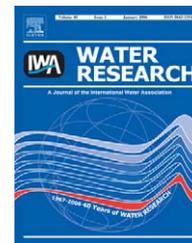


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# Comparative effectiveness of solar disinfection using small-scale batch reactors with reflective, absorptive and transmissive rear surfaces

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## ABSTRACT

This study investigated the enhancement of solar disinfection using custom-made batch reactors with reflective (foil-backed) or absorptive (black-backed) rear surfaces, under a range of weather conditions in India. Plate counts of *Escherichia coli* ATCC11775 were made under aerobic conditions and under conditions where reactive oxygen species (ROS) were neutralised, i.e. in growth medium supplemented with 0.05% w/v sodium pyruvate plus incubation under anaerobic conditions. While the addition of either an absorptive or a reflective backing enhanced reactor performance under strong sunlight, the reflective reactor was the only system to show consistent enhancement under low sunlight, where the process was slowest. Counts performed under ROS-neutralised conditions were slightly higher than those in air, indicating that a fraction of the cells become sub-lethally injured during exposure to sunlight to the extent that they were unable to grow aerobically. However, the influence of this phenomenon on the dynamics of inactivation was relatively small.

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## 1. Introduction

On a global basis, around 2 million deaths per year are attributed to water-borne diseases, and especially to diarrhoea in children (Gordon et al., 2004). In India, almost three-quarters of a billion people live in rural areas without access to safe drinking water and water-borne infections are a major component of morbidity (Patil et al., 2002). Water treatment methods used at the household level in rural communities include boiling, small-scale filtration, and chemical disinfection; however, their application may be limited by factors such as high cost and/or inconsistencies in the availability of fuel or chemical disinfectants (Sobsey, 2002).

An alternative, sustainable approach for locations where sunlight is plentiful is small-scale solar disinfection, based on the exposure of water kept in transparent bottles to sunlight (Acra et al., 1980). Several groups have carried out laboratory and field studies to assess the potential of solar disinfection for treating drinking water, investigating different types of containers ('batch reactors'), including glass and plastic bottles and purpose-made plastic bags (e.g. Acra et al., 1984; Lawand et al., 1997). Additional experiments have been conducted to see whether solar disinfection can be enhanced by the addition of a non-transmissive backing to the container, e.g. an absorptive rear surface to enhance thermal inactivation (Sommer et al., 1997) or a reflective backing to return the sunlight through the water (Kehoe et al., 2001).

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However, there has been no systematic evaluation of the effectiveness of different backing surfaces under the various climatic conditions experienced in locations where solar disinfection may be applied.

An additional complication arises from the fact that almost all previous studies have measured the effectiveness of solar disinfection using conventional aerobic plate counts. Recent research (Khaengraeng and Reed, 2005) has demonstrated that this may underestimate the number of viable bacteria due to respiratory self-destruction (Aldsworth et al., 1999), where the antioxidant defences of sub-lethally injured cells are overwhelmed by reactive oxygen species (ROS) formed as by-products of respiration (Bloomfield et al., 1998): such cells are able to multiply and form colonies only under ROS-neutralised conditions, achieved by the addition of ROS-scavengers such as sodium pyruvate, and by growth under anaerobic conditions.

The current study provides comparative data for the effectiveness of solar disinfection using custom-made batch reactors with either transparent, reflective or absorptive rear surfaces under different weather conditions in sub-equatorial India as part of a broader study into the applicability of solar disinfection in India. The faecal indicator bacterium *Escherichia coli* was enumerated by plate counts under aerobic and ROS-neutralised conditions, to investigate the phenomenon of respiratory self-destruction under natural conditions.

## 2. Materials and methods

### 2.1. Batch reactors

In preliminary experiments, two types of polyethylene terephthalate (PET) container were used: firstly, a commercially available 1 l bottle used for mineral water (McDowell, Bangalore) and secondly, a custom-made solar disinfection reactor (CeeJay, Kochi: Fig. 1), designed with the following factors in mind:

1. *Size*: 1 l of water was selected as appropriate for individual use, especially for children, being easily transported to school/workplace.
2. *Air-space*: to achieve aeration (Reed, 1997), a small air-space is required after adding water, to allow the bottle to be shaken before illumination. Consequently, the internal volume of the reactor was set at 1125 ml.
3. *Weight of plastic*: it was decided to use 48 g of PET per batch reactor. While such a system has a greater wall thickness than most commercially available PET bottles (in India, typically 22 g PET), giving a slightly reduced UV transmission (Fig. 2), it was felt that this would be offset by increased strength/durability in long-term use under field conditions.
4. *Shape*: a flattened, rectangular cross-section was chosen (9.5 cm × 23.0 cm) to provide the largest surface area for sunlight penetration. In a horizontal position (Fig. 1), this provided a light path of 5.2 cm from the front surface to the rear, whereas the commercial (round) mineral water bottle had a maximum light path of 7.5 cm.



Fig. 1 – Photograph of custom-made solar disinfection reactor, 48 g PET (left) and stainless steel reflector (right) alongside a 30 cm ruler, for scale.

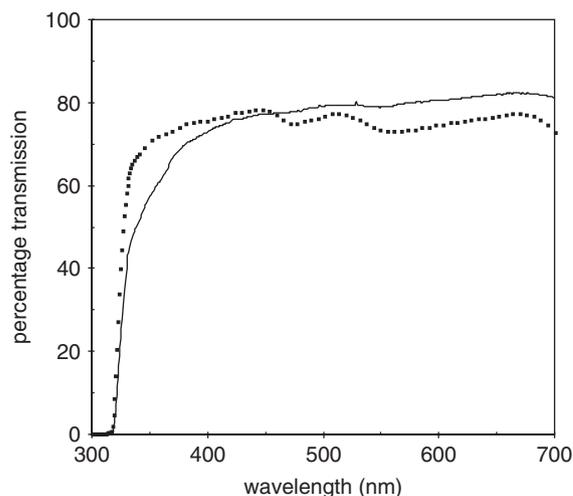


Fig. 2 – Transmission characteristics of plastic (PET) from commercially available mineral water bottle, 22 g PET (dotted line) bottle and custom-made solar disinfection reactor, 48 g PET (solid line).

5. *Style*: a standard 28 mm neck diameter was selected since wider necks proved to be less durable, leaking after a few experiments. The number of surface grooves and features designed to provide strength and rigidity to the reactor was kept to a minimum, to maximise UV transmission. Empirically, the front (exposed) surface was manufactured with 4 straight line grooves, with 5 similar grooves on the rear surface (Fig. 1).
6. *Backing surface*: for the absorptive rear surface, the back of the reactor was painted black. A reflective backing was

achieved by attaching a double layer of food-grade aluminium foil to the rear surface with two thin elastic bands, making sure that the reflective surface faced the container (Kehoe et al., 2001).

## 2.2. Bacterial cultures

*E. coli* ATCC11775 (American Type Culture Collection) was maintained on nutrient agar slopes (Himedia, Mumbai). Bacteria were prepared by loop inoculation of 10 ml nutrient broth (Himedia, Mumbai): after overnight incubation at 37 °C (stationary phase culture), the broth was centrifuged for 10 min at 2500g, the pellet suspended in sterile distilled water, recentrifuged and resuspended in sterile distilled water, to remove any traces of growth medium.

Experimental containers were sterilised by rinsing with 70% ethanol, repeatedly rinsed with sterile distilled water. As in earlier studies (e.g. Reed, 1997; Khaengraeng and Reed, 2005) sterile distilled water (pH 5.5–6.0) was then added to the commercial mineral water bottles (900 ml) and to the custom-made solar disinfection reactors (1050 ml). A rinsed pellet of *E. coli* was then added to each experimental bottle at an initial suspension density of  $\approx 10^6$  CFU ml<sup>-1</sup> measured by plate count, as described below, and representing a dilution of approximately 1000-fold compared to the original stationary phase overnight culture.

## 2.3. Experimental illumination

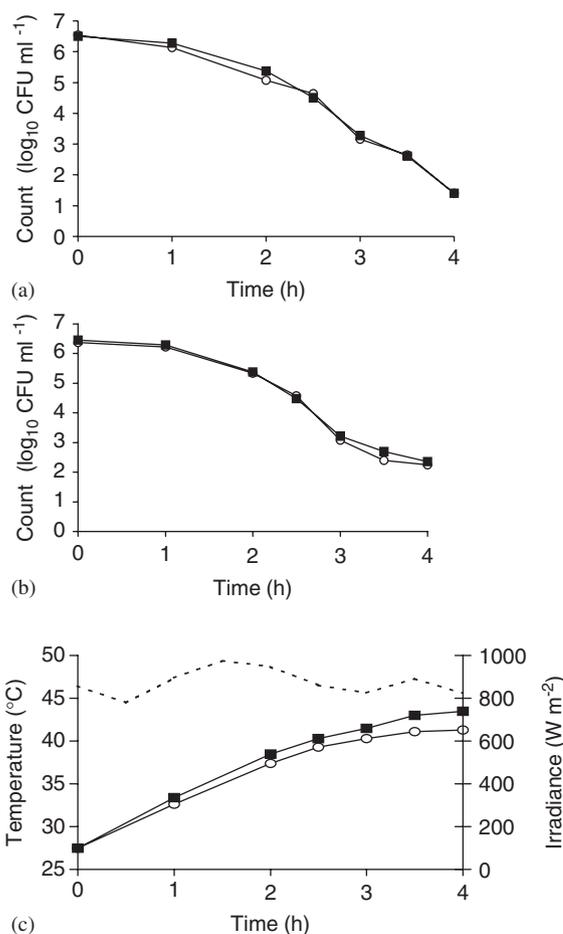
The inoculated containers were shaken well to ensure aeration and to disperse the bacteria, then placed horizontally on the flat roof of the environmental microbiology laboratory at Cochin University of Science and Technology and exposed to sunlight from  $\approx 9:30$  am onwards. At regular intervals (30–60 min), solar irradiation was measured using an SKS1110 pyranometer/SKT660 meter (Skye Instruments, Llandrindod Wells), while the water temperature was recorded using a digital thermometer (Sphincto, Bangalore). The initial water temperature was  $28.0 \pm 1.0$  °C across all experiments. Sampling times were selected according to the weather conditions, with shorter sampling intervals (30–60 min, up to 4–5 h) for strong sunlight, and longer intervals (2–3 h, up to 9 h) for low sunlight, with at least 4 time points per experiment. Average irradiances and maximum water temperatures provided comparative data for different weather conditions.

## 2.4. Enumeration

Samples were processed by serial decimal dilution to  $10^{-3}$ . Droplet plate counts were prepared in duplicate, using 20.0  $\mu$ l of each dilution, giving a minimum detection limit of 25 CFU ml<sup>-1</sup>. For enumeration under aerobic conditions, inoculated plates of unsupplemented nutrient agar were incubated at 37 °C aerobically for 24 h: follow-up incubation (48 h; 37 °C) showed no significant increase in colony counts. For ROS-neutralised conditions (Khaengraeng and Reed, 2005), plates of nutrient agar supplemented with the peroxide scavenger sodium pyruvate (0.05% w/v) were inoculated and immediately incubated at 37 °C under anaerobic conditions,

obtained using an Anaerocult<sup>®</sup> jar (Merck, Darmstadt) for the first 24 h and then aerobically at 37 °C for a further 24 h before counting (this ensured that small colonies obtained during the initial period of anaerobic growth were given sufficient time to reach a visible size): further incubation (48 h; 37 °C) gave no additional colonies.

Plate counts were then converted to CFU ml<sup>-1</sup>. Quantitative comparisons of the dynamics of inactivation were made by calculating the  $T_{90}$  values for each data set, based on log-transformed counts:  $T_{90}$  measures the time required to reduce the count by 90%, determined as the inverse of the slope of the line of best fit (linear regression) for a plot of log CFU ml<sup>-1</sup> against time (e.g. Fig. 3), assuming that the data fit a single exponential decay function (Reed, 1997). While such an approach may be an oversimplification in circumstances



**Fig. 3 – Data for solar disinfection of *E. coli* ATCC11775 in commercial and custom-made containers: plate counts made under (a) aerobic conditions and (b) ROS-neutralised conditions for the commercial mineral water bottle (open circles) and the custom-made solar disinfection reactor (closed squares), while (c) shows irradiance (dotted line) and water temperature for the commercial bottle (open circles) and custom-made reactor (closed squares).**

where the dynamics of inactivation are more complex (Reed, 2004), it provides a single number ( $T_{90}$ ) that summarises the average inactivation time across the entire experimental period.

### 2.5. Comparative evaluation of different backing surfaces

A series of 22 experiments compared custom-made reactors with transmissive, reflective and absorptive surfaces under different conditions throughout the annual cycle (2003–2004). Results were then grouped into three categories:

1. *Strong sunlight* (7 experiments): clear sky with little or no cloud; average irradiance  $>700 \text{ W m}^{-2}$ .
2. *Moderate sunlight* (7 experiments): light/high cloud; average irradiance  $300\text{--}700 \text{ W m}^{-2}$ .
3. *Weak sunlight* (8 experiments); heavy cloud and overcast skies, often with rain; average irradiance  $<300 \text{ W m}^{-2}$ .

## 3. Results

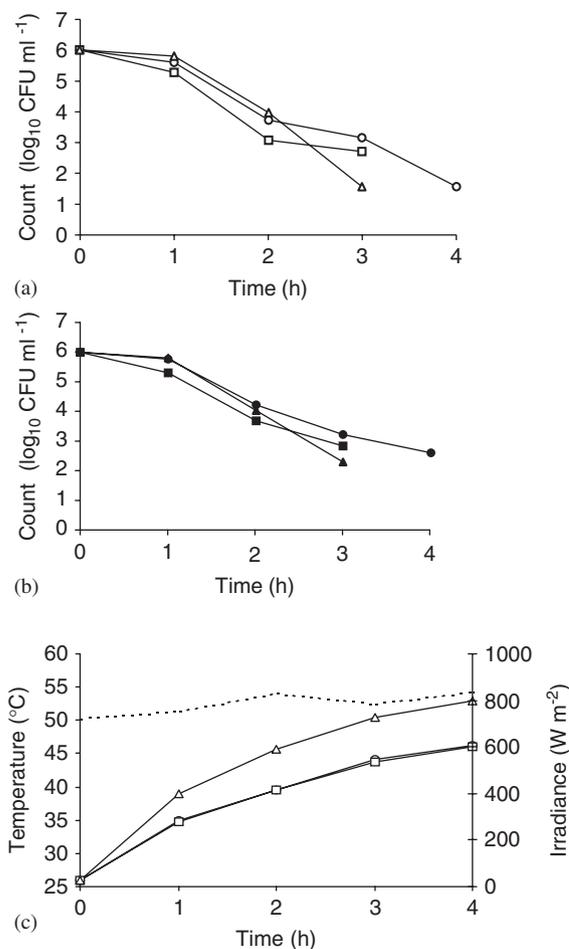
### 3.1. Comparison of commercially available and custom-made systems

Fig. 3 shows a representative experiment for inactivation of *E. coli* ATCC11775 in full-strength sunlight using the commercial bottle and the purpose-made batch reactor with transparent rear surface. In both cases, conventional aerobic plate counts showed an initial slow inactivation up to 1 h, with a steady decline after this period, becoming undetectable at 4 h (Fig. 3a). However, the counts obtained under ROS-neutralised conditions (pyruvate-supplemented medium with anaerobic incubation) were only slightly higher than the equivalent aerobic values towards the end of the time course, remaining above the detection limit at 4 h (Fig. 3b). The maximum temperature attained in both systems was comparable, at 42 and 44 °C respectively (Fig. 3c).

$T_{90}$  values calculated from regression analysis of the data in Fig. 1 showed that while there was little difference between the commercial bottle and the custom-made reactor, the apparent rate of inactivation observed in both systems was faster when counts were made under aerobic conditions, with both bottles giving  $T_{90}$  values of 46 min, while  $T_{90}$  values were around 10% higher under ROS-neutralised conditions, at 50 min for the commercial bottle and 52 min for the custom-made reactor. The commercial bottle also showed similar overall inactivation kinetics to the custom-made reactor in experiments performed at lower irradiances (data not shown).

### 3.2. Effects of different backing surfaces

Representative data for a single experiment using custom-made reactors with transmissive, reflective or absorptive rear surfaces in full-strength sunlight are presented in Fig. 4. Here, the reactor with the reflective backing was slightly more effective than the transmissive reactor, giving somewhat



**Fig. 4 – Data for solar disinfection of *E. coli* ATCC11775 in custom-made containers with different backings. Plate counts made under aerobic conditions (open symbols) are shown in (a) while (b) shows counts made under ROS-neutralised conditions (filled symbols) for reactors with transmissive (circles), reflective (squares) and absorptive (triangles) rear surfaces, and (c) shows irradiance (dotted line) along with water temperature for the reactors with transmissive (circles), reflective (squares) and absorptive (triangles) rear surfaces.**

lower counts under aerobic conditions (Fig. 4a) and ROS-neutralised conditions (Fig. 4b) at all time points during the experiment. This is also in agreement with the observation that the counts fell below the detection limit ( $25 \text{ CFU ml}^{-1}$ ) at 4 h for the reactor with the reflective backing, but not for the transmissive reactor. In contrast, the reactor with the absorptive rear surface gave a broadly similar pattern of counts to the transmissive reactor during the initial stages of illumination, up to 2 h, but then showed a more rapid decrease in counts between 2 and 3 h and was undetectable at 4 h. This decrease was accompanied by a sharp rise in the temperature of the treated water, reaching a maximum 5 °C higher for the reactor with the absorptive backing, compared to the reflective and transmissive reactors. When comparing the aerobic counts with the equivalent values obtained under

ROS-neutralised conditions, the latter generally showed only a slightly higher count, though this was somewhat variable. When the rate of inactivation was expressed in terms of  $T_{90}$ , based on regression analysis of the data in Fig. 4a and b, a slightly higher value was also obtained for the ROS-neutralised count data, with  $T_{90}$  values of 52, 48 and 38 min respectively for the reactors with the transmissive, reflective and absorptive backings based on aerobic plate count data while the corresponding  $T_{90}$  values for ROS-neutralised counts were 63, 52 and 45 min, representing an average increase in  $T_{90}$  of 16% overall.

### 3.3. Comparative effectiveness of reactors under strong, moderate and weak sunlight

To compare the three types of rear surface, average  $T_{90}$  values based on (i) aerobic counts and (ii) ROS-neutralised counts are shown in Table 1 for experiments grouped into (i) strong sunlight ( $>700 \text{ W m}^{-2}$ ), (ii) moderate sunlight ( $300\text{--}700 \text{ W m}^{-2}$ ) and (iii) weak sunlight ( $<300 \text{ W m}^{-2}$ ). For the standard transmissive reactor, it is clear that  $T_{90}$  was strongly influenced by irradiance, with the values obtained under weak sunlight being almost three times those under strong sunlight. As noted for Figs. 3 and 4,  $T_{90}$  values for counts made under ROS-neutralised conditions were slightly higher (9–14% greater) than the equivalent aerobic values and a one-sided paired t test confirmed that the higher average value under ROS-neutralised conditions was statistically significant ( $P = 0.045$ ).

When the  $T_{90}$  values for the reactor with the reflective backing are compared to those of the transmissive system, the former gave consistently lower values, with a broadly similar reduction (17–25% lower) across all illumination conditions. The absorptive reactor was clearly more effective than the transmissive reactor under strong sunlight (15% lower  $T_{90}$  for aerobic counts and 24% lower  $T_{90}$  for ROS-neutralised counts), but less so in moderate sunlight (5–6% lower), while under weak sunlight an increased  $T_{90}$  was observed (13–14% greater). The enhanced effectiveness of the absorptive reactor in strong sunlight is likely to be linked to the increase in maximum temperature observed in full sunlight (Table 2) which results mainly from the absorption of solar infra-red radiation by the back-painted rear surface. In contrast, the slightly reduced rate of inactivation under overcast conditions may be due to the fact that an unpainted (transmissive) rear surface may reflect back some UV/visible radiation, especially for sunlight at acute angles to the surface, whereas a black-painted rear surface would not. A one-sided paired t test showed that the higher average  $T_{90}$  obtained under ROS-neutralised conditions was statistically significant ( $P = 0.028$ ) for the reflective reactor, though not quite so for the absorptive reactor ( $P = 0.053$ ). A larger data set would be required to further investigate this discrepancy. Overall, while the effects of ROS-neutralisation may have been significant in some instances, the magnitude of the difference between ROS-neutralised and aerobic  $T_{90}$  values was relatively small.

Table 1 also allows the reactors with reflective and absorptive rear surfaces to be directly compared, showing clearly that the reflective reactor was more effective in weak

sunlight, where  $T_{90}$  values were around 33% lower for the reflective reactor, and in moderate sunlight, at around 19% lower. In contrast, the absorptive reactor was slightly more effective in strong sunlight, giving  $T_{90}$  values 2% (aerobic counts) and 6% (ROS-neutralised counts) lower than for the transmissive reactor. Thus, under non-optimal light conditions (overcast conditions), the reflective reactor gives the most rapid rate of inactivation, with  $T_{90}$  values around two-thirds of those observed for the reactor with the absorptive rear surface.

## 4. Discussion

The present study has shown that the dynamics of solar inactivation of *E. coli* in water kept in a mineral water bottle and a custom-made reactor of equivalent volume were broadly similar, suggesting that the greater thickness and reduced sunlight transmissibility of the plastic walls of the custom-made container (Fig. 2) are counterbalanced by the reduced light path. The custom-made reactors have also proved to be more durable in field trials with villagers in rural locations in India, justifying their use in preference to the lighter, commercially available bottles.

While, the extent of inactivation observed for *E. coli* ATCC11775 in full-strength sunlight is broadly similar to earlier studies (Reed, 2004),  $T_{90}$  values determined under ROS-neutralised conditions were slightly higher than those based on aerobic plate counts, in agreement with the findings of Khaengraeng and Reed (2005) for UVA-illuminated *E. coli* NCTC8912. While this disparity in  $T_{90}$  appears relatively small, it could amount to a somewhat larger difference in the extent of inactivation over the course of a day's exposure to sunlight, especially under conditions of low irradiance (Table 1). The fact that such bacteria might not be detected by aerobic plate counts begs the question as to whether conventional procedures are fully effective in enumerating indicator bacteria and potential pathogens: this is discussed further by Reed (2004).

The present study has clearly demonstrated that a reflective rear surface can enhance the solar inactivation of *E. coli*, irrespective of the strength of sunlight (Table 1), presumably due to the return of UVA and short-wavelength visible radiation through the reactor, leading to increased damage to cellular components and the consequent inactivation of the bacteria. This finding is in broad agreement with earlier work of Kehoe et al. (2001), who showed that a reflective foil backing could enhance the rate of inactivation of a Kenyan isolate of *E. coli* (based on aerobic plate counts) in a small-scale reactor (500 ml volume) under high levels of natural and simulated sunlight. In contrast to the current study (Table 2), Kehoe et al. (2001) also noted an increase in temperature within the reflective reactor, which may be linked to its smaller volume and/or different shape. Lawand et al. (1997) carried out experiments with plastic bags placed on reflective (white painted) surfaces, whereas our preliminary trials showed better results when using silvered aluminium foil as a reflector, rather than white paint. However, at a practical level, food-grade aluminium foil is not the most suitable material, as it tears readily in field conditions. Additionally,

**Table 1 – Average  $T_{90}$  data (in minutes) for *E. coli* ATCC11775 in custom-made solar disinfection batch reactors with transmissive, reflective and absorptive backings as a function of average solar irradiance, based on (i) aerobic counts and (ii) ROS-neutralised counts (ROS<sup>n</sup>), with standard errors shown in brackets ( $n = 7$  or  $8$ )**

Backing surface	Average solar irradiance					
	< 300 W m <sup>-2</sup>		300–700 W m <sup>-2</sup>		> 700 W m <sup>-2</sup>	
	Aerobic	ROS <sup>n</sup>	Aerobic	ROS <sup>n</sup>	Aerobic	ROS <sup>n</sup>
Transmissive	153 (34)	180 (52)	89 (16)	97 (15)	58 (3)	66 (4)
Reflective	117 (24)	135 (40)	68 (10)	75 (12)	48 (3)	53 (3)
Absorptive	175 (49)	204 (68)	84 (16)	92 (17)	49 (3)	50 (3)

**Table 2 – Average maximum temperatures (°C) attained by custom-made solar disinfection batch reactors with transmissive, reflective and absorptive rear surfaces as a function of average solar irradiance, with standard errors shown in brackets ( $n = 7$  or  $8$ )**

Backing surface	Average solar irradiance		
	< 300 W m <sup>-2</sup>	300–700 W m <sup>-2</sup>	> 700 W m <sup>-2</sup>
Transmissive	33.7 (1.5)	43.5 (1.3)	48.0 (0.6)
Reflective	33.6 (1.5)	43.4 (1.2)	47.7 (0.6)
Absorptive	36.4 (2.3)	48.9 (1.3)	54.6 (0.6)

the highly reflective surface dulls over the course of a month or two, due to oxidation, thereby reducing its effectiveness. In consequence, we have devised a purpose-built stainless-steel reflector (Fig. 1) which fits snugly onto the back of the reactor and which had a similar overall effect to aluminium foil; in comparative trials, the stainless-steel reflector gave  $T_{90}$  values (based on ROS-neutralised count data) of  $\approx 93\%$  of those of the foil-backed reactor. This system is proving to be extremely durable in on-going field trials with villagers in India.

While several studies have demonstrated that black-backed containers can enhance the solar inactivation of bacteria under strong sunlight (e.g. Sommer et al., 1997; Martin-Dominguez et al., 2005), other researchers have noted that under overcast skies such containers may take 2 days to completely inactivate the contaminant bacteria (Oates et al., 2003), which is in agreement with the results observed under low sunlight conditions during the present study. The temperature enhancement observed with black-backed reactors (Table 2) was also most notable at high irradiances, which means that synergetic interaction between thermal and optical effects noted at high temperatures (McGuigan et al., 1998; Wegelin et al., 1994) will not occur at low irradiances. In contrast, Joyce et al. (1996) have shown that highly turbid natural waters may show substantial temperature increases

due to absorption of solar radiation by suspended particulate material, without the need to use an absorptive rear surface. However, such waters may fail to reach high enough temperatures to give effective inactivation of bacteria under overcast conditions.

The study of Walker et al. (2004) appears to be the only previous research to specifically compare reflective, transmissive and absorptive rear surfaces, in a purpose-designed 1 l heat-sealed PET pouch format. When tested in declining (Autumn) and low (Winter) sunlight conditions (solar irradiances not determined), the reflective pouch was found to be more effective than the absorptive pouch, in agreement with the results of the current study for irradiances below 300 W m<sup>-2</sup> (Table 1). They also noted that the performance of the absorptive pouch was consistently poorer than the reflective pouch even when the temperature attained in the former was 52 °C compared to 36 °C in the latter. However, while such single-use, heat-sealed plastic pouches could have specific applications for short-term provision, e.g. in response to emergencies and disasters, the reusable bottle format used in the current study is a more practical and durable approach.

## 5. Conclusions

Custom-made small-scale batch reactors with a reflective rear surface gave better results for solar disinfection of *E. coli* than those with transmissive and absorptive backings under sub-optimal sunlight conditions where thermal effects are minimal and optical photo-oxidative inactivation is maximised by the return of solar radiation through the water under treatment. Given that solar disinfection is slowest under such conditions, the enhanced performance of a reactor with a reflective rear surface means that this design would be a preferred option for use in any location where seasonal variations in sunlight are significant, e.g. in countries affected by monsoon conditions, such as in India.

Counts of solar-irradiated *E. coli* performed under ROS-neutralised conditions were, on average, slightly higher than their aerobic counterparts, indicating that the antioxidant defences of some cells are damaged to the extent that they become sensitive to their own respiratory by-products. While

such a phenomenon has an effect on the overall dynamics of inactivation, leading to slightly higher  $T_{90}$  values for bacteria enumerated under ROS-neutralised conditions, it does not invalidate the findings of earlier studies, based solely on aerobic count data. Furthermore, the results of Smith et al. (2000) suggest that sub-lethally injured bacteria may be less virulent than their uninjured counterparts, which may mean that it is less important to enumerate this sub-group than the healthy cells.

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