Effect of solar water disinfection (SODIS) on model microorganisms under improved and field SODIS conditions

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ABSTRACT

SODIS is a solar water disinfection process which works by exposing untreated water to the sun in plastic bottles. Field experiments were carried out in Cochabamba, Bolivia, to obtain standard UV-A (320–405 nm) dose values required to inactivate non-spore forming bacteria, spores of *Bacillus subtilis*, and wild type coliphages. Inactivation kinetics for non-spore forming bacteria are similar under SODIS conditions, exhibiting dose values ranging between 15 and 30 $Wh\,m^{-2}$ for 1 $\log_{10}$ (90%) inactivation, 45 to 90 $Wh\,m^{-2}$ for 3 $\log_{10}$ (99.9%), and 90 to 180 $Wh\,m^{-2}$ for 6 $\log_{10}$ (99.9999%) inactivation. *Pseudomonas aeruginosa* was found to be the most resistant and *Salmonella typhi*, the most sensitive of the non-sporulating organisms studied here. Phages and spores serve as model organisms for viruses and parasite cysts. A UV-A dose of 85 to 210 $Wh\,m^{-2}$ accumulated during one to two days was enough to inactivate 1 $\log_{10}$ (90%) of these strong biological structures. The process of SODIS depended mainly on the radiation dose [$Wh\,m^{-2}$] an organism was exposed to. An irradiation intensity exceeding some 12 Wm^{-2} did not increase the inactivation constant. A synergistic effect of water temperatures below 50°C was not observed. Data plotting from various experiments on a single graph proved to be a reliable alternative method for analysis. Inactivation rates determined by this method were revealed to be within the same range as individual analysis.

Key words | home-based, low-budget water purification method, solar water disinfection

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ACRONYMS AND ABBREVIATIONS

SODIS	Solar Disinfection of Drinking Water
NTU	Nephelometric Turbidity Units
CFU	Colony Forming Units
PFU	Plaque Forming Units
UV	Ultra Violet radiation
PET	Polyethylene Terephtalate
FAD	Flavin Adenine Dinucleotide
ROS	Reactive Oxygen Species
UNICEF	United Nations Children's Fund
WHO	World Health Organisation
DIN	German Industrial Standard
EN	European Standard
OD	Optical Density
E. coli	*Escherichia coli*
St. faecalis	*Streptococcus faecalis*
P. aeruginosa	*Pseudomonas aeruginosa*

doi: 10.2166/aqua.2007.058

B. subtilis *Bacillus subtilis*
S. typhi *Salmonella typhi*
S. enteritidis *Salmonella enteritidis*
S. typhimurium *Salmonella typhimurium*

INTRODUCTION

Availability of safe drinking water for everyone throughout the year remains an urgent but unsolved problem. Major improvements are required as shown by UNICEF (Water, environment and sanitation statistics 2004), which reveal that 1.1 billion people still have no access to safe water. According to Wegelin *et al.* (1994), at least one third of the population in rural areas, poor suburbs and slums of developing countries and crisis areas are regularly exposed to numerous water-related diseases due to the consumption of pathogen-contaminated water. People rely on streams, rivers, ponds, rainwater from roofs, public cisterns, unreliable wells or fountain water (Rijal & Fujioka 2001). Since children are especially vulnerable to water-borne and diarrhoeal diseases caused by pathogens (e.g. typhoid, cholera, dysentery, norovirus, rotavirus, giardia, etc.), the transmitted pathogens are a major cause of infant and children mortality (Kehoe *et al.* 2001). Whereas, long-term operation of sophisticated water supply systems by qualified technical staff, and funds budgeted for long-term maintenance are common in urban centres and industrialised countries, low-cost water purification techniques are required to provide safe drinking water in developing countries and crisis areas. Such techniques, preferably applied at household level (e.g. boiling, chlorination, filtration), seem to be more feasible and their implementation more likely to be effective and sustainable. Solar Water Disinfection (SODIS), one of the home-based water treatment methods, takes advantage of solar energy abundant in many developing countries (Kehoe *et al.* 2001). The SODIS Manual "Solar Water Disinfection – A Guide for the Application of SODIS" of Meierhofer & Wegelin (2002) recommends exposing transparent polyethylene terephtalate (PET) bottles containing untreated raw water to direct sunlight for at least six hours. The raw water should not exceed 30 NTU (nephelometric turbidity units). The SODIS manual suggests SODIS application in regions between 35° N and 35° S, where SODIS users can generally rely on sufficient solar radiation throughout the year.

LITERATURE REVIEW

The aforementioned recommendations guarantee the efficacy of SODIS to inactivate non-sporulating bacteria and viruses (Acra *et al.* 1990; Wegelin *et al.* 1994; Sommer *et al.* 1997; Reed 1997*b*; Kehoe *et al.* 2001). The bactericidal effect of sunlight or of specific wavelength ranges of the sun's spectrum reaching the earth surface (e.g. UV-A 320–400 nm, UV-B 290–320 nm, so-called near-UV, 290–400 nm) have been known for decades (Kramer & Ames 1987; Reed 1997*a*, *b*). Results cited by Reed (1997*a*, *b*) revealed that even visible light is harmful to enteric pathogenic bacteria in water. These bactericidal sunlight properties can be applied in various contexts, ranging from laboratory and pilot scale water treatment plants, to designs for everyday usage (Webb & Brown 1979; Acra *et al.* 1984; Safapour & Metcalf 1999; Herrera Melian *et al.* 2000; Salih 2002, 2003; Solar Cooking 2003; Solar Water Pasteurization 2003). Since the ozone layer absorbs UV-C (100–290 nm) and PET absorbs the spectra below 320 nm (Wegelin *et al.* 2001), the DNA with its absorption maximum of about 250–260 nm is not the major point of impact (Moan & Peak 1989). Instead, UV-A ranging from 320 to 400 nm and wavelength ranges above 400 nm affect light-absorbing cell components, the so-called chromophores or their prosthetic groups (e.g. FAD, NAD, heme, quinons, porphyrins, Fe-S cores), the so-called endogenous photosensitizers (Webb & Brown 1979; Chamberlain & Moss 1987; Kramer & Ames 1987; Sammarton & Tuveson 1987; Eisenstark 1998). Absorption turns chromophores into an excited state and energy transmission occurs. If the energy acceptor is missing, energetic photons lead to conformational changes of chromophores and, consequently, to a loss of biological activity, culminating in the inactivation of the cell. Experiments by Chamberlain & Moss (1987) exemplify this supposition, showing membrane leakage of exponentially growing *Escherichia coli* irradiated with near-UV while maintaining a constant temperature of 27°C. Furthermore, excited choromophores can transmit absorbed energy to oxygen molecules, generating radicals, the so-called reactive

oxygen species (ROS). ROS react unselectively with cell components, damaging membranes (Chamberlain & Moss 1987) and the DNA by strand breakage and base changes (Kehoe *et al.* 2001). Some studies suggested an oxygen dependence of the SODIS process (Webb & Brown 1979; Kramer & Ames 1987; Reed 1997*a*) and led to recommendations to aerate water by vigorously shaking the bottles before solar exposure. Kramer and Ames reveal that overexpression of near-UV-absorbing proteins containing the aforementioned prosthetic groups increase the die-off rate of near-UV irradiated cells (Kramer & Ames 1987). Kowalski *et al.* (2002) could show a positive correlation of radiation intensity and *Aspergillus niger* spore inactivation, and the inactivation constant became larger when the spores where irradiated with increasing radiance. These findings led to the multi-target model where inactivation of a cell does not take place until enough sub-lethal damage to cell structures (organelles, membranes, enzymes, DNA) is accumulated. As a consequence, no measurable effect occurs until the so-called threshold dose is reached, reflected by the shoulder portion when inactivation is plotted on a graph of survival fraction against accumulated energy. This shoulder portion is known as the species and irradiance dependent threshold dose. Wegelin and coworkers could further demonstrate that direct absorption of radiation and the indirect impact of thermal water treatment (pasteurisation) above temperatures of 50°C result in a synergetic effect on microorganisms in the water, thereby leading to a more rapid inactivation compared to the isolated impact of radiation or heat (Wegelin *et al.* 1994).

RESEARCH OBJECTIVES

Frequent questions of health experts doubting the reliability of SODIS for all non-spore forming bacteria and phyisco-chemical resistant biological structures like viruses, spores or cysts were the motivation to do this research. Similarities and differences of the inactivation kinetics of six non-spore forming bacteria strains, one spore-forming bacteria type and one phage exhibits, are studied. This study aims at reaching two objectives: 1. To compare and classify the SODIS inactivation kinetics of common indicator organisms and pathogens. 2. To determine the effect of radiation intensity on the inactivation rate.

In addition, this publication provides further data on the effect of radiation intensities achieved during SODIS exposure, as well as data about organisms not previously studied in the SODIS research. Those were *P. aeruginosa*, a thermo-tolerant facultative pathogen which grows at temperatures as high as 42°C and *B. subtilis*, a spore-forming bacterium whose spores are difficult to inactivate. Several research groups (Payment 1998; Facile *et al.* 2000) and standards (DIN/EN 866-8, 2000) suggest spore-forming bacteria as conservative indicator organisms to evaluate the inactivation processes similar to the inactivation of parasite cysts (e.g. *Giardia lamblia*, *Cryptosporidium parvum*, worm eggs) by ozone and chlorination.

MATERIAL AND METHODS

This paper summarizes the results of two different series of experiments. After a first series, run between April 2002 and September 2003, experimental procedures were refined and different organisms were studied during a second series, carried out from April to October 2003.

Bacterial strains and growth conditions

In the first series, coliphages and *Salmonella* were studied along with *E. coli*. *Salmonella typhi* and *Salmonella enteritidis* which were biochemically reconfirmed Bolivian wild type isolates, purchased from the National Microbiology and Environmental Sanitation Institute INLASA in La Paz, Bolivia. *Salmonella typhimurium* was a lab strain ATCC 14028, made available by the microbial department of the Swiss Federal Institute of Technology (ETH). *E. coli* and all *Salmonella* species were cultivated under aerobic conditions in BHI (Brain Heart Infusion) by DIFCO for approx. 18 hours at 37°C. The coliphages were isolated and purified from the waste water lagoon of Cochabamba, Bolivia and proliferated on *E. coli* wild type plates. These samples were collected every two weeks in the early morning hours and processed as described by *Standard Methods* (APHA *et al.* 2000).

In the second series, the emphasis was on optimizing the exposure and the statistical relevance using larger

inocula and on minimizing the side effects of water quality.

E. coli, *Streptococcus faecalis* also classified as *Enterococcus faecalis*, and *P. aeruginosa* were grown under aerobic conditions overnight at 37°C in 100 ml tryptic soy broth (TSB) for approx. 15 hours. Some 40 ml of stationary phase cells, determined at OD 546 nm, were transferred into 50-ml Falcon plastic tubes. The tubes containing cultures of *E. coli*, *St. faecalis* and *P. aeruginosa* were subsequently centrifuged (10,290 *g*) for three minutes and washed twice with sterile water. Finally, the supernatants were discarded and the pellets resuspended in 40 ml distilled sterile water. *B. subtilis* spores were cultivated under aerobic conditions at 37°C for 24 hours, diluted in a phosphate buffer (APHA *et al.* 1995) and enriched with $MnCl_2$ (20 mg/l). A 10^{-2} dilution was then plated out on a tryptic soy sporulation agar enriched with $MgSO_4$ (0.01 M) and incubated for 15 days. Thereafter, the spores were harvested by rinsing the agar surface and scraping the spores off with a Drigalsky spatula. The spore-suspension was subsequently transferred into a 50-ml Falcon tube and dispersed in the suspension by a vortex. Finally, the spores were centrifuged three times at 10,290 *g* for 15 minutes, washed and resuspended. The first washing procedure was conducted with Tween-80 at 1% in order to mono-disperse the spores. Tween was then removed with sterile distilled water in two washing steps, before the spores were ready for use.

Exposure site and bottles

The experiments were conducted in the city of Cochabamba, Bolivia, a city located in the Andenean Cochabamba valley in central Bolivia at 17° 27′ S – 66° 08′ E and at an altitude of 2,553 m above sea level. Cochabamba has a dry and temperate climate and an annual mean precipitation of 482 mm, with marginal differences between winter and summertime. The average temperatures are 18.1°C in spring (September – December), 18.3°C in summer (December – April), 16.6°C in autumn (April – June), and 13.3°C in winter (June – September). South-east winds with an average velocity of 2 nodes are predominant in Cochabamba (Montes de Oca 1997).

The experiments were conducted with new, locally purchased smooth-surface 2-l PET bottles. The transmission properties of the bottles PET plastic are similar to the results published by Wegelin (Wegelin *et al.* 2001). The bottles were reused as long as they did not exhibit scratches which scatter and reflect the radiation. Prior to their reuse, the bottles were disinfected with a hypochlorite solution (4 mg/l) and washed with detergent. Thereafter, they were rinsed twice with distilled, sterile water and twice with the water used for the experiments. The inoculum concentrations for the improved and the field experiments are listed in Table 1.

Inoculation and exposure

For the first series, the water for inoculation of the test organisms and subsequent SODIS exposure was obtained from a cistern of public water supply services of the city of Cochabamba. The water quality of the first series was monitored in determined intervals. Turbidity was 0.8 NTU in the mean, with a standard deviation of 0.2 NTU and a pH value of 7.8 with a standard deviation of 0.4. Bacterial load was not measured because of the application of selective agar. In a first phase of the first series, the bottles were exposed to the sun from 9.00 am to 3.00 pm In a second phase the exposure time was extended from 8.00 am to 6.00 pm, due to the resistant nature of the coliphages against the solar disinfection. To simulate daily SODIS use by normal SODIS users, the corrugated iron sheet was placed in a horizontal position without regard to the sun's zenith. *E. coli* and coliphages (coliphages S) were exposed without any optimization. In the second phase, the arrangement was modified for coliphages in order to achieve an accelerated SODIS inactivation (coliphages B, BM). To this end, the bottles were exposed inside elliptic aluminium tubs commonly used for cooking in order to concentrate and reflect the incoming radiation. The tubs had a diameter at the base of 28 and 38 cm, and a diameter of 33 and 43 cm at the edge. The side height measured 10 cm and the side walls had an inclination of 70°. Such tubs were applied in unmodified shape (coliphages B) as well as in a modified version exhibiting almost a parabolic shape (coliphages BM). Each bottle contained a thermometer of ±1°C sensitivity and −20–110°C temperature range. Cell counts and temperatures were registered on an hourly basis.

Table 1 | Experimental conditions. Conditions: Common SODIS exposure (S), aluminium tub (B), modified aluminium tub (BM) with parabolic shape. Series: Series of experiments. Assays: number of assays. Samples: mean number of observations per assay

							Inoculum/100 ml				
Organism	**Conditions**	**Series**	**Method**	**Period**	**Assays**	**Samples**	**Mean**	**Median**	**Stdev**	**Min**	**Max**
Coliphages S	S	1	Plaque	04–10/02	42	4.7	5.1 E + 03	3.4 E + 03	4.6 E + 03	2.3 E + 03	1.6 E + 04
Coliphages B	B	1	Plaque	08–10/02	25	4.7	3.9 E + 03	3.8 E + 03	1.7 E + 03	1.8 E + 03	9.1 E + 03
Coliphages BM	BM	1	Plaque		20	4.8	4.3 E + 03	4.3 E + 03	1.7 E + 03	2.0 E + 03	9.1 E + 03
S. enteritidis	S	1	Membr	09/02–09/03	21	7	1.8 E + 07	1.7 E + 07	8.5 E + 06	5.6 E + 06	5.0 E + 07
S. typhi	S	1	Membr		21	7	1.8 E + 07	1.7 E + 07	1.1 E + 07	4.1 E + 06	6.1 E + 07
S. typhimurium	S	1	Membr		21	7	1.9 E + 07	1.7 E + 07	1.4 E + 07	3.2 E + 06	7.4 E + 07
E. coli (1^{st} series)	S	1	Membr	04–10/02	36	8.7	2.9 E + 03	2.1 E + 03	2.2 E + 03	1.0 E + 03	9.8 E + 03
E. coli (2^{nd} series)		2	Membr	04–10/03	10	8.7	9.1 E + 07	9.7 E + 07	6.4 E + 07	3.0 E + 06	2.1 E + 08
P. aeruginosa (F.M.)		2	Membr	08–10/03	8	9	3.7 E + 08	2.6 E + 08	2.8 E + 08	7.6 E + 07	9.4 E + 08
P. aeruginosa (P.C.)		2	Plate		7	9	1.9 E + 08	2.6 E + 08	1.4 E + 08	1.8 E + 06	3.8 E + 08
St. faecalis (F.M.)		2	Membr		10	9	5.6 E + 07	5.6 E + 07	1.5 E + 07	3.3 E + 07	8.8 E + 07
St. faecalis (P.C.)		2	Plate		11	9	5.3 E + 07	5.6 E + 07	1.1 E + 07	3.3 E + 07	6.6 E + 07
B. subtilis		2	Membr		7	9.3	7.0 E + 07	3.4 E + 07	8.9 E + 07	1.9 E + 07	2.5 E + 08

In the second series, the procedures were refined in the following manner. The inoculation water was purchased from Chacaltaya table water company (Chacaltaya, Cochabamba, Bolivia) originating from a 50-m deep groundwater aquifer. During the quality monitoring of the second series every individual assay was taken into consideration and the bacterial contamination was measured due to the parallel cultivation in selective and TSA agar. Turbidity stated at 0.4 NTU in the mean, with 0.3 NTU standard deviation, pH stated at 6.3 in the mean with a standard deviation of 0.2. Fecal coliforms were never detected; the mean concentration of total coliforms stated at 0.6, with a standard deviation of 1, a median of 0 and a maximum count of 4. Heterotrophic bacteria were detected prior to pre-treatment (245.6 in the mean, 30 median, 332.5 standard deviation, and 1,840 maximum count) and after pre-treatment (95.6 in the mean, 14 median, 146 standard deviation and 900 maximum count). After filling and prior to inoculation, the bottled water was exposed to the sun (SODIS) for the so-called pre-treatment during two full days to reduce potential bacteria occurrence. Before inoculation, the outer bottle surfaces were disinfected with ethanol (70%) and 1/4 of the bottle content was emptied into a sterile tank. Inoculation was conducted with the washed inoculum of *E. coli*, *St. faecalis*, *P. aeruginosa*, or *B. subtilis* spores. All the inoculated bottles were oxygenated by vigorous shaking for 20 seconds as described in the SODIS User Manual (Meierhofer & Wegelin 2002), except for two control bottles which were refilled without shaking. Subsequent to the shaking, the bottles were refilled with the saved water, leaving a space of 3 cm between lid and water surface. Duplicate bottles for every sampling point were prepared. The bottles were exposed to the sun on the corrugated stainless iron roof having a tilt of 22° facing the sun's zenith in the north. The exposure time throughout the experiments was from 9.00 am – 17.00 pm or, in the case of *B. subtilis*, until 17.00 pm of the following day. Six bottles containing clear water and thermometers of ± 1°C sensitivity and − 20 – 110°C temperature range were distributed on the roof, two on each side of the roof, and two between the bottles which contained the bacteria. The temperatures were registered on an hourly basis and the average of the six values was calculated. Cell counts were obtained every other hour for *B. subtilis*, and hourly for all other organisms.

Cell recovery and enumeration

Filter membranes incubated on selective agar and plate counts in tryptic soy agar (TSA) were applied in parallel as enumeration methods under improved conditions. In the first series, filter membranes incubated on selective agar and plaque forming assays for coliphages proposed by *Standard Methods* (APHA *et al.* 1995) were applied. Selective mFC agar (DIFCO) for *E. coli*, KF-Streptococcus agar (Oxoid) for *St. faecalis*, Cetrimide agar (Fluka) for *P. aeruginosa* and AgarSS (DIFCO) for *Salmonella* were used. The cellulose acetate membranes with 0.45 μm porosity and 47 mm diameter were incubated at 37°C for 48 hours, or 24 hours in the case of *St. faecalis*. The typical colonies were assumed to be *E. coli*, *St. faecalis*, *P. aeruginosa* and *Salmonella*. *B. subtilis* was enumerated only by membrane filtration. Samples were taken during exposure and diluted with sterile phosphate buffer (APHA *et al.* 1995). Prior to filtration, the filter was rinsed with sterile phosphate buffer followed by the sample filtration. After approximately three hours of exposure, dilution was not necessary anymore and filtration was done directly with the samples. For the last sampling point the entire bottle content was always filtered.

In the plate count method, 1 ml of the diluted or 1 – 5 ml of the undiluted sample were mixed with liquid TSA at 45°C and relayered with TSA after initial solidification. The Petri dishes were then incubated for 48 hours at 37°C. The assumed counts were confirmed biochemically by 5 randomly chosen colonies to offset the error margin. The results, expressed in CFU and PFU/100 ml, include dilution factors, processed sample volumes, correction factors, and statistical weighing of the results (Ettel *et al.* 2000).

Controls

Control bottles were stored at room temperature in the dark. They were processed at the end of an exposure day for experiments with non-spore forming bacteria and at the end of the second exposure day for experiments with coliphages and *B. subtilis.* Non-spore forming bacteria exhibited an average reduction of 5.1% with a standard deviation of 20.1%. The control bottles of the *E. coli* and the coliphage trials showed a reduction of 6.6% and 3.7%, with standard deviations of 5.6% and 3.3%, respectively. *B. subtilis*

experienced an average reduction of 2.8%, with standard deviation of 18.4%.

Radiation measurement

UV-A (320–405 nm) irradiance [Wm^{-2}] on a horizontal plane was measured with a MACAM SD 104 A-Cos sensor, exhibiting an optimal response at 375 nm, 5.98×10^{-8} $A(Wm^{-2})^{-1}$ sensitivity and 16.71 $A(Wm^{-2})^{-1}$ conversion constant. Global irradiance (400–1,800 nm) was measured in parallel with a LI-COR sensor and a conversion constant of $-8.94\,Wm^{-2}\,\mu A^{-1}$. Both sensors were placed in an open area, free from interferences such as shade, reflection or diffusion. The radiation intensities were measured every 15 minutes and the values converted and stored in a Data Logger using the ASCII code. The irradiance data, also known as flux, was converted according to Simpson's rule for numeric integration into dose values [$Wh\,m^{-2}$] or [kJm^{-2}] using Matlab.

Inactivation kinetics

A single-exponential decay-law is a suitable approximation of the real inactivation process:

$$N(t) = N_0 e^{-\kappa F(t)} \tag{1}$$

where N_0 is the initial bacterial load and $N(t)$ is the bacterial load after an irradiation period of length t [s]; $F(t)$ is the fluence, or accumulated radiation dose up to time t, [$Wh\,m^{-2}$] or [kJm^{-2}]; and κ [$m^2\,kJ^{-1}$] or [$m^2\,Wh^{-1}$] is the inactivation rate, which is characteristic for an organism under a determined disinfection process in consideration of the environment, settings and device. Equation (2) is obtained by taking logarithms and considering differences in order to obtain rates of change,

$$[\log(N(t+\Delta t)) - \log(N(t))]/\Delta t = -\kappa[F(t+\Delta t) - F(t)]/\Delta t = -\kappa I(t) \tag{2}$$

This equation relates the rate of bacterial inactivation, expressed in logarithms, to the intensity I of the irradiation. We will find evidence that the inactivation becomes independent of the irradiation intensity if the intensity exceeds some threshold. In this case, the right hand side of Equation (2) is replaced by a constant inactivation rate $-k$.

For practical applications, it is useful to consider the time until the number N is reduced to a fraction of 10^{-p}, with $p = 3$, say. This time is simply obtained as

$$T_p = p\log(10)/\kappa I \tag{3}$$

where I is a mean radiation intensity.

Some organisms can tolerate a certain amount of irradiation before they start decaying. This leads to an initial period with little change, after which the inactivation process can often be modelled again by the above exponential law. If α and $-\kappa$ are the intercept and slope of a straight line through the points of the decay phase, the time T_p is calculated as

$$T_p = [p\log(10) + \alpha - \log(N_0)]/\kappa I \tag{4}$$

Wegelin (Wegelin *et al.* 1994) and Kowalski (Kowalski *et al.* 2002) summarize the inactivation kinetics commonly used in UV inactivation research. UV disinfection results are equally published in W·h and kJ. Conversion allows a comparison of results published in kJ. Those must be divided by 3.6 to obtain W·h, as 3.6 kJ = 1 W·h.

Statistical analysis

An inactivation constant κ_b was determined for each bottle b as the slope of a straight line fitted to the pairs $[F(t_i), \log(N(t_i)]$. Measurements of 0 CFU cannot be used directly for the ordinary regression analysis corresponding to the model, since the logarithm of 0 is ill-defined. Therefore, these values were treated as censored at the "detection limit" of 1 CFU, and a line was determined by Tobit regression – the adequate estimation procedure for such data - if values of 0 were present. Means with confidence intervals and other summary statistics are calculated across the κ_b values for all bottles with the same conditions. For comparison purposes, a single straight line is fitted to all data points of all bottles of the group. Whereas its κ value agrees well with the mean of the κ_b values, adequate confidence intervals cannot be obtained from such an analysis.

In order to examine the adequacy of the model, the left hand side of Equation (2), the change rates, were plotted

against the right hand side, the intensity values. This revealed that the change rates were not proportional to the intensity, as suggested by Equation (2), but rather constant, as has already been mentioned.

RESULTS AND DISCUSSION

Data gathered during this study subjecting eight different organisms to real SODIS conditions in parallel, reconfirm published results obtained under laboratory and field conditions. Reed (1997*a*, *b*) detected a 6 $\log_{10}$ inactivation during two to three hours for *E. coli* and *St. faecalis* after subjecting the organisms in SODIS bottles to real sunlight. Fujioka *et al.* (1981) found a 2 $\log_{10}$ inactivation for faecal streptococci within three hours of exposing them to the sun suspended in seawater and phosphate buffer maintaining at 24°C. Wegelin *et al.* (1994) found a 3 to 4 $\log_{10}$ inactivation during about five hours for *E. coli* and *St. faecalis*, maintaining the temperature at 20°C. In contrast to our data, Wegelin *et al.* (1994) detected a 2 $\log_{10}$ inactivation for f2 coliphages during less then ten hours of exposure, maintaining the temperature at 30°C, while we measured about eleven hours of exposure for a 1 $\log_{10}$ inactivation of wild type coliphages. Safapour & Metcalf (1999) found a 2 $\log_{10}$ inactivation of a T2 phage within three hours of applying their "Cookit" reflector, exposing a transparent bottle to direct sunlight. Sammarton & Tuveson (1987) found a 4 $\log_{10}$ inactivation for *E. coli* with about 20 $Wh\,m^{-2}$ artificial UV-A light (320–400 nm, intensity 25 W/m^2). Kramer & Ames (1987) found a 4 to 5 $\log_{10}$ inactivation for *S. typhimurium* within approximately five hours of exposure to artificial UV-A light (320–400 nm, intensity 35 W/m^2). Organisms were irradiated maintaining them at 37°C in a minimal glucose medium. Smith *et al.* (2000) subjected *S. typhimurium* to artificial sunlight obtaining a 7 $\log_{10}$ inactivation for *S. typhimurium* within approximately eight hours.

Our data allows for classifying the organisms into two groups: persistent (coliphages, *B. subtilis* spores), and SODIS-sensitive (all vegetative bacteria). This is shown by the graphical display of the data in Figures 1 to 3 as well as the inactivation constants κ listed in Table 2.

Inactivation constants and times based on total irradiation

Except for *B. subtilis* to be discussed below, all organisms exhibited an exponential inactivation as modelled by Equations (1) or (2). Therefore, the inactivation constants are proportional to the times T_p needed to achieve a reduction to 1/10 (90%), 1/1,000 (99.9%), or 1 in 10^6 (99.9999%) for a given radiation intensity. Mean intensities at the experimental site were 25.6 Wm^{-2}, 16.3 Wm^{-2}, and 9.6 Wm^{-2} for an optimal, an average, and a cloudy day, respectively. The times given in Table 2 refer to a reduction to 1/10 on an average day. For a reduction to 1/1,000 or 10^{-6}, such a time is multiplied by 3 and 6, respectively. If the inactivation rate is proportional to the radiation intensity (Equation (2)), the time will be multiplied by 0.64 (= 16.3/25.6) and 1.7 for an optimal and cloudy day, respectively.

The table shows that the coliphages need a full optimal day for a reduction to 1/10. The improvement achieved by using the cooking tubs is noticeable, leading to times which are about 30% shorter.

As an advantage of the selective agar the organisms under study can be easily distinguished from potential contamination. On the other hand the selective agar can prevent the growth of weak organisms damaged by the SODIS procedure and the counts were slightly inferior in the case of *St. faecalis* in comparison to the TSA plating technique. A comparison of the two inactivation constants

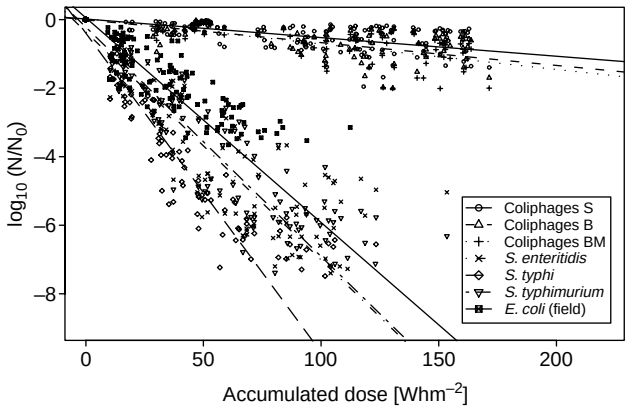

Figure 1 | Inactivation and cumulated dose for all data of the first experimental series. The lines stand for simple exponential inactivation. *E. coli* and the *salmonellas* degrade much more rapidly than the coliphages. "S" is the common exposure, "B" is the exposure inside of the tubs without -, "BM" with parabolic modification. The slope corresponds to the inactivation constant κ [m^2/Wh]. Zero counts not shown.

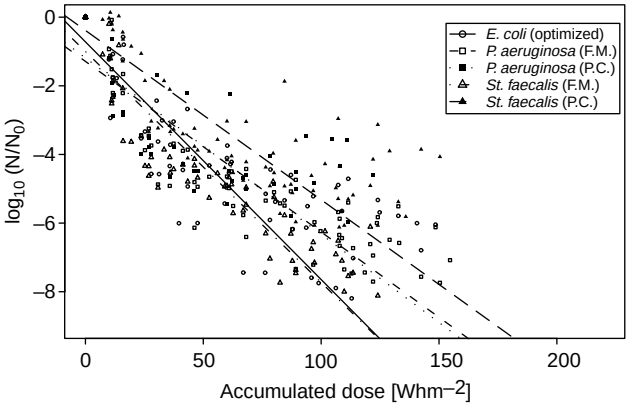

Figure 2 | Inactivation and cumulated dose for all data of the first experimental series. The lines stand for simple exponential inactivation. *E. coli* and the *salmonellas* degrade much more rapidly than the coliphages. Zero counts not shown.

in Table 2 exemplifies this thesis and was the reason for the application of a rich agar.

Relation between irradiation and decay

It is common and plausible to assume that the achieved reduction is a function of the total irradiation, as described by Equations (1) and (2). For comparison purposes, a single straight line is fitted to all data points of all bottles of the group. Whereas its κ value agrees well with the mean of the κ_b values, adequate confidence intervals cannot be obtained from such an analysis.

In order to examine the adequacy of the model, the left hand side of Equation (2), the change rates, were plotted against the right hand side, the intensity values (lowess fit).

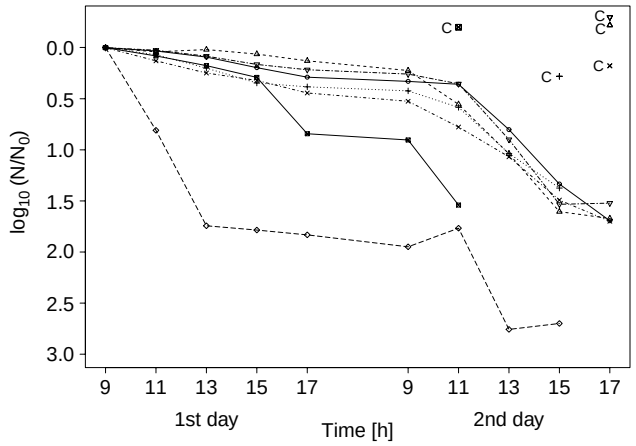

Figure 3 | Inactivation of *B. subtilis*. In 7 assays, slow inactivation on the first day was followed by a higher rate on the second day.

This revealed that the change rates were not proportional to the intensity, as suggested by Equation (2), but rather constant, as has already been mentioned.

For all the organisms under study except for the coliphages just discussed, the rate of inactivation does not seem to depend on the irradiation intensity as long as this intensity exceeds some 12 Wm^{-2} – and this is the case in our experiments, except for some morning hours on cloudy days given by Figure 4. Our results indicate maximum inactivation kinetics under sun irradiation, which is an indication of a determined inactivation process, depending on the destruction velocity of determined cell components (e.g. organelles, membrane proteins, prosthetic groups). Hence, it makes more sense to assume a constant inactivation rate and to calculate inactivation times from them directly, as shown in Table 2. These times apply under the condition of a sufficient radiation intensity $> 12\ Wm^{-2}$.

Relation between temperature and decay

Temperatures above 50°C have an important synergistic effect for solar disinfection (Wegelin *et al.*, 1994). Temperatures inside the bottle depend directly on the acquired radiation dose, on its spectra and the angle the sun irradiates the bottle. The dry and thin atmosphere, an average ambient temperature of 24 to 27°C between 11:00 and 17:00, and strong winds usually cooled the bottles down and resulted in maximum mean temperatures of 44°C. 50°C was exceeded in only 5% of the experiments and at most for an hour or two. Temperatures between 40 and 45°C commonly reached in the presented assays, theoretically accelerate the reaction kinetic of chemical and especially ROS reactions. A loss of biological activity of important structures (membranes, chromophores, proteins or lipid structures in general) should be a consequence. A plot of inactivation rates on temperature revealed no evidence for any influence of elevated temperatures on the inactivation process. Hence, the supposition of the importance of ROS reactions for the SODIS process, where dissolved oxygen forms radicals, could not be proved.

Inactivation times for high radiation intensities

The times for a reduction to 1/10 given in Table 2 show that *E. coli*, all *Salmonella* species, as well as *P. aeruginosa* and

Table 2 | Inactivation constants κ: Inactivation constant w.r.t. cumulated radiation. The mean and other statistics over the κ values times 1,000 for the individual assays are shown. Inactivation time, from cum. dose: Time by which the necessary dose for a 1 $\log_{10}$ inactivation is achieved on an average day (16.9 Wm^{-2}). From Mean: calculated from the mean κ. All data: calculated from κ based on pooled data. Direct: Time obtained from assuming a constant inactivation rate, independent of radiation intensity

	$1000 \cdot \kappa$ [m^2/Wh]					$1 \log_{10}$ Inact. time [h] by analysis: from dose		
Organism	**Mean**	**Median**	**Stdev**	**Min**	**Max**	**From Mean κ**	**All data**	**Direct**
Coliphages S	13	11	8	4	33	11.27	13.61	11.15
Coliphages B	15	13	7	6	29	9.19	11.06	9.52
Coliphages BM	17	15	8	6	37	8.23	8.86	8.35
S. enteritidis	153	154	41	80	266	0.92	1.11	0.80
S. typhi	215	206	52	115	304	0.66	0.84	0.58
S. typhimurium	154	152	40	102	267	0.91	1.07	0.79
E. coli (1^{st} series)	138	129	45	68	270	1.02	1.45	0.90
E. coli (2^{nd} series)	159	161	53	105	269	0.88	1.32	0.81
P. aeruginosa (F.M.)	114	114	23	84	153	1.23	1.35	1.13
P. aeruginosa (P.C.)	118	112	46	62	203	1.19	1.66	1.01
St. faecalis (F.M.)	155	155	22	134	203	0.91	1.02	0.80
St. faecalis (P.C.)	114	117	36	49	175	1.24	1.53	1.09
B. subtilis							12.4	14.82 *

St. faecalis are reduced to this level within less than 68 minutes. Thus, a reduction to less than $>10^{-5}$ is achieved within 6 hours of exposure.

The most rapid inactivation among the organisms studied here was observed for the *Salmonella* species followed by *St. faecalis*.

Results for *B. subitlis*

The data for *B. subtilis* exhibit a two phase process of decay, see Figure 3. On the first day of exposure, their count is reduced to about 46% (neglecting the one atypical bottle). On the second day, the inactivation is much faster and seems to follow the exponential law again. The inactivation time is therefore calculated differently, see the Methods Section. Within two full days of exposure, these bacterium spores are reduced to less than 1/35 of the initial concentration.

Remarks about statistical aspects

E. coli was studied in both series of experiments. The inactivation rates differed by some 15%, which turns out to be statistically insignificant ($p = 0.18$ for a Wilcoxon test).

These results suggest that the statistical error calculated from the repetitions of assays with identical conditions is overly optimistic. Its calculation may give a lower bound for the statistical uncertainty in such experiments.

We have estimated the degradation constant for each replicated assay and then averaged these estimates for obtaining a constant for given experimental conditions. Alternatively, the data may be pooled, and a straight line fitted

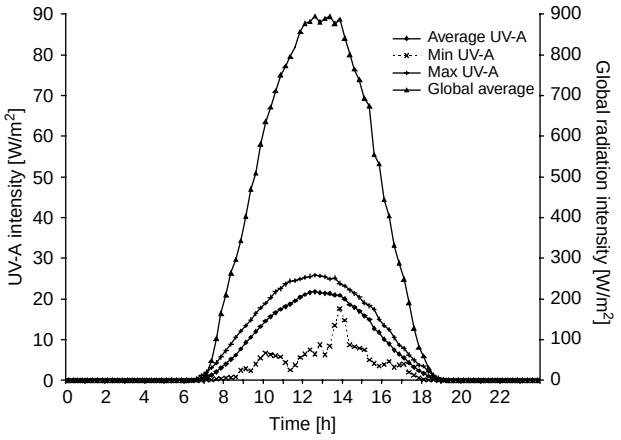

Figure 4 | Average irradiation intensity [W/m^2] of UV-A and global radiation (400–1800 nm) and the minimum and maximum UV-A values for UV-A for the period of August to October 2003. Integration over time results in the dose value. An intensity dependence of the inactivation constants could be observed up to a threshold intensity of 12 W/m^2.

Table 3 | Minimum exposure time to sun radiation with a UV-A portion >12 W/m^2 guarantees an inactivation of indicated bacterial loads

Exposure time to average sunshine (UV-A >12 W/m^2)	Inactivation levels for vegetative bacteria	Inactivation levels for phages and protozoan cysts
6 hours	$>5\log_{10}$	$<1\log_{10}$
12 hours (2 days)	not necessary	$>1\log_{10}$, $<2\log_{10}$

to all pertinent data. The respective results are given in the last column of Table 2. The values obtained by this somewhat simpler analysis are very similar to those obtained from averaging over the assays. The standard errors which may be formally obtained from the analysis of the pooled data would however be much too small – smaller than the standard errors just discussed – since the data from the same assay are not independent in this framework.

CONCLUSIONS

Table 3 summarizes the results obtained during the study here presented. Inactivation kinetics of vegetative bacteria shows a remarkable similarity, leading to a general approach for the inactivation of vegetative bacteria subjected to SODIS. An entire day of exposure was enough to inactivate up to $6\log_{10}$ (99.9999%) of non-spore forming bacteria and therefore make the water safe for use, taking into consideration the recommendations of SODIS guidelines (e.g. turbidity, exposure site). The non-spore forming bacterial load could hence be reduced within nine hours for more than $3\log_{10}$ for all bacteria types, exposed even during unfavourable climatic conditions. Amplification of irradiation comparing inactivation times of coliphages S with coliphages BM improves the disinfection. Approaches to increase the disinfection prosperities of SODIS (direction of SODIS bottles to the suns zenith, applying reflective support, windbreakers, parabolic shaped exposure containers…) are difficult and their communication has to be done properly by an implementation specialist who is aware of misinterpretation and misunderstandings thus keeping SODIS easy. It appears that inactivation is not enlarged if radiation intensity increases beyond 12 Wm^{-2}, except for the coliphages. A full day of exposure of a SODIS bottle produces a reduction of less than 1/10 in coliphages and *B. subtilis*. Although we cannot assess the potential infectivity and the risk deriving from pathogens being exposed to one day of sunlight, we would highly recommend a second day of exposure, in order to improve the protection against *B. subtilis*- and coliphage-like biological structures. As far as the coliphages can serve as a model for viruses harmful to humans, and *B. subtilis* spores may stand for other parasite cysts, these results show that a two day exposure of SODIS bottles to radiation at a level common in many third world countries, increases the water quality by more than 90% for viruses, spores and cysts. Whether these concentrations are considered to be safe, depends on several factors such as initial pathogen concentration and infectious dose or the immune response of an individual. Hence, if a total inactivation of spores or viruses is required, boiling water or chlorination remain the methods of choice.

ACKNOWLEDGEMENTS

We are thankful for the support provided during the project by Thomas Egli and his group of the Microbiology Department of the Swiss Federal Institute for Environmental Science and Technology (EAWAG). We greatly appreciate the reviews conducted by Silvio Canonica, Michael Berney from the Swiss Federal Institute for Environmental Science and Technology and the group of Daniel Mäusezahl and his assistant Michael Hobbins from the Swiss Tropical Institute. We thank the Swiss

Embassy of La Paz, Bolivia, for their administrative work and the lectors Silvy Peter and Christof Elmiger.

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First received 15 September 2005; accepted in revised form 2 February 2007