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Solar water disinfection: scope of the process and analysis of radiation experiments

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ABSTRACT: The exposure of water to sunlight radiation improves the microbiological quality of water. This treatment process called solar water disinfection could be used at household level to treat small quantities of water for drinking purposes. The bactericidal effect of UV-A and violet light and their combined effects assumed as hypothesis by earlier research could be verified by the laboratory tests carried out at EAWAG. A 3-log reduction of *E. coli* requires a fluence close to 2,000 kJ/m^2 or 555 W·h/m^2 (dose of solar radiation integrated in the 350–450 nm wavelength range), which correspond to ~ 5 h of mid-latitude midday summer sunshine. The same dose inactivates also the bacteriophage f2 and a rotavirus to a similar order of magnitude, whereas a picornavirus (encephalomyocarditis virus) was observed to be twice as resistant. Water temperatures with a threshold of ~ 50 °C considerably increase the inactivation rate of bacteria induced by solar radiation whereas the inactivation rate of viruses steadily increases with temperatures in the range of 20–50 °C. The recorded synergetic effects of solar radiation and thermal water treatment favour a combined use of these two water-treatment processes.

Désinfection solaire de l'eau: efficacité du procédé et analyse des essais de radiation

RESUME: La qualité microbiologique de l'eau s'améliore lorsqu'elle est exposée au soleil. Ce procédé de traitement appelé désinfection solaire de l'eau peut être utilisé à un niveau domestique pour traiter des petites quantités d'eau à des fins de production d'eau potable. L'effet bactéricide du spectre des rayons ultraviolets A et de lumière violette et ses effets combinés présumés par les recherches antérieures, ont été être confirmés par des essais de laboratoire à l'EAWAG. Une réduction de *E. coli* de 3 unités logarithmiques requiert une fluence de presque 2.000 kJ/m^2 ou 555 W·h/m^2 (dose de radiation solaire intégrée dans des longueurs d'ondes de 350–450 nm), ce qui correspond à environ 5 heures de soleil estival à midi sous environ 45° de latitude. La même dose permet aussi une inactivation semblable du bactériophage f2 et d'un rotavirus. Tandis qu'un picornavirus (virus encephalomyocarditis) est deux fois plus résistant. Une température seuil de l'eau d'environ 50 °C augmente considérablement le taux d'inactivation des bactéries provoquée par la radiation solaire, alors que le taux d'inactivation de virus augmente progressivement avec des températures entre 20° et 50 °C. Les effets synergiques de la radiation solaire ainsi que du traitement thermique de l'eau favorisent l'usage combiné de ces deux procédés de traitement de l'eau.

INTRODUCTION

At least one-third of the population in developing countries has no access to safe and reliable drinking water supplies. The lack of adequate water supply and sanitation facilities seriously exposes this unserved population to numerous water-related diseases. A dominant communicable disease is diarrhoea that is largely transmitted through the consumption of contaminated water. There are about 875 million cases of diarrhoea per year of which 4.6 million end in death. Different levels of inadequate water supply contribute to this toll, e.g.:

- lack of public water supply implies that a large part of the rural population is still forced to use contaminated surface water;
- unreliable public water supply due to frequent interruptions or breakdowns drives people back to polluted water sources;
- public water supply which distributes water unsafe for consumption exposes the supplied population to a considerable health risk.

As a consequence, substantial investments will have to be made in order to achieve full coverage in the near future. Due attention must be paid to the operation and maintenance of existing water supply schemes in order to increase their reliability in supply and their treatment efficiency. However, public funds are not sufficiently available to cover the estimated investment costs of $150 billion for full water supply coverage in developing countries. Choice of inappropriate technologies, missing operation and maintenance work, difficulties in the procurement of fuel and spare parts, and weak management structures often account for the poor performance of many existing public water supplies in developing countries. In order to overcome these problems it is essential to:

- reduce substantially the costs of water supplies through increased use of appropriate low-cost technologies;
- install only water supply facilities whose operation and maintenance can be managed and sustained with local resources.

Self-help individual water supply systems operating at household level is certainly one approach that will fulfil these criteria. On this low management level, the choice of an adequate technology will be especially decisive for the viability of the system. Boiling of water, disinfection with chlorine and filtration through ceramic filters are treatment methods often propagated at household level. However, these methods generally face the following problems:

- boiling of water requires energy which in rural areas is usually supplied in form of firewood. This type of water treatment is no longer a good practice, particularly in areas where firewood is scarce and deforestation is in progress;
- disinfection by chlorine compounds is often rejected by the consumers due to the undesirable taste and odour acquired by the water. In addition to these complaints, the use of chemicals often poses great problems with respect to their reliable supply, timely distribution and correct dosage;
- filtration through ceramic filters is often an unaffordable treatment method. However, apart from being expensive, ceramic filter candles are subjected to frequent clogging and often leak through fine cracks caused by careless handling.

The stated problems call for the development of alternative treatment techniques that are effective, practical and simple enough to be applied by individuals or households. Solar water disinfection is considered to be such an alternative. The treatment process is a simple technology using solar radiation to inactivate and destroy pathogenic bacteria present in water. Essentially, the treatment consists in filling clean and transparent containers with water which are then placed in full sunlight for several hours. The use of solar energy, which is universally available and free of charge, is the basis of this low-cost and sustainable technology.

Solar water disinfection aims at treating small quantities of drinking water in the order of 2 L per person per day or of approximately 10–15 L per family per day. This technology is designed to resolve the water quality problems of those people in developing countries deprived of a safe drinking water supply.

However, solar water disinfection has its limits too. Solar radiation is dependent on the geographic location and climatic conditions, and undergoes diurnal and annual variations. The application of solar water purification is simple. However, exposing a small quantity of contaminated water to solar radiation is a complex interaction of physical, chemical and biological processes which are not yet clearly understood.

LITERATURE REVIEW

Solar radiation is an ancient disinfection practice used without profound understanding of the process. However, different research groups have recently started to study the process of solar water disinfection. The following brief literature review concentrates on information pertinent to the further development of this alternative water-treatment technique.

Bacteria inactivation by radiation

Specific research on solar water disinfection was initiated by Prof. Aftim Acra at the American University of Beirut, Lebanon, in the late 1970s. His group carried out some field tests to assess the effect of solar radiation on the quality of water used in oral rehydration solution. The field experiments were carried out as batch tests with transparent containers of 1–3 L in volume. The main results, reported by Acra *et al.* [1], can be summarised as follows:

- *E. coli* strains are slightly more resistant to the lethal effects of sunlight than other bacteria (*P. aerugenosa, S. flexneri, S. typhi, S. enteritidis*). *E. coli* strains therefore serve as indicators to assess the effect of sunlight on enteric bacteria. Under the test conditions, a 75-minute exposure time achieved a 3- log reduction of *E. coli*.
- The effective component of solar radiation involved in microbial destruction seems to be the near-ultraviolet (A) band (320–400 nm) and to a lesser extent the visible band of violet and blue light (400–490 nm) – a hypothesis requiring verification by specific tests.

Acra carried out additional field research in 1986/87 using continuous-flow solar disinfection units and reactor volumes of ~ 5–18 L. These experiments were run at constant flow conditions with exposure times between 8–66 min. The test results presented by Acra *et al.* [2] can be summarised as follows:

- Compared with *E. coli* and coliforms, *Str. faecalis* were slightly more resistant to solar radiation and required a UV-A fluence of ~ 27 W·h/m^2 for a 3-log reduction.
- High bacteria concentrations showed a lower sensitivity to solar radiation compared with those of low or moderate density. In order to avoid prolonged exposure times, the tests were carried out with relatively low concentrations (e.g. 2–6000 CFU/mL for *E. coli*) in contrast to the bacte-

rial density range of 10^6–10^8 cells/mL generally used in research.

- Results of experiments involving the use of a pure *E. coli* culture differ from those using sewage as source for water contamination by coliforms and *Str. faecalis*. The use of sewage-contaminated water is recommended for solar radiation tests. However, no detailed information is given on the observed differences (shoulder effects (?) as recorded in the EAWAG tests).
- No visible growth of phytoplankton occurred at the inner surfaces of the solar reactors. Loss of soluble gases and absence of sufficient quantities of nutrients seem to be some of the reasons for this observed phenomenon.

Motivated by Acra's work, the Integrated Rural Energy Systems Association (INRESA), an associated programme of the United Nations' University, initiated a network project in 1985 which encourages local institutions to start research and to contribute to the information dissemination and technology transfer of solar water disinfection. The Brace Research Institute (BRI) of the McGill University in Montreal, Canada, was selected to host INRESA's secretariat. The field results carried out by five research institutions located in Peru, Colombia, Nigeria, Egypt, and Sri Lanka, were presented and reviewed by a workshop held at BRI in August 1988. The results of this research, as well as the main findings and recommendations for further investigations are summarised in the workshop proceedings [3]. However, some of the field tests did not use standardised methods and were conducted by people of different professional background (engineers, microbiologists, biochemists or physicist). The results are hardly conclusive, and general guidelines for the design and operation of solar water disinfection installations could therefore not be developed. It became evident that a considerable amount of additional research is necessary before this technology can be effectively used under field conditions. Some of the principal findings of INRESA's network project were the following:

- Acra's laboratory results were confirmed qualitatively by field tests in different parts of the world. Solar radiation exerts a germicidal effect on bacterially contaminated water.
- Solar water disinfection can be applied effectively if a solar radiation intensity of at least 500 W/m^2 is available for about 5 h, and provided contamination of the raw water does not exceed 1000 faecal coliforms/100 mL.
- A water temperature variation between 12 and 40 °C does not seem to play a significant role in the inactivation of bacteria. Synergetic effects with solar radiation were not observed.

General qualitative recommendations, such as the use of clear and transparent containers and low water turbidity, complement the quantitative findings. It was concluded that further laboratory work is necessary to establish minimum solar radiation intensity and required exposure time for the inactivation of different types of pathogenic micro-organisms (bacteria, viruses, protozoa and helminths), as well as to identify the most lethal range of the light spectrum which seems to be effective in the near UV and violet light.

Apart from INRESA's network project, a considerable amount of research has been conducted by photobiologists in order to characterise the radiation damage induced by ultraviolet and visible light to living cells. Logarithmic inactivation curves of bacteria are generally concave-shaped and show an early shoulder, which corresponds to a delay in the lethal effect of light. This shoulder is mainly caused by DNA repair mechanisms active in most living cells and deactivated only at higher fluences. For repair-deficient bacterial strains, the initial shoulder in the inactivation curve becomes very small. Great efforts have been made to determine action spectra; i.e. inactivation parameters as a function of wavelength (e.g. [4–7]). The inactivation rate of micro-organisms increases by a few orders of magnitude with decreasing wavelength. It increases in the order of visible light < UV-A < UV-B < UV-C and reaches a maximum in the UV-C at ~ 260 nm, which corresponds to the maximum DNA absorption. Action spectra are measured by monochromatic radiation and should be used with caution to determine the inactivation parameters of polychromatic radiation, such as for example sunlight. Indeed, biological systems, contrary to simple photochemical systems, may respond synergetically to irradiation at different wavelengths, as was shown for *E. coli* by Webb *et al.* [8]. Another kind of synergism was observed between light irradiation and heat treatment [9]. Both synergisms are of practical importance as shown later in this paper. Sunlight was also used to irradiate bacteria. Some experiments with sunlight aimed for example at determining the damage produced by sunlight on living cells and organisms and at using some micro-organisms as biological dosimeter; i.e. as a standard for measuring the intensity of the biologically active portion of the solar spectrum [10–12]. The monograph by Jagger [13] provides more details on the action of light on bacteria and, more generally, on living cells. For the present investigations, this review may be regarded as up-to-date, whereas recent developments in this field have focused more on cells of multicellular systems, including human cells, than on bacteria [7].

Virus inactivation by radiation

Ultraviolet radiation at the wavelength of 254 nm (UV-C) is a potent germicide which can be used to disinfect drinking water and secondary wastewater effluents. The process has been receiving increasing attention as it does not produce hazardous by-products as seen with chlorine and provides a simple technology in terms of operation and maintenance. The germicidal effect of UV-C is directly related to the induction of changes in nucleic acids, primarily through the formation of thymine-dimers [14]. Excessive radiation with UV-C can additionally lead to conformational changes of essential structures, such as enzymes and immunogenic antigens [15].

Chang *et al.* [16] reported comparatively similar UV-C doses necessary for a 99.9% inactivation of cultured vegetative bacteria and total coliforms. In contrast, the tested viruses, bacterial spores, and amoebic cysts required 3–4 times, 9 times, and 15 times higher doses, respectively, to achieve the same effect. The problem posed by light or dark reactivation to achieve effective bacterial inactivation by UV-C does not exist with viruses as they are unable to directly repair the damage caused to them by radiation [14].

Sunlight has been identified as potential disinfectant of estuarine water, wastewater and drinking water. Cubbage *et al.* [17] reported on the inactivation of poliovirus in river water and noted the importance of solar radiation doses for efficient inactivation, which was, however, inversely influenced by the given turbidity. Kapuscinski & Mitchell [18] studied the inactivation by solar radiation of bacteriophages and *E. coli* in filtered coastal water. The authors found inactivation rate constants of 1.74 and 0.5–1.0 log units/h for *E. coli* and the phage MS2. When filtered sunlight of ≥ 370 nm wavelength was used, the respective inactivation rate constants were 0.07–0.08 log units/h. The experiments revealed that although visible sunlight inactivated bacteria and viruses to some extent, the major effect was to be expected from wavelength ranging from 300 to 370 nm.

Photosensitisation of natural substances such as lignins, humic or fulvic acid is known to significantly improve the sunlight's germicidal effect. Moreover, photoreactive dyes, such as methylene blue or phenol red, are well-known for noticeably improving the germicidal effect of sunlight through the formation of singlet oxygen molecules. Such early observations prompted the use of methylene blue as an additive in wastewater effluent for improved solar disinfection [19]. The authors found that in the presence of as little as 0.01 mg/mL methylene blue, visible sunlight of 670-nm wavelength exerted a significant inactivating effect on coliform bacteria and poliovirus in wastewater of even high turbidity. Based on the study, it was concluded that in Houston, Texas, natural fluence rates of 670 nm reached up to 5 times the fluence rate necessary to achieve a 90% reduction of indicator organisms within 150 seconds. Together with visible light, photoreactive dyes are currently evaluated to overcome the problem of virus-contaminated human blood products [20].

RESEARCH OBJECTIVES AND PROGRAMME

The reason for IRCWD/EAWAG to embark in solar water disinfection studies is to elucidate the processes and promote the technology once the potential of its treatment efficiency is assessed.

A project team consisting of sanitary engineers, photochemists, bacteriologists, and virologists was formed to study the following aspects of solar water disinfection:

1 Wavelengths of the terrestrial solar radiation responsible for the inactivation of micro-organisms.

2 Fluence (dose) of solar radiation required to inactivate a given concentration of specific micro-organisms. Determination of the indicator organisms necessary to monitor the efficiency of solar water disinfection.

3 Influence of the water temperature on the process of solar water disinfection, and determination of the threshold water temperature. Are the effects of solar radiation and water temperature of synergetic or of additive character?

4 Establishing whether the dissolved natural organic matter enhances or hinders solar water disinfection.

5 Determining if methylene blue used as photosensitiser supports the process of solar water disinfection.

In order to assess the potential of solar water disinfection, the above aspects focus on the photochemistry and microbiology of the process. Technical and socio-economic aspects (e.g. shape of container, mode of operation, acceptability and affordability), which are important factors for the implementation of this technology, are key questions to be answered once the basic potential of solar water disinfection is established.

Laboratory tests were carried out with the following micro-organisms:

bacteria: *E. coli*, *Str. faecalis*, enterococci and total counts (bacterial density range 10^2–10^7 CFU/mL)

viruses: bacteriophage f2, encephalomyocarditis virus and rotaviruses (viral density range 10^4–10^{10} PFU/mL and $TCID_{50}$/mL, respectively)

in order to assess the inactivation rate of solar water disinfection on the selected micro-organisms under different operating conditions. Several field and laboratory tests were run in parallel to draw a comparison between laboratory experiments and the natural sunlight irradiation. The considered conditions varied in the following ranges:

wavelength:	cut-offs at **320 nm**, 340 nm, 370 nm and band-pass at 366 nm
turbidity:	**< 1**, 6.8., 14.5. and 24 NTU
temperature:	**20**, 30, 40, 50 and 55 °C
natural organic matter:	**< 0.5**, 1.7, 3.2 and 8.4 mg/L DOC
methylene blue:	**0**, 0.01, 0.1 and 1 p.p.m.

(the standard test condition is marked in bold)

MATERIAL AND METHODS

Material used for irradiation

Laboratory irradiations were carried out using a merry-go-round photoreactor (MGRR) DEMA Model 125 (see Fig. 1) equipped with a Hanau TQ718 medium-pressure mercury lamp that was operated at 500 or 700 W. Alternatively, a gallium iodide-doped version of the lamp (TQ718-Z1) was used. The lamp was placed in a cooling jacket in the centre of the MGRR. The light emitted from the lamp passed through the jacket and through 28 mm of filter solution before reaching the samples contained in quartz tubes (15 mm internal diameter). The filter solution was recirculated at a flux rate of 1.4 L/

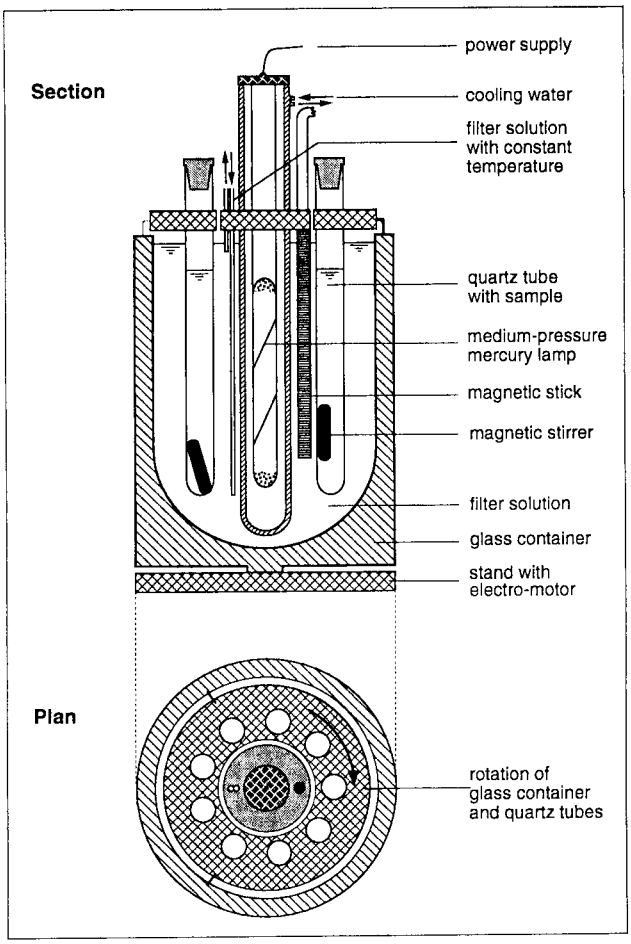

Fig. 1 Layout of photoreactor.

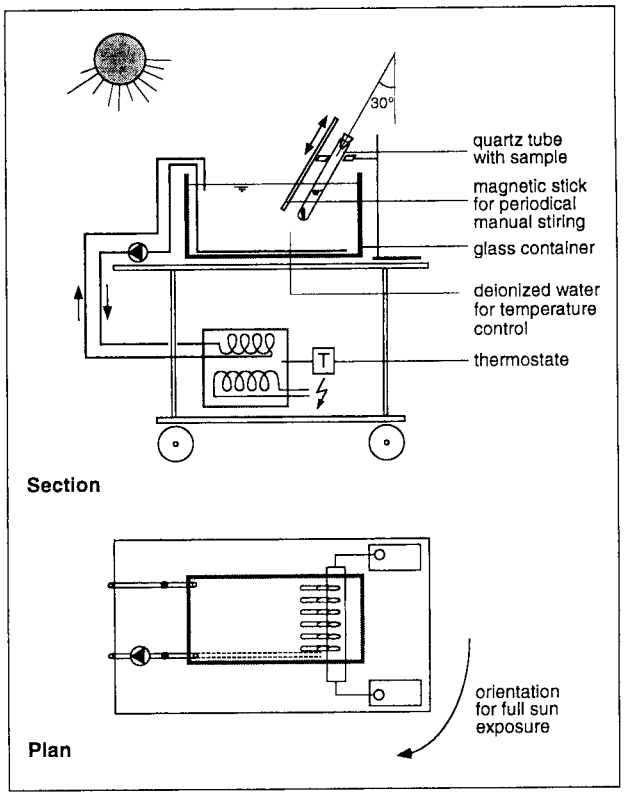

Fig. 2 Layout of field experiments.

min and led through a cooling thermostat Lauda Model RKS 20 that controlled the inside temperature of the MGRR.

The following combinations of filter glasses (cooling jackets) and filter solutions were used:

1 *Cut-off at 320 nm*, with half maximum at 340 nm: 4 mm of Duran 50 borosilicate glass and 12.75 g/L sodium nitrate solution.
2 *Cut-off at 340 nm*, with half maximum at 350 nm: 4 mm of Duran 50 borosilicate glass and 325 g/L sodium bromide with 1.5 g/L lead nitrate solution.
3 *Cut-off at 370 nm*, with half maximum at 390 nm: 4 mm of Duran 50 borosilicate glass and 21.25 g/L sodium nitrate with 3.45 g/L sodium nitrite solution.
4 *Band-pass at 366 nm:* 2 mm of quartz, UVW glass filter, 2 mm of Duran 50 borosilicate glass and 325 g/L sodium bromide with 1.5 g/L lead nitrate solution. The light transmitted through this filter is composed of a main contribution at 366 nm and a minor impurity at 406 nm (11% of the total intensity).

Chemical actinometry with *p*-nitroanisole/pyridine [21] was used to determine the fluence rate in the MGRR during the monochromatic irradiations, based on the method for weakly absorbing systems described by Zepp [22]. Experimental details are given by Canonica *et al.* [23]. Polychromatic fluences were calculated from monochromatic fluences, using the intensity ratios of the different emission lines of the TQ718 lamp given by the manufacturer and compensating for the transmission of the filters used. Fluences are given for the 350–450 nm wavelength range, unless otherwise specified.

For sunlight irradiations, a glass basin (width: 27 cm; depth: 41 cm; height: 27 cm) containing 25 L of deionised water was used to control the temperature of the sample quartz tubes that were mounted in a linear rack placed across the basin (see Fig. 2). The zenith angle of the long axis of the tubes was set at about −30°. The basin was reoriented horizontally during irradiation to avoid shadows on the sample tubes.

The emission spectrum of a TQ718 lamp (see Fig. 3) consists of discrete emission bands, many of which lie in the UV spectral range. Emission bands up to 313 nm are virtually completely cut off by the used filter combinations. The most important emission line in the UV-A range lies at 366 nm. The gallium iodide-doped lamp (spectrum not shown) exhibits an increased number of emission lines with respect to the undoped lamp. Particularly in the violet range (400–450 nm) light of the doped lamp is almost twice as intensive as that of the undoped lamp. Compared with the spectrum of sunlight reaching the earth surface, the TQ718 lamp spectrum (using the 320 nm cut-off filter) is deficient in UV-B and part of

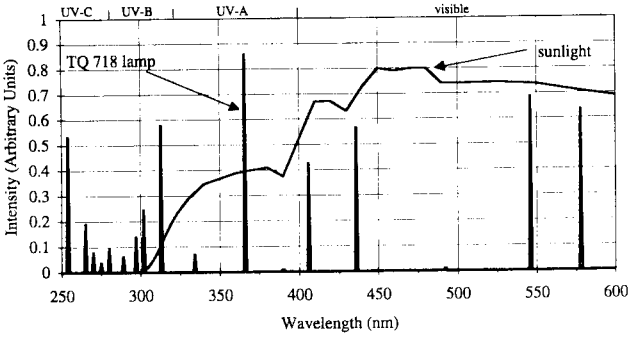

Fig. 3 Emission spectrum of the undoped TQ718 Hanau medium-pressure mercury lamp and typical spectrum of the solar radiation detected on a horizontal surface at noon at a mid-latitude location during a clear summer day. The spectra were scaled independently in order to fit in the same diagram.

UV-A radiation up to 350 nm. The coarse intensity distribution of the lamp spectrum in the UV-A and violet spectral range (350–450 nm) is similar to that of sunlight (the ratio of the intensity in the 350–400 nm range and in the 400–450 nm range for both sunlight and lamp is roughly the same). Thus, this lamp with the 320 nm cut-off filter may be used to simulate sunlight in the UV-A and violet spectral range. Laboratory irradiations were therefore performed to investigate the dependence of the inactivation kinetics on various parameters, and sunlight irradiations were used for calibration purposes.

During sunlight irradiations, the solar light intensity was measured by radiation sensors with different spectral responses. The UV-A light spectrum was recorded with a Macam sensor (SD 104A-COS; Macam Photometrics Ltd, Livingstone, Scotland), and the light spectrum of 400–700 nm wavelength with a quantum sensor (LI–192SA; LI-COR Ltd, Nebraska, USA). The average, maximum and minimum values were recorded by a data logger every 30 min. Simultaneous records with the quantum sensor and a pyranometer (LI-200SA), measuring the solar radiation intensity of 400–1100 nm wavelength range, were used to establish a correlation between the records of the two sensors. The mean solar radiation intensity during the irradiation of viruses ranged between 15.5 and 17.8 W/m^2 when recorded by the UV-A sensor, and between 780 and 890 W/m^2 when recorded by the pyranometer. The fluence values given in this work for sunlight irradiations refer to the wavelength range of 350–450 nm, and can thus be compared directly with those of laboratory irradiations. They were calculated in clear weather conditions at midday using the computer programme by Zepp & Cline [24]. These calculated values are roughly 10% higher than the actual values. Above 330 nm, the sunlight spectrum is practically independent of time of day and season (i.e. from the zenith angle of the sun), and therefore fluences (or fluence rates) of different wavelength ranges can be interconverted proportionally. 1000 kJ/m^2 (350–450 nm) correspond to 150 kJ/m^2 UV-A fluence measured by a calibrated SD 104-COS sensor, to 7600 kJ/m^2 measured by a calibrated pyranometer LI–200SA, and to 2.3 h for midday June sunlight radiation at Duebendorf under clear-sky conditions.

Methodology for bacteria analysis

Organisms

The bacterial colonies and mixtures used during the radiation experiments comprised not only the typical hygienic indicators for faecal pollution *E. coli* and enterococci, but also natural mixtures of bacteria found everywhere in surface water sources.

E. coli is a laboratory strain originally isolated on ECD agar from the Kriesbach, a small rivulet passing by the EAWAG. *Streptococcus faecalis* is an old specified laboratory strain. *Enterococcus* sp. was isolated from the Kriesbach on MEA agar. Bacterial mixtures were directly sampled from the Kriesbach. Bacterial mixtures with *E. coli* were directly sampled from the river Glatt, downstream from Duebendorf's wastewater treatment plant.

Substrates

To prepare, perform and evaluate the prescribed experiments, the following substrates were used:

- TSB, Trypticase soy broth BBL; TSA, Trypticase soy agar.
- PCA, plate count agar BBL (Standard methods agar); ECD, *E. coli* direct agar oxoid.
- MEA, *m-Enterococcus agar* BBL, buffer: 0.05 M phosphate (pH 6.8).

Cultivation and preparation of the experiments

All solutions, instruments and manipulations were kept or performed strictly under sterile conditions. The bacterial suspensions to be irradiated were prepared in such a way as to cultivate the strains until the end of their logarithmic growth phase, an interval which was experimentally verified in advance.

E. coli 5 h within TSA, *Streptococci* 4 h within MEA, then harvested by two 15-min centrifugations within 2-mL cups at 12000 r.p.m. and washed and suspended in buffer solution to achieve initial concentrations of 10^3–10^6 CFU/mL in order to meet the experimental prerequisites. After homogenisation by 1 min. ultrasonification, 20 mL of each of these bacterial suspensions were dosed in cotton plugged radiation tubes (quartz, 15 mm internal diameter, 240 mm long) using calibrated tipping pipettes.

The mixtures from the rivers Kriesbach or Glatt were filtered through cotton balls to remove polluting particles and then directly filled in the irradiation tubes. Before mounting in the merry-go-round photoreactor (MGRR), the samples were adapted for 15 min to the experimental temperatures in order to guarantee comparable temperature conditions.

To control temperature effects, one of the quartz tubes was wrapped in a light-impermeable black plastic foil. These dark controls were treated like the normal irradiation tubes and kept in the photoreactor during the whole experiment.

Sampling and evaluation

At the end of the chosen irradiation intervals, the samples were taken from the photoreactor, cooled under flowing tap water and immediately prepared for the plate counts. They were diluted in prepared buffer tubes to result in bacterial counts of ~ 100 CFU/mL and plate, mixed with the molten agar substrates, and cultivated in incubators: pure cultures of *E. coli* on TSA at 37 °C for 24 h, *Streptococci* on MEA at 37 °C for 24 h, mixed cultures on PCA at 20 °C for 72 h and then counted under a stereoglass.

E. coli from mixed cultures were membrane-filtered (pores 0.2 µm) after dilution of the samples in a buffer solution to obtain ~ 10 colonies per filter, which were transferred to agar substrates and cultivated on TSA at 37 °C for 4 h and on ECD at 44 °C for further 18 h. Colonies were counted after confirmation by positive indole test using p-dimethylaminobenzaldehyde (5% in 1n HCl). The overall bacterial parameter was CFU/mL (colony-forming unit).

A possible regrowth or revival of the treated suspensions was measured by periodic enumeration of the stored samples which had been transferred from the quartz tubes into Erlenmeyer flasks immediately following irradiation (for methods see [25]).

Methodology for virus analysis

The three viruses used in this study were (i) the bacteriophage f2, a single-stranded RNA virus with cubic capsid symmetry, (ii) encephalomyocarditis virus (EMCV), a member of the picornaviruses, and (iii) a bovine rotavirus, strain UK. The three viruses are widely used for reference purposes. The animal viruses were selected on account of their clinical relevance and their significant importance in water-related health microbiology, and the coliphage for its possible value as an indicator organism for quality control of drinking water and wastewater. The bacteriophage f2 and the host cell *E. coli*, K-12 Hfr CSH, used for phage propagation and quantal virus assay (PFU/mL) has been described [26]. For the propagation of EMCV (kindly supplied by the Institute of Virology, University of Zurich) and the rotavirus (Institute of Veterinary Virology, University of Berne), MA-104 cells, a continuous primate kidney cell line (Institute of Veterinary Virology, University of Berne), were used throughout. Growth medium consisted of Eagle's minimal essential medium (E'MEM) supplemented with antibiotics and 10% foetal bovine serum. For cell maintenance, the serum supplement was reduced to 2% with EMCV. The rotavirus was pretreated with 10 µm/mL of trypsin for 1 h at 37 °C and then propagated in MA-104 cells in the presence of 5 µm/mL of trypsin and of a serum-free maintenance medium. Virus stocks used for solar disinfection studies were grown in cells that were maintained in Dulbecco's modified MEM without phenol red (Sigma, Cat. D-2902). Virus stocks were stored at –80 °C. Infectivity titres were determined by the final dilution method ($TCID_{50}$/mL) using cells grown in 96-well microtitration plates. For each decadic virus dilution, four cultures were inoculated with 0.1 mL. The results were recorded by scoring monolayers for virus-induced cytopathology (EMCV) or immuno-stained cells (rotavirus).

To prepare samples for radiation experiments, 20 mL of a 1:20 dilution of the appropriate virus in phosphate buffered saline, pH 7.3, was dispensed into each of nine radiation tubes and kept at 4 °C until they were used. Prior to exposing the tubes to light, they were subjected for 15 min to the experimental temperature. At selected intervals, the single tubes were taken from the radiation apparatus, cooled under flowing tap water and stored at 4 °C until further processing. Residual infectivities were determined at the end of each experiment.

Inactivation kinetics

The simplest form of inactivation curve follows a single-exponential decay law:

$$N = N_0 e^{-kt} = N_0 e^{-\kappa F} \tag{1}$$

where N_0 is the number density of individuals prior to irradiation (mL^{-1}); t is the irradiation time (at constant fluence rate) (s); N is the number density of survivors at irradiation time t (mL^{-1}); k is the time-based inactivation rate constant (s^{-1}); F is the fluence (kJ/m); κ is the fluence-based inactivation rate constant (m^2/kJ).

Equation 1 may be expressed logarithmically as:

$$\log N = \log N_0 - kt \cdot \log e = \log N_0 - \kappa F \cdot \log e \tag{2}$$

In this work, all single-exponential decay curves were analysed by linear regression using eqn 2 and the statistics programme STATVIEW II (Abacus Concepts, Inc., Berkley, CA, 1987) on a Macintosh SE/30 computer.

Microbiological inactivation curves often deviate from a single-exponential law; i.e. log N as a function of t or F deviates from linearity.

Convex log N curves may be fitted by the equation:

$$N = N_0 \sum_{i=1}^{n} a_i \cdot \exp(-k_i t) = N_0 \sum_{i=1}^{n} a_i \cdot \exp(-\kappa_i F), \tag{3}$$

which corresponds to a model with mixed populations, each ith population undergoing a distinct exponential decay.

Concave log N curves, which are common for pure bacterial strains under laboratory and sunlight irradiation, may be fitted by the multi-target model:

$$N = N_0 \left[1 - \left(1 - \exp(-kt)\right)^m\right] = N_0 \cdot \left[1 - \left(1 - \exp(-\kappa F)\right)^m\right]. \tag{4}$$

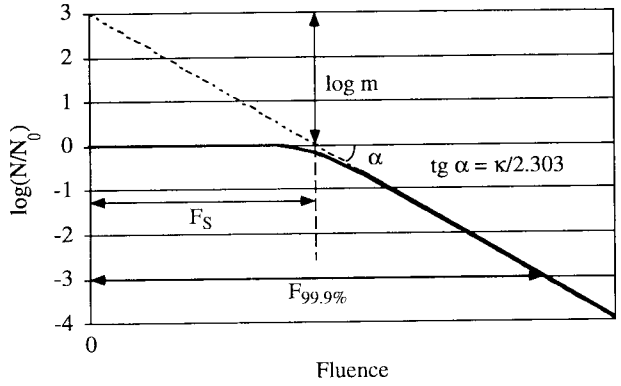

Fig. 4 Parameters describing a concave inactivation curve (see text for explanations).

According to the theory, m corresponds to the number of targets. In practice, however, eqn 4 is used as an empirical model to fit concave log N curves, and m loses its physical meaning. Concave inactivation curves (Fig. 4) are characterised by a shoulder (or threshold) time t_s or fluence F_s, given by:

$$t_s = \log m / (k \cdot \log e), \quad F_s = \log m / (\kappa \cdot \log e), \tag{5}$$

and by the final asymptotic slope (at high t) k·log e or κ·log e.

The data fits by eqns 3 or 4 were performed by nonlinear regression using the computer programme SYSTAT® on a Macintosh IIsi computer equipped with a mathematical coprocessor.

To characterise light sensitivity of a given microbiological system, the parameter $F_{99.9\%}$, which is the fluence required to inactivate 99.9% of the initial population, is defined. Depending on the type of inactivation curve, we obtain the following relations.

For a single-exponential function (eqn 1):

$$F_{99.9\%} = 3 / (\kappa \cdot \log e). \tag{6}$$

For a multiexponential function (eqn 3), $F_{99.9\%}$ has to be determined numerically or graphically. For a multitarget function (eqn 4):

$$F_{99.9\%} = -\log(1-0.999^{1/m}) / (\kappa \cdot \log e). \tag{7}$$

RESULTS AND DISCUSSION

Laboratory and sunlight irradiation results obtained with bacteria

Wavelength dependence

Figure 5 shows inactivation curves of *E. coli* at different irradiation conditions using the doped lamp (TQ718-Z1). The circles correspond to a cut-off filter of 320 nm; i.e. the UV-A bands and all visible bands are present in the spectrum. The squares correspond to a cut-off filter of 370 nm with only

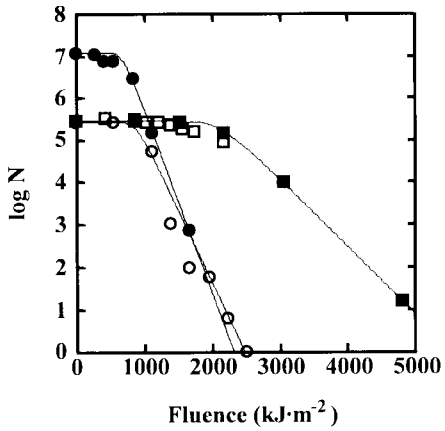

Fig. 5 Inactivation curves of *E. coli* at 20 °C using the doped TQ718-Z1 lamp. Circles: two different experiments with UV and visible light (cut-off at 320 nm). Squares: two different experiments with only visible light (cut-off at 370 nm).

visible bands present. With the use of visible light only, the shoulder in the survival curve is about twice as long as that obtained with UV-A and visible light. The reverse trend is observed as regards the final slope of the survival curves, with κ values of 3.66×10^{-3} m²/kJ and 7.82×10^{-3} m²/kJ for visible light only and for UV-A with visible light, respectively. The monochromatic inactivation constant at 366 nm (Fig. 6) is 4.49×10^{-3} m²/kJ and, thus, a little higher than that for violet light radiation. This value is in good agreement with the literature values obtained by Webb & Brown [4] for repair proficient and deficient *E. coli* strains: 2.4×10^{-3} and 5×10^{-3} m²/kJ, respectively. If the inactivation constant for UV-A and violet light is calculated as the weighed mean of the UV-A inactivation constant and of the violet light inactivation constant (i.e. the sum of inactivation constant times fluence rate for the 350–400-nm and the 400–450-nm range divided by the fluence rate for the 350–450-nm range), a

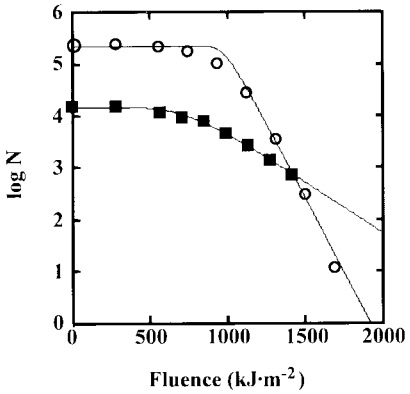

Fig. 6 Inactivation curves of *E. coli* at 20 °C using (○) the undoped lamp with the 320 nm cut-off filter or (■) monochromatic light at 366 nm.

value of 3.95×10^{-3} m²/kJ is obtained that is about half of the one observed. Therefore, UV-A and violet light act synergistically on the killing of *E. coli* (see also Fig. 6). If both UV-A and violet light are present (UV-A: violet intensity ~ 1:1), the action of violet light is increased by a factor of three. This observation concurs with the results of Webb *et al.* [8], who detected a strong sensitisation of the lethal action of 406 nm radiation by pre-exposure to high fluences (1000 kJ/m²) of 366 nm light in wild type and other strains of *E. coli*. In the same work, they observed a photoprotective effect of 406-nm radiation on 366-nm light. As regards our present experiments, the influence of such photoprotection seems rather limited as it was only observed with violet light fluences greater than 5000 kJ/m². A synergistic interaction of 334-nm and 366-nm radiation is also known from the literature [13], but does not play an important role in the present experiments: using the 320-nm and the 340-nm cut-off filters, we could show that *E. coli* survival is unaffected by the presence of the 334-nm radiation in the MGRR.

Streptococcus faecalis (Fig. 7) behaves differently. The action of violet light is very limited and no synergistic effect could be observed. The inactivation constant of *Str. faecalis* at 366 nm was determined at 20.0×10^{-3} m²/kJ; i.e. 4.5 times that of *E. coli*. Nevertheless, due to the synergism of UV-A and violet light, *E. coli* ($\kappa = 13.1 \times 10^{-3}$ m²/kJ) was found to be more sensitive than *Str. faecalis* ($\kappa = 5.8 \times 10^{-3}$ m²/kJ) when irradiated with broad-band light in the MGRR (Fig. 8). With broad-band irradiation, enterococci have a similar inactivation constant ($\kappa = 5.3 \times 10^{-3}$ m²/kJ) than that of *Str. faecalis*, but a longer shoulder fluence.

Wavelength dependence considerations, including synergistic effects, are important when transferring laboratory data to field conditions, where sunlight is used to radiate micro-organisms. As shown above, different micro-organisms behave very differently when subjected to different irradiation

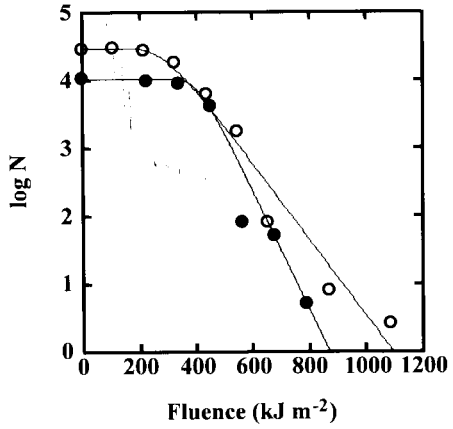

Fig. 7 Inactivation curves of *Streptococcus faecalis* at 20 °C (●) using monochromatic light at 366 nm or (○) using UV and visible light from the undoped lamp, whereby the fluence is integrated over the 350–400 nm range.

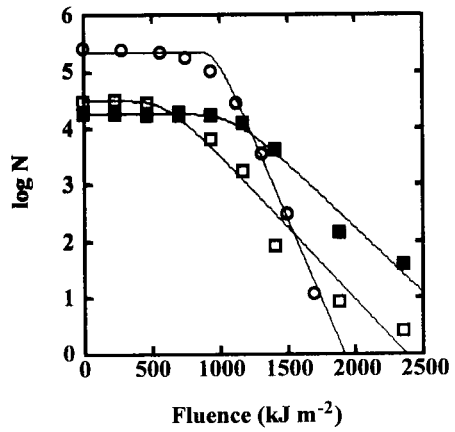

Fig. 8 Inactivation curves at 20 °C of (○) *E. coli*, (□) *Streptococcus faecalis* or (■) *Enterococcus*, using the undoped lamp with the 320 nm cut-off filter.

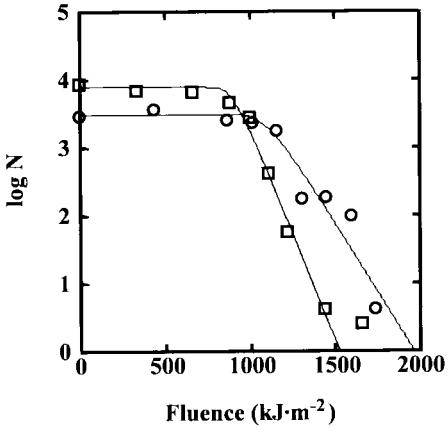

Fig. 9 Inactivation curves at 20 °C of (○) *E. coli* or (□) *Streptococcus faecalis*, using sunlight.

wavelengths. However, the survival curves of different micro-organisms are fairly similar with the used broad-band spectral light source (Fig. 8). They are also fairly similar under sunlight radiation (Fig. 9). Therefore, broad-band irradiation in the MGRR achieves a better simulation of the survival behaviour of the studied micro-organisms exposed to sunlight than irradiations using monochromatic light or only some spectral bands. The *E. coli* data also show that the use of action spectra to simulate the behaviour under solar radiation may be problematic as action spectra are derived from monochromatic experiments and do not take into consideration the synergism of the different wavelengths.

Temperature dependence

Inactivation parameters of various bacterial strains and mixtures at different temperatures under laboratory irradiation ($\lambda > 320$ nm) are summarised in Table 1. Survival curves of *E. coli* at different temperatures are given in Fig. 10. The ob-

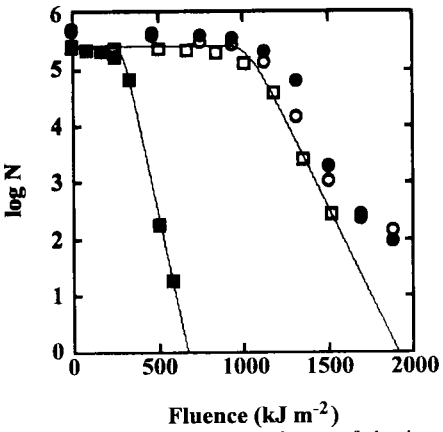

Fig. 10 Temperature dependence of the inactivation curves of *E. coli* using the undoped lamp with the 320 nm cut-off filter: (○) 20 °C, (●) 30 °C, (□) 40 °C, (■) 50 °C.

served survival behaviour remains basically unchanged at a temperature range of 20–40 °C, where shoulders F_s of approximately 1000 kJ/m^2 and inactivation constants κ of approximately 13×10^{-3} m^2/kJ are observed. At 50 °C, inactivation is faster, with a shoulder of 300 kJ/m^2 and an inactivation constant of 29.7×10^{-3} m^2/kJ. Thereby, fluences required to inactivate the same fraction of *E. coli* in the exponential part of the survival curve are at least three times smaller at 50 °C than at 20–40 °C. As documented in Table 1, *Str. faecalis* behaves similarly to *E. coli*, whereas wild-type Enterococcus do not show such a strong temperature effect; i.e. they are sensitised only by temperatures above 55 °C, the fluence enhancement factor being about 2. In all laboratory irradiations, even at elevated temperatures, the population of dark controls did not decrease during the course of an experiment. This means that radiation and temperature combined have a strong synergistic effect.

Table 1 Results of laboratory tests. Irradiations using the undoped TQ718 lamp with 320-nm cut-off filter. The fluence was integrated over the wavelength range 350–450 nm

Micro-organisms	κ (m^2/kJ)	F_s (kJ/m^2)	$F_{99.9\%}$ (kJ/m^2)
E. coli, lab. strain			
$T = 20$ °C	0.0131	984	1510
$T = 30$ °C	0.0098	997	1700
$T = 40$ °C	0.0143	1043	1530
$T = 50$ °C	0.0331	299	510
E. coli, Glatt			
$T = 20$ °C	0.00296		2330
$T = 50$ °C	0.010		670
$T = 55$ °C	0.02		350
Str. faec., lab. strain			
$T = 20$ °C	0.0058	659	1850
$T = 50$ °C	0.015	380	1000
Enteroc., lab. strain			
$T = 20$ °C	0.0053	1156	2460
$T = 50$ °C	0.0071	1317	2290
$T = 55$ °C	0.0163	656	1080
Total counts, Glatt			
$T = 20$ °C	0.00251		2750
$T = 40$ °C	*		~ 1500
Total counts, Kriesb.			
$T = 20$ °C	*		~ 5600
$T = 40$ °C	*		~ 4000

*Multiple exponential decay – graphical analysis.

Influence of natural organic matter

Natural organic matter (NOM), which is an important constituent of natural waters, is absent in the bacterial suspensions used in this work. NOM is known to act as photosensitiser for a variety of chemical reactions that are produced by energy transfer, singlet oxygen and radical species. It is also a well known fact that synthetic photosensitisers, such as methylene blue (see next section) or rose Bengal, are very effective in mediating the killing of micro-organisms, especially gram positive ones. Therefore, NOM was tested as a possible photosensitiser to inactivate *E. coli*. Two kinds of NOM were used in our experiments: Greifensee water NOM and the commercial Fluka humic acid. Greifensee water and the humic acid stock solution were filtered (0.45 µm). Compared to a buffer solution, *E. coli* suspended in Greifensee water (DOC = 3.2 mg/L) did not show any enhanced killing by light. No enhancement was observed with different concentrations of Fluka humic acid. Instead, a decrease of the lethal efficiency of light was observed with increasing NOM concentration. This decrease can be explained by a reduced transmittance of the cell suspension, since NOM was acting as an internal optical filter. Therefore, no photosensitisation by NOM was observed.

Methylene blue as photosensitiser

Methylene blue is already used on a technical scale for the disinfection of domestic wastewater [27]. Therefore, this photosensitiser was also employed in this study to verify its effect on the pure strain of *E. coli* used. Figure 11 shows that, compared with a sample without photosensitiser, the addition of 1 mg/L methylene blue to a bacterial suspension increases the killing rate dramatically. These irradiations were performed in the photoreactor with the 320-nm cut-off filter. The use of the 370-nm cut-off filter did not reduce the effectiveness of methylene blue. This is easily explained by the fact that methylene blue absorbs light mostly in the visible region (its absorption maximum lies at about 650 nm). The enhanced killing by methylene blue only worked for the first 2.5 decades of counts of the survival curve. After the rapid initial drop in population density, the survivors were inactivated at approximately the same rate as without photosensitiser.

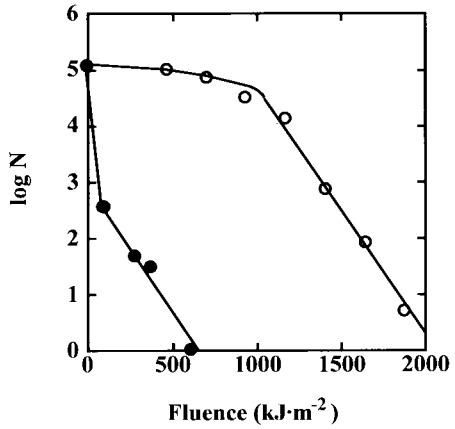

Fig. 11 Inactivation curves of *E. coli* at 20 °C using the undoped lamp with the 320 nm cut-off filter. (O) Without and (●) with 1 mg/L dissolved methylene blue.

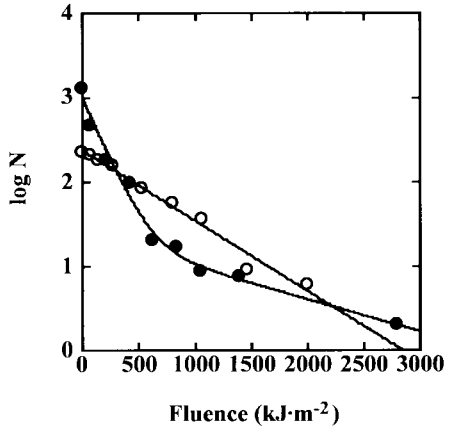

Fig. 12 Inactivation curves of a bacterial mixture from Kriesbach at 20 °C using (●) the doped lamp with the 320 nm cut-off filter or (O) sunlight.

Influence of turbidity

Turbidity is used as a parameter to characterise the optical properties of liquids containing absorbers and scatterers; i.e. suspended particles. The parameter defined by the ISO norm is very reductive since it is related to the wavelength of 546 nm. We think that this definition is rather inadequate for the UV spectral range. Therefore, transmittance of 1 cm sample with suspended particles at 400 nm was taken as turbidity measure. We employed a bentonite suspension that reduced the transmittance to 50%. No influence of the presence of bentonite was observed in the survival curves. This indicates that the effective UV intensity in the sample remains constant: the reduced transmittance of the sample is counterbalanced by the increased scattering. Hence, a small degree of turbidity (< 25 NTU) hardly reduces the disinfection efficiency of solar radiation. However, before applying solar water disinfection, it is recommended to reduce the raw water turbidity whenever possible.

Mixtures of micro-organisms and sunlight radiation

Survival curves of pure strains under laboratory and sunlight radiations have been described above. Here we focus our attention to mixtures of micro-organisms as found in two Swiss surface waters in order to assess the disinfection process under conditions close to reality. A bacterial mixture from Kriesbach (Fig. 12) showed a convex survival curve under laboratory irradiation, which indicates ageing of the mixture. First, the more sensitive bacteria were killed at a higher rate constant, followed by the less sensitive bacteria that were inactivated at a smaller rate constant. Under sunlight irradiation, a single-exponential survival curve was obtained within a good approximation for such a bacterial mixture isolated in an independent experiment. This might be due to the small dynamic range under observation (less than two decades) or to the different response of bacteria to sunlight and to TQ718 lamp light. One has to consider that the observed curves are not just a superposition of single-exponential decay curves but of concave curves (eqn 4), like the ones observed for pure cultures. Superposition of such curves, with different threshold fluences F_s and different inactivation constants k, may result in an approximately single-exponential curve within a limited time interval. Another case of inactivation of a bacterial mixture with only *E. coli* strains is presented in Fig. 13. The coliforms were isolated simultaneously from bacterial consortia out of River Glatt. The shoulders here are present in both laboratory and field experiments, although they are much smaller than for pure strains. These curves can also result from the superposition of many concave curves. Figure 14 shows survival curves of a natural bacterial mixture from River Glatt. In this case we have two apparently single-exponential curves. As regards the cases discussed in this section, the inactivation parameters are presented in Table 2. *E. coli*

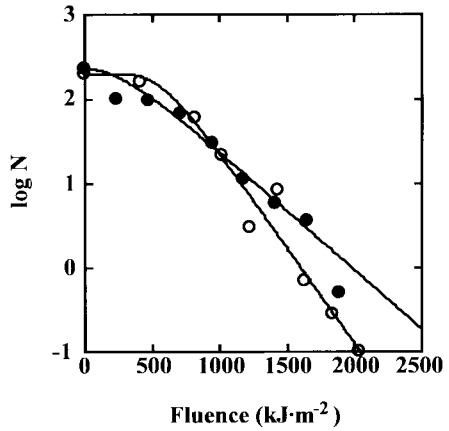

Fig. 13 Inactivation curves of a bacterial mixture (only *E. coli* strains) from River Glatt at 20 °C using (●) the undoped lamp with the 320 nm cut-off filter or (O) sunlight.

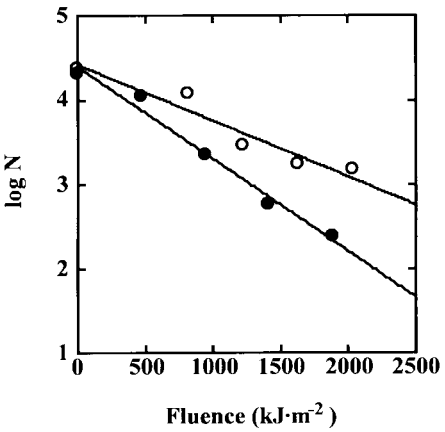

Fig. 14 Inactivation curves of a bacterial mixture from river Glatt at 20 °C using (●) the undoped lamp with the 320 nm cut-off filter or (○) sunlight.

Table 2 Results of sunlight irradiations. The fluence was integrated over the wavelength range of 350–450 nm.

Micro-organisms	κ (m^2/kJ)	F_s (kJ/m^2)	$F_{99.9\%}$ (kJ/m^2)
E. coli, lab. strain			
T = 20 °C	0.00741	1105	2040
T = 50 °C	0.0249	240	520
E. coli, Glatt			
T = 20 °C	0.0052	584	1900
T = 50 °C	0.014		500
Str. faec., lab. strain			
T = 20 °C	0.0144	911	1390
Total counts, Glatt			
T = 20 °C	0.00153		4500
Total counts, Kriesb.			
T = 20 °C	0.00192		3600

mixtures, like the pure strain, have $F_{99.9\%}$ values close to 2000 kJ/m^2, which correspond to approximately 5 h of mid-latitude midday summer sunshine. Natural bacterial mixtures, which are less relevant for defining the hygienic water quality, have $F_{99.9\%}$-values between 3600 kJ/m^2 (Kriesbach) and 4500 kJ/m^2 (Glatt), which correspond to ~ 9 to 12 h of mid-latitude midday summer sunshine.

Regrowth of bacteria

With respect to the observed revival of bacterial suspensions, inactivated by UV-C radiation (254 nm) at 20 °C [25,28,29] some experiments have been conducted to elucidate comparable effects by solar disinfection. Figure 15 shows the regrowth of *E. coli* and natural bacterial mixtures which have been extensively reduced by different irradiation procedures.

After irradiation within the laboratory photoreactor (MGRR) at a temperature of 20 °C for 32 min, the treated inactivated suspensions of *E. coli* regrew as described before,

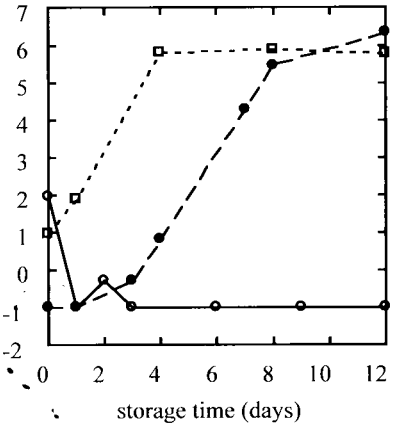

Fig. 15 Regrowth of *E. coli* and *bacterial mixtures* during increasing storage time of the irradiated suspensions. Temperature always at 20 °C. (●) *E. coli* irradiated using the undoped lamp and the 320 nm cut-off filter (fluence: 2240 kJ/m^2); (○) *E. coli* irradiated with sunlight (fluence: 1070 kJ/m^2); (□) bacterial mixtures irradiated with sunlight (fluence: 1470 kJ/m^2). For $N < 0.5$, log N was set at −1.

reaching the original cell density within a 1-week period. Contrary to this, solar irradiation never did result in any revival or regrowth even after prolonged storage periods of over two weeks of the treated suspensions. It seems that due to the relatively long exposure time of 160 min, which is necessary to reach a marked inactivation of the cells under solar irradiation conditions, viability of even the sensitive bacteria *E. coli* ceased completely. The observed regrowth of *natural bacterial mixtures* after solar irradiation (220 min) can be explained by the development of resistant saprophytic bacteria and their spores, respectively, which are widely abundant in open flowing waters.

Laboratory and field test results obtained with viruses

In a first series of experiments, the virucidal effect of artificial light at a wavelength range of ≥ 320 nm was assessed and the temperature maintained at a determined level. Among the three viruses tested, EMCV was the most resistant and the rotavirus the least (Table 3). To achieve a 99.9% reduction of infectivity titres at 20 °C (3-log units), fluences of 9000, 34 300, and 6800 kJ/m^2 were necessary for f2, EMCV, and rotavirus, respectively. This corresponds to 3.3, 12.5, and 2.5 h of radiation.

Table 3 also shows that ambient temperature exerts a net effect on radiation-induced virus inactivation. At ≤ 40 °C temperature the slopes of the inactivation curves essentially followed a first-order kinetic with the three viruses (Figs 16–18). With f2 and EMCV, the same was observed up to 50 °C. Control tubes kept at ≤ 40 °C in the dark showed no reduction of the initial infectivity titres (Table 3). Thus, radiation-induced viral inactivation was faster at 40 than at 20 °C.

Table 3 Inactivation by artificial sunlight of coliphage f2 and the animal viruses EMCV and rotavirus (laboratory experiments)

Virus	k	*R*	*D*	*F*	*TI*
f2					
20 °C	0.015	0.958	3.3	9 000	0
40 °C	0.027	0.995	1.9	5 100	0
50 °C	0.040	0.984	1.3	3 500	11.5
EMCV					
20 °C	0.004	0.959	12.5	34 300	0
20 °C	0.004	0.834	12.5	34 300	0
30 °C	0.007	0.956	7.1	19 500	0
40 °C	0.009	0.982	5.6	16 700	0
40 °C	0.010	0.982	5.0	13 700	0
50 °C	0.028	0.977	1.8	5 000	30.0
55 °C	0.080*	0.962	0.6	1 700	95.0
Rotavirus					
20 °C	0.020	0.974	2.5	6 800	0
30 °C	0.028	1.000	1.8	5 000	0
40 °C	0.071	0.999	0.7	1 900	11.8

k = Regression coefficient (reduction of log virus titre/mL/min).
R = Correlation coefficient.
D = Time (h) for a 99.9% reduction of the infectivity titre.
F = Fluence (kJ/m²) for a 99.9% reduction of the infectivity titre.
TI = Thermal inactivation, temperature effect on the overall inactivation (% of k)
*Phase two of inactivation only (see text for explanation).

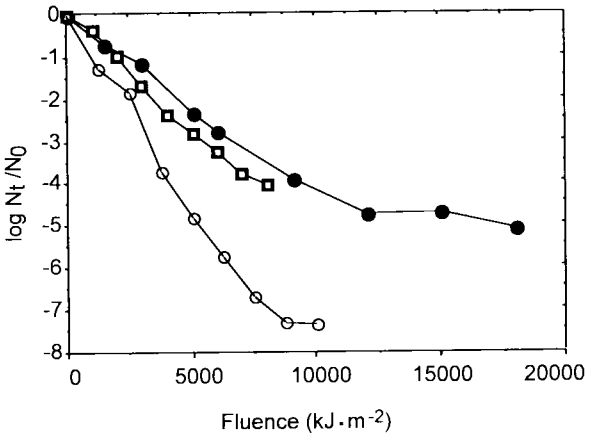

Fig. 16 Inactivation by UV-A of coliphage f2 at various temperatures (laboratory experiments): (●) 20 °C, (□) 40 °C, (○) 50 °C.

It can therefore be concluded that temperature alone was not effective in inactivating the viruses but amplified the effect of radiation. With EMCV, and temperatures ranging from 20 to 40 °C, the infectivity titre was observed to increase somewhat at the beginning of the experiments (Fig. 17). This could be explained, if pre-existing virus aggregates disintegrated under the influence of radiation.

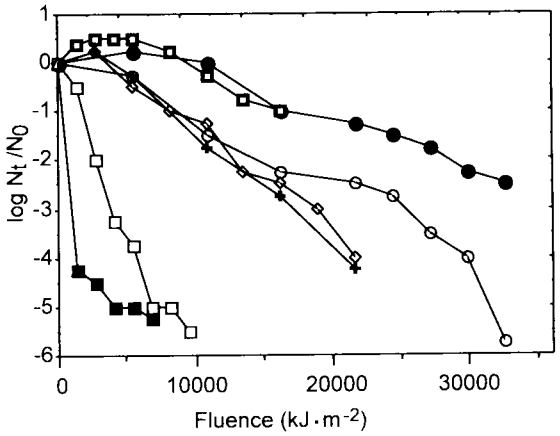

Fig. 17 Inactivation of EMCV by UV-A at various temperatures (laboratory experiments). (●) 20 °C, (■) 20 °C, (○) 30 °C, (◆) 40 °C, (✚) 40 °C, (□) 50 °C, (■) 55 °C.

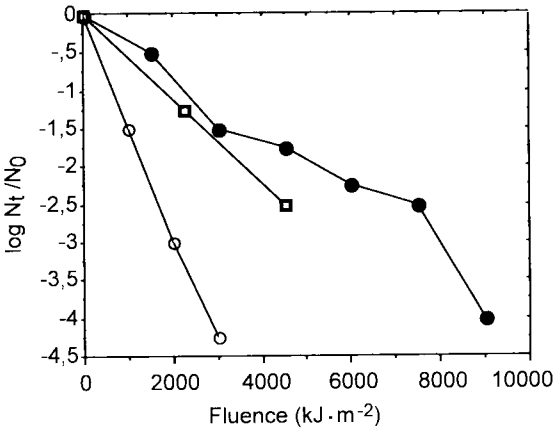

Fig. 18 Inactivation of rotavirus by UV-A at various temperatures (laboratory experiments). (●) 20 °C, (□) 30 °C, (○) 40 °C.

Since elevated temperature and radiation at ≥ 320 nm wavelength were found to increase the virucidal effect, we tested EMCV at 55 °C (Fig. 17). As can be seen, the inactivation curve no longer followed a first-order kinetic. During the first 30 min of radiation exposure, a sharp drop in the infectivity titre was observed, which was followed by a delay in virus decay. This second phase of inactivation, which can be explained by the slower inactivation of aggregated viruses, was used to calculate the k value given in Table 3. At 55 °C the overall inactivation of EMCV was attributed primarily to temperature alone (Table 3).

In a second series of experiments, sunlight radiation tests were carried out on different days at a constant temperature of 30 °C. When compared with the laboratory experiments, it is important to note that the wavelength spectrum was broadened to include radiation from 300 to 320 nm. It should also be

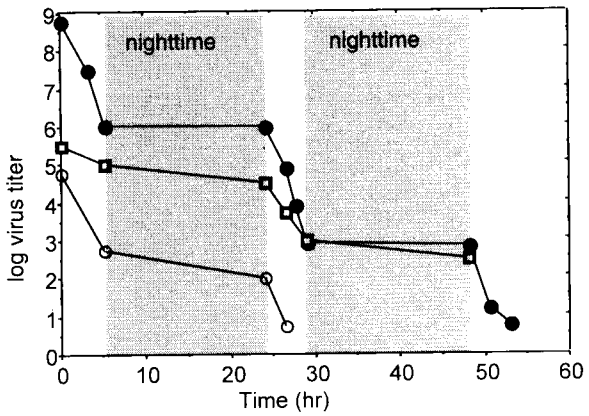

Fig. 19 Inactivation by sunlight of coliphage f2, and the animal viruses EMCV and rotavirus. (●) f2, (□) EMCV, (○) Rotavirus. Temperature was kept at 30 °C.

noted that the exposure was carried out on 2 consecutive days with EMCV and rotavirus, and on 3 consecutive days with f2 (Fig. 19). The exposure of EMCV was discontinued due to rainy weather. EMCV was more than twice as resistant to solar radiation as the other two viruses. With $F_{99.9\%}$-values of 2140 and 2480 kJ/m^2 the cumulative decay of f2 and rotavirus was similar. Infectivity titres of the contents of control tubes that were kept in the dark throughout the experiment, remained unchanged (data not shown). In contrast, some loss of infectivity was observed in the tubes that were stored at 4 °C after primary radiation exposure (Fig. 19). This was more pronounced with rotavirus than with EMCV.

When compared with the results of the laboratory tests, it was evident that the fluences needed to reduce infectivity titres by 3-log units were significantly lower in the field than with artificial light (Tables 3 and 4). This was attributed to the UV-B and lower part of UV-A light present in sunlight but not in the artificial light used for the laboratory experiments. The inactivation rate constants k obtained were 3 to 4 times higher than those reported by others [17,18]. This can be explained by the different characteristics of the viruses used in our experiments. However, it seems more likely that factors such as water turbidity, salinity, or pH are responsible for the observed differences.

This study has shown the virucidal effect of natural and artificial sunlight. Under field conditions, rotavirus and f2 were found to be two to three times more sensitive to the action of solar radiation than EMCV. To achieve a 3-log unit reduction of rotavirus and f2, a fluence of ~ 2300 kJ/m^2 was necessary. A similar value was obtained for *E. coli*. Provided good weather conditions, solar radiation may indeed disinfect drinking water in instances where no other water disinfection possibilities exist.

Table 4 Inactivation by daylight of coliphage f2 (30 °C) at , and the animal viruses EMCV and rotavirus. For abbreviations compare Table 3 and text

Virus	k	*R*	*D*	*F*	*TI*
f2	0.010	1.0	5.0	2140	0
EMCV	0.004	1.0	12.5	5270	0
Rotavirus	0.009	1.0	5.6	2480	0

CONCLUSIONS AND RECOMMENDATIONS

The laboratory and field tests that were carried out at EAWAG aimed at finding answers to the questions raised in the section on Research objectives. The results can be summarised as follows:

1 UV-A light (320–400 nm) is mainly responsible for the inactivation of micro-organisms. Violet light (400–450 nm) alone is hardly bactericidal. However, due to synergetic effects with UV-A light, its inactivation rate on *E. coli* is increased by a factor of three.

2 A fluence (dose of solar radiation integrated in the 350–450 nm wavelength range) of ~ 2000 kJ/m^2 or 555 W·h/m^2 is required to achieve a 3-log reduction of *E. coli* at water temperatures between 20 °C and 40 °C. The same amount of fluence reduces the bacteriophage f2 and a strain of rotavirus to a similar order of magnitude when exposed to solar radiation at 30 °C water temperature. EMCV, in contrast, was twice as resistant as *E. coli*, f2 or the rotavirus. The comparison of the required fluences for the inactivation of different micro-organisms indicates that *E. coli* and bacteriophage f2 may be used as indicator organisms to monitor the efficiency of solar water disinfection as regards the inactivation of bacteria and viruses.

3 Water temperatures between 20 and 40 °C do not affect the inactivation of bacteria by UV-A and visible light radiation. Synergetic effects were observed at a threshold water temperature of 50 °C. Compared to lower water temperatures, the fluence required to inactivate *E. coli* is more than three times smaller at this temperature. Viruses, however, are more sensitive to water temperature changes. The inactivation rate for the bacteriophage f2 increased by a factor 1.8 when the water temperature was raised from 20 to 40 °C. Enteroviruses and rotaviruses are even more sensitive to the same water temperature change; i.e. the inactivation rates increased by a factor 2.4 and 3.6, respectively.

4 The tested bacteria showed no photosensibilisation to natural organic matter (NOM). Instead, a decrease of the lethal efficiency of light was observed with increased NOM concentrations as a result of reduced light transmittance of the cell suspensions.

5 The efficiency of light in killing bacteria is considerably increased by methylene blue, which acts as a photosensitiser. The addition of 1 mg/L methylene blue to an *E. coli* suspension increases the killing rate for the first 2.5 decades of counts by a factor 20. The survivors, however, decay at approximately the same rate as without photosensitiser.

The use of *E. coli* or *faecal coliforms* as indicator organisms for faecal pollution is a well-established practice in drinking-water monitoring. The availability of field test kits enables to monitor the bacteriological water quality also in the field. According to the presented research results, the inactivation rate of *E. coli* has a similar order of magnitude as that of *Str. faecalis* and enterococci. Hence, *E. coli* or *faecal coliforms* are also appropriate indicators of the efficiency of solar radiation on the inactivation of bacteria of faecal origin.

In contrast to bacteria, virus concentrations in water are hardly ever monitored as their concentrations are generally low and their method of analysis sophisticated. The use of bacteriophages as indicator organisms for viral water contamination is now being considered in water treatment as bacteriophages occur at higher concentrations and their analysis is less complex. The inactivation rate of solar radiation for the tested bacteriophages f2 is similar to that of the investigated rotavirus whereas EMCV was twice as resistant. Nevertheless, the performed tests reveal that bacteriophages may be of value to assess the efficiency of solar radiation on the inactivation of bacteria and viruses.

The recorded sensitivity of the investigated bacteria and viruses to increased water temperatures paves the way for water disinfection by solar radiation. Two different processes using solar energy for water treatment have been developed independently so far, the first focusing on solar water disinfection by radiation and the second on solar water pasteurisation. Our radiation tests reveal a high sensitivity of viruses to water temperatures with synergetic effects at already low temperatures. Bacteria are more heat resistant; however, they showed remarkable synergetic effects with water temperatures above 50 °C. Hence, a combination of the two treatment processes – water disinfection by solar radiation and thermal water treatment by solar energy – seems to achieve best results. A simple test with half-side black coloured bottles proved that the water temperature could be raised over 50 °C within 5 h of exposure time. Water treatment by solar energy could indeed become an adequate and sustainable process for the treatment of small water quantities at household level.

However, additional field tests are necessary before solar water disinfection can be widely promoted. Further investigations are required to study on the influence of solid matter, present in surface water, on the inactivation of bacteria and viruses. The acquired information will contribute to the development of easily produced and operated solar water disinfection installations. Field tests will then be required to assess efficiency and affordability of the developed installations, as well as the sociocultural acceptability of the proposed treatment methods. Finally, large-scale demonstration projects will be necessary to disseminate the solar water disinfection treatment process to the most disadvantaged of our society in order to provide them with a simple and low-cost drinking-water treatment technique.

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