# Solar and UVA Inactivation of *Pseudomonas spp.*

**A project submitted in partial fulfilment of the requirements for the Degree of BSc/BSc (Hons) in Biomedical Sciences.**

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**Declaration:** I **Barry Hollinshead** confirm that I have read and understood the University regulations concerning plagiarism and that the work contained within this project report is my own work within the meaning of the regulations.

Signed…………………………………………………………………..

## Introduction.

In developing countries drinking water is very scarce due to a lack of funding for water treatment system development. In most eastern developing countries their main source of drinking water comes from surface waters such as rivers and lakes. This causes a major health problem as these rivers and lakes are not only used for drinking but other activities such as defecating near the water source, bathing and watering livestock. It is due to the multiple uses of the water source that results in the water becoming contaminated with pathogenic organisms with over one billion people being exposed to contaminated and unsafe water annually (Rojko, 2003). Due to the problem of these countries being unable to afford expensive water treatment and purification facilities has led to studies in to alternative and inexpensive methods of disinfecting and purification of water for safe drinking.

The oldest recorded purification method is the use of sunlight (Reed, 2004) which dates back to 2000 BC. It was not until 1887 when Downes and Blunt as reported by Reed (2004), who carried out the first scientific studies, in to the investigations of effectiveness of the disinfection and purification of solar radiation on bacterial organisms. Their results showed that urine could be made sterile by sunlight. However they also showed that mycelial fungi spores were more resistant to the sunlight inhibitory actions than bacterial cells. The practical application of solar disinfection on drinking water was introduced in 1980 (Acra et al, 1984) in a study that was promoted as a result of the understanding of the need for developing countries for safe drinking water supplies and as a result of the lack of safe drinking water the health problems that are associated with drinking contaminated water. The study was to assess the practicality of the use of solar disinfection of relatively small amounts of drinking water enough for the needs of individuals and families (Acra et al, 1984). The results of this study showed with exposure to sunlight for a good period of time water that was contaminated with faecal bacteria, the bacteria was susceptible to destruction and therefore disinfecting the drinking water and making it safe to drink. However Acra reported that the rate of which the destruction of the bacteria depended on certain important factors that had to be considered. These factors included the intensity of the sunlight and length of exposure time, the species of the bacteria which was being exposed and the type of containers used (Acra et al, 1984). This study also showed that not only bacteria could be affected in this way but also molds and yeasts were also susceptible and within three hours there was complete destruction of species such as *Aspergillus niger. Aspergillus flavus* and *Candida*. However it required 6-8 hours of exposure for complete destruction of *Penicillium* which proved to be the most resistant. The significance of this study showed that their preliminary findings could be of use for the preservation of stock solutions of oral re hydration fluids for distribution by world wide health care centres (Acra et al, 1984). The results of these studies suggested that the method of solar disinfection would be an inexpensive method of treating contaminated drinking water where an alternative was to expensive for developing countries and promoted the interest of this method and lead to further studies in this field of research (Reed, 2004).

**Methods and applications of solar disinfection.**

The methods of solar disinfection have been proven to be effective against faecal bacteria and water-borne pathogens and certain molds the method of applications are all the same but there are different styles and ways to carry out this method. The water is placed in plastic PET bottles with approximately 1 – 2 litres of water. In a report published in SODIS news (Anon, 1997), it showed that PET (polyethylene terephtalate) bottles were safer than PVC (polyvinyl chloride) bottles as there are less UV-stabilisers incorporated within PET (less than 1%). Although there is health risks concerned with these additives the risks are very minimal with PET as these very large molecules very rarely immigrate through the PET (Anon, 1997). When comparing the PET against glass bottles they found that ordinary window glass was opaque to UV- radiation, but did find certain glasses for example Pyrex, Corex and Quartz glass transmitted more UV- radiation. The problem with using such glass in large scale is the expense and availability of this glass in developing countries (Anon, 1997). The advantages for PET against glass are the PET bottles are low in weight, relatively unbreakable, transparent, they are taste neutral (don't leave taste of bottle in water where PVC bottles leave a plastic like taste in the fluid), and are chemically stable. The advantages of glass are that they are heat resistant where PET can deform at temperatures above 65$^{o}$C, they don't scratch as easy as PET. However the disadvantages of glass compared to PET are they are easily smashed, are more expensive and heavy in weight (Anon, 1997). Although the results of this showed that certain glass was more effective the advantages of the PET out weighed the advantages of the glass. Therefore it was recommended that PET bottles were the bottles of choice for use in solar disinfection. The water that is being disinfected should be strained and filtered through muslin or clean sand (Moulton, 2006) to reduce the turbidity of the water. If the water has to much turbidity this will reduce the absorbance of the UVA radiation through the bottle. Once the water is clear and free from turbidity the water has to be oxygenated by shaking the bottle vigorously to oxygenate the water for 15 – 20 seconds. In a study of the critical role of oxygen in solar disinfection (Reed, 1996) results showed that for rapid inactivation of *E. coli* and *Ent. faecalis* when exposed to sunlight dissolved oxygen was essential. In another study the importance of oxygenated water was also shown that the effects of oxygen should be considered at every stage of the procedure (Khaengraeng et al, 2005). This consideration was particularly important during the enumeration, where in order to gain a valid count neutralisation of the ROS (reactive oxygen species) inhibitory effects was essential. These inhibitory effects can be neutralised by adding pyruvate or catalase as these naturally occurring peroxide degrading compounds breakdown the ROS and enable the bacteria to grow (Khaengraeng et al, 2005). The bottles containing the contaminated water are then placed in a position upon where they would gain the most direct sunlight for a period of 4 to 6 hours. It is suggested that the bottles are inclined to receive the maximum amount of sunlight (Moulton, 2006). To achieve the maximum effect of the UV-radiation the bottles can be manipulated in to having specific qualities to enhance the solar disinfection. As well as using PET bottles by either blackening the under side of the bottle, the bottle can also be placed on a reflective backing such as tin foil or shiny metal plates. In a study to investigate this (Mani et al, 2006) bottles with painted black undersides and a custom made reflective backing were compared against a normal clear bottle. By carrying out a series of 22 experiments the bottles were compared under different conditions of sunlight throughout the annual cycle. When comparing the results they showed that the reflective backed bottles showed to be more effective than clear bottles and produced lower counts in either anaerobic or aerobic conditions (Mani et al, 2006). Initially the black painted bottle was at first showing similar results as the clear bottle then after two hours a rapid decrease in numbers was seen. It was also noted that the temperature of the water in the black painted bottle had raised 5$^{o}$C higher than the other bottles. In changes of sunlight from strong to weak the study showed that in strong sunlight the black painted bottle was much more effective than the other bottles which was suggested to be down to rise in temperature and in low sunlight the reflective bottles were the most effective (Mani et al, 2006). This study demonstrated irrespective of the strength of the sunlight reflective rear surfaces increases the effects of solar disinfection. In warm climates with strong sunlight the black painted bottles speed up the disinfection due to the temperature increase (Moulton, 2006). As well as using bottles a pouch has been developed using food grade packaging materials (Walker et al, 2004) which has shown also to be an effective system which is very inexpensive for use in application of solar disinfection.

**Further studies and applications of solar disinfection.**

As a result of the outcome and findings in previous studies in which small amounts of water were used to assess the effectiveness of solar disinfection a study into the possibility of using the same principles on a continuous flow system (Acra et al, 1990) a simple prototype was designed and its effectiveness in solar disinfection was assessed. Their results showed that prototypes worked satisfactorily when used in the experiments, but yield of safe drinking water could be increased by increasing the capacity of the reactor (Acra et al, 1990). In a series of studies (Conroy et al, 1996, 1999, 2001) the applications of solar disinfection were assessed to investigate whether this could reduce diarrhoea and cholera. The results of this study showed that solar disinfection was an effective method in the prevention of these illnesses and may significantly reduce the morbidity in counties that lacked the resources for safe drinking water (Conroy et al, 1996). In an update to the first study it was reported that solar disinfection proved to be an effective in vivo "free, low technology, point of consumption method of improving water quality" (Conroy et al, 1999). Due to an outbreak of cholera in the Kajiado District in Kenya during November 1997 to January 1998, the opportunity to test the effectiveness of solar disinfection arose (Conroy et al 2001). The results of this study showed out of the 155 children under 6 years of age who were drinking water treated by solar disinfection just 3 cases of cholera were reported in comparison of the 144 children under 6 years old in the control group where there was 20 cases reported (Conroy et al 2001). The results also confirmed solar disinfection was an effective method of reducing the risks of water borne diseases in children under 6 years old (Conroy et al 2001). The process of solar disinfection was applied to water drawn from contaminated wells in a study replicating a South African scenario of where villages in rural areas water is drawn from unlined wells and the water is contaminated (Meyer et al, 2001). The results that were obtained in this study demonstrated a 99.99% reduction of faecal and total coliform counts in a period of 4-6 hours. It also showed that there was no growth reactivation after a period of 24 hours. As a result of solar disinfection the water investigated complied with all bureaus for safe drinking water's terms of bacteriological quality (Meyer et al, 2001). The process was demonstrated in field trials in rural Rajasthan (Reed et al 2005), the aims of the study were to investigate the practical applications of solar disinfection and establish the effects of solar disinfection with the incidence of waterborne disease. The results of this study showed a reduction of gastroenteritis and diarrhoea in a trial over a 1 year period (Reed et al 2005). The study also showed that this procedure of disinfecting water "Can enable such households to widen their sources of treated drinking water" (Reed et al 2005).

**<u>The investigation in to effects of solar disinfection on the bacteria *Pseudomonas* species.</u>**

As it shows the studies and investigation in the effectiveness of solar disinfection on unsafe drinking water has all concentrated on its effect on fecal indicators and waterborne pathogens such as *Ent. faecalis* and *E. coli*. This study is to assess the effectiveness into another organism that is known to be an opportunistic pathogen which respires and can be found in water. Therefore my choice was the *Pseudomonas* species.

*Pseudomonas* is an aerobic gram –ve bacteria which have flagella and are able to move around. They are able to metabolise a variety of diverse nutrients and due to the ability to produce biofilms can live in unexpected places. They have been found in antiseptics such as ammonium compounds and bottled drinking water. They are resistant to penicillin and beta lactam antibiotics (Anon, 2007). *Pseudomonas* is an opportunistic pathogenic organism; the strains *Pseudomonas aeruginosa* and *P. maltophilia* are responsible for 80% of infections caused by the *Pseudomonas* species (Iglewski, 2007). This is a major problem and has a fatality case of 50% with patients who are hospitalised with conditions such as cancer and cystic fibrosis. It is also responsible for conditions such as endocarditis, pneumonia, and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin, and musculoskeletal system (Iglewski, 2007). This organism is aerobic and will produce ROS when respiring and aiding in the UVA inactivation. It is also known to live in water therefore my aim in this project is to investigate the effectiveness of solar disinfection when applied to water that contains the bacteria *Pseudomonas spp.* also to investigate the difference between injury and death.

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