COMPARATIVE STUDY OF THE IMMUNOMODULATORY EFFECT OF SOLAR AND PHOTONICALLY INACTIVATED *SALMONELLA* ENTERITIDIS ON DENDRITIC CELLS IN-VITRO

By

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DECLARATION

I hereby declare that the "Comparative study of the immunomodulatory effect of solar and photonically inactivated *Salmonella* Enteritidis on dendritic cells *in-vitro*" is my own work, except where specific citations has been quoted and are acknowledged by means of complete references. A bibliography is appended. This dissertation has not been submitted to any tertiary institution for the award of a degree.

DEDICATION

This work is dedicated to my amazing parents; my Dad Bisi Adeniran, who has always believed in me, supported, and encouraged me to become the best that I can be. My mother Elizabeth Adeniran, whose constant love and support has shaped me to become the woman I am today.

I would like to dedicate this to my wonderful life partner Bolaji Samuel for being there from the beginning and always pushing me to pursue my dreams. Your love and support show no boundaries and lastly my beautiful daughter Mercy Samuel you are a motivation.

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ABSTRACT

Salmonellosis is a food and water-borne disease that affects humans, especially those that are immunocompromised as well as children and the elderly. This disease is caused by a variety of *Salmonella* species. *Salmonella* Enteritidis (SE) is the most frequently isolated serovar in infections occurring in humans and from animals all over the world. *Salmonella* Enteritidis is found in many animals and can survive in environmental samples for several weeks under ideal conditions. The failure of waste water treatment plants, agricultural pollution, and storm water runoff into natural water sources has led to an increase in the presence of *Salmonella* in water. The possibility of fecal contamination of water remains high in resource poor communities where sanitary and hygienic practices are inefficient or insufficient. However, many resource poor communities are using solar disinfection (SODIS) as a means of treating water prior to consumption. The SODIS method is achieved by exposing bacterial contaminated water to the sun for the period of 6 to 8 hours. The reliability of the SODIS process depends on factors such as temperature, dissolved oxygen and most importantly UV-A radiation. These factors cannot be controlled in a natural environment due to fluctuations or climatic changes in weather conditions. Instead of relying only on SODIS, other methods such as the use of a photonic device to disinfect microbiologically water are being used.

The main aim of this study is to compare the immunomodulatory effect of solar irradiated and photonically inactivated *S.* Enteritidis on dendritic cells *in-vitro* and to provide supporting information on the immunological benefits on the consumers of SODIS drinking water through a SODIS mimicking device. To achieve this aim, there was a need to optimize the SODIS and photonic inactivation conditions of *S.* Enteritidis. *Salmonella* Enteritidis cultures were exposed to solar irradiation during spring, summer and winter as well as photonically using an ultraviolet

light. The result revealed that the inactivation efficiency of Solar ultraviolet radiation (SUVR) on *S.* Enteritidis was season dependent. A total loss of activity was observed in *S.* Enteritidis during summer and no regrowth was observed. With the photonic device, a combination of UV and oxygen inactivated the *S.* Enteritidis to below detectable limits.

This study compared the protein profiles of solar irradiated and photonically inactivated *S.* Enteritidis using SDS-PAGE. The results showed a gradual decrease in the concentration of the protein banding patterns with time in *S.* Enteritidis that was either solar irradiated or photonically inactivated.

The ability of the solar and photonically inactivated *S.* Enteritidis to induce maturation of dendritic cells *in-vitro* was also investigated. There was a significant increase in CD80 when the 8-hour solar inactivated samples of *S.* Enteritidis was used to stimulate the dendritic cells. The higher levels of co-stimulatory molecules observed suggested the possible involvement of these molecules in antigen uptake and presentation to produce a specific immune response. This finding will contribute towards the understanding of the immunological effects that may be generated from consuming SODIS water and whether it may result in an immune reaction or response. Although the current study shows that solar irradiated and photonically inactivated cultures of *S.* Enteritidis were able to induce the expression of key immunological surface makers by dendritic cells, further studies are required to corroborate the findings of this study.

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ABBREVIATIONS

APCs	Antigen presenting cells
CBB	Coomassie brilliant blue
B cells	B lymphocytes
CFU	Colony forming unit
CO_2	Carbon dioxide
Cu+	Cuprous ion
DCs	Dendritic cells
DFMs	direct-fed microbials
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EAWAG	The Swiss Federal Institute of Aquatic Science and Technology
FACS	fluorescence-activated cell sorting
FBS	Fetal bovine serum
FCS	flow cytometry standard
Fe2+	ferrous ion Iron
GMP	Good manufacturing practices

H_2O_2	Hydrogen peroxide
IMDM	Iscove’s Modified Dulbecco's Medium
LP	Lamina propia
LPS	Lipopolysaccharide
MFI	Mean Fluorescence Intensity
MHC	Major histocompatibility complex
2-ME	2-Mercaptoethanol
NTU	Nephelometric turbidity units
O_2	Superoxide
OD	Optical density
OH	Hydroxyl radical
OPV	Oral poliovirus
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffer saline
PET	Poly Ethylene Terephthalate
PVC	Polyvinyl chloride
RNA	Ribonucleic acid
RNS	Reactive nitrogen species

ROS	Reactive oxygen species
RPM	Revolutions per minutes
SDS	Sodium dodecyl sulphate
SEM	standard error of the mean
SODIS	Solar water disinfection
SUVR	Solar ultraviolet radiation
T CELLS	Thymus lymphocytes
TCR	T-cell receptors
TEMED	Tetramethylethylenediamine
TiO2	titanium dioxide
TLR	Toll-like receptor
UV	Ultraviolet
UV-DDB	UV- damaged DNA-binding
VBNC	viable but non-cultural state
WHO	World Health Organisation

CHAPTER 1

1. BACKGROUND

1.1 General background

Salmonellosis is a food and waterborne related infection that results in a copious loss of water from the infected individual through diarrhoea (Abulreesh, 2011). A variety of *Salmonella* species causes salmonellosis, the most notable being *Salmonella* Enteritidis. It is a motile, non-encapsulated, facultative anaerobe, Gram-negative bacillus of the family Enterobacteriaceae. Humans often contract *S.* Enteritidisthrough the consumption of livestock products, especially poultry and ground beef, as well as water harbouring the pathogen (Shellenbarger et al., 2008). Cross-contamination may also result from kitchenware, food preparation surfaces, and towels (Soares et al., 2012). Symptoms of an ongoing *S.* Enteritidisinfection include bloody watery diarrhoea, fever and abdominal cramps that usually occurs 18 to 48 hours after ingestion of the bacterium. *Salmonella* Enteritidisinfection generally lasts 2–5 days, but faecal carriage may continue for up to 12 weeks after recovery (Abulreesh, 2011).

The exogenous nature of *S.* Enteritidismakes this pathogen very dangerous because it often remains asymptomatic in the animal carrier, and only becomes symptomatic in the human host (Stevens et al., 2009). This qualifies *S.* Enteritidisas a zoonotic pathogen of great importance. *Salmonella* Enteritidishas been isolated from almost all types of aquatic environments, including lakes, rivers, ponds, run-off water, marine water, treated and untreated wastewater, globally (Abulreesh, 2011). Although *S.* Enteritidisis inherently found in animal hosts, its occurrence in an aquatic environment is often a signal of faecal contamination, often owing to agricultural run-off

and direct deposit of faecal materials from birds and wild animals into environmental water (Shellenbarger et al., 2008).

The contraction and spread of zoonotic water-related pathogens such as *S.* Enteritidis could be prevented through sanitary and hygienic practices. Such practices would ensure that only adequately sterilised water is consumed and used for domestic purposes. Several methods including chlorination, pasteurisation, filtration, boiling and solar water disinfection (SODIS) can be used to treat water before consumption. The disadvantage with all the mentioned methods, except SODIS, is the requirement of some form of financial input. In developing countries, for example, boiling of water is not ecologically friendly because trees are cut to produce the charcoal for the boiling process. However, solar water disinfection (SODIS) offers a cost-free means of disinfecting microbiologically contaminated water (Kalt et al., 2014). Solar water disinfection, unlike the other methods, relies on natural sunlight to sterilise microbiologically contaminated water filled into transparent vessels and exposed to direct sunlight for up to 7 hours (Borde et al., 2016). SODIS has successfully been utilised in various countries worldwide, especially in resource-poor communities in sub-Saharan Africa, South American regions and East Asia (Byrne et al., 2011b).

Currently, there has been substantial research directed towards understanding the mechanism through which SODIS can destroy the water contaminating pathogens (Tamas and Mosler, 2011), the impact of SODIS water consumption on humans (Gutiérrez-Alfaro et al., 2017) and upscaling the amount of water that could be treated (Bitew et al., 2018). However, few studies have tried to understand the impact of SODIS-treated water on the immunity of SODIS water consumers. A study by Ssemakalu et al. (2015) has shown that solar irradiated *Vibrio. cholerae* can induce dendritic cell maturation *in-vitro*. The maturation of dendritic cells is key to eliciting an immune

response. This observation shows that consumers of SODIS water may have immunological benefits. So the question we want to address is, could SODIS be used as a method for the preparation of inactivated whole-cell vaccines?

The techniques that underline the inactivation by SODIS is not fully understood, but previous studies by Bosshard et al. (2010) showed that solar radiation destroys protein through oxidative stress. The UVA component of solar irradiation causes damage to lipids, proteins and deoxyribonucleic acid (DNA) indirectly via the formation of reactive oxygen species (ROS). These ROS cause damage to the outer cell membrane and induce subsequent leakage which leads to dysfunctional cell processes followed by death (Berney et al., 2006). The photosensitizers tend to enter an excited state when UVA photons are absorbed during which they react with molecular oxygen to create ROS such as superoxide, hydroxyl radicals, and hydrogen peroxide (Abrahamse and Hamblin, 2016). The inactivation process is enhanced by these reactions increasing the rate 4 to 8 times for faecal bacteria in oxygenated water than that of deoxygenated water (Reed, 1997). The advantage of the consumption of SODIS water can be seen differently from the technique and biology of microbial inactivation. These antigens in SODIS water are obtained by antigen-presenting cells (APCs) and carried to the mesenteric lymph nodes together with the small isolated lymphoid follicles around the wall of the intestine for T-cells presentation (Ssemakalu et al., 2014). The activation of T-cells takes place with subsequent migration to all the non-lymphoid tissue following the presentation of the antigens by the APCs (Lefrancois and Puddington, 2006). One of the important components of the immune system of the intestinal mucosal environment is the lamina propia (LP) tissue. LP is a connective tissue located under a thin layer of tissues covering the large and small intestines. This tissue is rich in cells of both the innate and the adaptive immune system such as T-cells and APCs (Van Wijk and Cheroutre, 2010).

The nature of antigens derived from SODIS water and the influence it may have on the immune system is focused on the consideration of the number of factors (Pradeu and Edgardo, 2006) . A high dose of antigen is required before an immune response could take place. A low dose of antigen would not cause or trigger a satisfactory immune response, because the generation of antigen specific regulatory cells is favoured which results in the unresponsiveness in T-cell function through clonal deletion (Faria and Weiner, 2006). Such a phenomenon could be associated with SODIS users during outbreaks or epidemics when the bacterial load in untreated water is high enough to cause waterborne diseases. This leads to the rapid infection in the population. Solar irradiation has been shown to successfully inactivate a significant amount (6 log10, 99.9999% inactivation) of *Salmonella* cells from a bacteria dose (Dejung et al., 2007).

The use of SODIS for water disinfection of pathogens may be beneficial to consumers and provide a relevant immune response (Ssemakalu et al., 2014). Another factor that could be derived from SODIS water and its influence on the immune system is the speed of appearance of the infrequent antigenic determinants. SODIS may induce an extreme rapid or slow modification of the antigenic epitopes which can prevent its ability to cause an immune response (Ssemakalu et al., 2014). The right condition for the generation of critical modification on epitopes that could prompt an immune response may be possible by SODIS.

It is therefore important to investigate the immunological effects that may be generated from consuming SODIS water to see whether it may result in an immune reaction. Although SODIS can inactivate microorganisms that can eventually elicit an immune reaction, it may not be a reliable means of preparing a vaccine. The SODIS method can be unreliable due to some uncontrollable factors. For instance, solar radiation is unevenly distributed and varies in intensity from one geographical location to another, depending on seasons, latitude and the time of the day (Tamas

and Mosler, 2011). However, the SODIS process could be reproduced artificially through the use of artificial light. In this study, the Jaws II dendritic cell line was used to observe the immunomodulatory effect of SODIS.

1.2 Rationale and Motivation

Salmonellosis occurs mostly in children under the age of 5 and people who are immunocompromised including HIV/AIDS and patients receiving chemotherapy and organ transplant (Spickler and Leedom, 2013). *Salmonella* Enteritidis infection is associated with intensive animal husbandry. It is important to know that many animals may possess *Salmonella* and still appear healthy. Humans are the only natural host and reservoir for this organism (Stevens et al., 2009). Outbreaks associated with contaminated drinking water have been increasingly reported in South Africa, mostly in areas where flooding cause the sewer system to overflow (Jambalang et al., 2017).

The major outbreaks of *S.* Enteritidis in developed countries are connected to the use of untreated or inadequately treated water (Liu et al., 2018). It has been estimated that 1.1 billion people, globally, drink unsafe water (WHO/UNICEF, 2019). Despite global efforts to curb its spread *S.* Enteritidis infections persist, causing an ongoing challenge to both humans and animals. However, only vaccines provide protection against disease outbreaks and can guarantee long-term protection. The use of SODIS is ideal for treating drinking water in developing countries (Sift et al., 2017). The method is very simple, and its application is safe. It is particularly suitable for treating relatively small quantities of drinking water.

1.3 Problem statement

Salmonellosis is a major concern to human and animal welfare. The consumption of untreated environmental water, as well as poultry and meat products contaminated with *S.* Enteritidis results in the contraction of salmonellosis (Gantois et al., 2009b). *Salmonella* Enteritidis can assume a carrier state which does not elicit clinical disease in the host (Sanchez et al., 2002b). Furthermore, the ability of *S.* Enteritidis to cause disease in humans makes this pathogen zoonotic in nature. Therefore, there is a need for cost-effective means of controlling the spread of zoonotic pathogens. One way of achieving this is through the use of vaccines. However, it would be important to establish the ability of SODIS treated bacteria to activate dendritic cells. Dendritic cells play a critical role in priming the adaptive immune system. SODIS that has been used widely for disinfecting microbiologically contaminated water involves a complex interaction among factors such as temperature, dissolved oxygen and most importantly UV-A radiation (Tsydenova et al., 2015). These factors are difficult to control due to fluctuating weather conditions in a natural environment. Therefore, this study compared the immunomodulatory effects of solar and photonically inactivated *S.* Enteritidis on dendritic cells *in-vitro.*

1.4 Research aim

This study aimed to compare the immunomodulatry effects of solar and photonically inactivated *S.* Enteritidis on a dendritic cell line *in vitro*.

1.5 Research objectives

The research objectives were:

1. To optimise conditions to inactivate *S.* Enteritidis using solar irradiation.

2. To optimise conditions to inactivate *S.* Enteritidis using a photonic device.
3. To compare the proteomic profiles of solar irradiated and photonically inactivated *S.* Enteritidis using SDS-PAGE.
4. To determine the ability of solar and photonically inactivated *S.* Enteritidis to induce maturation of dendritic cells *in vitro*.

1.6 Research scope

1.6.1 Inclusion criteria

The JAWS II dendritic cell (DC) line was used in this study. JAWS II cells are immortalised, immature bone marrow-derived DCs, from p53-deficient C57BL/6 mice, which respond to stimuli similarly as the primary bone marrow-derived DC. This cell line has also been used for *in-vitro* studies and is a convenient cell line for the presentation of antigens (Jiang et al., 2008).

1.6.2 Exclusion criteria

This study was conducted strictly as an *in-vitro* study.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Salmonella enteritidis

Salmonella Enteritidis is a facultative, anaerobic, gram-negative, and rod-shaped bacterium. It is known to be one of the most important causal agents of a food-borne disease in developing and developed countries (Baudart et al., 2000). The genus *Salmonaellae* comprises approximately 2500 serovars, most of which are considered human pathogens. (Gorski et al., 2011). *Salmonella.* Enteritidis and *S.* Typhimurium have been responsible for infecting over 93.8 million people and 155,000 deaths each year worldwide (Majowicz et al., 2010). As such, both *S.* Enteritidis and *S.* Typhimurium are major public health problems. Ingestion of fewer than 1000 organisms can cause disease in a healthy adult human (Cabral, 2010). The period of incubation after ingestion can be 6 hours, with an average time to the illness of 12 to 36 hours.

2.2 Salmonella Enteritidis a zoonotic pathogen

Salmonella Enteritidis is often contracted through the consumption of contaminated water as well as meat and poultry products (Sanchez et al., 2002a). Unlike humans, animals specifically, poultry can harbour *S.* Enteritidis asymptomatically for either a short or an extended period. For instance, chicken and turkey are often colonized with Salmonella without any symptoms. This aspect makes *S.* Enteritidis a zoonotic pathogen (Revolledo and Ferreira, 2012), and salmonellosis a form of zoonosis. A zoonotic pathogen is a disease-causing microorganism that is naturally transmitted between animals and humans. Zoonotic pathogens such as *S.* Enteritidis break the animal-human barrier due to poor hygiene and unethical practices. Poor hygienic practices in poultry farms may

result in the introduction of *S.* Enteritidis into the environment. Improper disposal of diseased poultry carcasses or chicken droppings contaminated with *S.* Enteritidis into the water or underground water can compromise the quality of water (Spickler and Leedom, 2013).

Furthermore, eggs laid by birds harbouring *S.* Enteritidis may spread the pathogen through either vertical or horizontal transmission (Svobodová and Tůmová, 2015). During vertical transmission, *Salmonella* is introduced in eggs through pores in the eggshell. The horizontal transmission, on the other hand, occurs when the eggs make contact with faecal matter contaminated with *Salmonella* (Gantois et al., 2009a). Sometimes *S.* Enteritidis may go undetected in eggs with an unbroken clean and fresh eggshell. These inconspicuously contaminated eggs may directly or indirectly increase the chances of outbreaks in the human population when used to prepare mayonnaise, desserts, and salads (Kilroy et al., 2016).

Natural water also serves as a vehicle for the transmission of these microorganisms. Most outbreaks in developed countries are allied to the use of inadequately treated water. A study by levantesi et al (2012) demonstrated that the analysis of isolated serovars consistently showed a mixed human and animal origin of *Salmonella* in surface water environments. This statement demonstrated that *Salmonella* could be seen in a variety of aquatic environments and contamination originates from different sources.

2.3 Curbing the spread of *Salmonella* Enteritidis

Salmonellosis poses a significant threat to public health. Therefore, there is a need to prevent the contraction of *S.* Enteritidis in animals as well as curb the spread of this pathogen from animals to humans. The transmission of *S.* Enteritidis could be prevented through practicing proper hygiene and sanitation on farms and abattoirs and the use of antibiotics and vaccination (Nair et al., 2018).

2.3.1 Administration of antibiotics

Antibiotics are used in animal production to promote growth, treat, control, and prevent infectious diseases (Nair et al., 2018). Excessive use of antibiotics, especially for non- therapeutic uses, has contributed to the development of drug-resistant bacteria (McEwen and Fedorka-Cray, 2002; Sneeringer et al., 2015). There was a high prevalence of antibiotic resistance among strains of *Salmonella* serovars such as *S.* Enteritidis, *S.* Infantis, *S.* Typhimurium, and *S*. Heidelberg isolated from poultry meat compared to samples from beef and lamb. These serovars were found to be resistant to ampicillin, tetracycline, streptomycin, chloramphenicol, and sulphonamides (Chen et al., 2004; Parveen et al., 2007; Dutil et al., 2010)

There are intervention strategies practiced at the farm level currently to reduce antibiotic-resistant *Salmonella* in poultry and its spread to carcasses when processed. However, antibiotic-resistant strains of *Salmonella* such as *S.* Enteritidis have been isolated frequently from broiler carcasses (Moyane et al., 2013;Fair and Tor, 2014; Vargas et al., 2020). The conversion of a conventional farm into an organic farm was shown to have reduced the prevalence of antibiotic-resistant *Salmonella* (Hong et al., 2016). *Salmonella* isolates from the organic facility production presented significantly lower resistance to antibiotics such as ampicillin, cefoxitin, ceftiofur, and amoxicillin-clavulanate. Interventions such as the use of direct-fed microbials (DFMs), prebiotics, plant-derived compounds and organic acids that could be used to target antibiotic-resistant *Salmonella* colonization in food animals and poultry are considered for the improvement of preharvest microbiological safety (Nair et al., 2018).

2.3.2 Farm hygiene

The practice of good hygiene in farm management is of primary importance in curbing the spread of *S.* Enteritidis from animals to humans. However, it has been reported that most farms still lack

good hygiene practices, which tend to increase the prevalence of *Salmonella* (Hill et al., 2008). The primary source of human salmonellosis is by farm animals, particularly from pigs and poultry. The geographical location of a pig farm could influence biosecurity practices. That is, the bigger the density of pig farms in the environment, the higher the risk of introducing the disease, the stricter the biosecurity measures that have to be applied to avoid the spread of infections between herds (Alarcon et al., 2021). Due to the asymptotic nature of some of the zoonotic pathogens, infected animals often pass veterinary slaughterhouse inspection (Swai and Schoonman, 2012). Intestinal material from pig or poultry carcasses may harbor *Salmonella* and, as a result, contaminate the slaughterhouses (Arguello et al., 2013). Milk may also be contaminated by faecal material during collection (Reta et al., 2016). These examples of poor hygiene may increase the potential for introducing zoonotic pathogens through cross-contamination from unprocessed to processed foods (Andries and Davies, 2015). The spread of *Salmonella* in the environment can also be associated with irresponsible faecal excretions by humans, manures and slaughter offal disposal (Tine et al., 2012). This often leads to contamination of surface water.

Several measures need to be considered to solve problems associated with poor hygiene because zoonotic pathogens such as *Salmonella* could be acquired through the interaction between animals on the farm, the environment on the farm, and food processing facilities. Actions such as decontamination of animal feed and isolation are not sufficient or do not provide long lasting solutions (Deewal and Grooters, 2013). Measures such as proper hygiene practices on the farm and food processing and packaging plants should be considered. Microbial cross-contamination of food could be prevented through proper handling of food when cooking, regular washing of hands, surfaces and tools used to prepare and process the foods (Vineland, 2019).

2.3.3 Vaccination

The use of complex methods for the production of vaccines is required for making human vaccines available on a global scale. The process of creating, testing, and producing vaccines can take many years because the industry is greatly and rightfully regulated (Smith and Ryan, 2011). Before a vaccine can be formulated, researchers have to study the infectious agent. The type of vaccines that currently exist includes the live-attenuated (weakend) vaccines, killed-inactivated vaccines, and subunit vaccines (Ma et al., 2019). Live attenuated vaccines consist of a modified strain of pathogens that have been weakened but are capable of multiplying in the body and remain antigenic enough to induce a strong immune response. Examples of this type of vaccine are the yellow fever virus vaccine and the oral poliovirus (OPV) vaccine (Vetter et al., 2018). The killed-inactivated vaccine is a type of vaccine that requires the killing of bacteria or viruses as well as inactivation by chemical treatment or heat. This group of vaccines includes vaccines for hepatitis A virus, Rabies, and pertussis. Sub-unit vaccines contain a small part of a microorganism (bacterial or viral), which is selected for its ability to initiate a specific immune response. It is then isolated and purified (Dai et al., 2019). This procedure is used for the *Haemophilus influenza* type b vaccine and the acellular pertussis vaccine.

The production of a vaccine can be divided into the following steps:

- Generation and isolation of the antigen

  The first step in the production of vaccines is by generating the antigen which will trigger an immune response. This step involves the growth and harvesting of the pathogen's protein or deoxyribonucleic acid (DNA) (Giese, 2016). Firstly, viruses are grown on primary cells, for example, cells from a chicken embryo or using fertilized eggs or cells that repeatedly reproduce (e.g., hepatitis A). Recombinant proteins generated from the pathogen can be

generated either in bacteria, yeast, or cell cultures. Once the antigen is produced, it is separated from the cells and isolated from the proteins (Kumari, 2019).

- Purification of antigens

  Antigens are purified to generate a high purity or quality product by using different techniques such as ultrafiltration, chromatography, and chemical treatment for protein purification.

- Addition of other components

  This step involves the addition of an adjuvant, which is a material that boosts the recipient's immune system to a provided antigen. The formulation of vaccines is accomplished by the addition of stabilizers to prolong the preservatives or its storage life. The possibility of lack of interactions or incompatibilities between antigens and other ingredients sometimes causes a challenge when it comes to the development of combined vaccines (Giese, 2016). All the components used for the final production of vaccines are combined and mixed uniformly in a single vial or syringe.

- Packaging

  The vaccines are sealed in vials with sterile stoppers. All the steps involved in the production of vaccines will have to comply with standards defined for Good manufacturing practices (GMP) that include different quality control, adequate infrastructure, and separation of activities to avoid cross-contamination. The vaccine is then finally labeled and distributed worldwide (WHO, 2002).

The most practical measure to curb the spread of foodborne bacteria is through the use of vaccines. Vaccines offer a means of preventing the occurrence of a specific infection in animals, thus

negating the transmission of zoonotic pathogens such as *Salmonella* (Revolledo and Ferreira, 2012). Inactivated, live, and subunit vaccines such as Ty21a live-attenuated, Vi capsular polysaccharide, Polvac ST® have been used against *S.* Typhimurium, *S.* Dublin, *S.* Abortusequi, and *S.* Sholerasuis are used to prevent salmonellosis (Kilroy et al., 2016).Vaccines have been used to protect pullets against *Salmonella* during the rearing period to avoid fecal shedding, thereby reducing *salmonella* contamination of eggs (Gantois et al., 2009a) as well as in broilers. There are two problems associated with the administration of vaccines parenterally (Revolledo and Ferreira, 2012). The first problem is that they fail to elicit a cell-mediated immune response (Zhang et al., 2015). The other issues pertain to the lack of stimulation of IgA responses at mucosal surfaces, which is the key to protection against intestinal colonization (Cerutti et al., 2011). Nonetheless, vaccines have been shown to promote long-lasting immunity by manipulating the cytokine milieu to induce the appropriate effector mechanisms against a particular pathogen (Cerquetti et al, 2000).

2.4 Solar water disinfection (SODIS)

Solar water disinfection is a cheap and simple method that uses sunlight to improve the microbiological quality of water. During SODIS, microbiologically contaminated water is exposed to natural sunlight (Figure 2.1) for approximately 6 hours on a hot day and 2 days when it is cloudy (Tamas and Mosler, 2011).

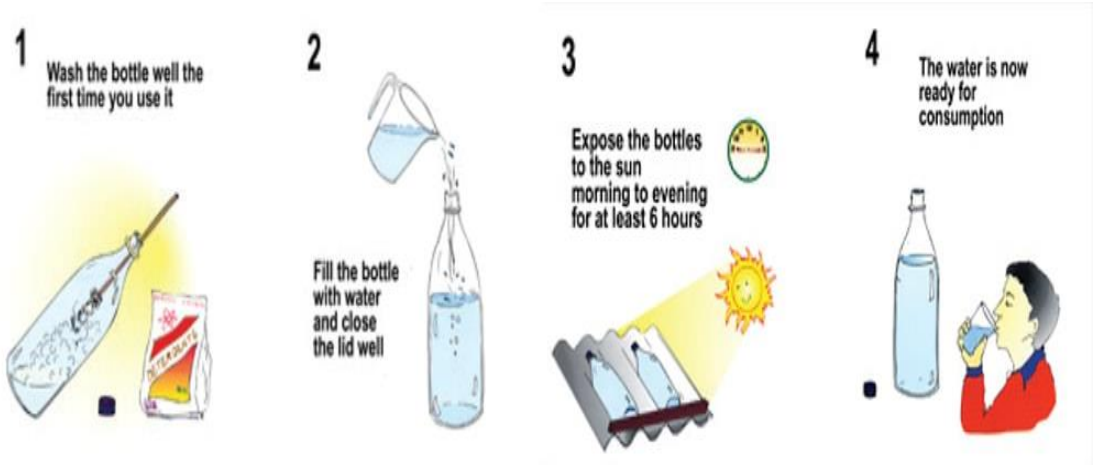

Figure 2.1 SODIS water treatment of drinking water (Sorlini et al., 2015). This diagram shows the protocol for the SODIS method as well as effective water treatment through solar disinfection.

The efficacy for SODIS to inactivate a variety of pathogens such as V*ibrio cholera* (Conroy et al., 2001), *Salmonella* Typhimurium (Wedel et al., 2005), *Shigella dysenteriae* (Kehoe et al., 2004) has been demonstrated by various research teams. Currently, more than 5,000,000 people in more than 24 African, American, and Asian countries use SODIS (Harding and Schwab, 2012).

The SODIS technique can reliably be used by communities that fall within 35° North and South of the equator (Lawand et al., 1988) due to prolonged sun hours all year round. Furthermore, communities located within this geographical area receive five or more hours of sunlight with at least 555 W-h/m^2 irradiance (Parsons, 2002). However, it is vital to consider the fact that weather patterns are variable even in regions close to the equator. So due to the variability in weather, EAWAG (The Swiss Federal Institute of Aquatic Science and Technology) recommends that on partially cloudy days, more than 6 hours of solar exposure is sufficient for the SODIS process (Haider, 2017). However, two days of exposure is required to achieve the desired inactivation

during cloudy days (Haider et al., 2013). It should be noted that the use of SODIS is not recommended during conditions of prolonged rainfall (Borde et al., 2016).

2.5 Factors influencing SODIS

Although SODIS may seem an ideal means to disinfect microbiologically contaminated water, it is influenced by several factors. One major factor that may affect the use of SODIS is the weather conditions (Byrne et al., 2011b; Borde et al., 2016). For instance, cloudy conditions have a significant influence on the amount of solar radiation received on earth regardless of location. Therefore, before the SODIS process can be implemented the availability of sunlight due to seasonal changes needs to be assessed to establish guidelines on the duration required to achieve the required solar radiation intensity (500 W/m^2) (Nwankwo et al., 2019).

Besides the weather conditions, water turbidity has a significant influence on SODIS. According to Myre and Shaw (2006 less than 1% of SUVR can penetrate water with a turbidity of 200 Nephelometric turbidity units (NTU). High water turbidity has been shown to reduce the efficacy of the SODIS process (Bitew et al., 2018). Highly turbid water reduces the ability of sunlight to penetrate through the water, therefore, protecting microbes from inactivation. According to the recommendation by EAWAG, water turbidity higher than 30 NTU needs to be pretreated before SODIS treatment (Pearce and Dawney, 2012). The turbidity of water to be solar irradiated could be reduced through filtration or simple settling. Turbidity can also be reduced by flocculation using minerals such as Alum (potassium sulfate) and seeds from plants such as *Moringa oleifera.* Both these flocculants have been studied as pre-treatment options to clarify water before the use of SODIS and have shown promising results (Asrafuzzaman et al., 2011). However, consideration

must be given to the fact that the addition of any form of pre-treatment step, extends the overall time required for disinfection and may have cost implications.

The amount of oxygen present in the water before SODIS has a significant influence on the outcome. Oxygen plays a key role in the formation of highly reactive forms of oxygen (oxygen free radicals and hydrogen peroxides) during solar irradiation. These reactive molecules react with cell structures and kill pathogens (Fisher and Nelson, 2014). SODIS is more effective in water containing high levels of oxygen (Cabiscol et al., 2000). Therefore the guidelines recommend that the vessel is vigorously hand-shaken to dissolve oxygen in the water (Cervantes, 2002). The material in which the vessel used for solar irradiation is made, also has a significant influence on the outcome of SODIS.

Different types of transparent plastic materials made from either polyethylene terephthalate (PET) or polyvinylchloride (PVC) are good transmitters of light in the UV-A and visible range of the solar spectrum (Borde et al., 2016;Cuerda-Correa et al., 2019). Transparent clear bottles such as empty soda and water bottles made from PET and PVC could be used for SODIS. There have been some concerns regarding the leaching of plastic such as antimony and phthalate but the study by Wegelin (1998) showed that chemicals were not present after SODIS.

2.6 Effect of SODIS on bacterial cells

The effect of sunlight on bacterial cells was firstly investigated by Downes and Blunt (1877). The seminal work on using sunlight to disinfect contaminated water for use in oral rehydration solutions was published by Acra et al. (1984). The bactericidal effect of UV involves both thermal and optical processes (Mcguigan et al., 1999). When DNA absorbs UV light, it causes thymine bases to bond covalently forming dimers. These thymine dimers terminates DNA replication

prematurely. Moreover, incorrect repair of thymine dimers can result in genetic mutations. The second mechanism for the inactivation of pathogens is that when dissolved organic matter in water absorbs UV light, highly reactive species such as hydroxyl radicals (OH), superoxides (O_2), and hydrogen peroxides (H_2O_2) are produced by photochemical reactions (Cuerda-Correa et al., 2019). The last mechanism in SODIS is through the elevation of the temperature due to the absorption of infrared and red light by water (Davarcioglu, 2015). Beyond the optimum growth temperature, any addition of heat causes denaturation, that impedes protein function and kills the organism (Lepock, 2003). This heat energy (temperatures above 45^{o}C) has a synergistic effect with the UV mechanism (Mackey et al., 1991). The inactivation of pathogenic microorganisms following SODIS is multifaceted because it stems from a combination of DNA damage, oxidative stress, and thermal destruction.

2.6.1 DNA damage due to SUVR

The absorption of UV light by DNA results in the covalent bonding of thymine bases to form thymine dimers, which interfere with DNA replication and transcription (Mathews and Holde, 1997;Johnson et al., 2001). Ideally, the DNA must be destroyed faster than microbes can repair to ensure pathogenic organisms are damaged or eliminated because bacteria could recover. Naturally, biological systems are designed to remedy the formation of thymine dimers through a process known as photoreactivation (Biswas, 2013). During photoreactivation, visible light activates DNA photolyase that breaks the bonds joining the thymine bases (Figure 2.2). DNA repair could also be achieved through excision where the damaged DNA is excised and replaced with new nucleotides (Rastogi et al., 2010). However, this process could go wrong. For instance, the affected organisms may replace thymine dimers with new but different nucleotides, thus

increasing the chances of acquiring mutations and resulting in the synthesis of faulty proteins (Salem, 2016).

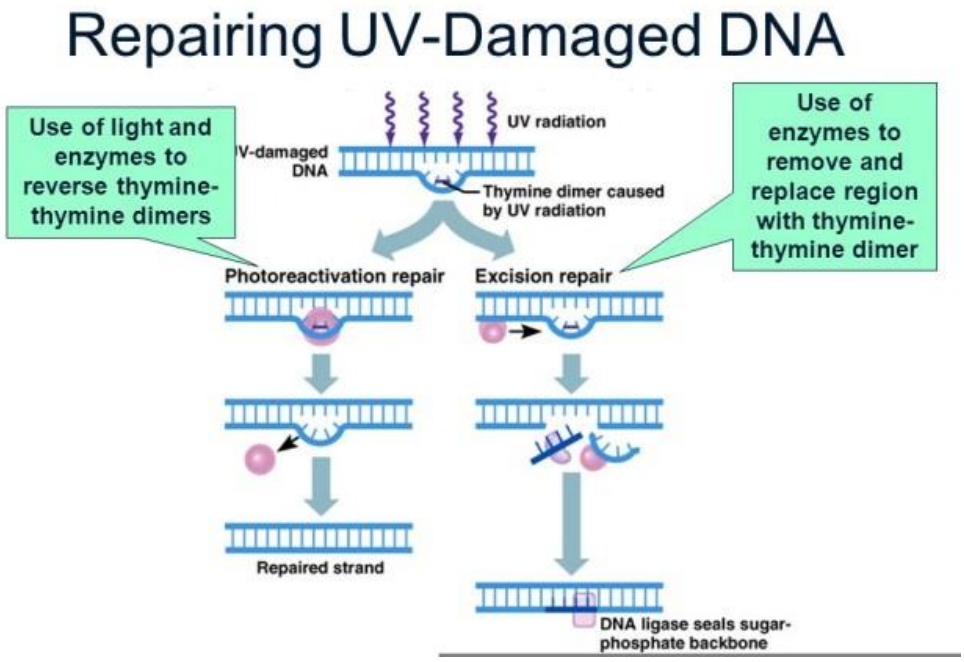

Figure 2.2. Illustration of DNA alteration by UV (Fields, 2015). The image shows the types and repair mechanism of DNA. The photoreactivation repair uses ultraviolet light causing thymine-thymine dimers while the excision repair begins with the identification and removal of the mutated base from the DNA helix by an enzyme.

2.6.2 Photo-Oxidative Disinfection

Oxidative stress is the imbalance between the production and accumulation of free radicals reactive oxygen species (ROS) & reactive nitrogen species (RNS) and antioxidants in a cell (Pizzino et al., 2017). Free radicals cause a chemical reaction in the cell due to its oxygen-containing molecule that consist of uneven number of electrons. Free radicals can either be harmful or beneficial. Antioxidants are molecules that donate an electron to a free radical that

allows the stabilization of the free radicals to be less reactive (Legg and Dix, 2017). Excessive production of free radicals and oxidants gives rise to oxidative stress, that is a harmful process that negatively affects different cellular structures such as proteins, membranes, lipids, DNA and lipoproteins (Pizzino et al., 2017). The most reactive among all the free radicals species *in-vivo* is the hydroxyl radical (OH•). Hydrogen peroxide acts as a channel to transfer free radicals through cell compartments and between cells. Some of the these radicals include O_2, H_2O_2, ferrous ion Iron (Fe_2+), or Cuprous ion (Cu+), (ZnO) and titanium dioxide (TiO_2).. Amongst these free radicals, the most commonly used for water treatment practice is (TiO_2), also called titania (McGuigan et al., 2012). The oxidative effect of TiO_2 photocatalysis occurs by direct contact of the catalyst particle with the bacteria. Other ROS like H_2O_2 and O_2 have been reported to be responsible for inactivation of microorganisms. ROS may cause fatal damage to microorganisms by disruption of the cell membrane or by attacking DNA and Ribonucleic acid (RNA).

The inactivation of microorganisms in water by sunlight is enhanced by an optical process (Acra et al., 1984; Kramer and Ames, 1987)**.** The bactericidal influence of sunlight depends on specific wavelength ranges of the sun's spectrum (e.g., UVA (320-400nm), UV B (290-300nm), visible (400-700nm) and infrared light (≥700nm) that reaches the surface of the earth (Figure 2.3). Reed (1997) demonstrated that visible light in the presence of dissolved oxygen can be harmful to enteric pathogenic bacteria in water.

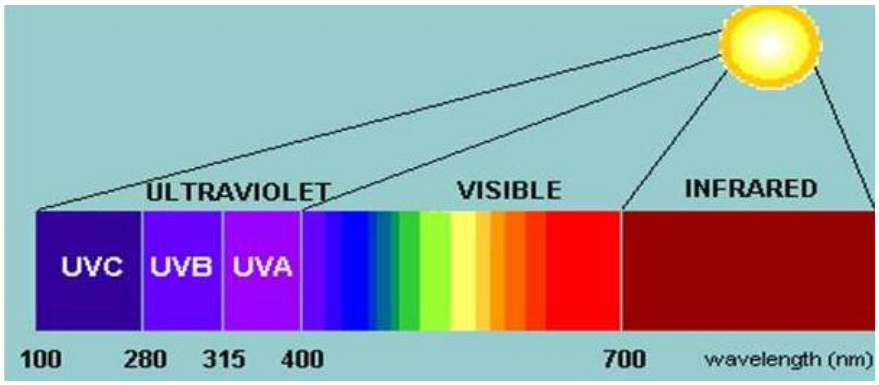

Figure 2.3. The spectrum of visible light from the ultraviolet range to the infrared (Lightworks, 2016). The UVA is a long wave, black light which is not absorbed by ozone layer. UVA wavelength ranges from 315-400. The UVB is a medium wave which is mostly absorbed by the ozone layer. UVB ranges from the wavelength of 280-315. The UVC is a short wave which is completely absorbed by the ozone layer and atmosphere. . The UVC wavelength ranges from 100-280.

Microorganism have developed mechanisms to defend themselves against various reactive oxygen species (Yun and Lee, 2001). For instance, defense against superoxide is attributed to a group of enzymes called superoxide dismutase, that decreases the lifetime of superoxide by a factor of 10^9 (Wang et al., 2018). Sufficient levels of oxygen need to be initially present before photo-oxidative disinfection can take place. Aerobic and anaerobic inactivation rate of *Escherichia. coli* and *Enterococcus faecalis* were compared and demonstrated by Reed (1997) using solar disinfection. The experiment showed that the presence of oxygen is essential for solar destruction of *E. coli* and *E. faecalis* as the aerobic rates of disinfection are much faster than the anaerobic rates (Reed, 1997).

2.6.2 Thermal Inactivation

The influence of temperature on SODIS has shown that water temperature above 45^{o}C shows a synergistic effect with UV radiation, speeding up the disinfection process (Wegelin et al., 1994;

McGuigan et al., 1998). A study conducted by Giannakis et al. (2014) revealed the potential antagonistic effect of temperature in SODIS. Their study concluded that temperature, treatment time, and intensity are critical parameters for the disinfection process, more especially with temperature above 50^{o}C and 60^{o}C which were able to increase inactivation efficiency.

Several enhancement**s** with the potential to enhance the thermal rate of microbial inactivation have been investigated. Thermal enhancement have been achieved by (i) circulating water over a black surface in an enclosed casing that was transparent to UV-A light (Dominguez et al., 2005), (ii) painting sections of the bottles with black paint, and (iii) using a solar collector attached to a double glass envelope container (Saitoh and El-Ghetany, 2002). However, the presence of UV-A on cloudy days can be used by a reflector to boost the optical inactivation of solar disinfection.

An increase in temperature past the optimum growth temperature results in the destabilization of the core structures of most proteins through denaturation. Denatured proteins are unable to carry out their critical biological tasks, and as a result, death of the affected microorganism may result (Berney et al., 2006; Pierik, 2012). The synergistic effect between SUVR and temperature has also been demonstrated in a study by Wegelin (1998). In this study, low irradiance of SUVR and relatively elevated temperature (50^{o}C) resulted in the inactivation of *E. coli*, bacteriophages, and enteroviruses. The increase in the water temperature has been attributed to infrared radiation from the sun.

2.7 Effects of SODIS water consumption on the immune system

It is important to assess the immunological effects and overall benefits that may arise from the consumption of SODIS water. The essence of microbial constituents in water following SODIS is uncertain, yet may introduce a variety of microbial antigenic determinants or epitopes. The consumption of SODIS water may trigger an immune response on the basis of how the microbial

epitopes are processed by the cells of the immune system (Bosshard et al., 2009). The antigen-antibody impact of SODIS happens in the intestinal mucosal environment. The probable antigens in SODIS water are obtained by antigen-presenting cells and moved to the mesenteric lymph nodes together with isolated lymphoid follicles for presentation to T cells (Lefrancois and Puddington, 2006). Would it be possible that the consumption of SODIS water may provide a noteworthy immunological impact on the consumers? Empirical proof is required to support all the hypotheses put forward about the immune response elicited by consuming SODIS water since the period of protection remains obscure. However, the consumption of SODIS water during a waterborne infection outbreak, for example, with *Salmonella species*, if at all immunogenic, may provide a significant immune response (Ssemakalu et al., 2014).

There are two major lines of defense to the immune system: these are innate and adaptive immunity. Innate immunity serves as the first line of defense against a foreign agent. This type of immunity is initiated within minutes or hours after aggression and has no immunologic memory, and is rapid (Marshall et al., 2018). The adaptive immune system on the other hand is specific and relies on the memory generated from previous encounters with a foreign agent (Bonilla and Oettgen, 2010). The development of adaptive immunity is triggered by the actions of the innate immune system. The following functions are involved in the adaptive immune response: firstly, it provides long-lasting defense and protection against recurrent infections due to its ability to learn and remember specific pathogens (Marshall et al., 2018). The generation of the adaptive immune response is initiated by clonal selection of lymphocytes, which are the basis for effective immunization against infectious diseases. The cells of the adaptive immune system are antigen-specific T cells that are activated to increase rapidly through the action of APCs (Antigen-presenting cells) and B cells (B lymphocytes), which differentiate into plasma cells to generate antibodies.

T cells (Thymus lymphocytes) obtained from the hematopoietic stem cells in the bone marrow following migration are generated in the thymus. These cells are programmed to be specific for a particular foreign agent (antigen) (Cavanagh and Findlay, 2019). T cells can express a series of unique antigen-binding receptors also known as T-cell receptors (TCR) found on their cell membranes (Figure 2.4).

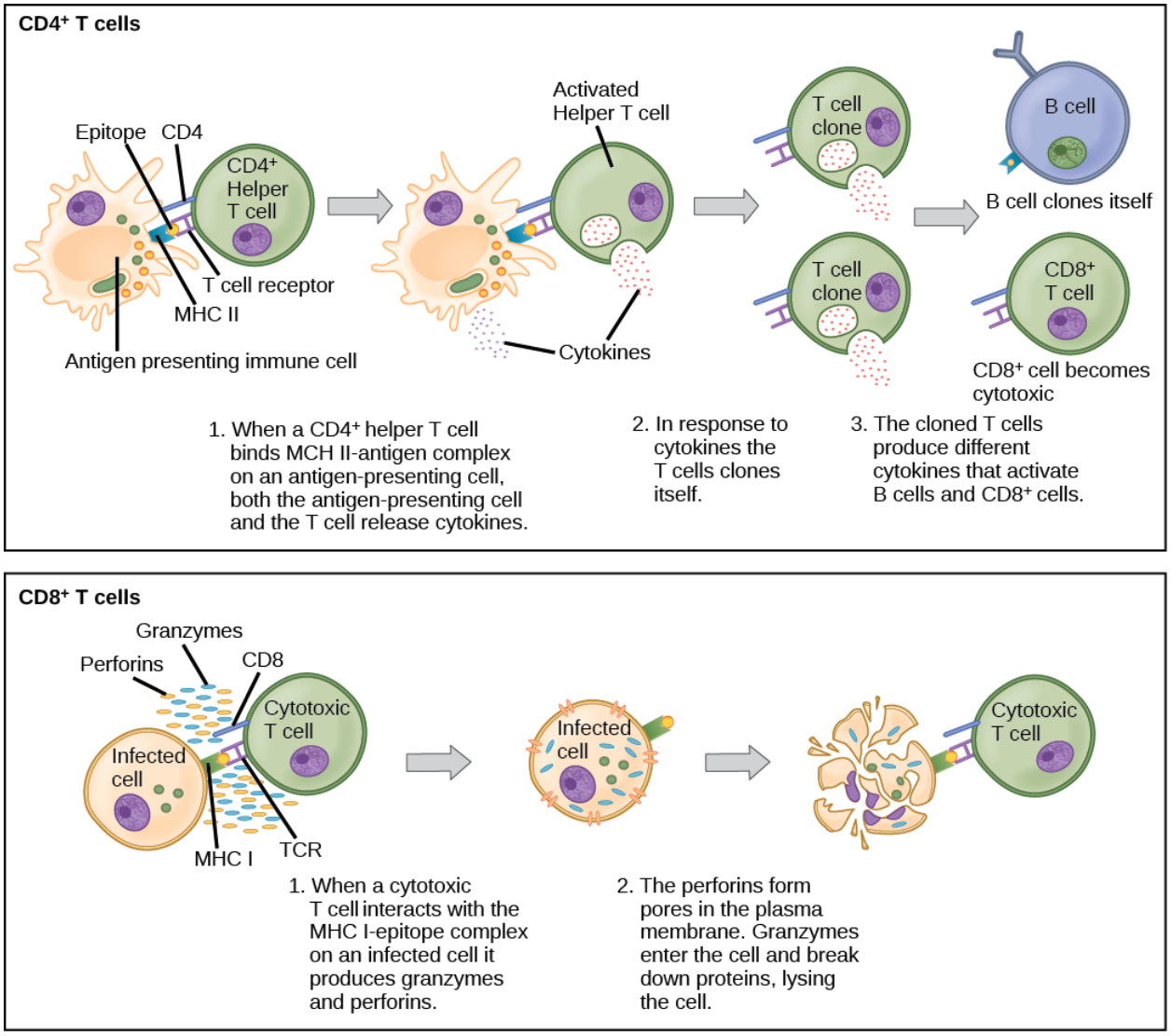

Figure 2.4. The illustration of how Naïve CD4+ T cells engage MHC II molecules on antigen-presenting cells (APCs) and become activated adapted from (Molnarand Gair, 2013).

Each T cell ($CD4^+$ helper T cells and $CD8^+$cytotoxic T cells) expresses a single type of TCR and binds to the antigen as it is held in a group of a protein called the major histocompatibility complex (MHC) on the surface of the APC, (Bonilla and Oettgen, 2010). This triggers the first activation of T cells.

The $CD8^+$cytotoxic T cells are mainly involved in destroying intracellular infectious agents such as viruses, and the killing of tumour cells expressing antigens (Bonilla and Oettgen, 2010). Clonal expansion of cytotoxic T cells aids in the production of effector cells that release a substance that induces the death of target cells (Janeway et al., 2001). Most of the effector cells die and are cleared by phagocytes upon the resolution of the infection. However, just a few of these cells remain as memory cells that can rapidly differentiate to an effector cell upon several encounters with the same antigen (Pennock et al., 2013).

The $CD4^+$ helper T cell plays a vital role in maximizing and establishing an immune response. These cells cannot directly kill infected cells or clear pathogens and have no cytotoxic or phagocytic activity. However, they initiate an immune response by directing other cells to perform these tasks and regulate an immune response that is produced (Alberts et al., 2002). T helper cells are activated by the recognition of TCR bound to class II MHC molecules. Once this is activated, the cells release cytokines that control the activity of many cell types, including the APCs (Alberts et al., 2002).

B cells are derived from the hematopoietic stem cells in the bone marrow. B cells can recognize antigen directly without the need for APCs through unique antibodies expressed on the surface of the cell (Marshall et al., 2018). The primary function of B cells is the production of antibodies against foreign antigens which requires their further differentiation. B cells undergo proliferation and differentiation into antibody-secreting plasma cells or memory B cells when activated by foreign

antigens to which they have an appropriate antigen-specific receptor (Bonilla and Oettgen, 2010). Memory B cells are long-lived cells that continue to express antigen-binding receptors.

2.8 Antigen-presenting cells

Antigen-presenting cells such as macrophages, dendritic, and B- cells are critical in bridging the gap between the innate and adaptive immune systems (Harding et al., 2003). These cells continuously survey their microenvironment, take up antigenic materials, process them, and present the resulting fragments to antigen-specific T-lymphocytes. The antigens are processed for presentation on major histocompatibility complex MHC class I and MHC class II to stimulate $CD8^+$ and $CD4^+$ T cells, respectively (Mantegazza et al., 2013).

Dendritic cells (DCs), which present antigens to T-cells, are known to be the bridge between adaptive and innate immune responses. DCs may occur in three different states which are; immature, semi-mature, and mature DCs (Hopp et al., 2014). The immature and the mature DCs differ distinctly based on the variations that occur on a phenotypic and functional level. The most productive APCs are the mature, immunologically competent DCs (Kim and Kim, 2019). The regulation of the immune system by DCs is dependent on their maturation (Al-Ashmawy, 2014). Many factors induce maturation following antigen uptake and processing within DCs. These factors include bacteria-derived antigens e.g., Lipopolysaccharide (LPS), ligation of select cell surface receptors (CD40), inflammatory cytokines and viral products (RNA or DNA) (Keselowsky and Lewis, 2017).

The process of DCs maturation generally involves; a redistribution of significant MHC from intracellular endocytic compartments to the surface of DCs, increase in the surface expression of costimulatory molecules, down-regulation of antigen internalization, cytoskeleton reorganization, morphological changes (e.g., the formation of dendrites), cytokines and proteases, secretion of

chemokines and surface expression of adhesion molecules and chemokine receptors (Mbongue et al., 2017). Mature DCs are potent stimulators of T cells *in vitro* (Larsson et al., 2000). An association between the appearance and insufficiency of various surface markers has been utilized to distinguish DCs subsets. DCs express costimulatory molecules, including CD86 (B7.2) and CD80 (B7.1), that are upregulated by DCs activation (Al-Ashmawy, 2014). CD86 is designated to be a marker of primary DCs maturation, in which CD80 only becomes high in mature DC. There are two additional methods for DCs markers in humans; CD83 and CMRF-44. CD83 is a significant marker for mature DCs. The CD83 appears to have regulatory roles for an immune response (Al-Ashmawy, 2014). While being actively upregulated during DCs activation and maturation. This marker is known to be a type 1 glycoprotein that belongs to the immunoglobulin superfamily (Li et al., 2019).

Main signals are brought to T cells during immune activation that defines the intensity and nature of the immune response to the specific antigen presented by DC. Dendritic cells can control the type of immune response through cytokine and chemokine cascades they secrete during their encounter with T-cells (Ssemakalu et al., 2015). The cascade of cytokines and chemokines, which are secreted in response to a microorganism, relies on the microbial cell's state or the microbial component that also depends on the condition under which the organism occurred prior to its consumption. Dendritic cells are provided with a vast battery of receptors that proficiently identify invading microorganisms. One of the vital receptor families is known as the Toll-like receptor (TLR). TLR is of crucial importance because its activation can induce both indirect and direct dendritic cell maturation (Tam, 2007).

The specific role of DCs as antigen-presenting cells in *Salmonella* has been well described (Yrlid and Wick, 2001). *Salmonella* cells are taken up by DCs and their antigens presented to T-cells (Kaluphana et al., 2005; Bueno et al., 2008). The antigens are captured by immature DCs and

undergo a complex maturation process, accompanied by the release of cytokine and chemokines as well as the high expression of co-stimulatory molecules (Figure 2.5). The study by Ssemakalu et al. (2015) showed that irradiated solar pathogens can induce dendritic cell maturation.

So SODIS could potentially generate inactivated microorganisms with the potential to induce an adaptive immune response. If so then the SODIS could be used to make an immunologic substance like a vaccine. But SODIS relies on the weather which is variable. Therefore, a follow-up question is could the SODIS process be replicated in a controlled environment using a photonic system?

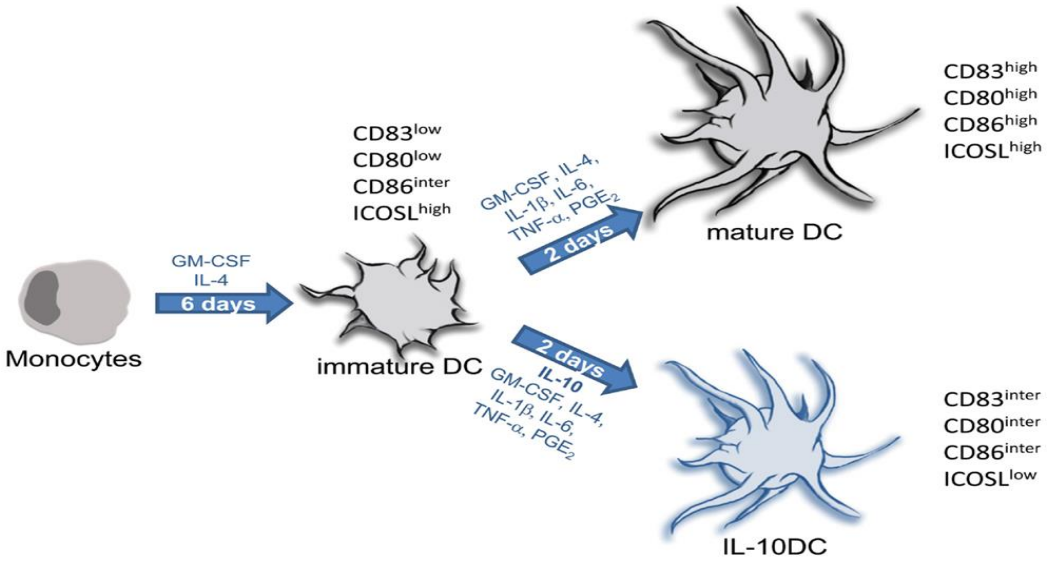

Figure 2.5. Mature and immature dendritic cell markers (Hubo et al., 2013)

2.9 Photonic inactivation

Photonic devices involve the use of radiant energy, such as light. Sunlight kills certain bacteria in water, and when water is exposed to UV light, pathogens are destroyed (Davarcioglu, 2015). UV light is electromagnetic radiation found in the spectral range of light between X-rays and visible light with a wavelength ranging from 200 to 390 nm (Figure 2.3). The most productive wavelength frequency for the disinfection of microorganisms is 254 nm, as this is where the optimum energy

intensity is found (Harley et al., 2008). A UV bulb can be designed using quartz glass which allows the UV radiation to pass through. The bulb is encased by a protective quartz glass sleeve which permits the water to be exposed to the disinfecting UV radiation. The quartz sleeve prevents water from reaching the UV bulb, and influences the pressure of mercury in the lamp and the level of UV output (Koca et al., 2018).

The extent to which microorganisms are destroyed relies upon the energy of the UV light, exposure time, proper wavelength, raw water quality, flow rate, and appropriate upkeep of the equipment. UV inactivates microorganisms by chemically damaging nucleic acids (Oram, 2014). A typical UV disinfection system involves the flow of water through a vessel containing a UV lamp. As the water goes through this vessel, microbes are exposed to high ultraviolet light energy which destroys the nucleic acids (DNA or RNA) required for reproductive functions. This damage prevents the microbes from multiplying or replicating. Since the microorganisms are not able to reproduce, no infection can occur. The disinfection of water is achieved when UV light causes the inactivation of microbes (Davarcioglu, 2015). However, UV radiation does not improve the smell, taste, and clarity of the water.

Short wavelength UV rays penetrate harmful pathogens inside the water and destroy them by attacking their genetic core (DNA). Absorption of UV-C photons by microbial DNA induces DNA-base damage through the formation of pyrimidine-pyrimidine 6-$_4$ photoproducts (6–4PP) recognition by UV- damaged DNA-binding (UV-DDB) in the nucleosome and cyclobutane pyrimidine dimers. These by-products obstruct DNA replication and, as a result, inactivate the microbial cells (Ssemakalu et al., 2013). This is extremely efficient in eliminating the microorganisms' ability to reproduce. Disinfecting water with UV light is exceptionally simple, effective, and environmentally

safe. Ultraviolet systems destroy 99.99% of harmful microorganisms without adding chemicals or changing the water's taste or odor (McGuigan et al., 2012).

UV-A, the active component of solar radiation, has been reported to indirectly and directly target different important microbial cell components and processes such as translation, metabolism, transcription, transport system, and chaperones that are responsible for counteracting dangerous oxygen radicals and thereby inducing cellular death of the microbes. UV-A also allows a single-strand break in DNA, and factors that lead to such damage have been shown to induce Cholera toxin bacteriophage (CTXΦ) multiplication that at the same time increases the expression of Cholera toxin AB (*ctxAB*) genes as a result of the SOS DNA repair response. The transmission of CTXΦ is dependent on its stability, which in turn depends on factors such as the bacteria viability, duration of UV exposure and other environmental factors such as oxygen, temperature, and pH (Ssemakalu et al., 2013). In this study, the photonic device using UVA light was used to mimic the SODIS process to inactivate *S.* Enteritidis.

CHAPTER 3

3 RESEARCH METHODOLOGY

3.1 Preparation of bacteria as glycerol stock on beads

A culture of *S.* Enteritidis (ATCC® 0345P) purchased from MediMark laboratories (Sevenoaks, UK) was used in this study. Luria broth (LB) (10 g of Tryptone, 5 g of Yeast extract and 10 g of sodium chloride in a liter of double distilled and autoclaved water) at pH 7.5 was prepared, autoclaved at 110°C for 30 min and used to grow the microorganism. The bacteria were inoculated into a 25 mL Erlenmeyer flask containing 20 mL of LB and incubated at 37°C on a rotary shaker at 150 rpm overnight (approximately 18 h). Thereafter, glycerol stocks of the microorganism were prepared as described elsewhere (Smith and Ryan, 2011). In brief, a 50% glycerol solution was heated on a magnetic stirrer to reduce viscosity. Then 300 µl of the 50% glycerol solution was aliquoted into 2 mL sterile cryovials containing glass beads. Afterwards, 700 µl of an overnight bacterial sample in LB broth was added to the cryovial followed by a brief vortex and storage at -80°C. All experiments in this study were conducted using stocks from the same lot.

3.2 Culture conditions of *S.* Enteritidis

S. Enteritidis cultures were prepared from the frozen glycerol stocks by spreading a couple of beads onto LB agar plates followed by 18 h of incubation at 37°C. The bacterial colonies were picked and streaked on fresh LB agar plates and then incubated at 37°C overnight. Thereafter, 2 to 5 colonies were inoculated in 200 mL of autoclaved LB broth and incubated at 37 °C on a rotary shaker at 150 rpm overnight until they reached the stationary growth phase by checking its optical density.

3.3 Preparation of *S.* Enteritidis for solar exposure

S. Enteritidis in the stationary phased was harvested (Day et al., 2009). The overnight culture was centrifuged at 4000 rpm for 10 min at room temperature. The pellet was then washed with 1X phosphate buffer saline (PBS) to remove all traces of LB broth. The bacterial pellet was then resuspended and diluted to an OD_{546} of 0.1 and 0.5 in autoclaved double deionised water. The bacteria suspension was transferred into transparent polystyrene unventilated T75 tissue culture flask. Prior to exposure, the samples were allowed to stand for 10 to 15 min to allow the bacterial cells to adapt to the water. The samples were then exposed to direct sunlight by placing them horizontally on the roof of VUT building (latitude 26^{o} 42'37.91"S and Longitude 27^{o} 51'39.35"E) for 30 min, 4 h, 6 h and 8 h. The temperature of the samples to be irradiated was recorded prior to exposure. The control samples were placed at the same location but covered with an opaque ventilated cardboard box to block exposure to solar irradiation. Following the solar exposure, the samples were prepared for enumeration by the plate count method. The samples were kept at room temperature in the dark for the observation of regrowth.

3.4 Exposure of *S.* Enteritidis in a photonic system

A bacterial suspension of *S.* Enteritidis was prepared at an OD_{600} of 0.1 and 0.5 as described in section 3.3. The bacterial suspension was then introduced into a glass canister where it was exposed to UVA light generated by a G5 Fluorescent T5, 8 Watt tube**.** Depending on the nature of the experiment some of the bacterial suspensions in the glass canister were purged with oxygen. In treatments where oxygen was used, the oxygen was supplied at ½ L/min in a tube. Also, the glass canister had a release valve to prevent pressure build-up in the system. The irradiated samples were collected for analysis after 30 min, 4 h and 8 h.

3.5 Enumeration of the *S.* Enteritidis

The solar and photonically treated bacteria, as well as their controls, were assessed for viability using the Miles and Misra technique (Miles and Misra, 1938) The samples were serially diluted and then 20 µl of each dilution was dropped onto a sterile LB agar plate in triplicate. The plates were incubated at 37°C. After 24 h the plates were observed for growth and sectors with less than 50 discrete colonies per drop were selected and counted. The total count was divided by the number of drops, multiplied by the dilution factor and then divided by the volume plated in 20 µl and converted to 0.02 mL, to give the number of Log CFU/mL.

3.6 Whole-cell protein extraction

Whole-cell lysates of *S.* Enteritidis were prepared as described by Nakamura et al. (2002) with modifications. The solar and photonically treated bacteria as well as their controls were centrifuged at 15,000 rpm for 15 min at 4^{o}C and the supernatant discarded. The pellet was suspended in 200 µl of 10% Sodium Dodecyl Sulphate (SDS) (Sigma, St. Loius. MO). An equal volume of Laemmli's loading buffer (Bio-Rad, Irvine. CA) was added. After vigorous shaking by vortex, the prepared samples were boiled for 10 min at 100^{o}C and centrifuged at 15,000 rpm at 20^{o}C for 1 min. The supernatants were stored at -20^{o}C until required.

3.7 Protein quantification and purification

The protein concentrations of the whole-cell lysates were measured using the Qubit®Fluorometer according to the manufactures instructions. The assay was standardized using standards supplied with the Qubit® Protein Assay Kits (Thermofisher Scientific, Waltham. MA). To 190 µl of Qubit® working solution, 10 µl of each sample was added, vortexed for 2 to 3 seconds and incubated at

room temperature (20-25ºC) for 15 min. Thereafter the protein was quantified in the Qubit®Fluorometer.

3.8 SDS PAGE

The whole-cell proteins extracts were separated on an SDS-PAGE following a method described by Laemmli, (1970). The electrophoretic gels were cast in two steps, starting with the preparation of the 12% resolving gel (Table 1). After adding Tetramethylethylenediamine (TEMED) the resolving gel solution was transferred to a gel caster system, and isopropanol was added on top of the gel to smoothen the surface and prevent the formation of bubbles. The 4 % stacking gel was prepared as stipulated in Table 3.1 and poured on top of the resolving gel after the isopropanol had been discarded. Gel pockets for sample application were formed by the insertion of a comb in the gel caster system.

Table 3.1 Preparation of Tris(hydroxymethyl)aminomethane -Glycine resolving and stacking gel

Reagent/Chemical	**Resolving gel (10 mL) Volume (mL)**	**Stacking gel (5 mL) Volume (mL)**
Distilled water	3.3 mL	3.4 mL
30% acrylamide mix	4.0 mL	0.83 mL
1.5 M Tris(hydroxymethyl)aminomethane (pH 8.8)	2.5 mL	0.63 mL
10% ammonium persulfate	0.1 mL	0.05 mL
TEMED	0.004 mL	0.005 mL
10% SDS	0.1 mL	0.05 mL

The protein samples from *S.* Enteritidis were heated at 95ºC -100ºC in a boiling bath for 4 min prior to electrophoresis. This was done to denature the proteins. The prepared gel was placed in the electrophoresis chamber. Then, 10 µl of each sample was added and the gel was run at 200

Volts (V), 200 milliampere (mA) and 50 Watts (W) for 45 min in a 1X Tris-Glycine running buffer. A protein molecular weight marker (ThermoFisher Scientific, Waltham. MA) was applied to one of the gel pockets to later serve as a size standard to which sample signals could be compared. At the end of the electrophoresis run, the gel was stained in 0.25% Coomassie Brilliant Blue R250 (Sigma) (Table 3.2) for 4 h with gentle shaking. The gels were then destained in coomassie blue destain solution (Table 3.2) overnight and visualised with the GelDoc EZ Imager (Bio-Rad) visualisation system (Spectronics Co. Irvine. CA).

Table 3.2 Coomassie blue stain and destaining working solution

Reagents/chemicals	**Coomassie blue staining**	**Coomassie blue destaining**
Ethanol	400 mL	50 mL
Coomassie blue R-250	1.25 g	
Distilled water	500 mL	1 L
Acetic Acid	100 mL	75 mL

3.9 Tissue culture and infection

3.9.1 Cell culture reagents

Iscove's Modified Dulbecco's Medium (IMDM), sterile 1X PBS was purchased from Life Technologies (Carlsbad, CA); antibiotics penicillin and streptomycin were purchased from Biowhittaker (Walkersville, MD) while gentamicin was purchased from Melford (Chelsworth, United Kingdom; Fetal Bovine Serum (FBS) was purchased from Thermo Scientific (Waltham, MA): 2-mercaptoethanol (2-ME) and 0.25% Trypsin-0.02% Ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma (St. Louis, MO); the rough form lipopolysaccharide (LPS) from *E. coli* serotype J5 was purchased from ENZO Life Sciences (Farmingdale, NY)

3.9.2 JAWS 11 dendritic cell culture

JAWS II dendritic cell line was obtained from the American Type Culture Collection (CRL-11904; ATCC Manassas, VA) and propagated as described by Ssemakalu et al.(2015). In brief, cells were transferred in a T75 flask and grown in a carbon dioxide (CO_2) incubator at 37ºC with 5% CO_2 in a complete culture medium consisting of IMDM supplemented with 10% FBS, 10 U/mL penicillin and 100 µg/mL streptomycin, 0.5 mM 2-ME and 5 ng/mL murine Granulocyte macrophage-colony stimulating factor (GM-CSF). The medium was pre-incubated at 37ºC for at least 15 min to allow it to reach the desirable pH (7.0 to 7.6) prior to addition of cells. The cell culture was maintained by transferring the non-adherent cells into a centrifuge tube. The adherent cells were washed with 1X PBS to remove any traces of FBS and then treated with a solution consisting of 0.25% trypsin and 0.02% EDTA at 37ºC for 10 min. The two cell suspensions were then combined and centrifuged together at 1000 rpm for 10 min in a single centrifuge tube, and the supernatant was discarded. The cell pellet was washed with 1X PBS and resuspended in a complete fresh medium and seeded as required.

3.9.3 Dendritic cell stimulation

Throughout the study, the infection was maintained at a multiplicity of infection of 10:1. The stimulation experiments constituted of three types of infection conditions: i) non-solar irradiated bacteria ii) solar irradiated bacteria and iii) photonically inactivated bacteria. On the day of stimulation, the 1.23 x 10^5 cells/ml pre-seeded dendritic cells were washed thrice with 1X Dulbecco's phosphate-buffered saline (DPBS), and 1000 µl of infection media without antibiotics (IMDM-I) containing *S.* Enteritidis (MOI 10:1) was added and placed in a humidified incubator at 37ºC, 5% CO_2 and incubated for 4 h. After the 4 h incubation, 5 µl of gentamycin was added and incubated for another 24 h. The supernatants were obtained by centrifugation at 1000 rpm for 10

min. The viability count of the bacterial cells was determined using trypan blue dye. The supernatants were stored at -80°C until further use.

3.9.4 Flow cytometry compensation

Phenotypic analysis of cells was performed on a Guava®EasyCyte 8HT Benchtop Flow Cytometer using EasyCyte software (Guava Technologies, Billerica, MA). Cells were incubated with (i) DCs alone, (ii) LPS (1 µg/mL), (iii) solar irradiated, and (iv) photonically inactivated *S.* Enteritidis for 24 h. Thereafter, the cells were washed with fluorescence-activated cell sorting (FACS) buffer (2% flow cytometry standard (FCS), 0.1% NaN3 in PBS). After washing, the number of cells was checked using the Zombie dye protocol. The compensation was carried out by looking at four different cell markers, and the flow channels were selected as follows; CD83 (PE, Yellow B), CD80 (BV 605, Yellow V), IA/IE (AF700 & BV 650, Red V), CD 40 (PE Cy 5, Red B). The selection of these specific monoclonal antibodies were based on their ability to express themselves well on dendritic cells. Zombie aqua dye was also used for the compensation and was set as (BV510, Green V). For the preparation of the cell compensation, eight tubes were labelled as follows: CD80, CD83, CD40, BV650, AF 700, Zombie dye, unstained, and mixed population dye. Then 200 µl of the untreated cell suspension was added in all the tubes, and the appropriate cell markers were added separately. However, a tube containing the cell suspension mixed with all the antibodies was also prepared. The cells were blocked with anti-mouse CD16/CD32 (2.4G2) on ice for 15 min followed by washing and dispensed as 2×10^5 cells in 50 µl of FACS buffer. The samples were then used for compensation.

3.9.5 Flow-cytometric measurements

Following the compensation adjustments, a mixture of four fluorescent dyes (5 µl of CD83, 20 µl of CD80, 5 µl of IA/IE, and 5 µl of CD40) per well mixed together with the stimulated DCs in U-

bottomed 96-well plates was prepared. The cells were stained on ice for 30 min. After 30 min, the cells were washed with cell staining buffer (FBS) and then resuspended in 100 µl FBS and subjected to flow cytometry. Data was collected with the Guava CytoSoft Data Acquisition and Analysis Software (Guava software) (Version 3.6) and analysed with the same software or WinMDI (version 2.9), where necessary.

CHAPTER 4

4 RESULTS

4.1 Solar ultraviolet radiation

4.1.1 Exposure of *S.* Enteritidis to natural sunlight in winter

The effect of solar irradiation on the culturability of *S.* Enteritidis was assessed during three different sunny days in winter (5th, 11th and 19th of June 2018). Following 30 min of solar irradiation, the CFU counts were significantly ($p<0.05$) reduced from an initial concentration of 7.48 log CFU/mL to 6.51 log CFU/mL in samples at an OD of 0.1 (Table 4.1).

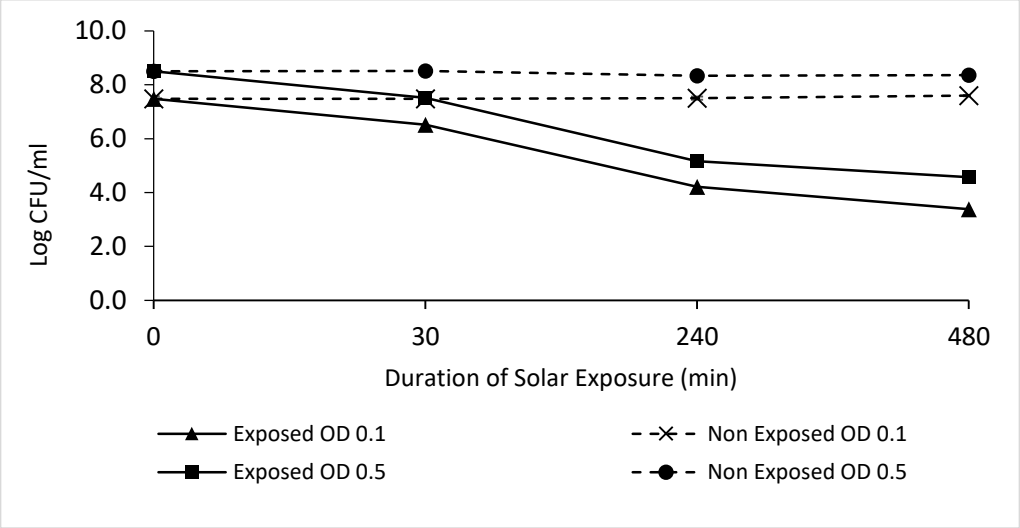

Figure 4.1. Log CFU/mL counts of solar irradiated and non-irradiated samples of S. Enteritidis in winter after 8 h. The solar irradiated samples are expressed as (exposed) while the non-solar irradiated ones are expressed as (Non exposed). Solar irradiated at 0.1 OD (▲),

non-solar irradiated at 0.1 OD (×), solar irradiated at 0.5 OD (■) and non-solar irradiated at 0.5 OD (●). Error bars indicate the standard error of triplicate experiments.

Similarly, after 30 min of solar irradiation, there was a reduction of CFU counts from an initial concentration of 8.50 log CFU/mL to 7.51 log CFU/mL in samples at an OD of 0.5. After 4 h of solar exposure a further highly significant *($p < 0.0001$*) decrease in the culturability of *S.* Enteritidis at both an OD of 0.1 (4.22 ± 0.03 Log CFU/mL; 240 min vs 0 min) and 0.5 (5.16 ± 0.02 Log CFU/mL; 240 min vs 0 minute) was observed. Following 8 h of exposure, *S.* Enteritidis at both an OD of 0.1 (3.38 ± 0.16 Log CFU/mL; 480 min vs 0 min) and 0.5 (4.56 ± 0.05 Log CFU/mL; 480 min vs 0 min) remained viable but was significantly (0.1OD *$p < 0.05$;* 0.5 OD *$p < 0.0001$*) lower in concentration compared to the initial concentration.

Table 4.1: Concentration [(Mean ± standard error of the mean (SEM)] and regrowth of solar irradiated S. enteritidis at 0.1 and 0.5 OD in winter

Exposure Duration (Min)	**Solar irradiation 0.1 OD (Log CFU/mL)**		**Solar irradiation 0.5 OD (Log CFU/mL)**	
	Exposed	**Re-growth**	**Exposed**	**Re-growth**
0	7.48 ± 0.09	8.17 ± 0.01	8.50 ± 0.02	9.60 ± 0.01
30	6.51 ± 0.01*	7.22 ± 0.01***	7.51 ± 0.06*	8.44 ± 0.04*
240	4.22 ± 003**	4.23 ± 0.30*	5.16 ± 0.02***	5.72 ± 0.29*
480	3.38 ± 0.16*	4.7 ± 0.19**	4.56 ± 0.05***	5.92 ± 0.04*

*Represents significance level at p <0.05 (comparisons between the solar irradiated and non-solar irradiated at 0 min); **Represents significance level at p <0.001 (comparisons between the solar irradiated and non- solar irradiated at 0 min); ***Represents significance level at p <0.0001 (comparisons between the solar irradiated and non- solar irradiated at 0 min); The mean and SEM values were derived from experiments run on three different days.

Therefore 8 h of exposure during the winter did not achieve total inactivation of *S.* Enteritidis at both an OD of 0.1 and 0.5 (Table 4.1 and Figure 4.1). The potential for the regrowth of the solar exposed *S.* Enteritidis at both an OD of 0.1 and 0.5 at all-time points was evaluated (Table 4.1). The results showed that the solar exposure of *S.* Enteritidis at both an OD of 0.1 and 0.5 for half an hour did not result in the sustained loss of

culturability (Table 4.1 & Figure 4.2). After 48 h of dark storage the viability of the 30 min solar exposed *S.* Enteritidis increased to almost the observed initial concentration prior to the solar exposure for samples at both an OD of 0.1 (7.22 ± 0.01 Log CFU/mL vs 7.48 ± 0.01 Log CFU/mL prior to exposure) and 0.5 (8.44 ± 0.04 Log CFU/mL vs 8.5 ± 0.02 Log CFU/mL prior to exposure).

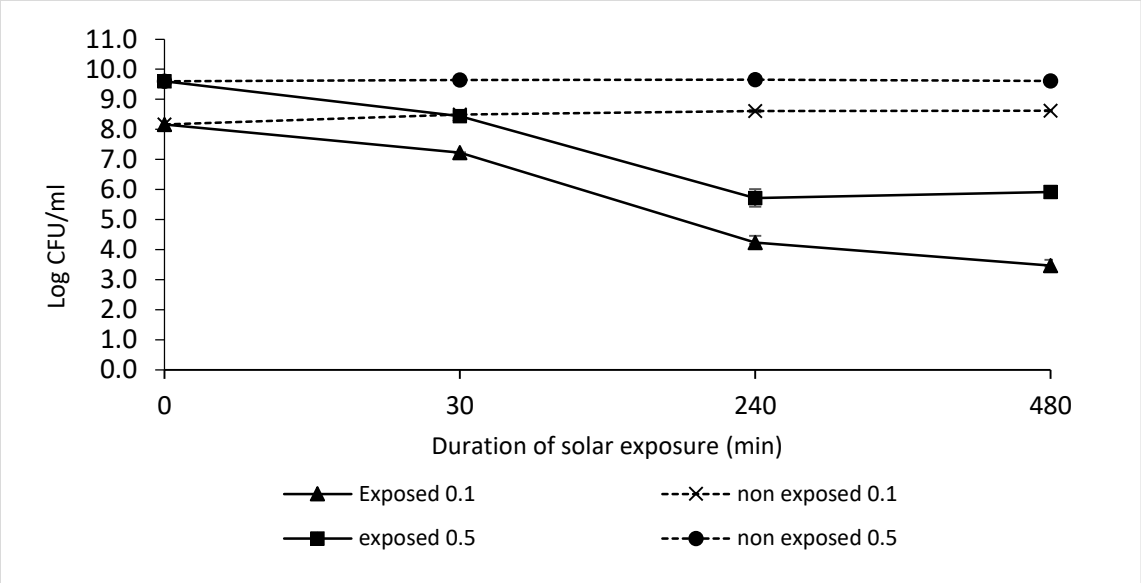

Figure 4.2. Log CFU/mL counts for the re-growth of solar irradiated and non-irradiated samples of S. Enteritidis in winter. The solar irradiated samples are expressed as (exposed) while the non-solar irradiated are expressed as (Non-exposed). Solar irradiated at 0.1OD (▲), non-solar irradiated at 0.1 OD (×), solar irradiated at 0.5 OD (■) and non-solar irradiated at 0.5 OD (●). Error bars indicate the standard error of triplicate experiments.

The culturability of the 240 and 480 min exposed *S.* Enteritidis at an OD of 0.1 following the dark storage showed a slight increase in Log CFU/mL (Table 4.1). A similar observation was made when *S.* Enteritidis was exposed at an OD of 0.5 (Table 4.1).

4.1.2 Exposure of *S.* Enteritidis to natural sunlight in spring

The effect of solar irradiation on the culturability of *S.* Enteritidis was assessed during three different sunny days in spring (3rd, 7th and 11th of September 2018). Following 30 min of solar irradiation, there was a reduction in the CFU counts from an initial concentration of 7.50 log CFU/mL to 6.39 Log CFU/mL in samples at an OD of 0.1 (Table 4.2).

Table 4.2 Concentration (mean ± SEM) of solar irradiated and re-growth of S. Enteritidis in spring

Exposure Duration (Min)	**Solar Irradiation 0.1 OD Log CFU/mL**		**Solar Irradiation 0.5 OD Log CFU/mL**	
	Exposed	**Re-growth**	**Exposed**	**Re-growth**
0	7.50 ± 0.01	8.41 ± 0.03	8.57 ± 0.02	9.57 ± 0.01
30	6.39 ± 0.06*	7.44 ± 0.08*	7.49 ± 0.03***	8.54 ± 0.01**
240	2.03 ± 0.72*	3.46 ± 0.12**	4.37 ± 0.15**	4.78 ± 0.04***
480	0.00 ± 0.00***	3.19 ± 0.09***	1.00 ± 0.82*	3.37 ± 0.12**

*Represent significance level at p <0.05 (comparisons between the solar irradiated and non-solar irradiated at 0 min); **Represent significance level at p <0.001 (comparisons between the solar irradiated and non- solar irradiated at 0 min); ***Represent significance level at p <0.0001 (comparisons between the solar irradiated and non- solar irradiated at 0 min); The mean and SEM values were derived from experiments run on three different days.

It was also observed that 30 min of solar irradiation, resulted in a reduction of CFU counts, from an initial concentration of 8.57 log CFU/mL to 7.49 Log CFU/mL in samples at an OD of 0.5. After 4 h of solar exposure, a significant (0.1 OD $p<0.05$; 0.5 OD $p<0.001$) decrease in the cultivability of *S.* Enteritidis at both an OD of 0.1 (2.03 ± 0.72 Log CFU/mL; 240 min vs 0 min) and 0.5 (4.37 ± 0.15 Log CFU/mL; 240 min vs 0 min) was observed. Following 8 h of exposure, *S.* Enteritidis at an OD of 0.5 (1.00 ± 0.82 Log CFU/mL; 480 min vs 0 min) remained viable but was significantly ($p<0.05$) lower in comparison to the initial concentration. As such, 8 h of exposure during the spring was significantly ($p<0.0001$) sufficient to result in the total inactivation

of *S.* Enteritidis at an OD of 0.1 (0.0.0 ± 0.00 Log CFU/mL; 480 min vs 0 min) Table 4.2 and Figure 4.3.

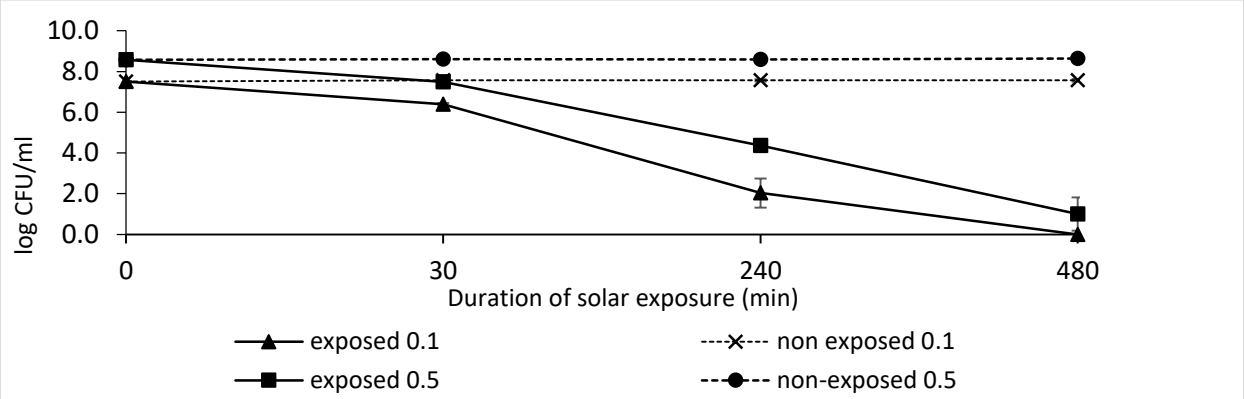

Figure 4.3. Log CFU/mL counts of solar irradiated and non-irradiated samples of S. Enteritidis in spring. The solar irradiated are expressed as (exposed) while the non-solar irradiated are expressed as (Non-exposed). Solar irradiated at 0.1OD (▲), non-solar irradiated at 0.1 OD (×), solar irradiated at 0.5 OD (■) and non-solar irradiated at 0.5 OD (●). Error bars indicate the standard error of triplicate experiments.

The potential for the re-growth of the solar exposed *S.* Enteritidis at both an OD of 0.1 and 0.5 at all-time points was evaluated (Table 4.2). The results showed that the solar exposure of *S.* Enteritidis for half an hour at both an OD of 0.1 and 0.5 did not result in the sustained loss of culturability (Table 4.2). After 48 h of dark storage, the viability of the 30 min solar exposed *S.* Enteritidis increased to almost the observed initial concentration prior to the solar exposure for samples at both an OD of 0.1 (7.44 ± 0.01 Log CFU/mL vs 7.50 ± 0.01 Log CFU/mL prior to exposure) and 0.5 (8.54 ± 0.04 Log CFU/mL vs 8.57 ± 0.02 Log CFU/mL prior to exposure).

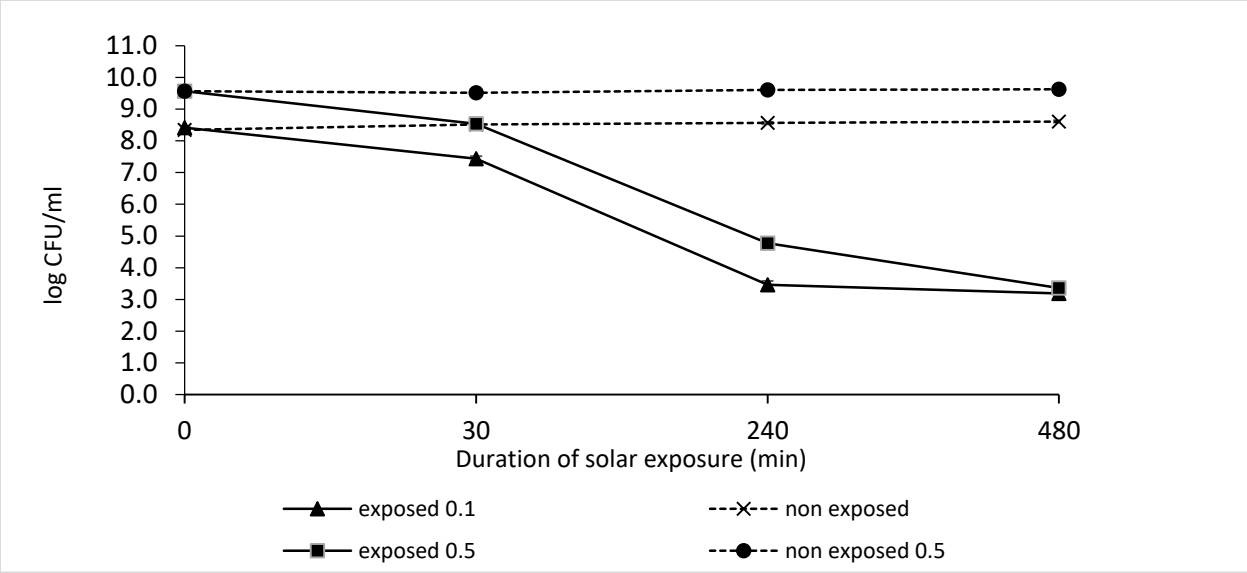

Figure 4.4. Log CFU/mL counts for the re-growth of solar irradiated and non-irradiated samples of S. Enteritidis in spring after 8 h. The solar irradiated are expressed as (exposed) while the non-solar irradiated are expressed as (Non-exposed). Solar irradiated at 0.1OD (▲), non-solar irradiated at 0.1 OD (×), solar irradiated at 0.5 OD (■) and non-solar irradiated at 0.5 OD (●). Error bars indicate the standard error of triplicate experiments.

The culturability of the 240 and 480 min exposed *S.* Enteritidis at an OD of 0.5 following the dark storage showed an increase in Log CFU/mL (Table 4.2). The 8 h solar exposed *S.* Enteritidis that was completely inactivated could not sustain it loss of cultivability following dark storage at an OD of 0.1 which suggest that the count was still viable but not culturable (Figure 4.4).

4.1.3 Exposure of *S.* Enteritidis to natural sunlight in summer

The effect of solar irradiation on the culturability of *S.* Enteritidis was assessed during three different sunny days in summer (11th, 26th, and 28th of November 2018). Following 30 min of solar irradiation, there was a reduction in the CFU counts from an initial concentration of 7.35 log CFU/mL to 6.31 Log CFU/mL in samples at an OD of 0.1 (Table 4.3).

Table 4.3 Concentration (mean ± SEM) of solar irradiated and re-growth of S. Enteritidis in summer

Exposure Duration (Min)	**Solar Irradiation 0.1 OD Log CFU/mL**		**Solar Irradiation 0.5 OD Log CFU/mL**	
	Exposed	**Re-growth**	**Exposed**	**Re-growth**
0	7.35 ± 0.05	8.37 ± 0.03	8.45 ± 0.04	9.48 ± 0.02
30	6.31 ± 0.06*	7.31 ± 0.03*	7.47 ± 0.03***	8.43 ± 0.03**
240	0.00 ± 0.00***	2.47 ± 0.01*	2.53 ± 1.03*	4.98 ± 0.08**
480	0.00 ± 0.00***	0.00 ± 0.00***	0.00 ± 0.00***	0.90 ± 0.73**

*Represent significance level at p <0.05 (comparisons between the solar irradiated and non-solar irradiated at 0 min); **Represent significance level at p <0.001 (comparisons between the solar irradiated and non- solar irradiated at 0 min); ***Represent significance level at p <0.0001 (comparisons between the solar irradiated and non- solar irradiated at 0 min); The mean and SEM values were derived from experiments run on three different days.

It was also observed that 30 min of solar irradiation, resulted in a reduction of CFU counts from an initial concentration of 8.45 log CFU/mL to 7.47 Log CFU/mL in samples at an OD of 0.5. After 4 h of solar exposure, a complete inactivation of *S.* Enteritidis in samples at an OD of 0.1 was observed (0.00 ± 0.00 Log CFU/mL; p <0.0001; 240 min vs 0 min; Table 3). After 4 h of solar exposure, a significant (p *<0.05*) decrease in the culturability of *S.* Eenteritidis at an OD of 0.5 was also observed (2.53 ± 1.03 Log CFU/mL; 240 min vs 0 minute). Following 8 h of exposure, *S.* Enteritidis at both an OD of 0.1 (0.00 ± 0.00 Log CFU/mL*;* 480 min vs 0 minute) and 0.5 (0.00 ± 0.00 Log CFU/mL*;* 480 min vs 0 min) was significantly (0.1 OD p *<0.000;* 0.5 OD p *<0.*0001) lower in comparison to the initial concentration. As such, 8 h of exposure during the summer was

sufficient to result in the total inactivation of *S.* Enteritidis at both an OD of 0.1 and 0.5 (Table 4.3 and Figure 4.5).

The potential for the re-growth of the solar exposed *S.* Enteritidis at both an OD of 0.1 and 0.5 at all-time points was evaluated (Table 4. 3 and Figure 4.6). The results show that solar exposure of *S.* Enteritidis at both an OD of 0.1 and 0.5 for half an hour did not result in the sustained loss of cultivability (Table 4.3). After 48 h of dark storage, the viability of the 30 min solar exposed *S.* Enteritidis increased to almost the observed initial concentration prior to the solar exposure for samples at both an OD of 0.1 (7.31 ± 0.01 Log CFU/mL vs 7.35 ± 0.01 Log CFU/mL prior to exposure) and 0.5 (8.43 ± 0.04 Log CFU/mL vs 8.45 ± 0.02 Log CFU/mL prior to exposure).

The non-culturability of the 240 min exposed *S.* Enteritidis at an OD of 0.1 following the dark storage showed an increase in Log CFU/mL (Table 4.3). The culturability of the 240 min exposed *S.* Enteritidis at an OD of 0.5 following the dark storage showed an increase in Log CFU/mL (Table 4.3). The results showed that the solar exposure of *S.* Enteritidis at an OD of 0.1 for the 480 min resulted in a sustained loss of cultivability (Figure 4.6). Regrowth was observed when *S.* Enteritidis was exposed to higher concentration at an OD of 0.5 (0.90 ± 0.73 Log CFU/mL*;* 480 min vs 0 minute) (Table 4.3).

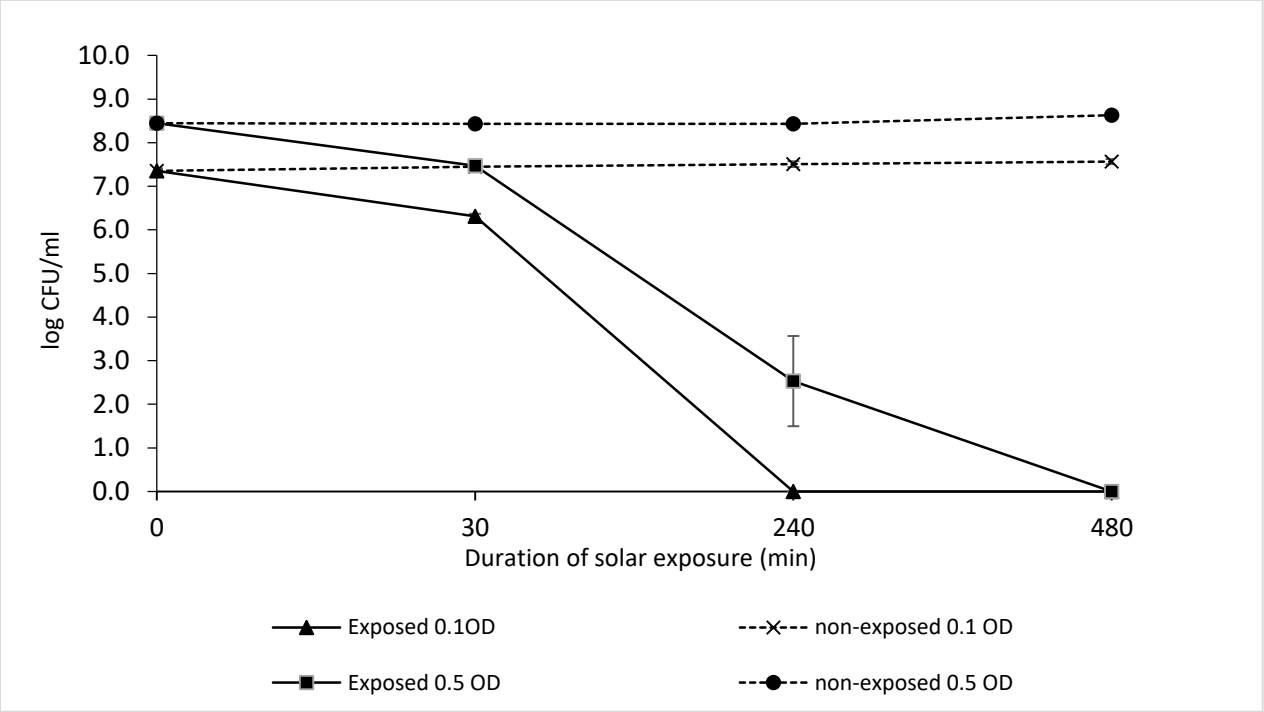

Figure 4.5. Log CFU/mL counts of solar irradiated and non-irradiated samples of S. Enteritidis in summer. The solar irradiated samples are expressed as (exposed) while the non-solar irradiated are expressed as (non-exposed). Solar irradiated at 0.1OD (▲), non-solar irradiated samples at 0.1 OD (×), solar irradiated at 0.5 OD (■) and non-solar irradiated at 0.5 OD (●). Error bars indicate the standard error of triplicate experiments.

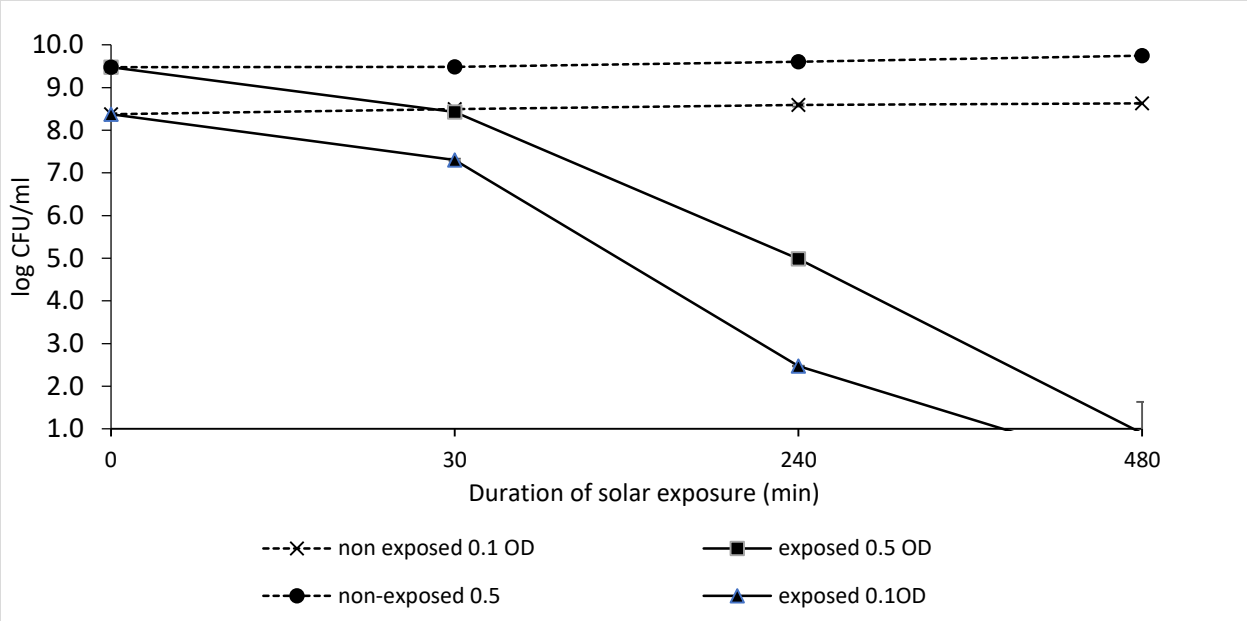

Figure 4.6 Log CFU/mL counts for the re-growth of solar irradiated and non-irradiated samples of S. enteritidis in summer after 8 h. The solar irradiated are expressed as (exposed) while the non-solar irradiated are expressed as (Non-exposed). Solar irradiated at 0.1OD (▲), non-solar irradiated at 0.1 OD (×), solar irradiated at 0.5 OD (■) and non-solar irradiated at 0.5 OD (●). Error bars indicate the standard error of triplicate experiments.

4.2 Exposure of *S.* Enteritidis to photonic device

The inactivation of *S.* Enteritidis at an OD of 0.1 was evaluated using a UVA lamp for 30 min, 4h and 8 h. The results were generated from 3 different experiments as follows: (1) treatment with a combination of UV and oxygen, (2) treatment with UV only, and (3) no UV treatment, which served as the control. In this experiment, only *S.* Enteritidis suspensions at an OD of 0.1 were used. This choice was based on the inactivation process from the SODIS experiment which demonstrated better inactivation of *S.* Enteritidis at an OD of 0.1.

4.2.1 Exposure of *S. enteritidis* to a photonic device at 26ºC

Following 30 min of exposure, there was a reduction in the CFU counts from an initial concentration of 7.60 log CFU/mL to 5.74 Log CFU/mL in the samples treated with a combination of UV and oxygen. A similar observation was made in samples treated with UV only where the CFU counts reduced from an initial concentration of 7.60 log CFU/mL to 6.39 Log CFU/mL (Table 4.4).

Table 4.4 Concentration (Mean ± SEM) of Photonic irradiated S. Enteritidis at room temperature.

Exposure Duration (Min)	**UV and Oxygen Log CFU/mL**		**UV Only Log CFU/mL**	
	UV and Oxygen	**Re-growth**	**UV Only**	**Re-growth**
0	7.60 ± 0.02	8.66 ± 0.02	7.60 ± 0.02	8.66 ± 0.02
30	5.74 ± 0.19*	6.47 ± 0.06**	6.39 ± 0.06*	6.66 ± 0.06**
240	2.94 ± 0.22*	3.57 ± 0.91*	3.87 ± 0.08**	5.01 ± 0.60*
480	0.00 ± 0.00***	0.00 ± 0.00***	3.42 ± 0.18***	3.61 ± 0.06**

*Represent significance level at p <0.05 (comparisons between samples treated with UV and Oxygen to the non-treated *S.* Enteritidis at 0 min); **Represent significance level at p <0.001 (comparisons between samples treated with UV and Oxygen to the non-treated *S.* Enteritidis at 0 min); ***Represent significance level at p <0.0001 (comparisons between samples treated with UV and Oxygen to the non-treated *S.* Enteritidis at 0min); The mean and SEM values were derived from experiments run on three different days.

After 4 h of exposure, there was a significant ($p < 0.05$) decrease in the culturability of *S.* Enteritidis in both samples treated with a combination of UV and oxygen (2.94 ± 0.22 Log CFU/mL; 240 min

vs 0 min). There was also a decrease in the culturability of *S.* Enteritidis for samples treated only with UV (3.87 ± 0.08 Log CFU/mL; $p < 0.001$; 240 min vs 0 min). Following 8 h of exposure, the culturability of *S.* Enteritidis treated only with UV remained viable (3.42 ± 0.18 Log CFU/mL; 480 min vs 0 min) but significantly ($p < 0.0001$) lower in comparison to the initial concentration. However, 8 h of exposure to a combination of UV and oxygen was sufficient to result in a significant ($p < 0.0001$) total inactivation of *S.* Enteritidis (0.00 ± 0.00 Log CFU/mL; 480 min vs 0 min) Table 4.4 and Figure 4.7.

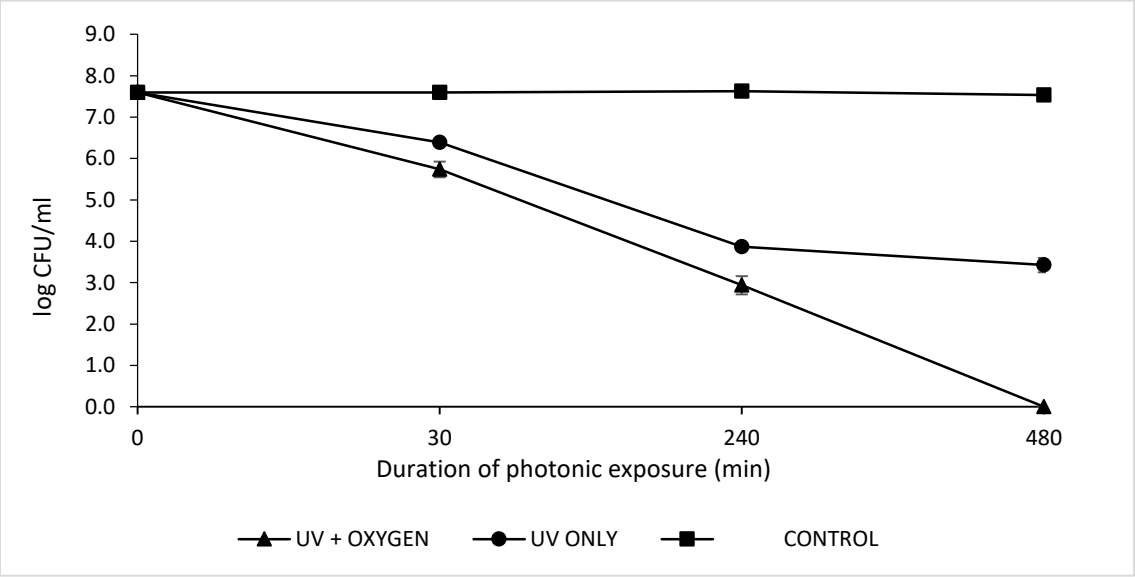

Figure 4.7. Log CFU/mL counts for the exposure of S. Enteritidis to photonic device. The samples treated with both oxygen and UV irradiation are expressed as (UV+ Oxygen), the samples treated only with UV are expressed as (UV only). The unexposed are the controls. Error bars indicate the standard error of triplicate environment.

The potential for the regrowth of the photonic treated samples of *S.* Enteritidis at all-time points was evaluated (Table 4.4). The results showed that the photonic treated samples of *S.* Enteritidis at both conditions for half an hour did not result in a sustained loss of cultivability (Table 4). After

48 h of dark storage, the viability of the 30 min exposed *S.* Enteritidis increased to almost the observed initial concentration prior to exposure for samples treated with both UV and oxygen (6.47 ± 0.06 Log CFU/mL vs 7.60 ± 0.02 Log CFU/mL) and samples treated only with UV (6.66 ± 0.06 Log CFU/mL vs 7.60 ± 0.02 Log CFU/mL). The culturability of *S.* Enteritidis treated with UV only for 240 and 480 min showed an increase in Log CFU/mL following the 48 h of dark storage (Table 4.4). A similar observation was made when *S.* Enteritidis was treated with UV and oxygen at 240 min. However, *S.* Enteritidis treated with a combination of oxygen and UV for 480 min resulted in a sustained loss of cultivability after 48 h of dark storage (Figure 4.8).

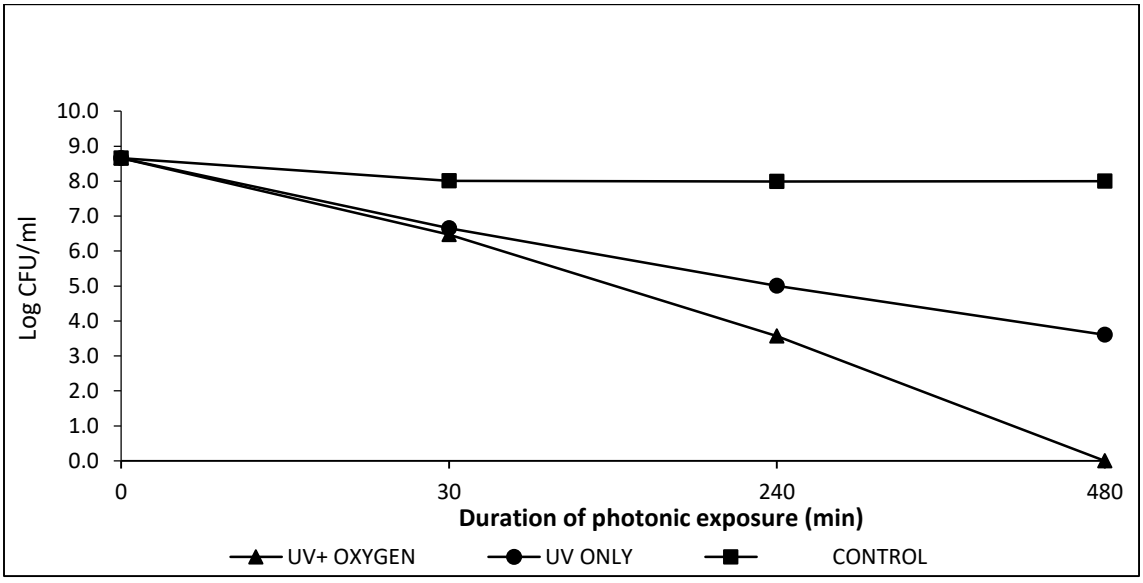

Figure 4.8. Log CFU/mL counts for the re-growth of S. Enteritidis to photonic device. The sample treated with both oxygen and UV irradiation are expressed as (UV + Oxygen), the samples only treated with UV are expressed as (UV only). Error bars indicate the standard error of triplicate environment.

4.2.2 Exposure of *S.* Enteritidis to photonic device under 37°C

The inactivation of *S.* Enteritidis with the use of the photonic device was further analyzed in an incubator at a temperature of 37ºC to determine the effect of temperature on the inactivation of the microorganisms. Following 30 min of exposure, there was a reduction in the CFU counts from an initial concentration of 7.57 log CFU/mL to 5.73 Log CFU/mL in the samples treated with the combination of UV and oxygen. A similar observation was made in samples treated only with UV where the CFU counts reduced from an initial concentration of 7.57 log CFU/mL to 5.60 Log CFU/mL (Table 4.5).

After 4 h of exposure, there was a non-significant ($p > 0.05$) decrease in the culturability of *S.* Enteritidis in both samples treated with a combination of UV and oxygen (4.16 ± 0.08 Log CFU/mL; 240 min vs 0 min). There was also a decrease in the culturability of *S.* Enteritidis in samples treated only with UV (4.49 ± 0.06 Log CFU/mL; $p > 0.05$; 240 min vs 0 min). Following 8 h of exposure, *S.* Enteritidis treated with UV and oxygen (3.49 ± 0.01 Log CFU/mL; $p < 0.05$; 480 min vs 0 min) and *S.* Enteritidis treated only with UV (3.90 ± 0.01 Log CFU/mL; $p < 0.05$; 480 min vs 0 min) remained viable but was significantly lower in comparison to the initial concentration. Photonic exposure at 37ºC for 8 h was not sufficient to result in the total inactivation of *S.* Enteritidis after treatment with UV and oxygen, and with UV only (Table 4.5 and Figure 4.9).

The potential for the regrowth of the photonic treated samples of *S.* Enteritidis at all-time points was evaluated (Table 4.5). The results showed that the photonic treated samples of *S.* Enteritidis at both conditions for half an hour did not result in the sustained loss of cultivability (Table 4.5). After 48 h of dark storage, the viability of the 30 min exposed *S.* Enteritidis increased to almost the observed initial concentration prior to exposure for samples treated with both UV and oxygen (6.27 ± 0.01 Log CFU/mL vs 7.57 ± 0.01 Log CFU/mL) and samples treated only with UV (6.36

± 0.04 Log CFU/mL vs 7.57 ± 0.01 Log CFU/mL). The culturability of *S.* Enteritidis treated with UV and oxygen at 240 and 480 min showed an increase in Log CFU/mL following the 48 h of dark storage (Table 4.5). A similar observation was made when *S.* Enteritidis was treated with only UV at 240 and 480 min (Figure 4.10).

Table 4.5. Concentration (Mean ± SEM) of Photonic irradiated S. Enteritidis at 37ºC

Exposure Duration (Min)	**UV and Oxygen Log CFU/mL**		**UV Only Log CFU/mL**	
	UV and Oxygen	**Re-growth**	**UV Only**	**Re-growth**
0	7.57 ± 0.01	8.57 ± 0.02+	7.57 ± 0.01	8.57 ± 0.02
30	5.73 ± 0.03*	6.27 ± 0.01*	5.60 ± 0.07	6.36 ± 0.04*
240	4.16 ± 0.08	4.58 ± 0.05*	4.49 ± 0.06	4.66 ± 0.01*
480	3.49 ± 0.01*	4.40 ± 0.03*	3.90 ± 0.01*	4.54 ± 0.04*

*Represent significance level at p <0.05 (comparisons between samples treated with UV and Oxygen to the non-treated *S.* Enteritidis at 0 min); **Represent significance level at p <0.001 (comparisons between samples treated with UV and Oxygen to the non-treated *S.* Enteritidis at 0 min); ***Represent significance level at p <0.0001 (comparisons between samples treated with UV and Oxygen to the non-treated *S.* Enteritidis at 0 min); The mean values were derived from experiments run on three different days.

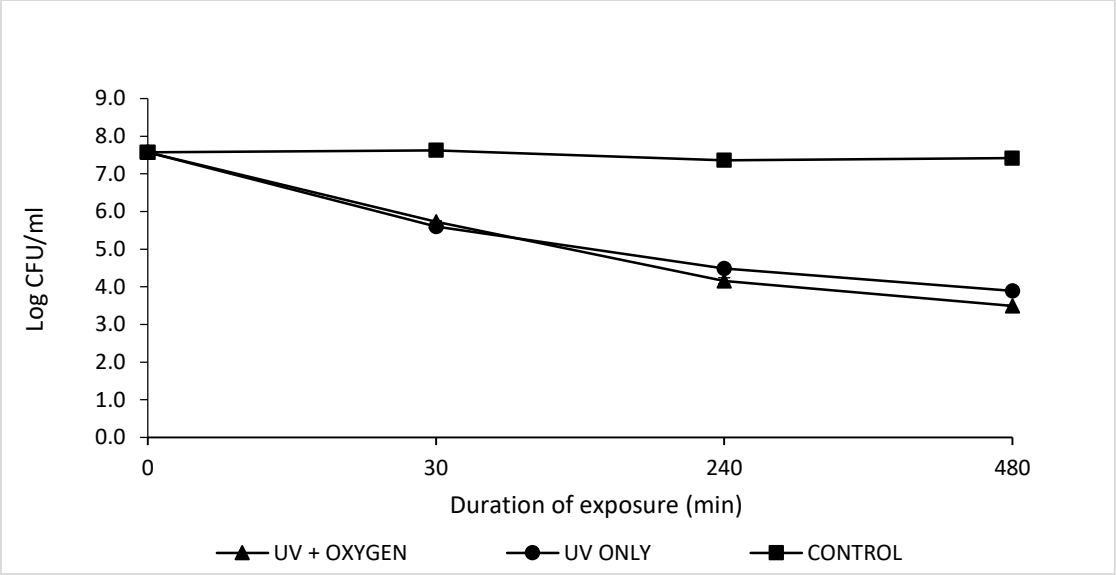

Figure 4.9. Log CFU/mL counts for the exposure of S. Enteritidis to photonic device at 37ºC. The samples treated with both oxygen and UV irradiation are expressed as (UV+ Oxygen), the samples treated with only UV are expressed as (UV only). The unexposed are the controls. Error bars indicate the standard error of duplicate experiments.

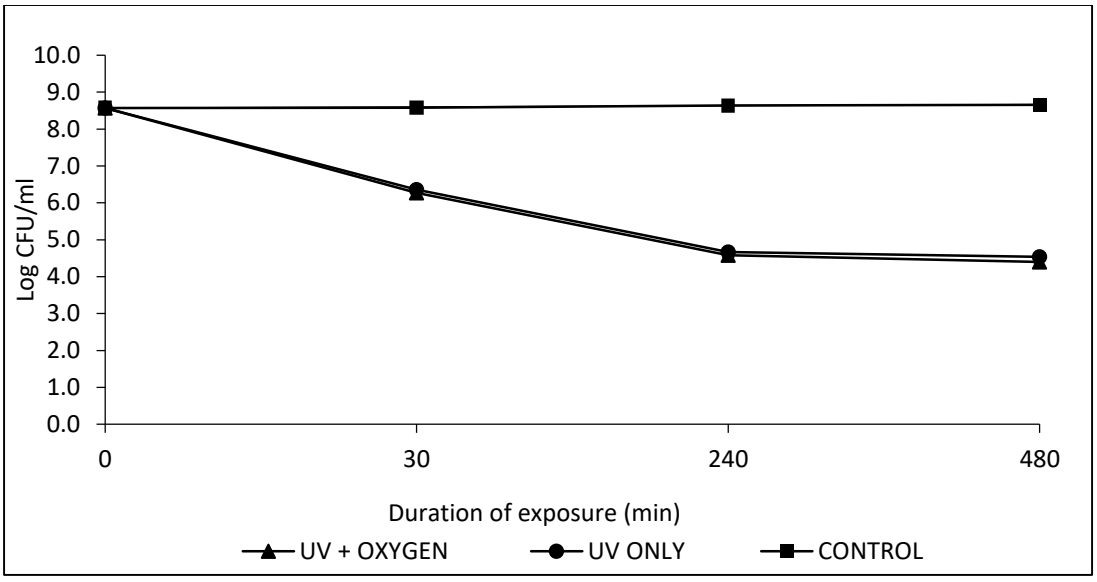

Figure 4.10. Log CFU/mL counts for the re-growth exposure of S. Enteritidis to photonic device. The sample treated with both oxygen and UV irradiation are expressed as (UV+ Oxygen), the samples only treated with UV are expressed as (UV only). The unexposed are the controls. Error bars indicate the standard error of duplicate experiments.

4.3 Whole cell protein profiling of *S.* Enteritidis using SDS-PAGE

4.3.1 Whole cell protein profiling from the samples treated with solar irradiation

The whole-cell protein profiles of the irradiated and non-irradiated samples of *S.* Enteritidis at an OD of 0.1 were analysed using SDS-PAGE and stained with Coomassie brilliant blue R-250 dye-(CBB) staining.

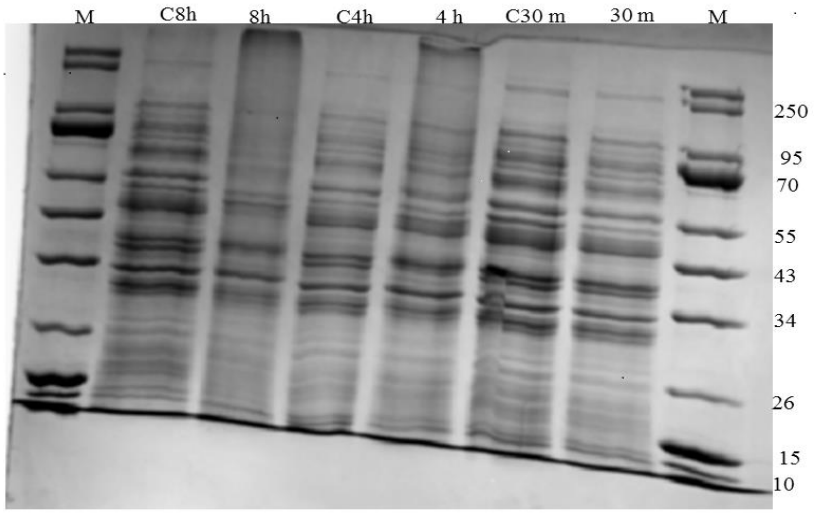

Figure 4.11: Representative Coomassie brilliant blue (CBB) stain gel SDS-PAGE illustrating the whole-cell protein profiles of solar irradiated and non-irradiated S. Enteritidis strain exposed in summer. From left to right. Lane 30 m, 4 h and 8 h are the irradiated samples. Lane C8h, C4h- and C30m- represent the controls 8 h, 4 h and 30 min. Lane M-molecular weight is the Precision PlusProtein^{TM} Unstained Standards, (Biorad).

The banding pattern of the solar irradiated samples showed a progressive decrease in the intensity of the protein bands as the duration of SUVR exposure increased (Figure 4.11). The protein banding profile of *S.* Enteritidis that was solar irradiated for 30 min shared a similar banding pattern as that observed in all the controls (Figure 4.11). The protein bands for the 30 min SUVR

exposed samples had more prominent bands between 34 KDa to 250 KDa. Following 4 h of SUVR exposure, there was almost complete elimination of the protein bands of 70 KDa, 95 KDa and 250 KDa in comparison to the protein bands of similar molecular weight in the 30 min solar irradiated samples. After 8 h of exposure, there was a distinct elimination of some of the protein bands including the 55 KDa, 70KDa. 95 KDa and 250 KDa bands (Figure 4.11). Generally the protein gel showed that increasing SUVR exposure time decreased the prominence of some of protein banding patterns.

More than 20 protein bands could be resolved from the control sample after 30 min of solar irradiation. These bands ranged in size from 10 KDa to 250 KDa as determined by the visual assessment of their approximate molecular masses (Figure 4.11). The protein profile of all the controls exhibited similar banding patterns. It was also observed that the protein profiles of all the controls were not altered and shows consistency throughout the experiment.

4.3.2 Proteomic profiling from the samples treated photonically

The whole-cell protein profiles of photonically inactivated (UV + Oxygen) and non-treated samples of *S.* Enteritidis at an OD of 0.1 were analyzed using SDS-PAGE with Coomassie brilliant blue R-250 dye (CBB) staining. The banding pattern of the photonic inactivated samples showed a progressive decrease in the intensity of the protein bands as the duration of UV exposure increased (Figure 4.12). The protein banding profiles of *S.* Enteritidis that was photonically inactivated for 30 min with the combination of UV and Oxygen shared a similar banding pattern to that observed in all the controls (Figure 4.12). The protein bands for the 30 min exposure were more prominent between 43 KDa to 250 KDa. Following 4 h of photonic exposure, there was almost complete elimination of the protein bands between 43 KDa to 70 KDa, leaving the protein bands above 70 KDa prominent. After 8 h of photonic exposure, there was a distinct elimination

of some of the protein bands such as the 26 KDa, 43 KDa, 55 KDa, 70KDa. The protein bands at 95 KDa were almost completely eliminated after 8 h. The protein bands at approximately 80 KDa remained prominent in all the photonic inactivated samples (Figure 4.12). The protein gel showed that increasing exposure time could actually decrease the prominence of the protein banding pattern.

More than 20 protein bands could be resolved from the control sample after 30 min of solar irradiation. These bands ranged in size from 26 KDa to 250 KDa as determined by the visual assessment of their approximate molecular masses (Figure 4.12).

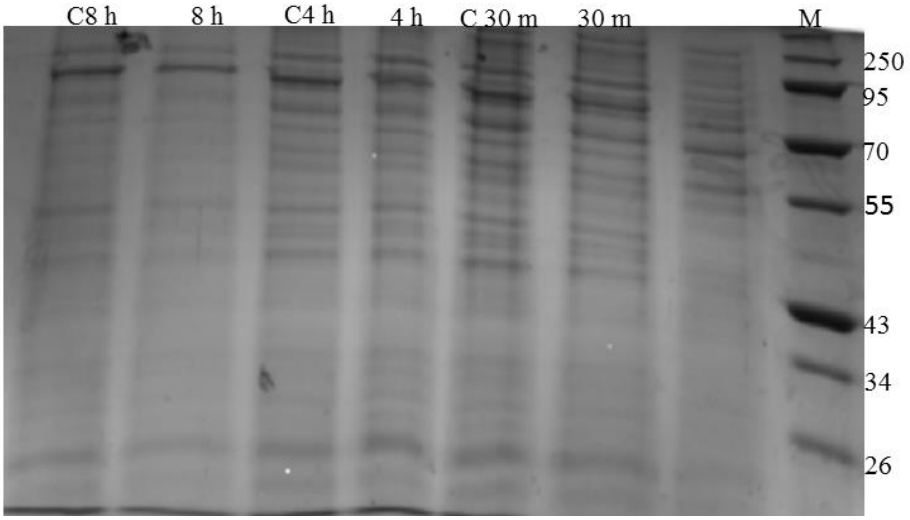

Figure 4.11. Representative Coomassie brilliant blue (CBB) stain gel (Bio-rad). SDS-PAGE illustrating the whole-cell protein profiles of S. Enteritidis strain exposed to the combination of UV and oxygen photonic inactivation. Lane 30m, 4h and 8h are the exposed samples while C8h- C4h- and C30m- represent the control for 8h, 4h and at 30 min. Lane M-molecular weight marker (Precision PlusProtein^{TM} Unstained Standards).

The protein profile of all the controls exhibited similar bands. It was also observed that the protein profiles of all the controls were not altered and showed consistency throughout the experiment except for the control at 8 h which was slightly altered.

4.4 Flow Cytometry Analysis

In this study, flow cytometry was used to assess the potential for solar irradiated and photonically inactivated *S.* Enteritidis at 0.1 OD to induce the maturation of dendritic cells *in-vitro*. The cell surface markers assessed were CD80, CD83 and MHC-II. A two-way ANOVA was used to compare the effects of solar irradiated, photonically inactivated, non-irradiated and LPS on the expression of the maturation markers on the dendritic cells. Differences at $P < 0.05$ level were considered statistically significant. The data were expressed as means of the Mean Fluorescence Intensity (MFI) ± standard error of the mean (SEM) obtained from three biological replicates for each experiment.

4.4.1 Expression of CD80 cell surface marker

The dendritic cells that were stimulated with 30 min solar irradiated cultures of *S.* Enteritidis showed an increased expression of CD80 (168.51 ± 7.48). A similar observation was made when dendritic cells were stimulated with 4 h inactivated *S.* Enteritidis (162.67 ± 2.42). But there was a decrease in the expression of CD80 (150.86 ± 2.47) by the dendritic cells when the 8 h solar irradiated cultures of *S.* Enteritidis were used as a stimulant (Table 4.6).

Table 4.6. Expression of CD80 (expressed as the Mean Florescence Intensity ± SEM) by dendritic cells stimulated with solar irradiated and photonically inactivated S. Enteritidis for 72h.

Duration of irradiation (time)	Mean flourescence intensity (± SEM) for solar irradiated samples	Mean fluorescence intensity (± SEM) for photonic irradiated samples
Non-treated	155.79 ± 1.63	155.79 ± 1.63
30 minute	168.51 ± 7.48	157.04 ± 3.71
4 hour	162.67 ± 2.42	176.63 ± 6.78
8 hour	150.86 ± 2.47	160.37 ± 5.95

Other dendritic cells treatments

LPS	174.64 ± 0.00
Unstimulated (DC only)	161.55 ± 0.25

Unstimulated refers to dendritic cells treated only with PBS (Negative control)

LPS refers to the positive control

Non-treated refers to *S.* Enteritidis cultures that were not treated by solar irradiation or photonic inactivation.

Photonic irradiated sample refers to *S.* Enteritidis cultures that were treated with the combination of UV and oxygen.

When the dendritic cells were stimulated with *S.* Enteritidis that had been photonically irradiated for 30 min, a decrease in CD80 on the cell surface was observed (157.04 ± 3.71) relative to the

unstimulated dendritic cell (161.55 ± 0.25). However, the expression of CD80 increased (176.63 ± 6.78) when the dendritic cells were stimulated with the 4 h photonically irradiated *S.* Enteritidis. This increase was not sustained because a decrease in the expression of CD80 was observed in dendritic cells stimulated with the 8 h photonically irradiated samples of *S.* Enteritidis (160.37 ± 5.95).

4.4.2 Expression of CD83 cell surface marker

The dendritic cells that were stimulated with 30 min of solar irradiated cultures of *S.* Enteritidis showed an increased expression of CD83 (47.13 ± 4.99). A different observation was made when dendritic cells were stimulated with 4 h and 8 h inactivated *S.* Enteritidis. The expression of CD80 at both 4 h and 8 h was minimal (40.17 ± 0.00) and (42.48 ± 2.41, respectively) and close to that observed in the untreated dendritic cells (Table 4.7).

When the dendritic cells were stimulated with *S.* Enteritidis that were photonically irradiated for 30 min, an increase in CD83 on the cell surface was observed (44.33 ± 4.18) relative to the unstimulated dendritic cell (42.82 ± 1.56). However, the expression of CD83 decreased (35.88 ± 11.96) when the dendritic cells were stimulated with the 4 h of photonically irradiated *S.* Enteritidis. This decrease was sustained because a decrease in the expression of CD83 was also observed in dendritic cells stimulated with the 8 h of photonically irradiated samples of *S.* Enteritidis (37.58 ± 0.88).

Table 4.7. Expression of CD83 (expressed as the Mean Fluorescence Intensity ± SEM) by dendritic cells stimulated with solar irradiated and photonically inactivated S. Enteritidis for 72h.

Irradiation duration time	Mean fluorescence intensity (± SEM) for solar irradiated samples	Mean fluorescence intensity (± SEM) for photonic irradiated samples
Non-treated	41.89 ± 2.11	41.89 ± 2.11
30 min	47.13 ± 4.99	44.33 ± 4.18
4 h	40.17 ± 0.00	35.88 ± 11.96
8 h	42.48 ± 2.41	37.58 ± 0.88

Other dendritic cells treatment

LPS	37.73 ± 0.00
Unstimulated (DC only)	42.82 ± 1.56

Unstimulated refers to dendritic cells treated only with PBS (negative control)

LPS refers to the positive control

Non-treated refers to *S.* Enteritidis cultures that were not treated by solar irradiation or photonic inactivation.

Photonic irradiated sample refers to *S.* Enteritidis cultures that were treated with the combination of UV and oxygen.

4.4.3 Expression of MHC-class II cell surface marker

The dendritic cells that were stimulated with 30 min of solar irradiated cultures of *S.* Enteritidis showed an increase in the expression of MHC-II (269.54 ± 2.14). A similar observation was made when dendritic cells were stimulated with 4 h inactivated *S.* Enteritidis (268.49 ± 8.27). But a decrease in the expression of MHC-II (248.48 ± 1.13) by the dendritic cells was observed when the 8 h solar irradiated cultures of *S.* Enteritidis was used as a stimulant (Table 4.8).

When the dendritic cells were stimulated with *S.* Enteritidis that were photonically irradiated for 30 min, an increase in MHC-II on the cell surface was observed (255.59 ± 2.92). A similar observation was made when dendritic cell was stimulated with 4 h inactivated *S.* Enteritidis (268.49 ± 8.27). This increase was sustained because an increased expression of CD80 was observed in dendritic cells stimulated with the 8 h photonically irradiated samples of *S.* Enteritidis (265.54 ± 11.90).

Table 4.8 Expression of MHC-II (expressed as the Mean Florescence Intensity ± SEM) by dendritic cells stimulated with solar irradiated and photonically inactivated S. Enteritidis for 72h.

Irradiation duration time	Solar irradiated samples (MFI ± SEM)	Photonic irradiated samples (MFI ± SEM)
Non-treated	264.90 ± 8.31	264.90 ± 8.31
30 min	269.54 ± 2.14	255.59 ± 2.92
4 h	268.49 ± 8.27	259.19 ± 18.28
8 h	248.48 ± 1.13	265.54 ± 11.90

Other dendritic cells treatment

LPS	256.72 ± 11.61
Unstimulated (DC only)	251.34 ± 6.15

Unstimulated refers to dendritic cells treated only with PBS (Negative control)

LPS refers to the positive control

Non-treated refers to *S.* Enteritidis cultures that were not treated by solar irradiation or Photonic inactivation.

Photonic irradiated sample refers to *S.* Enteritidis cultures that were treated with the combination of UV and oxygen.

CHAPTER 5

5 DISCUSSION AND CONCLUSION

5.1 Solar and photonic irradiation and the inactivation of *S.* Enteritidis

Solar irradiation of water was shown to inactivate and eliminate microbial pathogens including *S.* Enteritidis. However, not enough focus has been placed on using a photonic device to treat contaminated water. In this study, *S.* Enteritidis was prepared and exposed to either natural solar radiation or UV-A photonic irradiation in combination with oxygen. The rate of inactivation of *S.* Enteritidis observed within the first 30 min of solar exposure in winter was noticeably less than that observed in the spring and summer (Tables 4.1, 4.2, 4.3). The experimental period of 8 h was found to be insufficient for the total inactivation of *S.* Enteritidis under the weak and moderate sunlight in winter (Figure 4.1). A similar observation was made by Karim et al. (2021) where a significant amount of *E*. *coli* was found to be present in the solar treated water after 8 h of exposure. One way to prevent the culturability of *S.* Enteritidis in winter is, perhaps, to increase the time of exposure to SUVR. This assumption requires further research. A 4 h solar exposures in spring did not inactivate *S.* Enteritidis completely (Figure 4.3). However, 8 h of exposure resulted in the total inactivation of *S.* Enteritidis at an OD_{600nm} of 0.1 (0.00 ± 0.00 Log CFU/mL) but not at an OD_{600nm} of 0.5 (1.00 ± 0.82 Log CFU/mL). These results suggest that the solar conditions during spring were sufficient to completely activate *S.* Enteritidis at a concentration of (7.50 ± 0.01Log CFU/mL for OD546nm 0.1). However, total inactivation did not occur in the more concentrated sample (8.57± 0.02Log CFU/mL for OD_{600nm} 0.5). The higher concentration of *S.* Enteritidis may have hindered

light penetration into the water. Furthermore, the inconsistent weather conditions experienced during the spring influenced the duration needed to achieve total inactivation (Figure 4.3). This is due to a scatter pattern of solar irradiance observed during this season as the sky remained cloudy for most of the time during the experimental days. One of the solar exposures carried out in spring was accompanied by rainfall and it appears that SODIS is inefficient during days of continuous rainfall as observed by (Karim et al., 2021). This implies that weather conditions does affect SODIS and should be considered when using this method for water disinfection. These results are supported by findings of Asiimwe et al. (2013) which showed that bacterial inactivation under cloudy conditions was achieved after 6 h of exposure. This may suggest that two consecutive days of exposure may be sufficient to inactivate *S.* Enteritidis under cloudy conditions. Support for this assumption is provided by the findings of other studies that demonstrated that solar inactivation of a water borne pathogens under cloudy conditions should be carried out for two consecutive days (Oates et al., 2003; Navntoft et al., 2008; Ssemakalu et al., 2012).

In summer, complete inactivation of *S.* Enteritidis from a starting concentration of 7.35 Log CFU/ mL (OD_{600nm} 0.1) to 0.00 Log CFU/mL was achieved within the first 4 h of solar irradiation (Figure 4.5). This complies with the WHO (2004) guidelines that states that microbial standard water should be 0 CFU for the maintenance of microbiological quality of water. However, total inactivation of *S.* Enteritidis after 4 h of solar exposure did not occur at a higher bacterial concentration of 8.44 Log CFU/mL (OD_{600nm} 0.5). It took 8 h of exposure for total inactivation of *S.* Enteritidis at this concentration. This observation suggests that the efficiency of solar inactivation depends on the microbial concentration, which explains why the suspension of *S.* Enteritidis at an OD_{600nm} 0.5 remained culturable after SODIS treatment throughout the three seasons analysed. In addition, the bacterial sample at a high OD_{600nm} was more turbid; hence a

higher amount of UV and greater exposure time was needed to achieve the same level of inactivation in comparison to that required by the sample at an OD_{600nm} of 0.1. The data gathered from this experiment, agrees with published results obtained under field and laboratory conditions. For example, a study by Dessie et al. (2014) demonstrated complete inactivation of *E. coli* within 4 h of exposure using adjusted parameters for water disinfection. Another study conducted by Asiimwe et al. (2013) reported that the inactivation of the pathogen *E. coli* was achieved after 6 h of solar exposure.

This study assessed the potential for the irradiated samples of *S.* Enteritidis to re-grow following a dark-storage period for 24 h. This experimental approach was important because bacteria have repair mechanisms that allow recovery following stress/injury. Bacteria recover using some of the documented cellular repair mechanisms such as nucleotide excision repair, photoreactivation, recombination DNA repair and mutagenic DNA repair, therefore permitting their recovery and the manifestation of the regrowth phenomenon (Jacobs and Sundin, 2001).

The results of this study showed that total inactivation of *S.* Enteritidis occurred following 8 h of solar irradiation during spring re-growth after the dark storage occurred. A similar observation was made following 4 h of solar irradiation of samples at an OD_{600nm} of 0.1 (Figure 4.5 and 4.6). These results suggest that the microorganism may have existed in a viable but non-cultural state (VBNC) meaning that the cells were characterized by a loss of culturability on a routine agar plate and could not be detected by conventional plate count. This may result in an underestimation of the total number of viable cells in an environment or clinical sample (Laam et al., 2014). This could explain the analogy that the ability of SODIS to inactivate water pathogens such as *S.* Enteritidis, has been attributed to the effect derived from the interaction of SUVR and temperature. Less SUVR will enable the microorganisms to recover from solar irradiation effects.

This observation was similar to the study carried out by Karim et al. (2021) that showed complete disinfection of *E. coli* in water after 8 h of exposure during summer, but a significant microbial count was detected after 12 and 24 h of post-irradiation periods, possibly due to repair of partially damaged cells. The study by Karim et al. (2021) used a lower concentration of an initial count of 5×10^5 CFU/100 mL (3.7 Log CFU/mL). The current study used a very high bacterial dose (7.88 Log CFU/mL) that is generally not found in the environment. It is important to maintain sufficient exposure time to control microbial re-growth in treated water when storing the water before drinking. Re-growth of bacteria after overnight storage at room temperature following SODIS was reported by other researchers (Hirtle, 2008; Byrne et al., 2011a; Wilson, 2010).

Based on the observations made following the solar inactivation experiments, all the photonic based inactivation experiments used *S.* Enteritidis at an OD_{600nm} of 0.1. The results showed that 4 h of photonic exposure in the presence and absence of oxygen resulted in a decrease in the concentration of *S.* Enteritidis (Figure 4.7) with total inactivation occurring after 8 h. The inactivation of *S.* Enteritidis by photonic treatment with UV light in the presence of oxygen at 4 h effectively reduced the *S.* Enteritidis in water by inactivating a total of 4 to 5 $\log_{10}$ from an initial concentration of 7.60 ± 0.02 to 2.94 ± 0.22 Log CFU/mL when compared to the samples in the absence of oxygen with an initial concentration of (7.60 ± 0.02 to 3.87± 0.08 Log CFU/mL (Figure 4.7). However, increasing the treatment time to 8 h showed no microbial counts when compared to the 4 h treatment. The lack of efficacy at 4 h may be due to the limited amount of oxygen. Treatment of *S.* Enteritidis for 8 h showed total inactivation of this bacteria (Figure 4.7). In another experiment, *S.* Enteritidis contaminated water was treated with a higher flow rate of oxygen for up to 4 h. This treatment showed a reduction in total microbial counts and no colonies were detected on growth plates. UV radiation inactivates microorganisms by instigating a cross-link between

pyrimidine nucleotide bases in the DNA; this inhibits transcription and DNA replication mechanism and later leads to microbial death. In addition, the outcome of this experiment revealed that UV radiation effectively reduced *S.* Enteritidis in water in the presence of oxygen. The sample of *S.* Enteritidis exposed only to UV radiation for 8 h showed a decrease in microbial count whereas total loss of microbial activity was noticed in the sample exposed to both UV radiation and oxygen. A previous study by Meyer (2001) also reported that the efficiency of photo-oxidative disinfection was indeed better when a combination of solar UV radiation and dissolved oxygen was used for disinfection rather than using these factors individually. There were no indications that the microorganism DNA repair took place during the 24 h storage period. This makes the reactivation of *S.* Enteritidis highly unlikely. The photonic device at 37^{o}C did not inactivate the *S.* Enteritidis (Figure 4.9) even after 8 h. The reason for this anomaly remains unknown and requires further investigation.

5.2 Proteomic profiling of solar irradiated and photonic inactivation of *S.* Enteritidis

SDS-PAGE is the most widely used technology to separate mixtures of proteins. Hence, the evaluation of protein expression, assessment of the purity of protein samples, quantification and immunochemical identification of proteins are techniques that make use of SDS-PAGE.

The whole-cell protein profiles of *S.* Enteritidis were different for those treated with solar radiation and photonic exposure. In both cases a decrease in some of the protein bands were observed after treatment with an increase in SUVR and photonic exposure (Figure 4.11 and 4.12). This may be due to the denaturation of some the cellular proteins because of UV exposure. This is expected if the DNA of the bacteria are damaged. A great similarity in protein profiles was observed between the solar irradiated and photonically inactivated *S.* Enteritidis. High similarity in the protein profiles of the bands labelled 4 h and 8 h (Figure 4.11, Figure 4.12) were subjected to oxidative

stress (formation of protein-protein cross-links, amino acid modifications, carbonyl group formation, and formation of S-S bridges). This may be attributed to the increase in exposure time to UV. Proteins are crucial targets of radiation, and it appears that the cell's capacity to prevent protein denaturation during radiation is determined by its resistance or susceptibility properties (Chatgilialoglu et al., 2011).

Previous studies on proteomic profiles proved that the modification of proteins is subjected to large number of reactions which involves reactive oxygen species. Among these reactions, proteins that are damaged oxidatively result in increased susceptibility to proteolysis and exposure to hydrophobic patches than their normal counterparts. Carbonylation is one of the reactions that has attracted a great deal of attention. It has been recommended that carbonylation, being an irreversible modification, leaves the modified protein to degradation and also to lose antigen-recognition site (Bota and Davies, 2002; Dukan et al., 2000; Grune et al., 2004).

SDS-PAGE of the various samples of *S.* Enteritidis at different treatments produced reproducible protein profiles (Figures 4.9 and 4.10). The profiles of all the controls at different time points also exhibited similar bands. A distinct observation was made between the protein profiles of *S.* Enteritidis samples at 4 h and 8 h. For instance, the protein profiles at 4 h and 8 h were similar to those at 30 min but were distinguished by the presence of a double specific band. Studies by (Bosshard et al., 2010) demonstrated that UVA radiation targets cellular functions such as ATP synthesis, transcription and translation, chaperone functions, respiration, catalase, degradation and amino acids synthesis. The absorption of UV radiation between 290 to 315 nm by cellular constituents are considered to lead to the denaturation of proteins.

5.3 Flow cytometry analysis of solar irradiation and photonic inactivation of *S.* Enteritidis

The maturation of dendritic cells based on their cell surface markers following their stimulation with solar irradiated and photonic inactivation and non-solar irradiated and cultures of *S.* Enteritidis was assessed. The use of SODIS as a water disinfecting method has been previously reported to induce the secretion of co-stimulatory receptor molecules such as CD80. CD83 and MHC-class II by dendritic cells *in-vitro* (Ssemakalu et al., 2020).

Co-stimulatory receptors are a group of cell surface molecules expressed by antigen presenting cells that regulate T-cells activation and the generation of effector T-cells responses (Magee et al., 2012). Knowledge of their pathways involved in the various stages of the immune response is crucial. CD80 plays a crucial role in the initiation and maintenance of the immune response. The results from this study showed that the non-solar irradiated and non-photonic treated cultures of *S.* Enteritidis at 30 min significantly ($p < 0.05$) induced the dendritic cell to express a higher level of CD80 when compared to the unstimulated dendritic cells. This observation could be explained in relation to the ability of CD80 to play a role in the initial inflammatory proliferation (Tormanen et al., 2020). When the dendritic cells were stimulated with solar irradiated and photonic inactivated cultures of *S.* Enteritidis, a significant ($p<0.05$) expression of dendritic cells was observed when compared to the unstimulated dendritic cells.

The stimulation of both solar irradiated and photonically inactivated samples of dendritic cells did not stimulate a detectable amount of CD83 and MHC- class II (Table 7 and 8) when compared to untreated dendritic cells. The stimulation of the solar irradiated sample of *S.* Enteritidis at 8 h showed a significant response to CD80 co-stimulatory marker when compared to the LPS stimulated cells (Table 7).

In conclusion, the results from this study showed that solar irradiated cultures of *S.* Enteritidis induced the dendritic cells to express more CD80 than that observed when photonically inactivated cells and LPS were used as stimulants. Moreover, the concentration of CD80 produced by the dendritic cells in response to the solar irradiated cultures of *S.* Enteritidis was significantly ($p < 0.05$) higher than that observed in the untreated dendritic cells. This is similar to the result reported by (Ssemakalu et al., 2020). Another study reported that solar irradiated microorganisms are capable of inducing the secretion of pro-inflammatory cytokines and chemokines (Ssemakalu et al., 2015). The result of this study showed that solar irradiated and photonic cultures of *S.* Enteritidis induced the maturation of dendritic cells. The results obtained from the co-stimulatory molecules suggested the possible involvement of these molecules in antigen uptake and presentation to produce a specific immune response. The present investigation provided further insight that the consumption of SODIS and photonically inactivated pathogens in water is likely to induce an immune response.

5.4 Conclusion

The use of solar irradiation, when compared to other household water treatments, has a huge limitation since it relies on weather conditions for disinfection of microorganisms (Clasen et al., 2009). The goal of this research was to understand the immunomodulatory effect of SODIS and photonic application on inactivated *S.* Enteritidis on dendritic cells *in-vitro*. The results of this study suggested that the effect and inactivation efficiency of SUVR on *S.* Enteritidis was season dependent. The experiments carried out in summer showed a total loss of activity in *S.* Enteritidis and no re-growth was observed compared to the experiments carried out in spring and winter. We can also conclude that the mimicking of SODIS by using a photonic device was found to produce an enhancement in inactivation kinetics, but at relevant environmental conditions. Disinfection by

this photonic device will be faster in the presence of both UV and oxygen. An increase in the regulation of oxygen flow may enhance the inactivation process to emphasize the key role of oxygen in addition to sunlight. The protein profiles of solar irradiated and photonically inactivated *S.* Enteritidis appeared to be subjected to oxidative stress as the results showed a gradual decrease in the protein banding pattern intensities with time in *S.* Enteritidis. The immunomodulatory effect of solar and photonically inactivated *S.* Enteritidis on dendritic cells revealed that the solar irradiated and photonically inactivated cultures of *S.* Enteritidis were able to induce the stimulation of CD80.

5.5 Recommendations

The use of photonic inactivation for disinfecting water should be reviewed by future research to generate a consolidated overall-applicable instruction protocol for achieving greater efficacy under field conditions.

Given that current study shows that solar irradiated cultures of *S.* Enteritidis were able to induce the stimulation of dendritic cells, the process may still need optimisation. Further studies should be conducted under various settings and conditions to determine the immunomodulatory effect of solar and photonically inactivated *S.* Enteritidis on macrophages.

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