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ORIGINAL ARTICLE

Efficacy of solar disinfection of *Escherichia coli*, *Shigella flexneri*, *Salmonella* Typhimurium and *Vibrio cholerae*

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Escherichia coli, mild heat, reciprocity, resistance, *Salmonella* Typhimurium, *Shigella flexneri*, solar disinfection (SODIS), *Vibrio cholerae*.

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Abstract

Aims: To determine the efficacy of solar disinfection (SODIS) for enteric pathogens and to test applicability of the reciprocity law.

Methods and Results: Resistance to sunlight at 37°C based on F_{99} values was in the following order: *Salmonella* Typhimurium > *Escherichia coli* > *Shigella flexneri* > *Vibrio cholerae*. While F_{90} values of *Salm.* Typhimurium and *E. coli* were similar, F_{99} values differed by 60% due to different inactivation curve shapes. Efficacy seemed not to be dependent on fluence rate for *E. coli* stationary cells. Sensitivity to mild heat was observed above a temperature of 45°C for *E. coli*, *Salm.* Typhimurium and *Sh. flexneri*, while *V. cholerae* was already susceptible above 40°C.

Conclusions: *Salmonella* Typhimurium was the most resistant and *V. cholerae* the least resistant enteric strain. The reciprocity law is applicable for stationary *E. coli* cells irradiated with sunlight or artificial sunlight.

Significance and Impact of the Study: *Escherichia coli* might not be the appropriate indicator bacterium to test the efficacy of SODIS on enteric bacteria and the physiological response to SODIS might be different among enteric bacteria. The applicability of the reciprocity law indicates that fluence rate plays a secondary role in SODIS efficacy. Stating inactivation efficacy with T_{90} or F_{90} values without showing original data is inadequate for SODIS studies.

Introduction

The World Health Organization (WHO) estimated in 1996 that every 8 seconds a child dies from a water-related disease and that each year more than five million people die from illnesses linked to consumption of unsafe drinking water or inadequate sanitation (WHO 1996). Daily, there are numerous reports of outbreaks worldwide due to the consumption of untreated or improperly treated drinking water contaminated with bacterial, viral or parasitic micro-organisms (WHO 1993, 1996, 2001; Ford and Colwell 1996; Hunter 1997). WHO and the United Nations Children's Fund (UNICEF) recently claimed that improvement of drinking water quality and basic sanitation can cut this toll and, furthermore, that simple, low-cost household water treatment can save lives as it cuts the primary transmission route for diarrhoeal diseases (WHO/UNICEF 2005). Solar disinfection (SODIS) is such a water treatment method whereby exposure of drinking water in poly(ethylene terephthalate) (PET) bottles to sunlight (≥ 6 h) causes the enteric bacteria in the water to get inactivated (Acra *et al.* 1984; Wegelin *et al.* 1994). The two primary sources for bacterial inactivation in this method are believed to be mild heat and ultraviolet A (UVA) light (Wegelin *et al.* 1994). SODIS was demonstrated to be effective against bacteria and higher organisms (Wegelin *et al.* 1994; Joyce *et al.* 1996; McGuigan *et al.* 1998; Lonnen *et al.* 2005) and its benefit was shown in a recent health impact study (Hobbins *et al.* 2003). Today, SODIS is one of the recommended methods for household drinking water disinfection (WHO/UNICEF 2005).

The inactivation mechanism of SODIS is complex and not yet fully understood. The central hypothesis is that

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UVA light produces reactive oxygen species, which can damage nucleic acids, proteins or other life-supporting cell structures (Jagger 1985; Eisenstark 1989). It was also found that broad-spectrum UVA light blocks the electron transport chain, inactivates transport systems, interferes with metabolic energy production and can cause a general increase in permeability of the membrane (Koch *et al.* 1976; Jagger 1981; Berney *et al.* 2006a). Furthermore, direct inhibition of certain enzymes (e.g. catalase) has also been observed (Eisenstark 1998).

Although SODIS is a recommended and regionally used drinking water treatment method, experimental data about its effect on pathogenic bacteria in drinking water are still scarce. The lethal action of mild heat on enteric bacteria, which adds another inimical component to SODIS was mostly investigated in complex media and at temperatures above 52°C, but for bacteria in water challenged with constant heating at temperatures between 40 and 52°C, very little data are available (Humpheson *et al.* 1998; Benito *et al.* 1999). Also, reciprocity (i.e. that total applied fluence produces the same response regardless of the fluence rate) was studied only for monochromatic UVA irradiation (365 nm), but not for broad-band UVA or sunlight (Peak and Peak 1982; Lang *et al.* 1986). Reciprocity is very important for SODIS because solar irradiation can vary considerably during a day due to clouds or other factors. Hence, in this study, we investigated the sensitivity of *Escherichia coli* and three pathogenic enteric strains, namely *Salmonella enterica* serovar Typhimurium, *Shigella flexneri* and *Vibrio cholerae*, to the lethal action of sunlight and mild heat. Furthermore, we tested whether or not the reciprocity law is valid for SODIS.

Materials and methods

Bacterial strains

The following strains were used in this study: *E. coli* K-12 MG1655 (ATCC 700926), *Salm. enterica* serovar Typhimurium ATCC 14028, *Sh. flexneri* ATCC 12022 and *V. cholerae* 01 Ogawa biotype El Tor (Nent 720-95). For selected experiments, an isogenic *rpoS* mutant of *E. coli* K12 MG1655 (*rpoS13*::Tn*10*) (Wick *et al.* 2002) was used.

Growth media and cultivation condition

Batch

Luria-Bertani (LB) broth (10 g tryptone, 5 g yeast extract, 10 g NaCl per litre) was used for batch cultivation (Miller 1972). LB medium was always filter-sterilized with Millipore syringe filters (Millex GP, 0·22 μm; Millipore, Volketswil, Switzerland) and diluted to 33% volume/volume (v/v) of its original strength (unless indicated otherwise) with ultrapure water (deionized and activated carbon filtered). Precultures were prepared for each individual batch experiment from the same cryo-vial stored at −80°C by streaking out a loopful onto LB agar plates. After 15–18 h of incubation at 37°C, one colony was picked and loop-inoculated into a 125-ml Erlenmeyer flask containing 20 ml of LB broth. This was incubated at 37°C on a rotary shaker at 200 rev min^{-1}). At an OD_{546} between 0·1 and 0·2 [measured spectrophotometrically at 546 nm in glass cuvettes with 1 cm light path using a JASCO V550 UV/VIS spectrophotometer; Tokyo, Japan] cells were transferred into 500-ml Erlenmeyer flasks containing 50 ml of prewarmed LB broth. In this way, no lag phase of the culture was observed. The culture volume to be inoculated into the fresh medium was calculated beforehand to attain an initial OD_{546} of 0·002. These flasks were then shaken at 200 rev min^{-1} in a temperature-controlled water bath (SBK 25D; Salvis AG, Reussbühl, Switzerland) at 37°C for about 18 h until stationary phase (specific growth rate, $\mu = 0$ h^{-1}) was reached. The specific growth rate μ was calculated from five consecutive OD_{546} measurements.

Sample preparation and plating

Cells were harvested by centrifugation from batch culture (at 13 000 ***g***, Biofuge fresco; Kendro, Zürich, Switzerland), washed three times with filter-sterilized (Nuclepore Track-Etch Membrane, 0·22 μm; Sterico AG, Dietikon, Switzerland) commercially available bottled water (EVIAN, Danone, Paris, France) and diluted to an OD_{546} of 0·01 (corresponding to 1–5 × 10^{7} cells ml^{-1}). Exposure of bacterial suspensions was started 1 h after dilution, to let the cells adapt to the mineral water. Aliquots were withdrawn at different time points and diluted in decimal steps (10^{-1} to 10^{-5}) with sterile-filtered (0·2 μm) mineral water (EVIAN). Following dilution, 1 ml of test solution was withdrawn and mixed with 7 ml of liquid tryptic soy agar (TSA) (Biolife, Milano, Italy) at 40°C (pour plate method). After 20 min, the solidified agar was covered with another 4 ml of liquid TSA (40°C). Plates were incubated for 48 h at 37°C until further analysis. Plate counts were determined with an automatic plate reader (Acolyte, SYNBIOSIS, Cambridge, UK).

Mild heat exposure

A bacterial suspension of 20 ml was transferred into 30-ml quartz glass tubes (WISAG AG, Zürich, Switzerland), and placed into a temperature-controlled water bath at defined temperatures between 40 and 52°C as indicated later. Care was taken that vials were entirely submersed to prevent temperature gradients in the vials. The final temperature of the liquid was reached in

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≤ 2 min. During exposure, samples were stirred with a magnetic stirrer. Unstressed control samples were kept in the dark at 37°C.

Sunlight exposure

Samples of 10 ml of bacterial suspension were exposed to solar light in 30-ml quartz tubes, which were placed into a temperature-controlled acrylic glass container with a quartz front glass, holding 25 tubes in total (Berney *et al.* 2006b). A circulating water bath was used to control the temperature of the sample tubes in the container. The container was adjusted regularly so that sunlight met the tubes at an angle of 90 ± 2°. At each time point, one tube was withdrawn and its contents immediately processed as described earlier. Irradiation intensity data were obtained from a weather station, which is located 300 m away from the exposure site (BUWAL/NABEL, EMPA Dübendorf, Switzerland). The fluence rates for sunlight irradiation given in this work refer to the wavelength range of 350–450 nm, which reflects the wavelength range of the UVA lamps also used (Berney *et al.* 2006b). Conversion factors and calculations were used as in Wegelin *et al.* (1994). The light spectra were recorded with a calibrated LI-1800 portable spectroradiometer (LI-COR, Lincoln, Nebraska, USA), 8-nm bandwidth, fitted with a model 1800-10 detector head (Berney *et al.* 2006b).

Artificial UVA exposure

Samples of 10 ml of bacterial suspension were exposed to UVA light in 30-ml quartz tubes, placed in a carousel reactor (holding ten tubes) [adapted from Wegelin *et al.* (1994)] equipped with medium-pressure mercury lamps Hanau TQ 150, TQ718 or TQ718 Z4 (doped) (WISAG AG), which were operated at 150, 500–700 W and 500–700 W, respectively. The fluence rates varied from 56 to 734 W m^{-2}. The lamps TQ 150 and TQ 718 exhibit the same wavelength spectrum, but differ in power output. The doped lamp TQ 718 Z4 exhibits a broader wavelength spectrum [see Berney *et al.* (2006b)]. The lamp was placed in a cooling jacket (Duran 50 borosilicate glass) in the centre of the carousel reactor. The light emitted from the lamp passed through the glass jacket and through 35 mm of filter solution before reaching the cells in the quartz tubes. The temperature of the filter solution was maintained at 37°C and consisted of 12·75 g l^{-1} of sodium nitrate with a cut-off at 320 nm and a half maximum at 340 nm. The transmission property of the filter solution was measured before each experiment. Chemical actinometry with *p*-nitroanisole/pyridine was used to determine the fluence rate at the tube position (Wegelin *et al.* 1994). Bacterial solutions were mixed intermittently on a magnetic stirrer. At each time point, one tube was withdrawn and its contents immediately processed as described earlier.

Table 1 T_{90}, F_{90} and F_{99} values [time or fluence until 90% (99%) of the population is inactivated] from sunlight exposure of four different enterobacterial strains. Bacterial cells were harvested from stationary phase Luria-Bertani (LB) batch cultures, washed three times and diluted in bottled mineral water. The values were derived from best-fit curves modelled with the program Gearaerd and Van Impe Inactivation Model Fitting Tool (GinaFiT). SD was calculated from three independent measurements

Strain	T_{90} (min)	F_{90} (kJ m^{-2})*	F_{99} (kJ m^{-2})*
E. coli MG1655	182 ± 15	1210 ± 188	1530 ± 70
S. Typhimurium	187 ± 37	1238 ± 341	2431 ± 425
S. flexneri ATCC 12022	136 ± 37	932 ± 233	1194 ± 142
V. cholerae 01 Ogawa biotype E1 Tor	24 ± 5	165 ± 32	305 ± 35

*Fluence was calculated from solar irradiation data for the wavelength range between 350 and 450 nm.

Modeling with Gearaerd and Van Impe Inactivation Model Fitting Tool

The Gearaerd and Van Impe Inactivation Model Fitting Tool (GInaFIT) was used for testing six different types of microbial survival models on our data (Geeraerd *et al.* 2005). The following models were used: log-linear regression (Bigelow and Esty 1920), log-linear + tail (Geeraerd *et al.* 2000), log-linear + shoulder (Geeraerd *et al.* 2000), log-linear + shoulder + tail (Geeraerd *et al.* 2000), Weibull model (Mafart *et al.* 2002), Biphasic model (Cerf and Metro 1977), biphasic + shoulder (Geeraerd *et al.* 2005). 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 most informative measure of goodness-of-fit for linear and nonlinear models (Geeraerd *et al.* 2005). The model with the smallest RSME was considered the best fit for the respective inactivation curve. When the magnitude of RMSE was much smaller than experimental precision the model was considered to over-fit the data and a simpler model was chosen. If two models had the same or very similar RMSE values the simpler model was considered to fit best. T_{90} and F_{90} values (time or fluence to reduce plate counts by 90%) were calculated using the best-fit model of GInaFIT (Table 1).

Results

SODIS works on the basis of two major facts, the lethal action of solar UVA light, and the synergistic effect which is created when water temperature rises above 50°C (Wegelin *et al.* 1994). Unfortunately, it is not always possible to reach such high water temperatures in the

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bottles. SODIS field trials in different geographic regions carried out by SANDEC (Department of Water and Sanitation in Developing Countries, Eawag, Switzerland) have shown that temperatures above 50°C are rarely reached (R. Meierhofer, personal communication). Therefore, we investigated the effectiveness of sunlight (factoring out the temperature effect) to inactivate *E. coli* and three pathogenic strains, namely *Salm.* Typhimurium, *Sh. flexneri* and *V. cholerae*. In order to estimate a possible synergistic effect between solar irradiation and mild heat, we also measured temperature sensitivities for all four strains at temperatures relevant to the SODIS method between 40 and 52°C.

Sunlight sensitivity

Sensitivity to sunlight was investigated at a constant temperature of 37°C. For each strain, three experiments were conducted on three different days (Fig. 1). The fluences in the sunlight experiments were calculated for the wavelength range between 350 and 450 nm (Wegelin *et al.* 1994) in order to compare results with experiments carried out with the medium pressure lamps. A fluence of 2400 kJ m^{-2} (350–450 nm) corresponds to about 6–7 h of sunlight exposure in Switzerland from June to August. *Escherichia coli* and *Sh. flexneri* exhibited very similar inactivation behaviour with *Sh. flexneri* being more sensitive than *E. coli* (see also Table 1). The inactivation curves of these two strains both showed a distinct shoulder effect as indicated from the best fitting models when analysed with GInaFiT. Both *Salm.* Typhimurium and *E. coli* had very similar F_{90} values, but the shape of the curve was totally different. This discrepancy is also indicated by the F_{99} values (Table 1). The slow inactivation of *Salm.* Typhimurium indicates low sensitivity of this strain to sunlight. On the other hand, *V. cholerae* was a lot

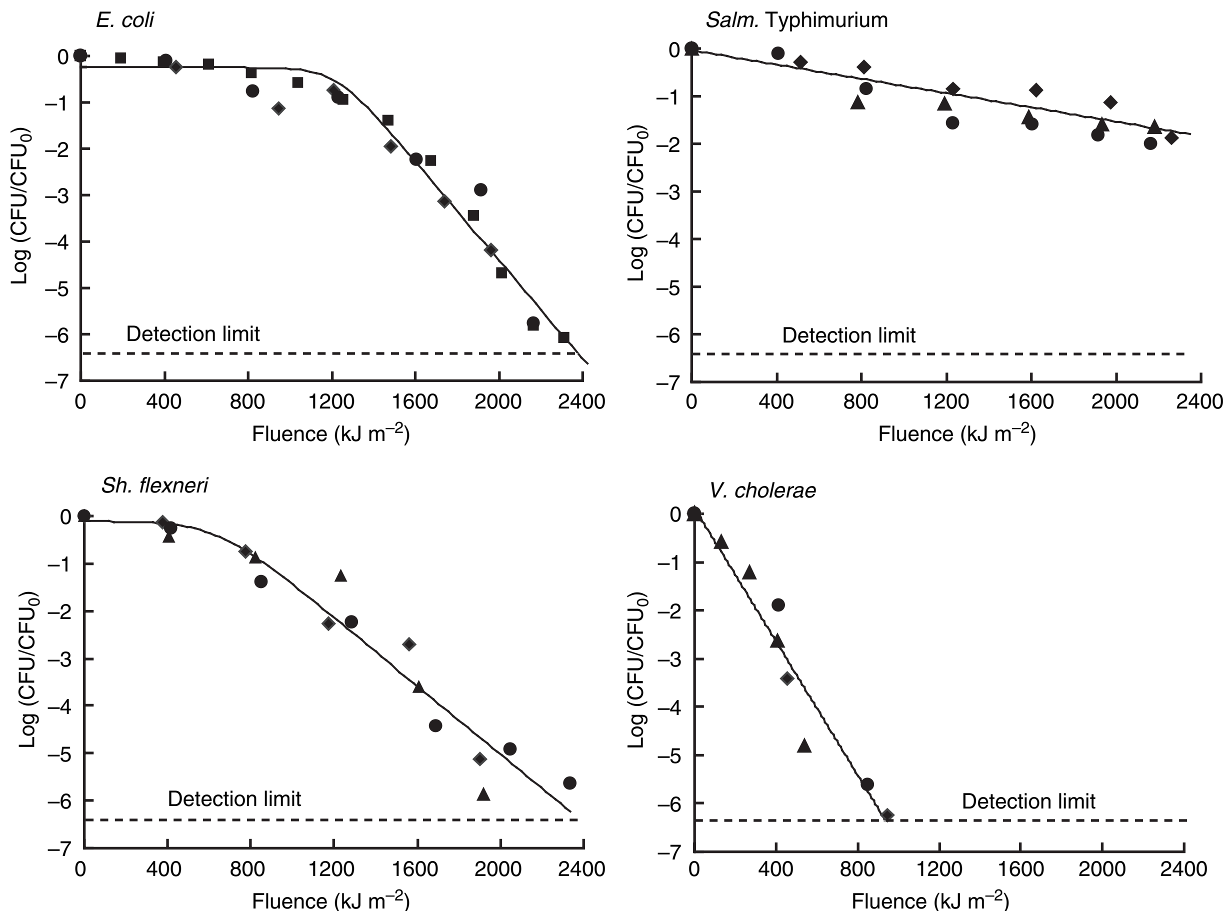

Figure 1 Inactivation curves of *Vibrio cholerae*, *Salmonella* Typhimurium, *Shigella flexneri* and *Escherichia coli* exposed to sunlight (350–450 nm) on three different days. Temperature was held constant at 37°C. Bacterial cells were harvested from stationary phase Luria-Bertani (LB) batch cultures, washed three times and diluted in bottled mineral water before exposure. Colony forming units were measured by pour plating and sensitivity was recorded as CFU/(CFU at time zero). Lines represent modelling results obtained with the software Gearaerd and Van Impe Inactivation Model Fitting Tool (GinaFiT) (Geeraerd *et al.* 2005).

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more sensitive to sunlight. For the data of *V. cholerae*, the log-linear model fitted best.

Mild heat sensitivity

Sensitivity to mild heat was investigated in order to elicit the role of temperature in SODIS experiments (Fig. 2). Again the pathogenic enteric bacterial strains (*Salm.* Typhimurium, *Sh. flexneri* and *V. cholerae*) were compared with *E. coli*. Stationary phase bacteria ($\mu = 0\ h^{-1}$) were exposed to temperatures between 41 and 52°C. *Salmonella* Typhimurium, *Sh. flexneri* and *E. coli* reacted similarly to mild heat with *E. coli* being less sensitive at higher temperatures. However, *V. cholerae* exhibited sensitivity already at temperatures above 40°C. When inactivation curves were modelled with the Program GInaFIT, the model log-linear + shoulder or log-linear model fitted best.

Reciprocity

Till now it was assumed that fluence (dose) is the appropriate parameter to compare different irradiation experiments. This is based on the multihit and multitarget theories (Harm 1980). As far as we know it has not been shown yet that this assumption holds true for *E. coli* exposed to sunlight or simulated sunlight. Therefore, we performed irradiation experiments at ten different fluence rates with three different medium pressure mercury lamps (Fig. 3) (see also in 'Materials and methods'). The two lamps with the same wavelength spectrum showed the same inactivation efficiency with an average F_{90} value of $1123 \pm 121\ kJ\ m^{-2}$ for *E. coli* cells in six experiments with different fluence rates (56, 91, 352, 483, 542, 569 $W\ m^{-2}$). Also the doped lamp showed no difference in inactivation efficiency when operated at different intensities (four

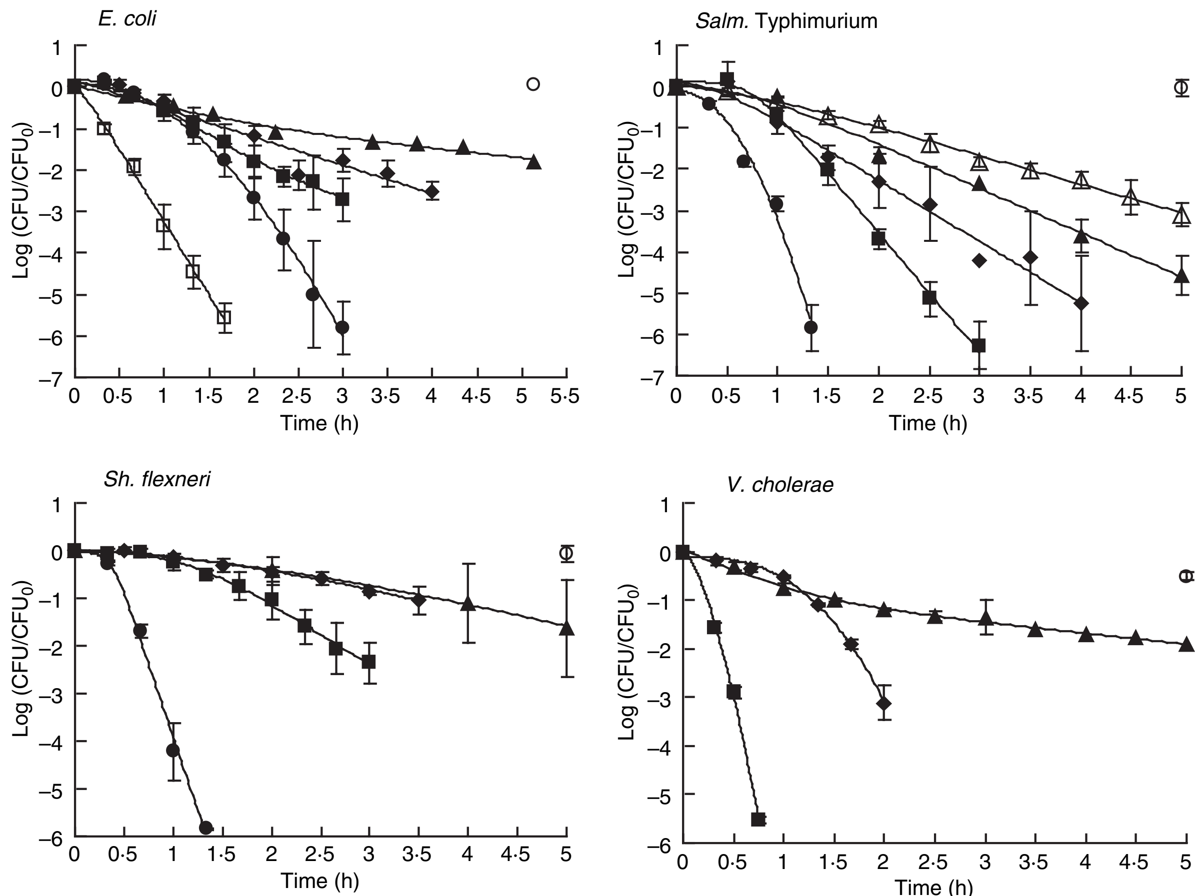

Figure 2 Inactivation curves of *Vibrio cholerae*, *Salmonella* Typhimurium, *Shigella flexneri* and *Escherichia coli* exposed to mild heat between 40 and 52°C. Symbols for *E. coli*, *Salm*. Typhimurium and *Sh. flexneri* are: △, 47°C; ▲, 48°C; ◆, 49°C; ■, 50°C; ●, 51°C; □, 52°C; and for *V. cholerae*: ▲, 41°C; ◆, 42°C; ■, 43°C. Bacterial cells were harvested from stationary phase Luria-Bertani (LB) batch cultures, washed three times and diluted in bottled mineral water before exposure. Colony forming units were measured by pour plating and sensitivity was recorded as CFU/(CFU at time zero). Error bars represent SD of triplicate measurement. Lines represent modelling results with the program Gearaerd and Van Impe Inactivation Model Fitting Tool (GinaFiT) (Geeraerd *et al.* 2005). Control samples were held at 37°C (○).

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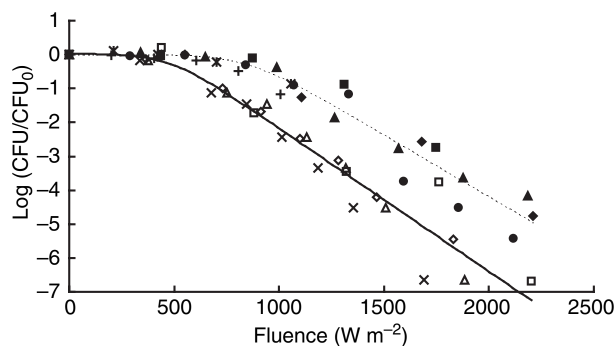

Figure 3 Inactivation curves of *Escherichia coli* exposed to 11 different UVA intensities [TQ150: (+) 56 and (■) 91 W m^{-2}; TQ718 Z1: (⋇) 352, (●) 481 W m^{-2}, (◆) 542 W m^{-2}, (▲) 569 W m^{-2}; TQ718 Z4: (×) 282, (◇) 305, (△) 314 and (□) 734 W m^{-2}]. Bacterial cells were harvested from stationary phase Luria-Bertani (LB) batch cultures, washed three times and diluted in bottled mineral water. Colony forming units were measured by pour plating and sensitivity was recorded as CFU/(CFU at time zero). Lines represent modelling results with the program Gearaerd and Van Impe Inactivation Model Fitting Tool (GinaFiT) for experiments with undoped (dashed line) and doped (solid line) lamps (Geeraerd *et al.* 2005).

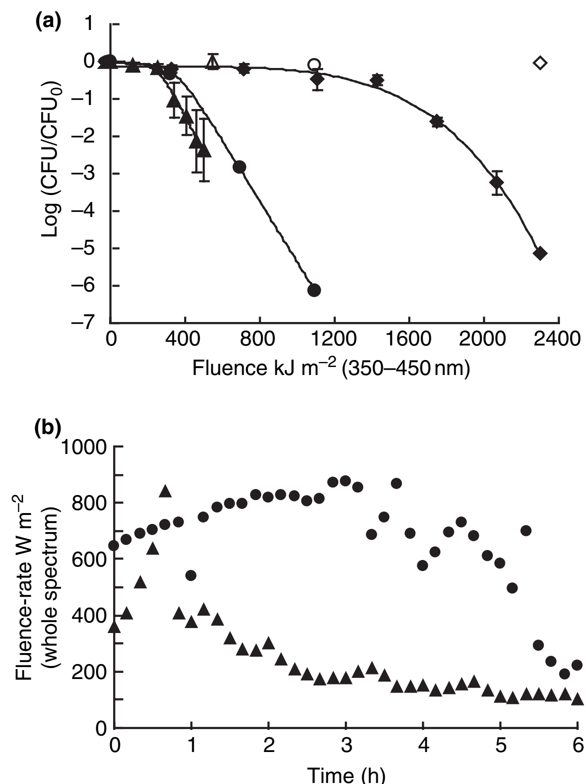

Figure 4 (a) *Escherichia coli rpoS*-minus exposed to sunlight on a cloudy (▲) and on a sunny day (●). For comparison, the inactivation curve of wild-type *E. coli* on a sunny day was added (◆). Bacterial cells were harvested from stationary phase Luria-Bertani (LB) batch cultures, washed three times and diluted in bottled mineral water. Colony forming units were measured by pour plating and sensitivity was recorded as CFU/(CFU at time zero). Error bars represent SD of triplicate measurements. Unstressed control samples are displayed with open symbols. (b) Average sunlight intensities (10 min average) on the corresponding days during the experiment. Fluence was calculated from solar irradiation data for the wavelength range between 350 and 450 nm.

experiments, 282, 305, 314, 734 W m^{-2}), but compared with the undoped lamp it seemed to be more effective for inactivation of *E. coli* ($F_{90} = 759 \pm 39$ kJ m^{-2}). Interestingly, *E. coli* cells exhibited similar sensitivity to irradiation with the undoped lamps as with sunlight ($F_{90} = 1210 \pm 188$ kJ m^{-2}).

In the field, *rpoS*-minus mutants of *E. coli* were exposed on sunny and cloudy days (Fig. 4a). On the sunny day, plate counts of *E. coli rpoS*-minus mutants were already below detection limit after 4 h. During this time, the solar irradiation intensity was always above 600 W m^{-2}. On the cloudy day, average intensity was mostly below 300 W m^{-2} except during a short spell of sunlight in the first hour of irradiation (Fig. 4b). When inactivation curves were compared with regard to fluence, no significant difference was observed. Furthermore, as expected, the *rpoS*-minus mutant was more sensitive to sunlight irradiation than the parent strain (Berney *et al.* 2006b). *rpoS* is an alternative sigma factor in *E. coli* and *Salm.* Typhimurium and is involved in global stress response to various environmental stresses (Hengge-Aronis 1999).

Discussion

For a sound application of the SODIS method it is vital to know whether the reciprocity law applies for bacterial inactivation by sunlight. The method is partly based on the assumption that each bacterial strain is inactivated above a certain threshold fluence. For example, it is assumed that a 3-log reduction of *E. coli* requires a fluence of about 2000 kJ m^{-2} (dose of solar radiation integrated in the 350–450-nm wavelength range), which corresponds to about 5 h of mid-latitude midday summer sunshine (Wegelin *et al.* 1994). The reciprocity law states that the effect of radiation is the function of the total radiant energy, and is independent of intensity and time (Zetterberg 1964). Although this assumption holds true for cells irradiated with monochromatic UVC (254 nm) (Zetterberg 1964), it was shown to be incorrect for *E. coli* cells irradiated with monochromatic UVA light at a wavelength of 365 nm (Peak and Peak 1982). These authors demonstrated that *E. coli* WP2 followed the reciprocity law only above fluences of about 750 W m^{-2} and that sensitivity increased with lower fluence rates (if the same

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fluence is applied). However, our results show that for stationary phase *E. coli* diluted in mineral water and irradiated with artificial sunlight the reciprocity law holds true (Fig. 3). We did not find any tendency of increased sensitivity of *E. coli* towards lower fluence rates. Also the field experiments did not indicate otherwise. Although the average fluence rate was considerably lower on the cloudy day (250 W m^{-2}) than on the sunny day (756 W m^{-2}), *E. coli rpoS*-minus mutants did not show different inactivation behaviour. Furthermore, we found that the lamp with the broad wavelength spectrum (TQ 718 Z4) was slightly more effective in inactivating *E. coli* cells. This is probably due to the increased wavelength intensities in the UVA range (300–400 nm), which cause more damage to the cells than the longer wavelength (Kramer and Ames 1987).

Most studies about sensitivity of enteric bacteria to solar or artificial UV-light have been conducted with *E. coli*. It is assumed that *E. coli* is the most suitable bacterium for such experiments. It is accepted worldwide as an indicator bacterium for detecting a potential contamination of drinking water with faeces (WHO 1996). Despite this, its use as the indicator bacterium for SODIS should be considered carefully because the inactivation mechanism in this particular treatment is not known yet and may be different in *E. coli* than in other enteric pathogens.

In this study, we compared the inactivation efficiency of SODIS for the three different entero-pathogenic strains *Salm*. Typhimurium, *Sh. flexneri* and *V. cholerae* and compared it with *E. coli*. Resistance to sunlight at a constant water temperature of 37°C of these four organisms based on F_{90} values (fluence needed to reduce plate counts by 90%) was in the following order: *Salm.* Typhimurium ATCC 14028 > *E. coli* MG1655 > *Sh. flexneri* ATCC 12022 > *V. cholerae* 01 Ogawa biotype El Tor. Although the F_{90} values reflected the observed order, it has to be mentioned that due to the different shape of the inactivation curves, some are shouldered, others are linear, one can not extrapolate from these values to, e.g. F_{99} values (Table 1). For example, the inactivation curve of *Salm.* Typhimurium is log-linear during the time measured as opposed to clearly shouldered curves for *E. coli*. The different curve shapes can either indicate different sensitivities to the same damaging effect, different inactivation mechanisms in the two strains or the capability of *Salm.* Typhimurium to adapt to sunlight stress. Even less appropriate are the T_{90} values because they do not take into account different irradiation intensities. Unfortunately, the display of T_{90} values has become very common in SODIS publications, which makes a comparison among different studies very difficult.

Evison (1988) also found that *Salm.* Typhimurium had an increased resistance to sunlight (artificial source) compared with *E. coli*. If this is true for all *Salmonella* strains, especially also for *Salmonella typhi*, this species should be considered as an indicator organism for SODIS effectiveness. However, it was shown that *Salm.* Typhimurium C5Nx^r exposed to artificial sunlight (probably ~700 W m^{-2}) for 8 h and a temperature regime temporarily reaching 55°C failed to produce detectable infections in *BALB/c* mice (Smith *et al.* 2000). Even culturable cells that had been irradiated for 1·5 h were less infective (virulent) than their nonirradiated counterparts. Furthermore, *V. cholerae* seems to be very susceptible to SODIS. This might be due to its preference for water with higher salt concentrations. Its natural niche is believed to be in brackish water (Louis *et al.* 2003). The influence of salt concentrations on the sunlight sensitivity of *V. cholerae* still has to be tested. In another study, it was shown that *V. cholerae* was more resistant to simulated sunlight than *Salm.* Typhimurium or *Sh. flexneri* (Kehoe *et al.* 2004). When one deduces from these data F_{90} values for the UVA light range, one finds slightly higher values for *V. cholerae* (≈230 kJ m^{-2}), but more than ten times lower values for *Salm.* Typhimurium and *Sh. flexneri* (≤ 100 kJ m^{-2}) compared with our study (see Table 1). The higher resistance of *V. cholerae* in the study of Kehoe *et al.* (2004) could be due to the use of phosphate-buffered saline (PBS), which probably had a higher salt concentration than our dilution medium, while the much lower resistance of *Salm.* Typhimurium and *Sh. flexneri* in their study might be due to different cultivation conditions or again the dilution medium, which might be less favourable for these organisms. A list of T_{90} values collected from different studies for inactivation of various bacteria by sunlight or artificial sunlight was provided by Reed (2004). The two values given for *V. cholerae* are 35 and 171 min, respectively, which differ by a factor of five. Unfortunately, it is not possible to deduce the reasons for these discrepancies between different studies; it might be either due to the reasons discussed here, or the different experimental methods, or the differences between strains. In a recent study, we demonstrated the influence of cultivation condition, particularly specific growth rate, on the sensitivity of bacteria to sunlight. Perhaps much of the discrepancies might be due to using cells cultivated differently. Furthermore, our results for *E. coli* compared well with those of previous reports (Evison 1988; Wegelin *et al.* 1994; McGuigan *et al.* 1998).

Vibrio cholerae was more susceptible to mild heat than the other three strains. This might be due to the same reason as described earlier. For the other three strains, temperatures above 47°C seemed to become lethal over a period of 5 h. Very striking was the fact that already a temperature increase of 1°C led to a measurable difference in sensitivity. Wegelin *et al.* (1994) reported earlier

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that a synergistic effect between sunlight and mild heat can be observed for *E. coli* at temperatures above 50°C. Our results confirm this observation and suggest a slight synergistic effect even at 48°C. McGuigan *et al.* (1998) reported a synergy between light and thermal inactivation already above 45°C for *E. coli*. The latter authors used HPLC grade water for dilution, which might have 'weakened' the cells before exposure. The synergistic effect between mild heat and sunlight is not well studied. Temperature regime can vary greatly depending on irradiation intensity, ambient temperatures and underlying surface. A recent field study confirmed that temperature is not a predominant factor in the elimination of bacteria with sunlight but that it is mainly radiation, which determines the efficiency of the method (Martin-Dominguez *et al.* 2005). However, we propose that the mild heat effect should not be neglected in SODIS experiments as a very small increase in temperature can immensely increase SODIS effectiveness for certain strains. Therefore, all possible and available measures should be taken to increase water temperatures in the bottles during SODIS.

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