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Solar inactivation of faecal bacteria in water: the critical role of oxygen

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R.H. REED. 1997. Suspensions of the faecal indicator bacteria *Escherichia coli* and *Enterococcus faecalis* were incubated in full sunlight in plastic bottles containing either (i) air-equilibrated (oxygenated) water or (ii) anaerobic (deoxygenated) water. A rapid decrease in cfu ml^{-1} was observed for actively-growing and stationary phase cells of both types of faecal bacteria when illuminated under aerobic conditions, with *Ent. faecalis* showing the greater enhancement in the rate of inactivation in air-equilibrated water. The demonstration of an oxygen requirement for the inactivation of faecal bacteria using sunlight indicates that solar-based water disinfection systems are likely to require fully aerobic conditions in order to function effectively.

INTRODUCTION

The lack of satisfactory drinking water is a major problem for a significant proportion of the global population, resulting in the spread of water-borne diseases due to the consumption of contaminated water. Diarrhoeal diseases are major contributors to morbidity and mortality in developing countries, resulting in up to 6 million deaths per year (Thielman and Geurrant 1996) and there is an urgent need for simple, effective, low-cost methods for the production of drinking water free of pathogenic microbes. Visible light is known to be an important factor affecting the survival of enteric pathogenic bacteria in natural waters (Gameson and Saxon 1975; Evison 1988) and Acra and co-workers have demonstrated that water may be decontaminated by illumination in sunlight, either on a small scale, using plastic bottles or bags (Acra *et al.* 1980), or on a larger scale, using continuous flow systems (Acra *et al.* 1990). This process, usually termed solar water disinfection, has been proposed as a means of preparing drinking water and oral rehydration solutions for use in developing countries (Acra *et al.* 1984). However, some studies have shown that faecal bacteria are not always inactivated in sunlight (e.g. Miller 1988; MacKenzie *et al.* 1992), suggesting that factors other than solar illumination may be involved in the process.

The present study was conducted to assess the significance of aerobic conditions, since previous research has indicated that oxygen may be involved in the inactivation of u.v.-irradiated *Escherichia coli* (Webb and Brown 1979) and γ-irradiated *Salmonella typhimurium* (Kim and Thayer 1996). This paper shows that the photoinactivation of *E. coli* and *Enterococcus faecalis* exposed to sunlight is markedly sensitive to the oxygen status of the suspension medium, suggesting that solar disinfection will be most effective in fully oxygenated water.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Stock cultures of *E. coli* NCTC 8797 and *Ent. faecalis* NCTC 775 (National Collection of Industrial and Marine Bacteria, Aberdeen, UK) were maintained by repeated subculture on nutrient agar and brain-heart infusion agar, respectively. Experimental cultures were prepared by loop inoculation of 250-ml flasks containing 100 ml of either nutrient broth (*E. coli*) or brain-heart infusion broth (*Ent. faecalis*), respectively, followed by incubation at 37°C, either to early-log phase (A_{550} 0·3, 2 h) or to stationary phase (A_{550} 1·6, 18 h). Prior to experimentation, 25-ml aliquots of cell suspensions were centrifuged (2500 *g*, 15 min), resuspended in sterile distilled, deionized water and re-centrifuged, to remove all traces of the growth medium.

Illumination in sunlight

Rinsed, centrifuged cells were transferred to separate 2-l plastic bottles (made from polyethylene terephthalate), filled

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to the brim with either filter-sterilized distilled, deionized water (pH 6·8) prepared either under aerobic conditions (bubbled with sterile air for 60 min) or under anaerobic conditions (vacuum-filtered, then bubbled with sterile helium for 60 min) to give a cell count of $\approx 10^6$ cfu ml^{-1}. Intermediate oxygen concentrations were obtained by mixing various volumes of aerated and deoxygenated water. Each bottle was screw-capped with a rubber septum, taking care to exclude all air bubbles, and then incubated either in direct sunlight, on the roof of the Department, or in darkness in a laboratory cupboard, with intermittent shaking (every 30 min). Illuminated bottles were kept in continuous full sunlight throughout the experimental period (typically 9 a.m. to 12 noon on selected cloudless days in June–July, typical irradiance 600–750 W m^{-2} during illumination, based on data supplied by the Photovoltaics Group, University of Northumbria, UK). Aerobic and anaerobic bottles were illuminated simultaneously, to allow valid comparison between colony count data. The oxygen status of each bottle was confirmed at the end of the experiment using a Jenway 9010 probe (Dunmow, UK); no detectable oxygen was present in any of the anaerobic water samples. Water temperatures remained at 20–28°C throughout all incubation periods, in sunlight and in darkness.

Enumeration

Timed samples were taken by syringe, immediately shielded from sunlight and then processed in a dimly lit room, to avoid photoinactivation. Samples were then processed by serial decimal dilution in quarter-strength Ringer's solution, followed by plating on either nutrient agar medium (for *E. coli*) or brain-heart infusion agar (for *Ent. faecalis*), using the surface spread method (0·05–1·0 ml, depending on sample, dilution and time). Inoculated media were incubated at 37°C for 18 h; colony counts of appropriate dilutions were converted to cfu ml^{-1} by correcting for volume and dilution (Collins *et al.* 1989). Each experiment was performed using duplicate bottles for each treatment. All experimental and growth media were obtained from Merck (Poole, UK).

RESULTS

As shown in Fig. 1, the incubation of actively-growing cells of *E. coli* and *Ent. faecalis* in sunlight resulted in a rapid loss of culturability, with a more pronounced decrease in cfu ml^{-1} in the presence of oxygen than under anaerobic conditions, while incubation in darkness caused no significant change in bacterial counts, irrespective of oxygen status. The data shown in Fig. 1 are for a single day's experiment; a similar enhanced rate of decrease in culturable count on illumination under aerobic conditions has been obtained on four separate occasions for exponential phase cells of both micro-organ-

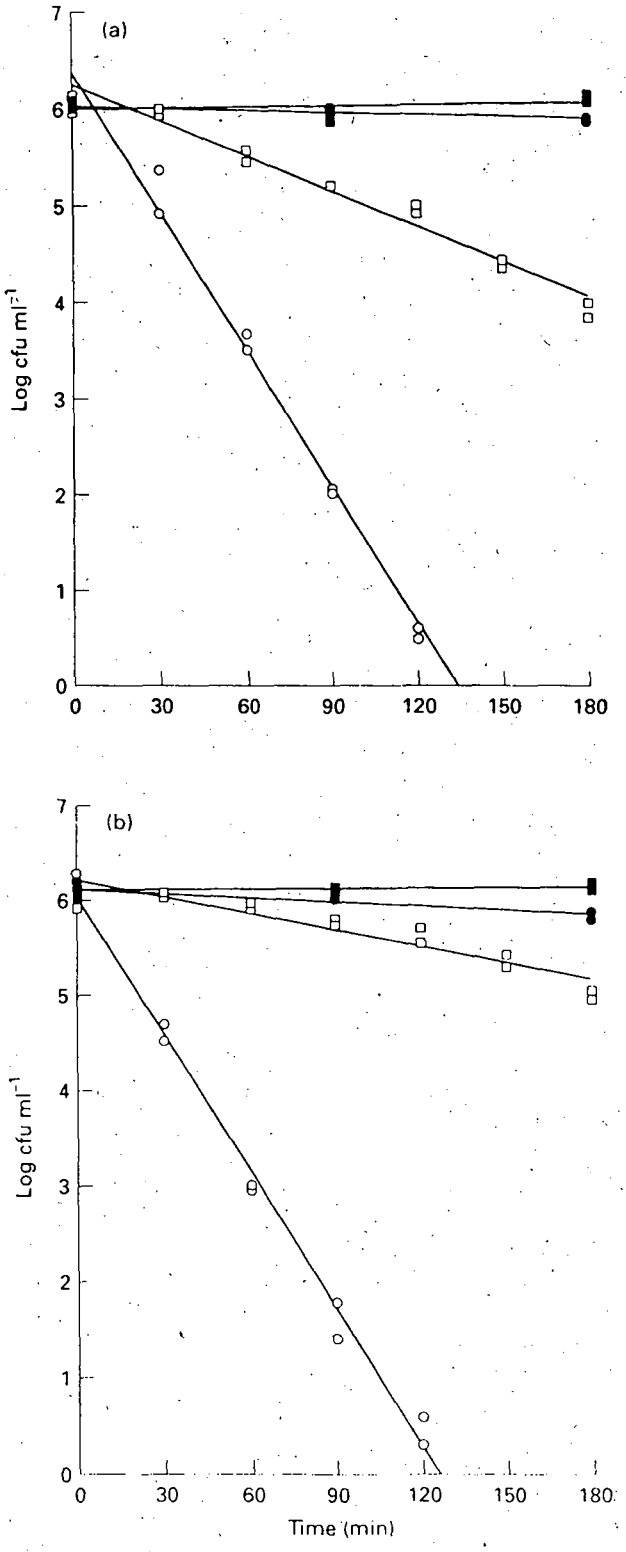

Fig. 1 Inactivation of exponential phase (a) *Escherichia coli* and (b) *Enterococcus faecalis*, either in full sunlight under aerobic (○) or anaerobic (□) conditions, or in darkness under aerobic (●) or anaerobic (■) conditions

isms. The decreases in cfu ml^{-1} of exponential phase cells broadly follow first order kinetics, based on log-linear plots. Inactivation rate constants for the observed decreases in cfu ml^{-1} were calculated by linear regression analysis. For both organisms, the mean inactivation rate constants (based on four experiments) were greater in sunlight under aerobic conditions, with *E. coli* having a mean value of $-0{\cdot}115\ min^{-1}$ under aerobic conditions and $-0{\cdot}0285\ min^{-1}$ under anaerobic conditions, while the corresponding mean values for *Ent. faecalis* were $-0{\cdot}119\ min^{-1}$ (aerobic) and $-0{\cdot}0113\ min^{-1}$ (anaerobic).

The dynamics of inactivation of stationary phase cells were slightly different to those of actively growing cells, typically with a short initial period up to 30 min, where the bacteria showed some resistance to solar inactivation, followed by an exponential decrease in culturable count (Fig. 2). Consequently, inactivation rate constants were calculated by linear regression analysis of data values between 30 and 180 min. Mean inactivation rate constants were greatest in sunlight under aerobic conditions, with *E. coli* having a mean vale of $-0{\cdot}071\ min^{-1}$ in sunlight under aerobic conditions and $-0{\cdot}0187\ min^{-1}$ in the absence of oxygen while the mean values for stationary phase *Ent. faecalis* were $-0{\cdot}079$ (aerobic) and $-0{\cdot}006$ (anaerobic), based on three separate experiments.

The effect of oxygen concentration on survival is shown in Fig. 3. For *E. coli* and *Ent. faecalis*, a similar result was obtained, with a broadly linear relationship between oxygen concentration and the rate of inactivation. There was no evidence of any requirement for a threshold oxygen concentration, or any other non-linear effect; the results are consistent with the involvement of reactive oxygen species, including superoxide and hydroxyl free radicals and hydrogen peroxide, formed in direct proportion to the concentration of dissolved oxygen in the medium (Whitelam and Codd 1986).

A number of research studies have shown that enteric bacteria may be sub-lethally injured when subjected to moderate environmental stress (Kapuscinski and Mitchell 1980; Fujioka and Narikawa 1982; Barcina *et al.* 1989). The possibility that the inactivated bacteria were injured, rather than killed, by the combination of sunlight and oxygen was assessed by keeping the illuminated samples for 24 h after completion of the experiment, and then performing further spread plate counts on each medium; no increases in cfu ml^{-1} were obtained, suggesting that the inactivation was irreversible.

DISCUSSION

The results of the present study indicate that dissolved oxygen is essential for the rapid inactivation of *E. coli* and *Ent. faecalis* exposed to sunlight. Previous studies have shown that the responses of these indicator microbes are likely to be representative of a broader range of faecal bacteria, including

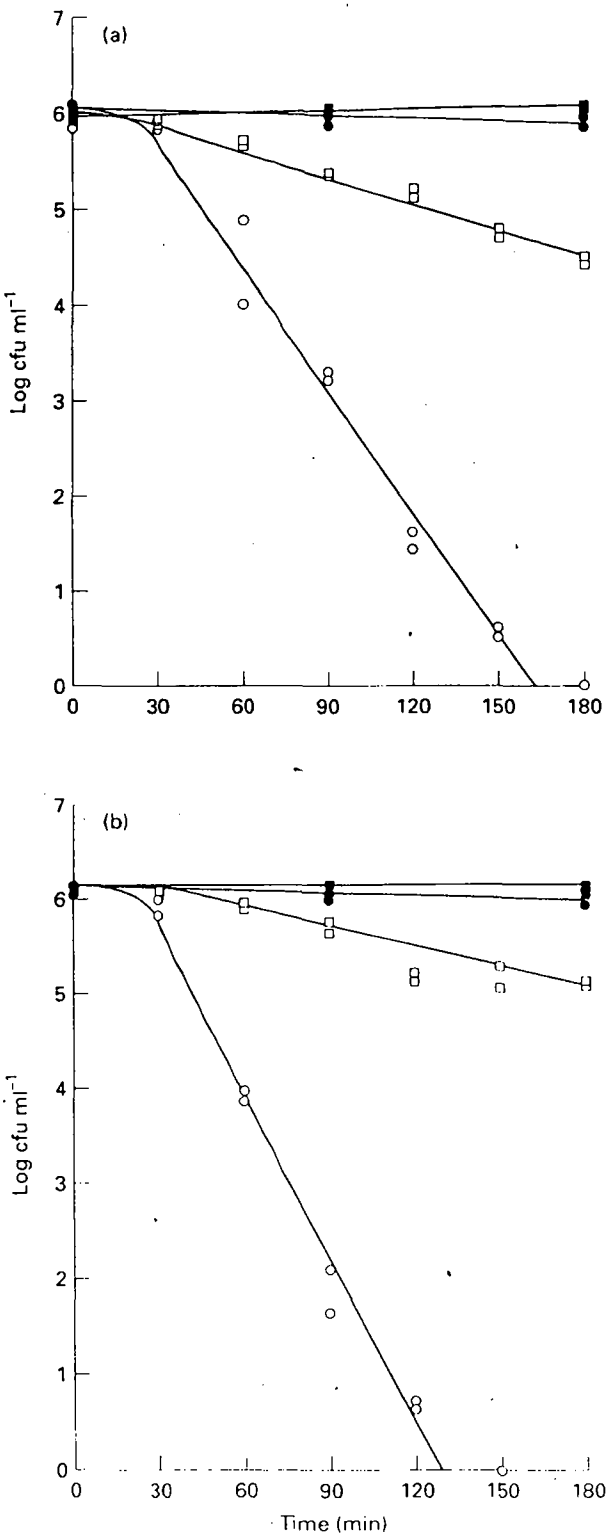

Fig. 2 Inactivation of stationary phase (a) *Escherichia coli* and (b) *Enterococcus faecalis*, either in full sunlight under aerobic (○) or anaerobic (□) or in darkness under aerobic (●) or anaerobic (■) conditions

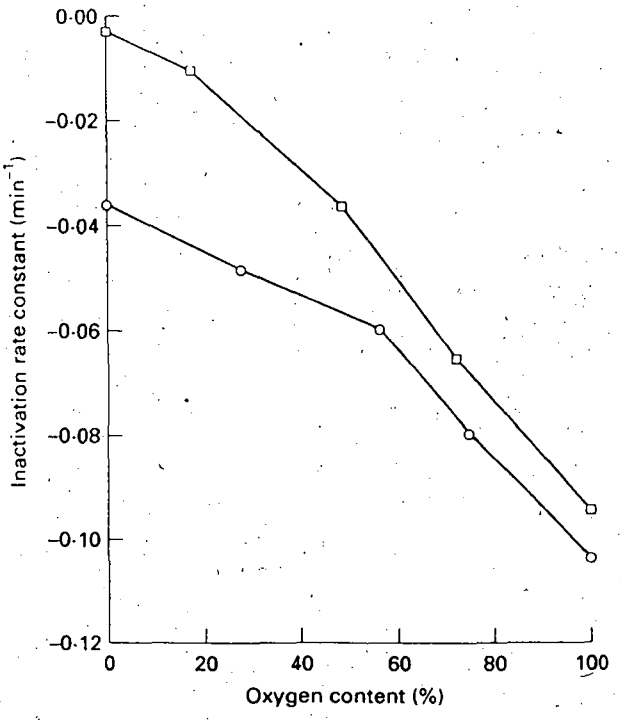

Fig. 3 Effect of oxygen concentration on the rate of inactivation of *Escherichia coli* (○) and *Enterococcus faecalis* (□). Air-equilibrated water (100%) contained oxygen at 8·4 mg l^{-1}

enteric pathogens (Acra *et al.* 1990). While other research has focused on the use of dye solutions (e.g. methylene blue, rose bengal; Acher 1985) or heterogeneous catalysts (e.g. titanium dioxide; Wei *et al.*, 1994; Watts *et al.* 1995) in the photo-oxidative inactivation of faecal bacteria exposed to sunlight, the present study clearly demonstrates that a similar effect can be observed without the need for added sensitizers, indicating that cellular components may play a significant role in photo-oxidation. The different rates of inactivation observed in aerobic and anaerobic water also provide a possible explanation for the variable results of previous workers, who used natural water samples of unknown oxygen status (e.g. Miller 1988; MacKenzie *et al.* 1992, cf. Acra *et al.* 1989).

The comparable rates of inactivation of actively-growing *E. coli* and *Ent. faecalis* in aerobic water exposed to sunlight are broadly similar to those of some earlier studies (e.g. Alkan *et al.* 1995), although others have suggested that enterococci are generally more resistant to sunlight (Gameson and Gould 1975; Davies-Colley *et al.* 1994), possibly reflecting differences in strain history and/or experimental conditions. In the present study, the mean inactivation rates were slightly lower for stationary phase cells, when compared with their growing counterparts, reflecting the decreased sensitivity of stationary phase bacterial cells to physico-chemical stress (Gauthier *et al.* 1992). The responses of stationary phase cells are more likely to be representative of enteric bacteria in natural waters, under conditions of restricted growth and nutrient limitation.

It is worth noting the greater enhancement in the rate of solar inactivation of *Ent. faecalis*, compared with *E. coli*. The data shown in Fig. 1 indicate an oxygen enhancement ratio (OER) for solar illumination of 8·4 for exponential phase *Ent. faecalis*, with an OER of 3·9 for actively growing *E. coli* in sunlight, while stationary phase cells shown a similar trend (*Ent. faecalis* OER = 7·9, *E. coli* OER = 4·3). The greater resistance of *Ent. faecalis* to solar inactivation under anaerobic conditions indicates that this organism is intrinsically less sensitive to oxygen-independent photoinactivation than *E. coli*. The larger proportional increase in solar inactivation of *Ent. faecalis* under aerobic conditions may reflect the lack of measurable catalase activity in *Ent. faecalis*, in contrast to *E. coli* which utilizes this enzyme to increase the rate of decomposition of H_2O_2, a key intermediate in photo-oxidative damage (Ahmad 1981).

The present study indicates that solar disinfection of water will only be fully effective under aerobic conditions. On a practical level, this could be achieved by the agitation of small-scale systems, either by hand or by using an air pump. The next step will be to determine the responses of faecal microbes, including enteric pathogens, under appropriate field conditions and in sewage-contaminated natural waters, so that inactivation can be optimized. In future, it would seem appropriate to refer to this water treatment process as solar photo-oxidative disinfection, to emphasize the key role of oxygen in addition to sunlight.

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