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Solar disinfection of drinking water contained in transparent plastic bottles : characterizing the bacterial inactivation process

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K.G. MCGUIGAN, T.M. JOYCE, R.M. CONROY, J.B. GILLESPIE AND M. ELMORE-MEEGAN. 1998. A series of experiments is reported to identify and characterize the inactivation process in operation when drinking water, heavily contaminated with a Kenyan isolate of *Escherichia coli*, is stored in transparent plastic bottles that are then exposed to sunlight. The roles of optical and thermal inactivation mechanisms are studied in detail by simulating conditions of optical irradiance, water turbidity and temperature, which were recorded during a series of solar disinfection measurements carried out in the Kenyan Rift Valley. Optical inactivation effects are observed even in highly turbid water (200 ntu) and at low irradiances of only 10 mW cm^{-2}. Thermal inactivation is found to be important only at water temperatures above 45 °C, at which point strong synergy between optical and thermal inactivation processes is observed. The results confirm that, where strong sunshine is available, solar disinfection of drinking water is an effective, low cost method for improving water quality and may be of particular use to refugee camps in disaster areas. Strategies for improving bacterial inactivation are discussed.

INTRODUCTION

A primary concern of many communities in developing countries is that of obtaining clean drinking water. It has been suggested that solar energy might be of use in improving water quality in those parts of the developing world that experience hot, sunny climates (Acra *et al.* 1980; Ciochetti and Metcalf 1984; Lawand *et al.* 1988; De Lorenzi *et al.* 1989; Acra *et al.* 1990; Joyce *et al.* 1992; Reed 1997; Sommer *et al.* 1997). A previous paper (Joyce *et al.* 1996) showed that the heating effect of Kenyan sunlight could produce complete inactivation of high populations of the faecal indicator organism, *Escherichia coli*, in highly turbid water (approximately 200 nepholometric turbidity units (ntu)) within 7 h if the water temperature reached at least 55 °C. Furthermore a recently reported clinically controlled field trial of solar disinfected drinking water in the reduction of diarrhoea in Kenyan Maasai children between the ages of 5 and 16 years (Conroy *et al.* 1996) showed that children who stored their drinking water in 1·5 l plastic (polyethylene terephthalate (PET)) bottles that were placed in direct sunlight for continuous periods of not less than 6 h experienced a 9% reduction in incidences of diarrhoea and a 26% reduction in incidences of severe diarrhoea (which was defined in the study as diarrhoea severe enough to prevent performance of daily duties) over the 3 month duration of the trial, compared with the control group of children who kept their water bottles out of the sun.

In this paper, results of an investigation of the roles of optical irradiance, water temperature and turbidity in the bacterial inactivation processes that occur under simulated Kenyan solar disinfection conditions for populations of wild type *E. coli* in water samples contained in plastic containers are reported. The optical irradiances and water temperatures reproduced in the simulations are those measured for 1·5 l water samples contained in PET plastic bottles during solar disinfection trials in Maasailand, Kenya (Joyce *et al.* 1996).

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The bacterial inactivation effect of heat and light is examined both in isolation and in combination, and the contribution of turbidity as a confounding factor is studied.

MATERIALS AND METHODS

Solar simulation apparatus

The apparatus used to reproduce the Kenyan solar irradiance and water temperature regimens used in the simulations consists of two systems : one to reproduce the optical irradiances, the other to reproduce the water temperatures. Both systems can be used either independently or simultaneously.

The system for controlling the water temperature consists of a platinum resistance temperature sensor, which was calibrated against a Type J Iron-Constantan thermocouple, and immersed in the sample within the container. The output from this sensor is monitored by a temperature controller unit (model E5CS-X, Omron, The Netherlands) that controls the mains supply to an ordinary 2·2 kW domestic fan heater (model CE793, Cascade, China) which blows heated air onto the sample bottle. The sample container is surrounded by a cubic plastic housing that has a hole cut into two sides. A large hole faces the fan heater and a smaller hole allows the irradiating light through. The purpose of the housing is to trap as much of the heated air as possible around the bottle to speed up the heating process.

The irradiating light source is a 150 W Xenon arc lamp (model 150W/1 XBO, Osram, Germany) fitted with a rear reflector and U.V.-Grade fused silica F1 collecting optics. The light from the lamp passes through a schott glass heat-absorbing filter (model KG02, Melles-Griot, Cambridge, UK) which helps approximate the short wavelength transmission properties of the atmosphere. The filter also attenuates the strong infrared (IR) emissions produced by the Xenon lamp between 0·8 and 1·05 μm. A U.V. Grade fused silica converging lens is placed between the filter and the sample container to improve the homogeneity of the illuminating field and to facilitate the production of a range of irradiances.

Figure 1a shows the irradiance spectra of the filtered Xe arc lamp and of solar radiation after it has passed through the earth's atmosphere at equatorial latitudes measured at sea level (Watson 1993). The Xenon spectrum depicted is a convolution of the arc spectrum (Oriel Corp. 1994) and the transmission spectrum of the bandpass filter (Melles Griot 1995). The filtered Xe lamp light is a good approximation to the solar spectrum for the U.V. and visible regions of the spectrum. The similarity ends in the IR region. However, both Acra *et al.* (1990) and Mancini (1978) have shown that these IR wavelengths do not play a significant role in the bactericidal action of both real and simulated sunlight. The filtered lamp system can produce irradiances up to 100 mW cm^{-2} but beyond 70 mW cm^{-2}, the illuminating spot size becomes too small for uniform sample coverage. All irradiances were measured using a calibrated optical power meter (model 200/10+, Coherent, Cambridge, UK) which is sensitive over the range 0·3–10·2 μm.

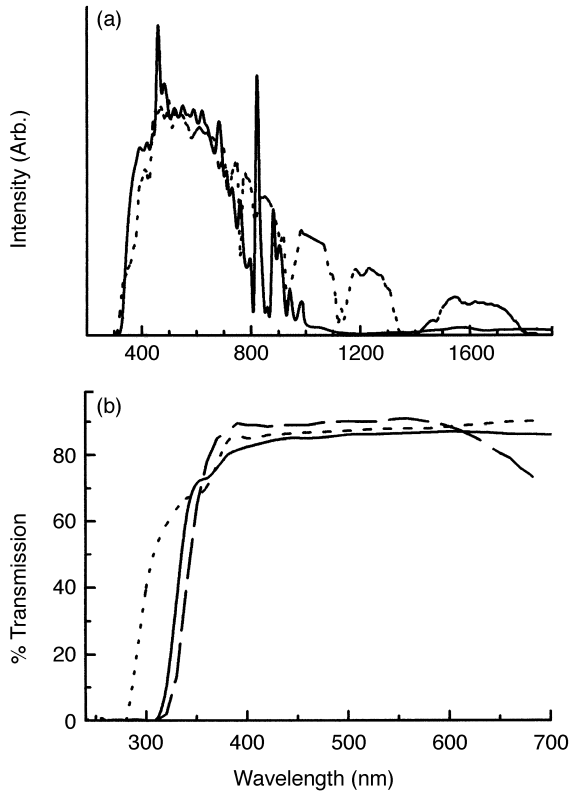

Fig. 1 (a) Comparison of irradiance spectra of the solar filtered Xe arc lamp output (solid line) and of ground level solar spectrum (Watson 1993) (dotted line). (b) Transmission spectra of the Kenyan water container material (PET, solid line), the polystyrene Universal container material (dotted line) and the heat-absorbing solar filter (dashed line)

Container material

The solar disinfection field measurements taken in Africa, and the subsequent field trials, used 1·5 l transparent, PET soft-drink bottles as water containers. The Xe lamp used in the solar simulation system cannot irradiate such a large surface with the required power density. Consequently, the water samples in the simulations were held in transparent polystyrene 20 ml Universal containers (BDH, Leicester, UK). A comparison of the transmission spectra of polystyrene, PET and the solar bandpass filter used in the simulator lamp system is shown in Fig. 1b. Although polystyrene allows more of the highly bactericidal U.V.-B (290–320 nm) to pass through than PET, these wavelengths are highly

attenuated by the atmosphere which is simulated using the sharp cut-off of the bandpass filter at 320 nm. By convolving the transmission spectrum of the filter with that of each of the container materials, the total energy content of the lamp light reaching the water in the polystyrene container is calculated to be only 3% higher than that passing through the PET, and this advantage is evenly distributed across the visible wavelengths.

Bacterial preparation and enumeration

A wild type strain of *E. coli*, isolated from the stool of a Maasai child living near Esonorua (the Kenyan solar irradiation site; latitude 1 29S, longitude 36 38E), was inoculated into 25 ml of sterile nutrient broth (Oxoid CM67) and incubated overnight at 37 °C. The culture was washed the following morning to remove nutrients. To do this, the culture was transferred into a sterile Universal container and centrifuged at 855 *g* for 10 min. The supernatant solution was then discarded and the pellet resuspended in 20 ml of HPLC analytical reagent (Analar) grade sterile water. This washing procedure was repeated three times. Finally, the pellet was resuspended in 8 ml of sterile water to form the stock solution. A viable bacteria count of this prepared stock was performed using the Miles and Misra drop count technique (Miles and Misra 1938) and standard plate count agar plate (Oxoid CM463). Stock prepared in this manner consistently produced a viable count of approximately 10^7 cfu ml^{-1}.

Turbidities were measured in nephelometric turbidity units (ntu) using a standard turbidity tube (Robens Institute, Guildford, UK). Test samples of varying turbidities (200 and 0 ntu) with bacterial concentrations of 10^5 cfu ml^{-1} were used to simulate the complete range of water conditions encountered in Kenya. Dust and soil collected from around the Esonorua River site was gradually added to 300 ml of HPLC Analar grade sterile water until the required turbidity was achieved. This solution was then sterilized by autoclaving for 15 min at 20 lb pressure and 120 °C. Then, 3 ml of the *E. coli* stock solution were added to 297 ml of the sterile turbid sample to make a test sample with a bacterial concentration of 10^5 cfu ml^{-1}. The control solution was prepared in the same manner. Once prepared, 7 ml of the test sample were placed in a 30 ml Universal container. Volumes of 100 μl were taken from the test and control samples at the beginning of each simulation and hourly after that for 8 h. The final volumes were taken 24 h after the start of the experiment. These volumes were diluted in a series of 10-fold dilutions. A 20 μl volume was taken from each dilution and dropped onto a Standard Plate Count Agar plate (Oxoid CM463). Each dilution was sampled three times to ensure accuracy. Plates were incubated overnight at 37 °C and counted the following day. Only those plates that showed discrete colonies in the drop area, preferably those that gave less than 40 colonies per drop, were selected and counted. The count was divided by the number of drops, multiplied by 50 to convert to 1 ml, and then by the dilution itself to give the number of cfu ml^{-1} (Miles and Misra 1938).

A marked reduction in the size of some colonies was observed as the optical and combined simulations progressed. As this was not observed for any of the thermal simulations, it is assumed that it resulted from damage caused by the incident optical radiation. There was therefore a risk that bacterial populations might be underestimated because of their small size. To avoid this problem, samples were diluted in a maximum recovery diluent (CM733, Oxoid, Basingstoke, UK) instead of sterile water and left standing at room temperature for 45 min before being plated. However this produced no significantly different recovery from the original sterile water diluent. In fact, the maximum recovery diluent was found to promote growth in samples that were left standing for periods above 1 h. Consequently, this intervention was stopped. Samples were thus inoculated onto plates and left at room temperature for the duration of the 8 h exposure experiment, and then incubated overnight. If, when reading the colony counts from the optical experiments, the colonies were small or just a shadowy background, they were reincubated for a further 8–12 h. This usually increased the colony size, thereby facilitating reading.

Simulations

Three types of simulations were performed: (1) thermal only, (2) optical only and (3) combined thermal and optical. For thermal simulations, the sample water temperature was adjusted hourly to reproduce one of the high, medium or low water temperature regimens recorded in Kenya (Fig. 3a). These regimens were recorded in Kenya for 1·5 l water samples exposed, on different days, to high, medium and low levels of solar radiation (Joyce *et al.* 1996) under conditions of cloud cover which roughly corresponded to cloudless, scattered cloud (approximately half cloud cover) and completely overcast, respectively.

In optical simulations, samples were irradiated at room temperature (22 °C) for 8 h at one of three irradiances: 70, 40 and 10 mW cm^{-2}, corresponding to Kenyan conditions of strong sunlight, hazy sunlight and completely overcast, respectively. The Kenyan field measurements of irradiance varied from a maximum of 89 mW cm^{-2} to a minimum of 3 mW cm^{-2} (Joyce *et al.* 1996). However, the upper irradiance limit of the solar simulator was 70 mW cm^{-2} for the reasons explained earlier. These irradiance values produce corresponding optical doses of 2·02, 1·15 and 0·29 kJ cm^{-2}, respectively, during each optical simulation and are in good agreement with doses used elsewhere (Fujioka *et al.* 1981; McCambridge and McMeekin 1981; Zemke *et al.* 1990). The maximum water temperature recorded in the Kenyan

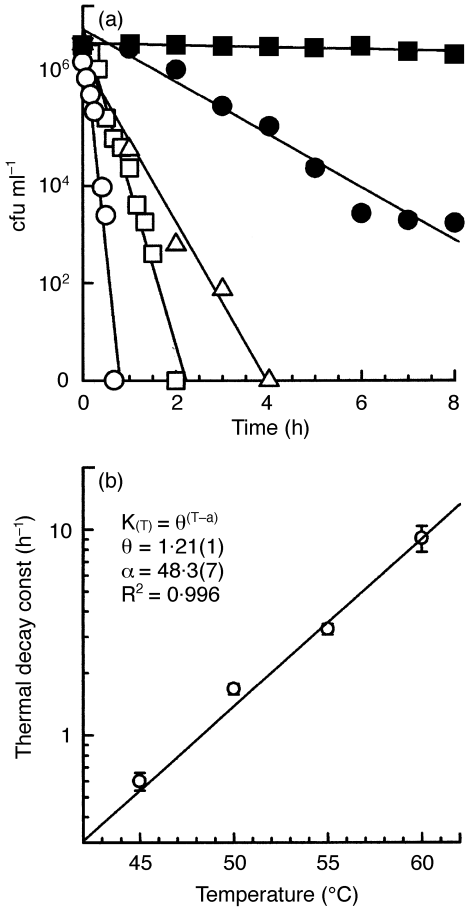

Fig. 2 (a) Thermal inactivation of wild type *Escherichia coli* (10^6 cfu ml^{-1}) maintained at fixed water temperatures. The solid lines are first order regression curves. ●, 40 °C; ■, 45 °C; △, 50 °C; □, 55 °C; ○, 60 °C. (b) Variation of thermal decay constant (see Equations 1 and 2) as a function of water temperature

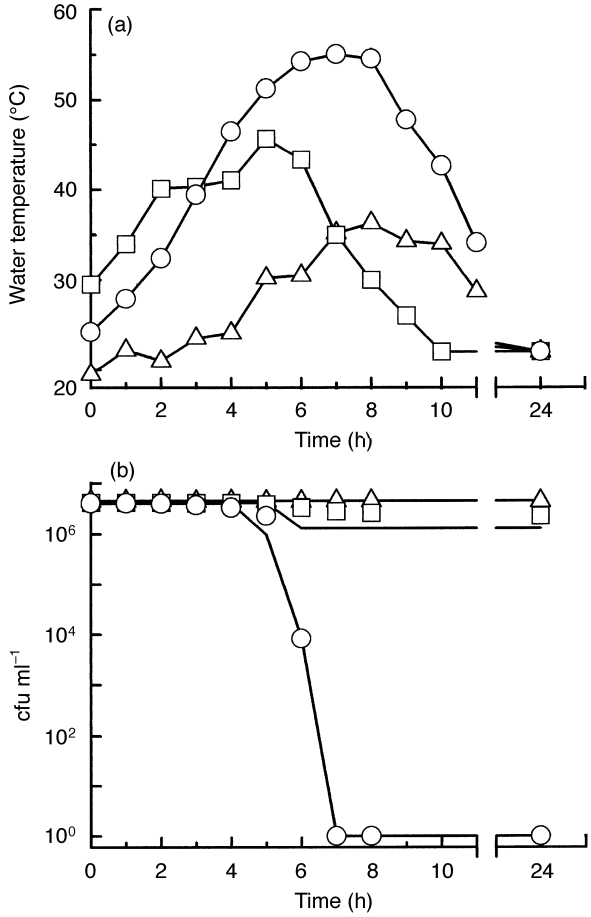

Fig. 3 (a) Water temperature regimens used in the thermal simulations. These measurements were recorded for 1·5 l samples in PET plastic bottles exposed to Kenyan sunshine conditions (Joyce *et al.* 1996). (b) Thermal simulation results. ○, □ and △ denote high, medium and low temperature regimen simulations, respectively. The solid lines are theoretical fits to the data based on Equations 1 and 2

measurements (55 °C) (Joyce *et al.* 1996) occurred on a day when the sample was exposed to irradiances between 76 and 80 mW cm^{-2} for seven consecutive hours. Consequently, the irradiances during each simulation were held at a constant value.

Combined simulations were carried out by maintaining the sample water temperature at one of the three temperature regimens while simultaneously irradiating the container with the filtered Xenon arc light at one of the predetermined irradiance levels.

Control samples for all simulations were kept at room temperature (23–25 °C) in the dark.

RESULTS

Thermal inactivation

Measurements of thermal inactivation of bacterial populations at fixed temperatures were made before simulating the thermal regimens. These bacterial inactivations are displayed in Fig. 2a. First order thermal decay constants (k_T) (Chamberlin and Mitchell 1978) were calculated by regression analysis from these data, and the variation of k_T as a function of temperature is shown in Fig. 2b. The data have been fitted to a modified form of the equation suggested by Mancini (1978):

$$N_{(t)} = N_0 \cdot e^{-k_T \cdot t} \qquad \text{Eqn 1}$$

$$k_T = \theta^{(T-\alpha)} \qquad \text{Eqn 2}$$

where $N_{(t)}$ = the instantaneous total bacterial population (cfu ml^{-1}), N_0 = the original bacterial population (cfu ml^{-1}), k_T = thermal decay constant (h^{-1}), t = time (h), θ = species

specific fit parameter, and α = nominal activation temperature (°C).

The solid line in Fig. 2b is a fit to the data using values of $\theta = 1{\cdot}21(1)$ and $\alpha = 48{\cdot}3(7)$. Temperatures below 45 °C have negligible inactivating effects on the bacteria whereas inactivation rates increase with temperatures above this value. This is in good agreement with the variation of the thermal decay constant reported by Mancini (1978) for other unspecified coliforms.

Reasonable agreement between measured and predicted (solid lines, Fig. 3b) thermal inactivations are obtained if the definition of k_T in equation 2 is altered such that:

$$k_T = 0 \quad \text{for} \quad T < 45\,°C$$

$$k_T = 1{\cdot}21^{(T-48{\cdot}3)} \quad \text{for} \quad T > 45\,°C$$

Simulations of the temperature regimens show that the low temperature regimen (maximum temperature = 36·3 °C, see Fig. 3a) produces no inactivation, which is to be expected since the temperature never exceeds 45 °C. The medium temperature regimen produces an inactivation of less than one order of magnitude over the 8 h simulation period. However, all the high temperature regimen simulations produce total and permanent inactivations within 7 h, as previously reported (Joyce *et al.* 1996). The inactivation achieved for each thermal simulation was independent of the turbidity (data not shown); consequently, only the 200 ntu measurements are displayed in Fig. 3b.

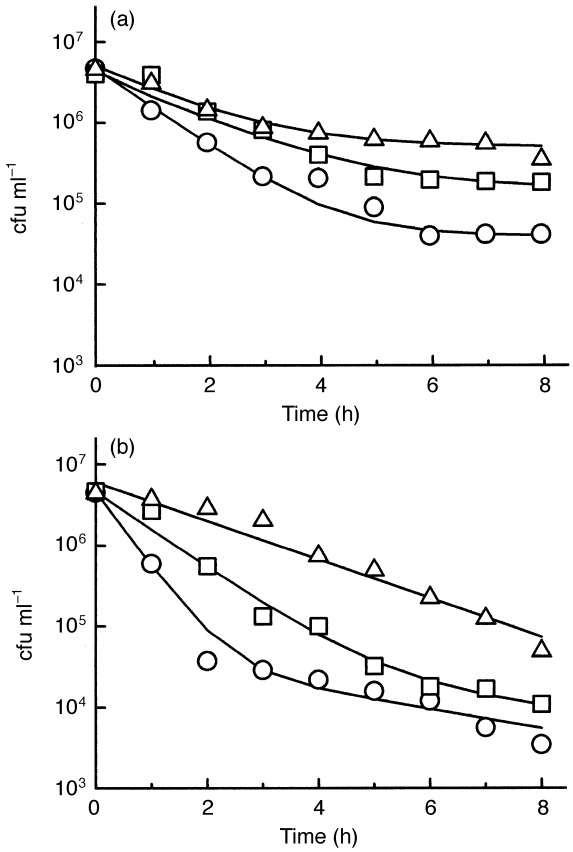

Fig. 4 Optical simulation results for water samples with turbidities of (a) 200 ntu and (b) 0 ntu, respectively. (○), (□) and (△) denote optical irradiances of 70, 40 and 10 mW cm^{-2}, respectively

Optical inactivation

The bacterial inactivations produced by optical irradiation only are shown in Fig. 4. The decreases in bacterial population were permanent and no subsequent increase was observed in the 24 h period after the simulation ended. All but the 10 mW cm^{-2}, 0 ntu simulation show evidence of a population decrease consistent with a double-exponential decay. This behaviour is attributed to the presence of both light resistant and light sensitive bacteria among the starting population. The solid lines in Fig. 4 are theoretical fits to the data, assuming first order decay kinetics of the form:

$$N_{(t)} = N_s \cdot e^{-k_s \cdot t} + N_R \cdot e^{-k_R \cdot t} \qquad \text{Eqn 3}$$

where $N_{(t)}$ = the instantaneous total bacterial population (cfu ml^{-1}), N_s = the original light sensitive bacterial population (cfu ml^{-1}), N_R = the original light resistant bacterial population (cfu ml^{-1}), t = time (h), k_s = decay constant for the light sensitive population (h^{-1}) and k_R = decay constant for the light resistant population (h^{-1}).

Non-linear regression analysis (Jandel Scientific 1992) was used to obtain parameter values for k_s, k_R and N_R and these values are listed in Table 1. For the sake of simplicity, N_s is assumed to be equal to N_0. Optical inactivation is clearly dependent on turbidity. Comparison of the 0 ntu k_s values with those reported by Mancini (1978) shows good agreement for the 40 and 10 mW cm^{-2} data. The 70 mW cm^{-2} values are lower than those predicted by Mancini (1978), but his calculations were based on assumptions of light falling upon a large body of water and make no allowance for either reflective losses of incident radiation from the curved surface of the bottle, or absorption losses within the container material itself. Simulations using 0 ntu water samples achieve approximately one order of magnitude greater inactivations than the corresponding 200 ntu samples for irradiances between 40 and 70 mW cm^{-2}.

The decision to analyse the observed optical inactivations on the basis of a double exponential decay corresponding to separate populations of light resistant and light sensitive bacteria is reasonable, and is further supported by the observation, mentioned earlier, for the optical and combined simulations, of colonies of both normal and reduced size. This effect was not observed in any of the thermal only simulations.

Table 1 Parameter and coefficient of determination values for theoretical fits to the optical irradiance simulation measurements using Equation 3 and non-linear regression. These fits are displayed as solid lines in Fig. 4

Optical irradiance ($mW\,cm^{-2}$)	Turbidity (ntu)	k_S (h^{-1})	k_R (h^{-1})	N_R ($cfu\,ml^{-1}$)	Coefficient of determination R^2
70	200	0·32 (3)	0·00 (1)	3×10^4	0·967
40	200	0·27 (2)	0·020(4)	3×10^5	0·958
10	200	0·25 (2)	0·07 (1)	0	0·963
70	0	1·04 (9)	0·17 (1)	1×10^5	0·971
40	0	0·45 (4)	0·14 (3)	1×10^5	0·986
10	0	0·25 (2)	0·00 (1)	1×10^6	0·962

Combined thermal and optical inactivation

The entire set of possible combined simulations can be subdivided into nine groups (three temperature regimens at three irradiance values). Each group contains two simulations taken at different turbidity values (0 and 200 ntu) but subjected to the same optical irradiance and temperature regimen. In effect, each group corresponds to a specific solar exposure, or meteorological environment. All nine groups of simulations, which are listed in Table 2, were carried out. None of these simulations produced an increase in bacterial population. Irrespective of the irradiance level, all samples that experience the highest water temperature regimen (maximum temperature 55 °C) are totally inactivated within, at most, 7 h of the start of the simulation, and in each case, no subsequent bacterial recovery is observed after leaving the samples to cool down overnight. For all values of thermal regimen and turbidity, an increase in the irradiance produces a corresponding increase in bacterial inactivation.

Table 2 Temperature regimen and optical irradiance values for the combined simulations and the corresponding meteorological conditions that these most closely match. Note each group of simulations was carried out at 0 and 200 ntu turbidity

Combined simulation group	Optical irradiance ($mW\,cm^{-2}$)	Temperature regimen*	Corresponding meteorological conditions
1	70	High	Sunny, hot
2	70	Medium	Sunny, warm
3	70	Low	Sunny, cool
4	40	High	Hazy, hot
5	40	Medium	Hazy, warm
6	40	Low	Hazy, cool
7	10	High	Overcast, hot
8	10	Medium	Overcast, warm
9	10	Low	Overcast, cool

* See Fig. 2a.

Groups 4, 7 and 8 in Table 2 do not correspond to solar exposure conditions that can realistically be expected in the field and so are not discussed further. Of the remaining groups, those combined simulations that utilize the low temperature regimen (groups 3, 6 and 9) produce inactivations that are not significantly different (paired Student *t*-test at the $P = 0{\cdot}05$ significance level (Jandel 1992)) to those exposed to light only (data not shown). Plots of the viable bacterial populations for the remaining three groups of simulations (1, 2 and 5) are shown in Fig. 5. The results of these combined simulations (triangles) are displayed in each panel alongside the corresponding thermal only (circles) and optical only (squares) simulation results for comparison purposes.

Attempts to model the population dynamics of the combined simulations were made using an equation derived from Equations 1, 2 and 3, of the form:

$$N(t) = N_s \cdot e^{-(k_s+k_T)\cdot t} + N_R \cdot e^{-(k_R+k_T)\cdot t} \qquad \text{Eqn 4}$$

where each term retains its previous meaning. Equation 4 assumes a non-synergistic relationship between the optical and thermal components of the observed combined inactivation. However, this consistently underestimates the measured inactivation (dashed line in Fig. 6), showing that the relationship between optical and thermal inactivation mechanisms is more than additive in the combined simulations. The synergistic relationship between the optical and thermal processes can be modelled by multiplying the decay constants in Equation 4 by an extra synergy term S in the manner shown in Equation 5. Values of S larger than 1·0 indicate that synergy occurs.

$$N(t) = N_s \cdot e^{-S\cdot(k_s+k_T)\cdot t} + N_R \cdot e^{-S\cdot(k_R+k_T)\cdot t} \qquad \text{Eqn 5}$$

The solid lines in Fig. 6 are fits to the data made using this equation, and from the values of the correlation coefficients, it can be seen that they are reasonable. The parameter values of N_R, k_R and k_s that were used in both the non-synergistic (dashed line) and synergistic (solid line) fits in Fig. 6 are identical and are listed in Table 3. The coefficients of determination values (R^2) listed in each panel of Fig. 6 refer to the

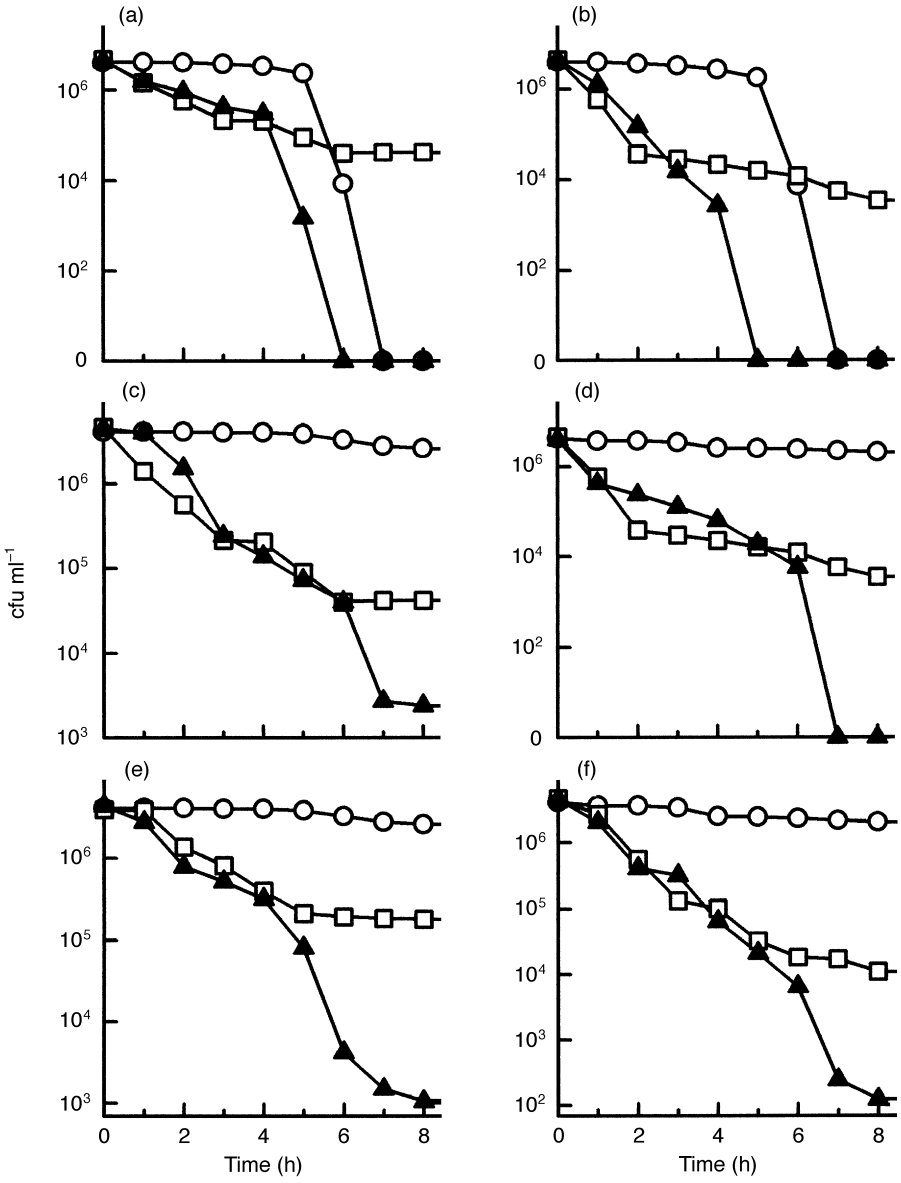

Fig. 5 Comparisons of corresponding thermal (○), optical (□) and combined (▲) bacterial inactivations achieved during simulations. Note: solid lines are not fitted. See Table 3 for simulation parameters

synergistic fits only and are calculated using the R2.XFM transform from the Sigmaplot (Jandel Scientific 1992) suite of curve-fitting software.

DISCUSSION

Exposure of bacteria to artificial and natural sunlight has been shown, sometimes, to cause a transformation in bacteria from a viable and culturable state to a viable but non-culturable (VNC) state (Tyrell 1976; Colwell *et al.* 1985; Barcina *et al.* 1989). The time required for this transformation to occur varies with species and nature of the stressful environment (Barcina *et al.* 1989; Davies and Evison 1991; Medema *et al.* 1992; Jacob *et al.* 1993). Gourmelon *et al.* (1994) report an optically induced VNC state in *E. coli* after 10 h of exposure to artificial light sources. However, the neon light source used in that study was approximately 7·5 times more intense than the highest irradiance used in our simulations and has all its optical energy compressed into five narrow spectral bands within the U.V./visible spectrum (Oriel Corp. 1994). In addition, the reduction in bacterial population after 10 h exposure, as measured by the direct viable count technique

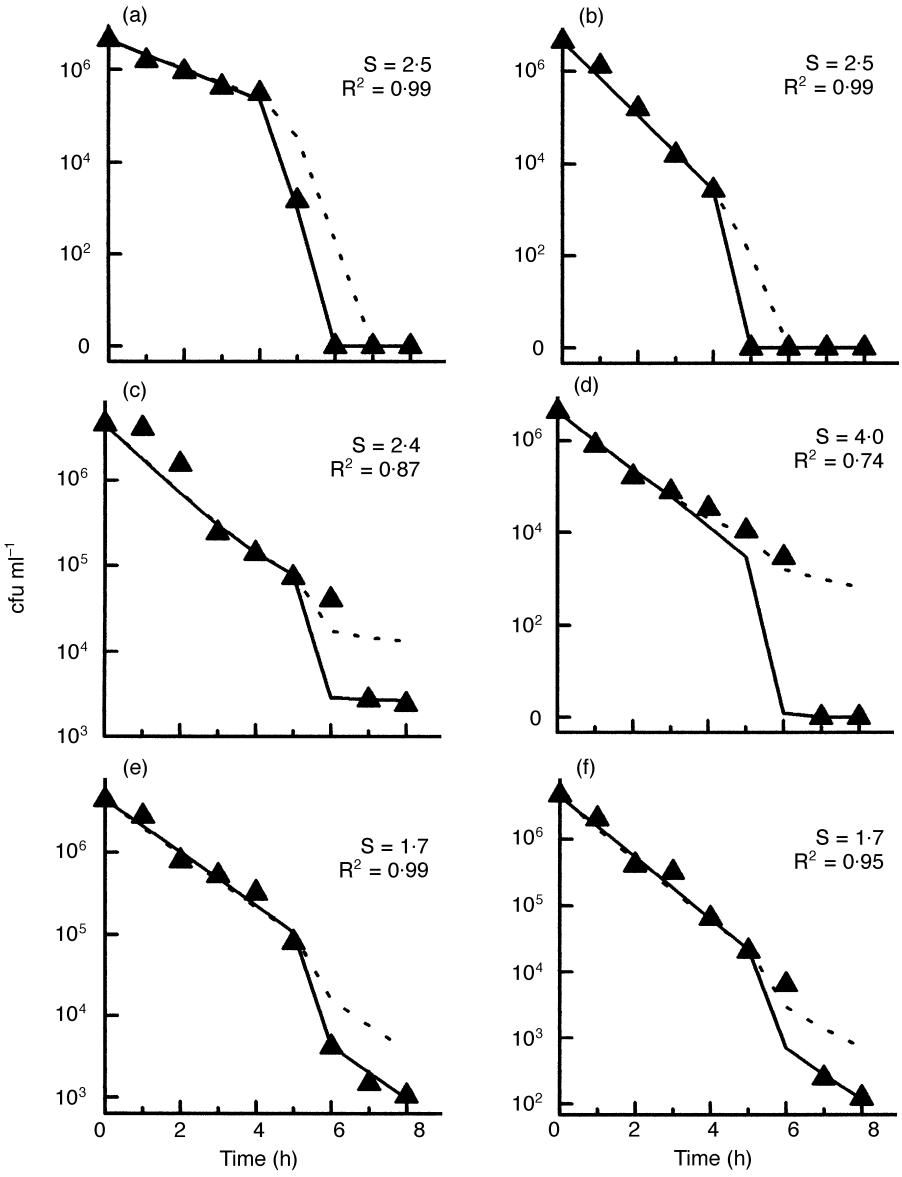

Fig. 6 Comparison of synergistic (solid line, see Equation 5) and non-synergistic (dashed line, see Equation 4) theoretical fits to the combined simulation measurements shown in Fig. 5

Table 3 Theoretical fit parameters for the combined simulations in Fig. 5 using Equation 4

Combined simulation*	Temperature regimen	Optical irradiance (mW cm^{-2})	Turbidity (ntu)	Resistant population (cfu ml^{-1})	k_R (h^{-1})	k_S (h^{-1})
a	High	70	200	0	0	0·75
b	High	70	0	0	0	1·85
c	Medium	70	200	4×10^4	0	0·95
d	Medium	70	0	5×10^4	0·4	1·5
e	Medium	40	200	4×10^4	0·4	0·75
f	Medium	40	0	4×10^4	0·4	1·1

* As in Fig. 5.

(Kogure *et al.* 1979) was only 1 log unit lower than that measured using the Acridine Orange Direct Count (AODC, Hobbie *et al.* 1977) method for measuring VNC bacteria. Davies and Evison (1991) observed no evidence of a VNC state being induced in *E. coli* exposed to solar irradiances of 25 mW cm^{-2} in fresh water for periods up to 10 h, and reported that the number of bacteria estimated by the AODC method was consistently higher than those counted by culturable methods, even at the start of each simulation. Concern has been expressed regarding the accuracy of the AODC method (Al-Hadithi and Goulder 1989; Singh *et al.* 1989). In addition, all of the decreases in bacteria populations that were measured in the simulations were of a permanent nature, and no subsequent re-activation after 24 h was observed on any occasion. For these reasons, it was deemed reasonable to use the Miles and Misra drop count enumeration method (Miles and Misra 1938) for our simulations.

The relative importance of thermal inactivation in comparison to optical inactivation depends very much on both initial water quality and prevailing weather conditions. The transmission properties of the PET container material (see Fig. 1b) are such that most of the U.V.B wavelengths (280–320 nm), whose bactericidal properties are well documented (Chamberlin and Mitchell 1978; Davies and Evison 1991), are filtered out of the incident light before they can reach the bacteria. The optical inactivation mechanisms in operation during solar disinfection must result from the remaining U.V.A (320–400 nm) and visible light. Turbidities above 200 ntu absorb approximately 99% of the incident radiation within the first centimetre of optical path (Joyce *et al.* 1996). While a sizeable optical inactivation under such circumstances might not be expected, the opacity associated with turbid water will change the optical absorption characteristics, which in turn affects the thermal inertia of the sample since darker surfaces have higher emissivities than lighter surfaces and the turbid agent (dust, algae, etc.) will usually have a lower specific heat capacity than the water itself. Consequently the temperature dynamics of highly turbid water samples can be faster than those of clearer samples which would help thermal inactivation processes.

Our measurements show that the inactivation of *E. coli* during solar disinfection results from a synergistic relationship between the optical and thermal inactivation mechanisms, if, and only if, the water temperature exceeds 45 °C. All the combined simulations displayed in Fig. 5 show similar population dynamics to the corresponding optical only simulations until the water temperature exceeds 45 °C, at which point the inactivation rate increases beyond that expected for the addition of the thermal decay constant (k_T) and the optical decay constants (k_S or k_R) in Equation 4 (dashed lines in Fig. 6). This is in broad agreement with Wegelin *et al.* (1994) who reported that water temperatures between 20 and 40 °C do not affect the inactivation of bacteria by UVA and visible light but synergistic effects are observed at a threshold water temperature of 50 °C.

Furthermore, a complete inactivation of high populations of *E. coli* can be produced in drinking water, even of high turbidity (200 ntu), by exposing 1·5 l volumes contained in plastic soft drink containers to strong to medium solar irradiances for periods of at least 7 h. If a water temperature greater than 55 °C is reached in the sample, thermal stress is primarily responsible for the observed inactivation. The inactivated bacteria do not recover as the water cools following solar exposure. Bacteria in samples that achieve intermediate water temperatures (about 45 °C) can still be fully and permanently inactivated if the water is of low turbidity and is exposed to high irradiances (70 mW cm^{-2}) for periods of up to 7 h.

The benefits of such a simple and cheap method of improving water quality are obvious for developing countries. The technique can be improved considerably by the inclusion of any of several simple steps to the technique. Such steps might be pre-filtration or reduction of the turbidity using locally available flocculation agents (Folkard *et al.* 1996). Reed (1997) has shown that inactivation increases as dissolved oxygen concentration levels increase, and suggests that periodic agitation of the bottle during exposure would speed up the disinfection process. If the water is of sufficient clarity, the optical inactivation process can be improved by wrapping the rear half of the bottle with reflective aluminium foil. Alternatively, the thermal inactivation process can be enhanced by coating the rear half of the bottle with a dark opaque substance (mud or paint) and keeping the bottle sheltered from the convective cooling effects of the wind.

None of the above interventions were included in the work reported here because the aim was to determine whether the basic solar disinfection process, using the clear bottle only and no pre-treatment for the water, would be effective under conditions that might exist in a refugee camp or disaster situation. Our results show that solar disinfection of drinking water taken directly from a contaminated source, stored in transparent plastic bottles and exposed to the sun, would be an effective first option under such situations if strong sunshine is available. Even if strong sunshine is not available, our results show that solar exposure will, at best, improve the water quality and at worst, leave it no worse than it was before. This is further confirmed by the results of our controlled field trial of solar disinfected drinking water in the reduction of diarrhoea in Kenyan Maasai children between the ages of 5 and 16 years (Conroy *et al.* 1996) which showed a 26% reduction in incidences of severe diarrhoea over a 3 month period compared with the control group. This reduction in the incidence of diarrhoea also suggests that even if it is not certain whether the water-borne pathogens are killed, inactivated or transformed into a VNC state, solar dis-

infection does reduce the risk of diarrhoeal disease. It is reasonable to assume that the thermal and optical inactivation mechanisms observed in our simulations play some role in this process.

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