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Inactivation of enteropathogenic *E. coli* by solar disinfection (SODIS) under simulated sunlight conditions

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Abstract. Solar Disinfection (SODIS) is a low cost water treatment method currently used in communities that do not have year round access to safe water. However, there is still reluctance in widespread adoption of this treatment method due to a number of limitations. An important limitation is the lack of SODIS inactivation studies on some waterborne pathogens in the developing world. SODIS inactivation of enteropathogenic *E. coli* (EPEC), a major cause of infantile diarrhoea is reported for the first time under simulated sunlight conditions and following a natural temperature profile. EPEC was exposed to simulated sunlight ($885 Wm^{-2}$) for periods up to a cumulative time of 4 hours. Inactivation was determined by a log reduction in growth of the organisms. The temperature (^{o}C) of the water was taken at every time point. After 4 hours exposure EPEC was completely inactivated (7 log reduction) by SODIS. Imposing a realistic water temperature profile (min-max) concomitant with irradiation produces a greater kill of EPEC. Maintaining simulated sunlight experiments at a high fixed temperature may result in over –estimation of inactivation. Following a natural water temperature profile will result in more reliable inactivation comparable with those that might be obtained under natural sunlight conditions.

1. Introduction

In areas where people do not have-year round access to safe drinking water, solar disinfection (SODIS) is a low cost, environmentally friendly method of treating contaminated water. The method involves exposing microbially contaminated water in transparent containers ≥ 6 hours of direct sunlight resulting in the inactivation of enteric bacteria, through the synergistic effect of ultraviolet A (UVA) radiation and an increase in water temperature [1] .The most commonly used transparent containers are plastic bottles made of poly (ethylene) terephthalate (PET) with a maximum volume of 5 liters per bottle. However, there is still reluctance in adoption of the SODIS technique due to a number of limitations including the lack of SODIS inactivation studies regarding some waterborne pathogens that are prevalent in developing countries.

Enteropathogenic *Escherichia coli* (EPEC) is the leading cause of infantile diarrhea in developing countries. Infants contract this bacteria through consumption of contaminated water and ingestion of milk formula which is prepared with contaminated water. EPEC still remains untested against SODIS [2].

The aims of the following study were, firstly, to determine if solar UV, generated through the use of a solar simulation apparatus, can be used to disinfect drinking water contaminated with EPEC. Secondly, to conduct inactivation of EPEC by following a natural temperature profile that one expects

for fixed volumes of water instead of maintaining the water temperature at a constant maximum as was done for inactivation studies of other organisms.

2. Methods

2.1. Bacterial Growth

EPEC *E. coli* O157 (ATCC 23631) and *E.coli* K-12 were obtained from frozen stocks and streaked on to Luria Broth (LB) agar and incubated at appropriate conditions for growth. Single colonies were then inoculated in 5 ml of sterile Luria broth (Sigma; L 3522) and incubated at 37 ^{O}C for 18 h to obtain a stationary phase culture. Cells were harvested by centrifugation at 2000 x *g* for 10 minutes and washed three times with phosphate buffered saline (PBS).

2.2. Bacterial Inactivation

The pellet was resuspended in sterile de-ionised water to a final concentration of 10^6 colony forming units CFU/ml). Volumes of 10ml of this preparation were placed in lidded 6 –well transparent polystyrene tissue culture plates. Optical transmission properties of polystyrene are comparable to those of PET as both transmit UVA (320nm – 400nm) and both are opaque to wavelengths below UVB [3, 4]. Control wells were covered with foil to exclude sunlight. Tissue culture plates were then placed in position E (Figure 1). The temperature of the water in the PS well was taken at time point 0 and at each time point thereafter to obtain a natural temperature profile (minimum to maximum). Bacterial inactivation experiments were conducted in triplicate for each time point.

2.3. Bacterial Enumeration

Bacterial enumeration of water samples exposed to sunlight was conducted through the standard plate method. 20 µl of the approximately diluted sample was spread on agar plates in triplicate and incubated 37 ^{O}C overnight. The log kill of organisms was plotted with standard error of the mean for each time point according to the formula (Log Nt/N0) where Nt is the viable count at the experimental time point and N0 is the initial viable count at the start of the experiment. The limit of detection for the plate count method was 7 CFU/ml.

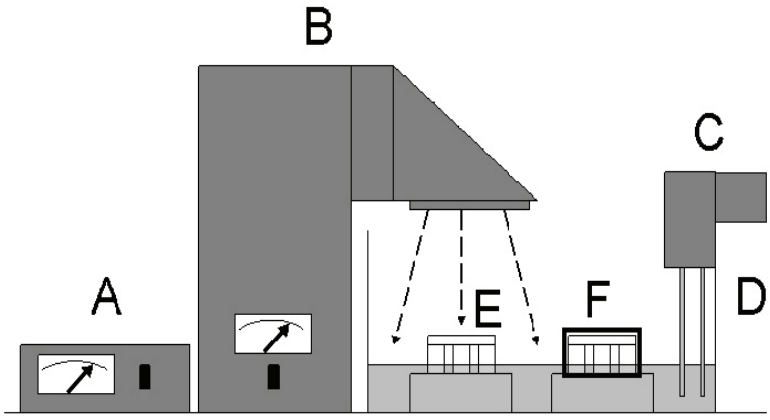

Figure 1 - Solar- filtered 1000W xenon arc lamp solar simulator apparatus: (A) = 1000 W Arc lamp power supply; (B) = 1000 W xenon arc lamp housing; (C) = Water heater; (D) = Water Bath; (E) = Test microbe suspension sample in a lidded 6-well microtitre plate; (F) = Control microbe suspension samples in a lidded 6-well microtitre plate wrapped in aluminium foil

3. Results and Discussion

Within 4 hours exposure of EPEC to simulated sunlight conditions (885Wm^{-2}), the bacteria are below the limit of detection (Figure 2). This demonstrates the susceptibility of the organism to SODIS as previously reported for enterobacteriacea, such as *Salmonella typhimurium*, *Shigella dysenteria*, *Escherichia coli*, *Vibro cholera* and *Pseudomonas aeuriginosa [5-8] E. coli* K-12 is not a waterborne pathogen but is a laboratory strain organism that shares similarities to EPEC and acts as a positive control as it is known to be susceptible to SODIS. However, considerable differences in the time required and pattern inactivation by SODIS of these organisms requires that every organism be tested individually [6]. This is to ensure that overestimation of the inactivation of an organism does not occur based on previous inactivation studies of similar organisms.

Under natural sunlight conditions, water gradually heats up to reach a maximum temperature. Previous solar simulated inactivation studies have been conducted at a fixed maximum temperature and might therefore have overestimated the inactivation time required by a given pathogen. This is due to the synergistic effect that occurs during SODIS when temperatures are greater than 45^{o}C [7]. For the inactivation of EPEC instead of having a fixed temperature, a natural temperature profile was followed (Fig. 2). At low temperatures inactivation of EPEC still occurred showing that SODIS inactivation of microbial pathogens is not dependent on temperature. The role of UV irradiation in inactivation is not only important at low temperatures but is also required even at higher temperatures as resistance to greater temperatures has been observed in faecal coliforms [9]. Even though a temperature ≥ 45^{o}C was not obtained in this experiment, a greater reduction in log kill was observed when the temperature of the water increased (Fig 2.)

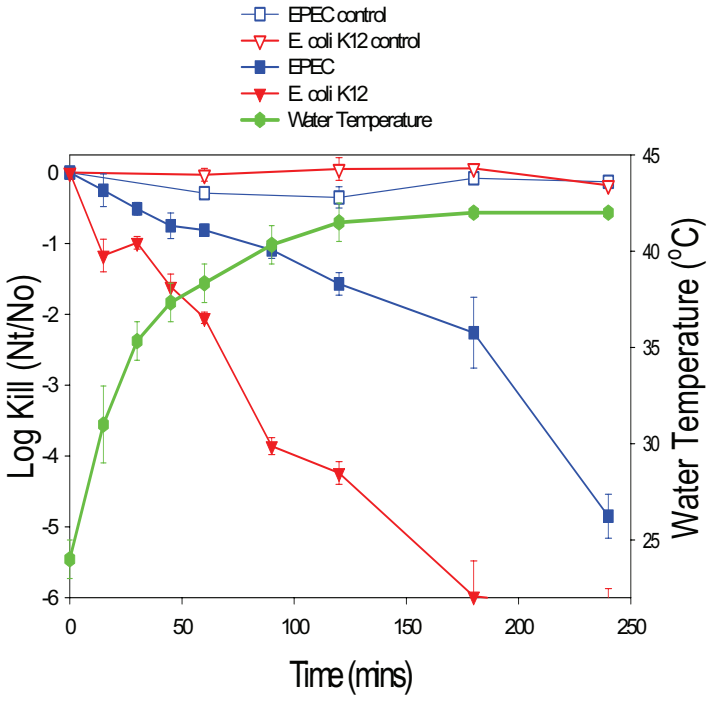

Figure 2 - Inactivation kinetics of EPEC and E. coli K12 exposed to simulated sunlight conditions, following a natural temperature profile

4. Conclusion

By demonstrating the inactivation of EPEC by SODIS under simulated conditions, we have shown the potential of SODIS to be a household water treatment method for a highly infectious waterborne pathogen.

Following a natural temperature profile during SODIS inactivation under simulated conditions will ensure that inactivation times obtained for EPEC are comparable to those obtained under real sunlight conditions. However, other factors such as turbidity and the use of natural water need to be further investigated to determine their effect on the inactivation of EPEC.

5. Acknowledgements

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6. References

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