of organisms present in the water. The spontaneous mutation that may naturally occur in controls with water stored in normal containers (e.g. barrels) will also be assessed and compared to mutation rates occurring in PET bottles. Other intensive genotoxicity assay methods such as the Comet assay which assesses DNA damage on human leukocytes by toxic compounds offer interesting and alternative investigative routes.

6.5 Conclusions

1. Under standard SODIS conditions, which included daily re-use of plastic bottles over a 6-month period, water contamination by genotoxic compounds was not observed with the *Salmonella* Ames- Fluctuation assay in unconcentrated samples.
2. The Ames-fluctuation test was successful in detecting potential genotoxicity in no-refill samples after 2 months for both dark-control and solar exposed samples. However, the mutagenic ratio was higher for the solar exposed samples (3.1±0.2) compared with the dark controls (2.3±0.2).
3. Further study is required to evaluate the genotoxicity of SODIS water over a range of sample concentrations, for a variety of different PET containers, with a more realistic microbiological profile and the use of intensive genotoxicity assay methods.

Chapter 7

General Discussion

7.1 Solar Disinfection

7.1.1 Microbial Inactivation

Due to the simplicity of SODIS, questions still arise concerning the effectiveness of the method to inactivate bacteria as well as more structurally complex organisms such as viruses, parasites and fungi. Both laboratory and field studies have shown solar inactivation of common enteric bacteria such as *V. cholera*, *S. typhimurium*, *Sh. dysenteria* Type 1 and *E. coli* [20, 25, 80]. However, over the recent years, other bacteria have become significant contributors to waterborne disease. These include a group classified as prominent emerging pathogens made up *Y. enterocolitic*a, *C. jejuni* and enteropathogenic *E. coli*, which share the similarity of having low infective doses as well as the ability to persist in the environment for long periods of time. In this project, the inactivation of an enteropathogenic *E. coli* under stimulated sunlight was determined, in order to show that SODIS is effective in activating other important waterborne pathogens. Successful inactivation under solar simulation of a clinical isolate of *E. coli* O157 was achieved after 4 h, during which a dose of 885 W/m^2 was received by the bacteria. Solar inactivation of *Y. enterocolitica* and *C. jejuni* has also been observed under natural sunlight conditions. *C. jejuni* was more susceptible to SODIS having a T_{90} value of 2.1 min, while *Y. enterocolitica* had a T_{90} value of 78.6 min [45]. The faster inactivation time of *C. jejuni* was attributed to the sensitivity of the bacteria to oxidative stress due to its microaerophilic nature [45].

In the general population, young children (up to 5 years old), the elderly and pregnant women are considered to have a high risk to microbial infection [175]. Young children who are infected by common enteric bacteria are at the highest

risk for waterborne microbial infections that result in gastrointestinal disease. Solar disinfection has proven to be highly successful inactivation of these common enteric organisms. However, due to the presence of severely immunocompromised people within the population, a group of organisms that are also found in drinking water but do not generally cause infections in healthy individuals are now responsible for opportunistic infections. An opportunistic microorganism is defined by Von Graevenitz as an organism that inflicts damage on its host by taking advantage of the weakened defence mechanisms of the host [175, 176]. Organisms that fall into this category include *Pseudomonas*, *Acinotobacter*, *Aeromonas*, *Legionella*, and *Mycobacterium avium* complex (MAC) [175]. In AIDS patients, protozoan infections caused by *Cryptosporidium*, *Giardia*, *Isospora*, and *Cyclosporia* which are also opportunistic microorganisms, are very common, as well as fungal organisms such as *Aspergillus*, *Cladosporium* and *Penicillium* [175]. Among these opportunistic organisms, SODIS has proven to be effective against *Pseudomonas aeruginosa*, oocysts of *Cryptosporidium parvum* and cysts of *Giardia muris* [3, 27, 31].

Though not a waterborne pathogen, *Bacillus subtilis* endospores, which are highly resistant to solar disinfection need two days of strong sunlight exposure to achieve a > 90% reduction in concentration, thereby exemplifying the limitations of the water treatment method [22, 45]. As can be seen from the above mentioned list, there still remains a number of important organisms to be evaluated in order to determine their susceptibility to SODIS. However, SODIS has proved to be effective in inactivating common enteric bacteria, prominent emerging pathogens as well as some opportunistic microorganisms. Furthermore, there will always be a need to evaluate the efficiency of SODIS as changes in the population and environment bring to the forefront new organisms that contribute to waterborne disease. By following a natural temperature profile as demonstrated in the inactivation of *E. coli* O157, solar simulation will provide a more accurate predictor of inactivation of a given pathogen under real sunlight conditions.

7.1.2 Microbial Regrowth after SODIS

Unlike other water treatment methods such as chlorination, there is no residual disinfection that occurs once a SODIS reactor is removed from sunlight. This places great importance on ensuring that microorganisms have been fully inactivated without the chance of recovery.

The use of 0.05% sodium pyruvate supplemented media and anaerobic incubation (ROS neutralised conditions) by Kehoe *et al*. [14] as well as Khaengraeng and Reed [80], encouraged the growth of injured bacteria after solar treatment. Subsequent work by Mani *et al*. [57] on three solar disinfection systems also revealed slightly higher counts on the supplemented media. However, the researchers concluded that the higher counts observed on the supplemented media did not override previous counts obtained with experiments conducted with aerobic media. In this study, regrowth of *E. coli* O157 and *E. coli* K-12 was assessed by plating samples after 24 h and 48 h post solar exposure on to aerobically incubated media. Once bacteria had received a sufficient amount of UV dose, regrowth was not observed 24 h or 48 h later. The occurrence of no regrowth after 24 h was also observed during initial SODIS work in PET bottles conducted by McGuigan *et al*. [18]. Other studies have evaluated regrowth over a longer period of time. Berney *et al*. [16] did not observe regrowth 5 days after solar exposure and Wegelin *et al*. [21] also did not observe regrowth 14 days after solar exposure.

In contrast to studies that have observed no regrowth after solar exposure, are studies conducted with photocatalysts, specifically Titanium dioxide (TiO_2), which have observed regrowth [177]. In these experiments, regrowth occurred in solar exposed samples, while in samples that had been exposed to light and photocatalyst, regrowth did not occur. However, flow cytometry analysis of cellular changes during solar inactivation provide further evidence that if an organisms has received sufficient UV dose there is a loss in membrane potential and it is impossible to repair the damage from solar exposure. In the case of *E. coli* K-12, this lethal UV dose was shown by Berney *et al*. to be greater than 1500 kJ/m^2 [16]. Most of the detailed works on regrowth studies have focused on bacterial inactivation by sunlight; however, SODIS inactivates parasites, fungi

and viruses. For these more structurally complex microorganisms, infectivity studies involving different biological models will provide the best information as to whether solar exposure has led to complete inactivation and whether after solar exposure subsequent reactivation or regrowth of the organism has occurred.

7.2 Enhancing Solar Disinfection

Areas that benefit from the use of solar disinfection receive on average 2500-3000 h of sunshine per year [3], there are times in the year when the rainy season occurs and even on a given day, there could be intervals of cloudy weather dispersed on an otherwise sunny day. Under those conditions, SODIS is compromised. To achieve disinfection of indicator bacteria on cloudy days, it is recommended that bottles are exposed for 48 h [129, 137], but in some cases 2 days exposure has proved an insufficient exposure time during the rainy season and as a result another disinfection method will have to be used. Furthermore, even under sunny conditions, users complain about the work and time involved in solar disinfection [178] and as a result the longer period of time required for solar exposure under cloudy conditions may prompt SODIS users to revert back to drinking unsafe water.

The turbidity of water is another factor that affects the ease of use of SODIS. Currently, recommendations are that water should be of low turbidity (< 30 NTU). If the turbidity is greater than 30 NTU then water has to be filtered before been exposed to sunlight [129].

Finally, the volume of water that can be disinfected at a given time is limited to 1- 3 L and will require a number of bottles to be placed in the sun in order to obtain sufficient quantities of disinfected water for the average household. The enhancement technologies assessed in this study, seek to accelerate solar disinfection under optimum conditions (sunlight and low water turbidity). But more importantly these technologies try to address the inadequacies of SODIS - slow or incomplete microbial inactivation, the negative effect of highly turbid water on inactivation and the restriction of the volume of water inactivated in a given time.

7.2.1 Compound Parabolic Concentrators

If there is sufficient sunshine, then enhancement of SODIS by blackening PET bottles and the use of absorptive material in SODIS reactors are a reliable means of increasing the pasteurisation effect of sunlight. The use of absorptive material has proved to be successful in reaching temperatures that inactivate not only bacteria, but viruses and protozoan cysts which have a greater resistance to solar inactivation [56, 57, 179]. Unfortunately, the effects of enhancements that serve to increase the temperature of water are diminished on partly sunny days and will not work on cloudy days.

However, on cloudy days, UV-A is still available for disinfection even though it is predominantly in a diffuse form. A technology that is able to utilise the little available UV-A has the potential to provide enhancement to SODIS not only under sunny conditions but cloudy conditions as well. In previous work, compound parabolic concentrators (CPCs) were fitted onto continuous-flow solar reactors and were shown as an enhancement for solar photocatalysis in these reactors under sunny conditions [85, 96]. The main advantage of the CPC is that it does not rely solely on direct solar radiation but concentrates diffuse radiation.

In this work, the ability of a CPC to enhance solar disinfection in batch reactors containing both clear and turbid water under sunny conditions and cloudy conditions was evaluated. CPCs proved to be an enhancement to SODIS for both clear and turbid water under sunny conditions, where all systems achieved complete inactivation of bacteria but systems fitted with a CPC had a faster inactivation rate. More importantly, under cloudy conditions, only SODIS reactors fitted with CPCs resulted in complete inactivation of bacteria [132]. Furthermore, even after experiencing non-homogenous degradation from being used in the field for 3 years, complete inactivation was still observed in the system fitted with the old CPC [132].

The enhancement that a CPC system provides to SODIS is still to be assessed with other less SODIS sensitive organisms; however, the use of the CPC illustrates that it is worthwhile investigating technologies that enhance the optical properties of sunlight. There still remains a major limitation of assessing whether the amount of UV photons needed to inactivate pathogens have reached the

contaminated water irrespective of what type of SODIS reactor (PET bottle or system fitted with a CPC). In this regard, technologies that focus on optimising the thermal effects of sunlight have an advantage, in that simple indicators such as the use of soybean wax which melts at a temperature of 70ºC can indicate that pasteurisation temperatures have been reached in a SODIS reactor [56]. A simple cheap technology that can assess the amount of UV photons entering a system will help ensure that complete inactivation of organisms has occurred under conditions of low solar intensity and low ambient temperature.

7.2.2 Disinfection of Large Volumes of Water

Continuous-Flow Solar Reactors

In the initial work conducted by Acra *et al*. [51] on solar disinfection, the use of simple continuous-flow reactors made up of only borosilicate glass tubes were explored as a possibility to enhance the disinfection of larger volumes of water. During experiments conducted by Acra *et al*. [51], 99.9% inactivation of both *E. coli* and *E. faecalis* was achieved during exposure conditions but maximum volumes of water inactivated was only up to 18 L [51]. Continuous-flow reactors developed by other researchers that combined optical and thermal inactivation or that were based solely on thermal inactivation were also efficient in the disinfection of water as pasteurisation temperatures of 65–70ºC were reached [63, 64]. However, as indicated earlier, the reliance on thermal inactivation meant that on cloudy days inactivation was severely compromised.

This work explored the use of continuous-flow reactors fitted with CPCs which had been identified by Vidal *et al*. [84] as a promising technology for disinfecting large volumes of water. However, unlike previous experiments conducted in continuous- flow reactors where most studies in reactors had a flow rate of < 2 l/min, this work, determined the effects of 2 l/min and 10 l/min flow rates on disinfection of large volumes of water (14 L and 70 L). Furthermore, the water used in the reactors was well-water unlike distilled water used in other continuous-flow experiments. The use of well-water meant that bacteria were in a more realistic osmotic environment and therefore not prematurely weakened

before subjected to solar disinfection. Using well-water also gives a better approximation of inactivation under field conditions [86]. Lastly, the continuous-flow reactors used in this study had a temperature controlled system where temperature could be regulated and set to temperatures as low as 25ºC, which would not result in activation of bacteria and therefore the disinfection potential of optical inactivation of bacteria on its own could be assessed.

The experiments conducted on continuous-flow reactors in this study, revealed that increase in flow rate had a negative impact on inactivation of bacteria. After a 5 h period only a batch system SODIS reactor with no flow rate had achieved complete inactivation while the 14 L and 70 L continuous-flow reactors had a stable residual concentration of 2 log bacteria remaining after 5 h [133]. This led to more detailed work exploring the delivery of UV dose into SODIS reactors, since in a batch reactor water is constantly illuminated by UV, while in continuous-flow reactors, water receives UV in an interrupted manner. Since experiments were conducted with *E. coli* K-12, the UV-dose required for complete inactivation of a 10^6 CFU/ml was > 108 kJ/m^2 or > 9 kJ/l irrespective of high or low solar intensity conditions [133].

Despite the negative impact of increasing flow rate, the future of continuous-flow solar reactors as an enhancement of solar disinfection still remains promising. As this work illustrates, it is possible to use sunlight alone to inactivate 4 log bacteria in a large volume of water (70 L) within a 5 h period and with a water temperature < 30ºC during solar exposure. Identifying the lethal UV-dose of less SODIS sensitive pathogens will help ensure that new prototypes of continuous-flow reactors are designed in way that the lethal UV-dose is delivered each time the water is illuminated in the reactor instead of sub-lethal doses over a period of time. Designing the reactor according to the lethal UV doses of less SODIS sensitive pathogens will mean that more sensitive SODIS organisms will definitely be inactivated. Avoiding the delivery of sub-lethal doses over a period time will not only ensure that all pathogens are inactivated and water is safe for consumption but will also prevent the selection of pathogens in a given microbial population that might be more SODIS resistant.

Enhanced Batch SODIS Reactors

Solar disinfection in continuous-flow solar reactors offers the possibility of disinfection volumes of water (70 L) that will not only be sufficient for families but for small communities as well. However, these reactors will require more technically skilled knowledge to operate and maintain and therefore it would be necessary to designate people within a community to receive this specific training. In this study a CPC enhanced batch reactor (EBR) was designed and evaluated with the focus of maintaining the simplicity of SODIS in PET bottles. Solar disinfection studies involving batch disinfection of volumes of water > 3 L are very few. Reed *et al*. [11] demonstrated successful disinfection of 22 L and 25 L volumes of water in plastic containers [11]. In this work a through study of batch disinfection of 25 L of water over a period of 7 months, proved that disinfection of large volumes of water within the recommended six hours of strong light is possible. Inactivation studies were only conducted with *E. coli* K-12 and hence the reactor will need to be tested with other pathogens.

7.3 Genotoxic Assessment of Solar Disinfected Water

In light of the current interest in the toxicity and safety of plastics in relation potable water, there has been the need to evaluate the possibility of genotoxic substances leaching from PET bottles into solar disinfected water. Extensive chemical analysis of substances emanating from PET bottles under SODIS conditions has been evaluated [53, 54, 154]. Some studies showed there was no difference in the chemical composition of water in PET bottles with or without sunlight exposure [53]. For other studies where plasticisers were identified, their concentrations were well below the limits set by the WHO in the Guidelines for Drinking-water Quality [54].

In this study, a biological approach was used to evaluate the possibility of genotoxic release from PET bottles under SODIS conditions. Genotoxic tests have been used to evaluate water from PET bottles in other studies but never under the conditions that occur during SODIS. Genotoxic results obtained with the *Salmonella* Ames-Fluctuation assay revealed that solar disinfected water over

a six month period was negative for genotoxic substances, while for PET bottles that were continuously exposed to sunlight for the same six month period, genotoxicity was detected after two months in both sunlight exposed and dark control samples. However, sunlight exposed samples had a greater mutagenic ratio (3.1±0.2) than dark controls (2.3±0.2).

As this study was a preliminary investigation, there remains more detailed analysis of genotoxic content of solar disinfected water to be performed using different PET bottles of different ages. This will involve conducting concentration-response curves, identifying concentration methods which will not jeopardise the genotoxicity level of the original sample by contamination or by the loss of potential genotoxic substances during concentration such as highly volatile chemicals. Concentrating the water from a reasonable volume (10-20 L) will enable the samples to be evaluated in a wide concentration range (50-5000x concentration factor). A microsuspension method can be used to increase the resolving power of the genotoxic assay thereby increasing the detection of potential genotoxic compounds at low concentrations [180, 181]. Finally, there are number of TA strains (TA 98, 100, 104,) with different gene targets that can be used for the *S. typhimurium* assay. The TA 100 strain with the gene target *hisG46* was used in this work since this strain has been shown to reverse when exposed to compounds that are likely to leach from PET material [174]. Based on previous experiments other strains do not show as much sensitivity to compounds that might leach from PET bottles. However, future experiments on genotoxicity will involve the use of at least 2 strains since testing different PET bottles from various sources might result in the leaching of compounds where one of the strains mentioned above might be more sensitive to a given compound than the TA 100 strain.

7.4 Concluding Remarks

The aim of this work was to show that SODIS is an appropriate, effective and acceptable intervention against waterborne disease. In the field SODIS is performed in plastic bottles where water (< 3 L) with a turbidity of less than 30

NTU is exposed to strong sunlight conditions for 6 h and on cloudy days for a recommended 48 h.

In light of the results obtained during the course of this work, SODIS in PET bottles still remains an effective, low cost, point-of-use water treatment technology during periods of strong sunlight. In this work, SODIS was demonstrated to be effective in the inactivation of enteropathogenic *E. coli*, a highly infectious waterborne pathogen under simulated sunlight conditions. Given that a natural water temperature profile was followed during the course of the simulated solar experiment, inactivation results of EPEC under field conditions will be comparable to those obtained under simulated light. Successful inactivation of *E. coli* K-12 occurred in PET bottles under real sunlight conditions and with water turbidity of up to 100 NTU. Furthermore, the safety of using PET bottles as SODIS reactors was evaluated. Negative genotoxicity results were obtained for water samples that had been in PET bottles and exposed to normal SODIS conditions (strong natural sunlight) over 6 months.

The drawback of the SODIS method however, is the very long exposure times needed under cloudy conditions and the restriction in the volume of water treated in a given time. Both factors affect the appropriateness and acceptability of the method. In this work CPC mirrors were found to be an effective way to accelerate the inactivation rate of *E. coli* K-12 not only on sunny days but on cloudy days and furthermore for water with a turbidity of up to 300 NTU. This work also established that large volumes of water could be solar disinfected, and this proved to be true in the successful inactivation of *E. coli* K-12 in a 25 L CPC enhanced batch reactor. However for continuous-flow solar reactors which could treat 14 L and 70 L volumes of water, an increasing flow rate was detrimental to inactivation.

Inactivation studies in SODIS reactors will not only be carried out with *E. coli* K-12 but with *E. faecalis*. The use of a gram-positive waterborne pathogen which is known to be more resistant to water treatments than *E. coli* K-12 will give better prediction of inactivation of more highly resistant pathogens. Furthermore

nactvation studies involving water containing a mixture of different relevant waterborne pathogens as is sometimes found in the natural environment will be studied. This is to determine if a protective effect against SODIS is observed for certain pathogens, due to the presence of other organisms within the same volume of water.

Future work deducing the response of different organisms to UV dose and UV intensity would help in the construction of continuous-flow solar reactors. This would ensure that when water is exposed to sunlight in these reactors, organisms are fully inactivated. The development of a low cost UV dose indicator which measures how much UV dose a SODIS reactor has received would prove invaluable for SODIS users. Under strong sunlight conditions, the UV dose could signal to users that water is safe to drink well before the recommended 6 h of exposure time has elapsed, while on cloudy days and during the disinfection of turbid water it could alert users that the water is still not safe to drink, since not enough UV dose has been received.

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APPENDIX 1

PEER REVIEWED PUBLICATIONS IN INTERNATIONAL JOURNALS RESULTING FROM THIS RESEARCH