Purpose of visit

The aim of the project was to access the effectiveness of batch process SODIS (solar disinfection) using natural sunlight against the bacterial pathogens *Campylobacter jejuni*, an entropathogenic strain of *Escherichia* coli and *Staphylococcus epidermidis*. The Solar Disinfection of drinking water involves storing contaminated drinking water in transparent plastic containers that are placed in direct sunlight for periods of up to 8 hours before consumption.

Work Carried out

Bacterial samples were irradiated using natural sunlight at the Plataforma Solar de Almerıa (PSA) solar research facility in Almeria, Spain (Latitude 37° 05′ 54″ ,Longitude 2° 2′ 32″) in May 2006. Measurements of solar dose were made using a 300–400 nm broadband UV radiometer.

C*. jejuni* NCTC 11168 was grown on Campylobacter blood-free selective agar base in anaerobic conditions((modified CCDA-Preston); CM0739, Oxoid, UK) *C. jejuni* isolates were inoculated into 10ml. of sterile Brucella Broth Base (Sigma, Germany) and incubated in a sealed, air-tight container with an anaerobic Gas Generating Kit (BR0038B, Oxoid, UK) overnight at 37^{o}C, on a rotary shaker at 200rpm. 1.5 litre Polyethylene Terephthalate (PET) bottles were filled with sterile deionised water or a sterile 0.9% (w/v) saline solution. A standard solution of bacteria was made in order to obtain an inoculum level of $\geq 10^{6}$ colony-forming units (CFU) $ml^{-1.}$ Samples were left at room temperature for 15 min to allow *C. jejuni* cells adjust to their new nutritional and osmotic environment. Samples were shaken at the start of an exposure to guarantee maximum starting oxygen levels.

S. epidermidis RP62A was grown on BHI agar (Oxoid UK) and an overnight was prepared from a single colony and inoculated into 5 ml of BHI broth (CM0025, Oxoid UK).*E. coli* (enteropathogenic strain) was routinely grown on Luria Betani (LB) agar (Sigma, Germany) and a single colony inoculated into 5mls of LB broth (Sigma, Germany).)

Both cultures were incubated at 37ºC, on a rotary shaker at 150rpm overnight. The cultures were then spun at 3000 rpm for 10 minutes and washed three times with distilled water. The pellet was then resuspended in distilled water and used to create a standard solution of approximately 10^6 CFU ml-1, this solution was made in 1.5 L PET bottles which left at room temperature for 15 minutes and then shaken prior to exposure. Bottles were placed down on a flat surface exposed to strong natural sunlight and the intensity of light and temperature monitored.

For all experiments were performed in triplicate and 1 ml samples were taken at different time points and serially diluted in their respective broths, the 20ul of each dilution was then plated using the Miles Misra drop count technique onto their respective agars.

S. epidermidis and *E. coli* were incubated aerobically at 37ºC for 24 hours whilst *C. jejuni* was incubated in a sealed anaerobic container for 42-48 hours before counting. *Solar disinfection of enteropathogenic E. coli in both distilled water and saline*

Results Obtained

The results obtained from the solar disinfection experiments with E. coli exposed in 1.5L PET bottles (fig. 1), showed a 5.5 log reduction in viable cells after 90 minutes when the samples were exposed in water, the exposed saline solution achieved the same reduction after 240 min , after this time no detectable viable cells were found.

After exposure samples were stored in the dark for 48 h and then replated in order to assess possible regrowth after disinfection, no regrowth was found for any of the samples exposed to solar radiation. The maximum temperature reached for this experiment was 37ºC and the peak UVA/B reached was 4.7 W/M2.

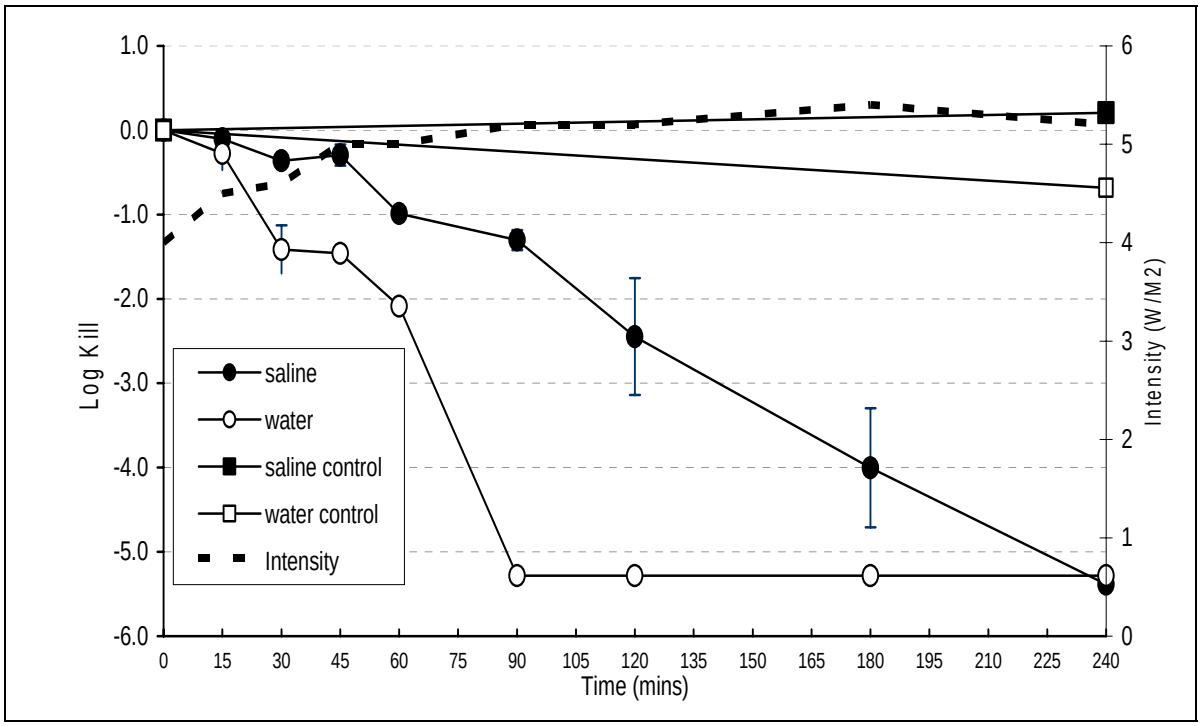

Figure 1. Solar disinfection of enteropathogenic *E. coli* in 0.9% sterile saline and distilled water

1.1 Solar disinfection of Staphylococcus epidermidis RP62A in both distilled water and saline

The results obtained from the solar disinfection experiments with S. epidermidis exposed in 1.5L PET bottles (fig. 2), showed a 5 log reduction in viable cells after 45 minutes when the samples were exposed in water, the exposed saline solution achieved a 5 log inactivation after 90 minutes, after this time no detectable viable cells were found.

After exposure samples were stored in the dark for 48 h and then replated in order to assess possible regrowth after disinfection, no regrowth was found for any of the samples exposed to solar radiation. The maximum temperature reached for this experiment was 31.7 ºC and the peak UVA/B reached was 4.9 W/M^2.

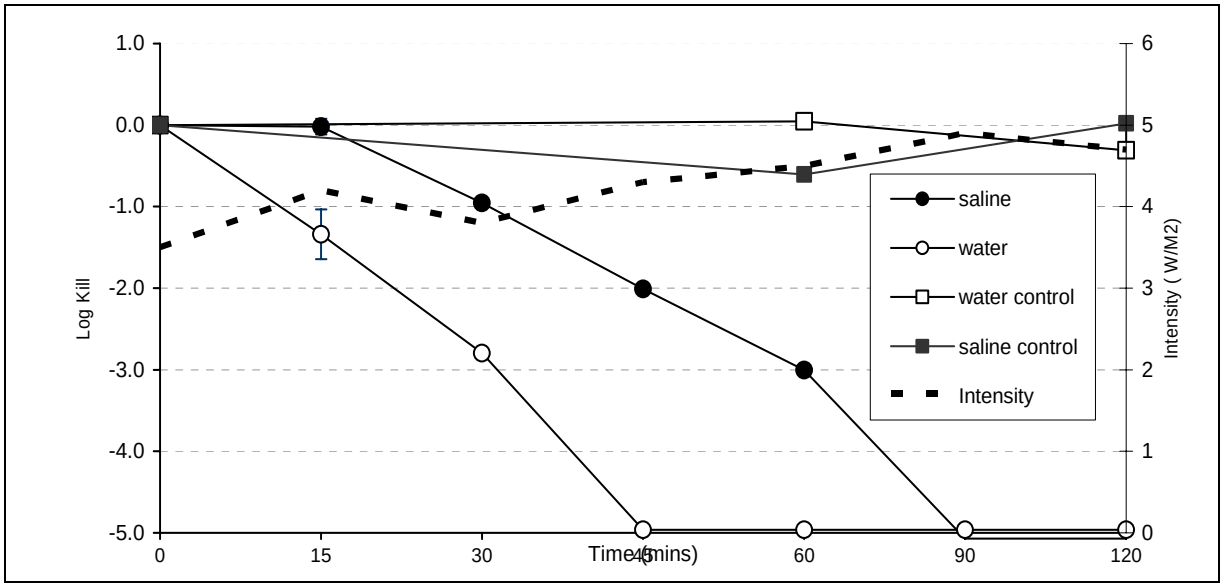

Figure 2. Solar disinfection of *S. epidermidis* RP62A in distilled water and in 0.9% sterile saline.

1.2 Solar disinfection of Campylobacter jejuni NTCT 11168 in distilled water

The results obtained from the solar disinfection experiments with C. jejuni exposed in 1.5L PET bottles (fig. 3), showed a 5 log reduction in viable cells after 15 minutes when the samples were exposed in water, after this time no detectable viable cells were found. After exposure samples were stored in the dark for 48 h and then replated in order to assess possible regrowth after disinfection, no regrowth was found for any of the samples exposed to solar radiation.

The maximum temperature reached for this experiment was 26.8 ^{o}C and the peak UVA/B reached was 4.8 W/M^{2}.

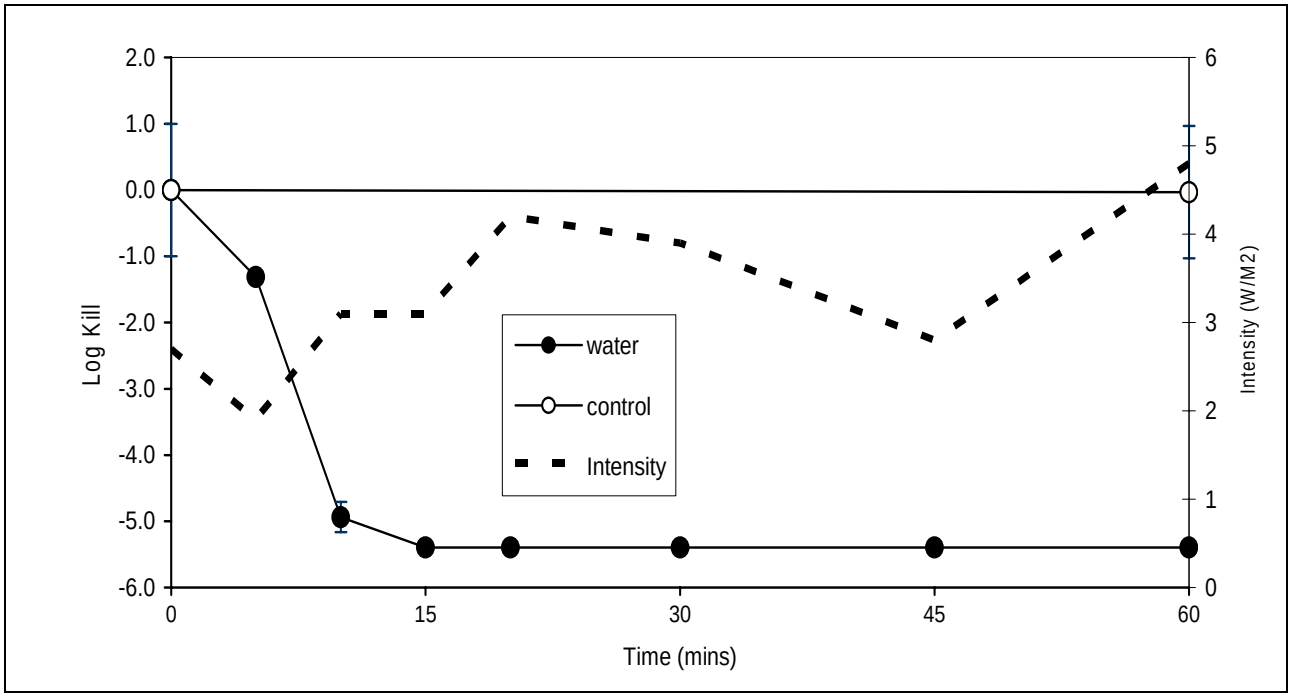

Figure 3. Solar disinfection of *C. jejuni* in distilled water

Conclusions:

- Batch Process solar disinfection is effective against
  - An entropathogenic strain of *E. Coli*
  - Gram positive *S. epidermidis*
  - Anaerobic *C. jejuni.*

- A slower inactivation is achieved with saline than distilled water.

Future Collaborations with Host institution

There will be a future collaboration involving the evaluation of solar disinfection enhancement technologies through the use of photocatalysts and also experiments involving SODIS of bacteria using natural waters and its effect on the survival of the microorganisms.

Projected publications/articles resulting or to result from the STSM (if applicable).

1. publication in Solar Energy Journal: Establishing the range and limits of SODIS: Inactivation of *C. jejuni*, EPEC, *Y. enteroclitica* and Spores of *B. subtilis*: Boyle, Sichel, Fernandez, Bolivians, McGuigan

Confirmation by the host institute of the successful execution of the mission

In my opinion this work performed in Plataforma solar de Almería (PSA) on May 2006 has been carried out successfully. The aim of the experimental series and the obtained results are clearly of the best scientific quality. In fact, they are going to be published in an international scientific journal due to the innovation and originality of this work. I am very satisfied with the work and results obtained by Maria Boyle at PSA.

Dra. Pilar Fernánez Ibáñez

Supervisor of the work at Plataforma Solar de Almería