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Enhancing the Solar Water Disinfection (SODIS) Method Using a Fresnel Lens

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by

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1. INTRODUCTION

Currently, about one in seven people worldwide lack access to clean water, and meeting the needs of a growing population will only further stress our existing resources (Gleick 2002). It is for these reasons that sustainable, cost effective, efficient water disinfection systems are needed. Therefore, the subject of this research is to explore a possible method of enhancing the Solar Water Disinfection (SODIS) method by using a Fresnel lens to concentrate solar energy, with the goal of attaining pasteurization temperatures to reduce holding times for disinfection. This method will be referred to as "PULSE" – Pasteurization Using a Lens and Solar Energy. The hypotheses are that pasteurization temperatures can be reached using a Fresnel lens and, secondly, that the Fresnel lens will accelerate the rate of disinfection by causing a rapid temperature rise during the solar disinfection process.

To test the first hypothesis, a Fresnel lens was used under varying weather conditions to determine its heating capabilities and conditions under which it is able to reach pasteurization temperatures. In testing the second hypothesis, disinfection rate as a function of temperature increase was established using a hot plate. Following demonstration that disinfection increases with temperature increase, an experiment utilizing PET bottles (typically employed in the SODIS method) (SODIS: How does it work? 2011) filled with water spiked with *E. coli,* was carried out to determine bacterial inactivation rates. Each condition (Dark, Hotplate, SODIS, and PULSE) was

tested, but SODIS and PULSE were done over the summer months, when weather conditions would be similar, to ensure reproducibility of results. Temperature and *E. coli* numbers were monitored as a function of time. The rates of bacterial inactivation in each of the PET bottles were compared to confirm how temperature affects disinfection rates and to determine whether the Fresnel lens will speed up the solar disinfection process. The goal was to demonstrate that by achieving pasteurization temperatures, a Fresnel lens could significantly increase disinfection rates, thereby providing clean water in a much shorter amount of time.

In exploring this technology approach, this research sought to lay a foundation in which the mechanism, application, and relevance of lens-enhanced solar disinfection can be more closely studied. Better designs, better materials, and a better understanding of the fundamental principles governing disinfection can be brought to the forefront with the examination of existing designs, materials, and current data that this research looks to elucidate. Ultimately, it is towards this end in which this research is directed – to help mitigate human suffering due to something well within our capability.

2. BACKGROUND

Water quality issues and problems are highly related to the economic well-being of the region. In this chapter, issues surrounding water quality in developing nations, current alternatives to those issues, and potential alternatives are discussed.

Water Issues in Developing Nations

LACK OF INFRASTRUCTURE

The cost of poor water quality affects people throughout the world. For this reason, subsidized services are implemented to provide utilities such as water, electricity, etc. In some places, these services are made possible through public expenditures and tax revenues. Some examples of government-funded subsidies include countries of the former Soviet Union, where 10% of the GDP was estimated to account for power sector subsidies in Kazakhstan, Tajikistan, and Uzbekistan. Power is an important resource as it can be consumed to move water from one location to another. In contrast, although an extremely important utility, power sector subsidies represent only about 1% of the total GDP in countries such as India and Mexico, as the fiscal limitations in those countries are significant. Further, in India, drinking water subsidies were estimated at 0.5% of the GDP (Komives, et al. 2005).

Clearly, the provision of adequate infrastructure truly matters. Productivity and living standards are associated with having a reliable source of water, sanitation, and electricity. There's a strong correlation associated with infrastructure (namely improved water supply, sanitation, and electricity) and reduced poverty by increasing the standard in living and contributing to improved health outcomes. Water related illness accounts for substantial problems of disease in developing countries, leading to high costs related to death, malnutrition, and reduced productivity.

Improved water and sanitation have shown to reduce those costs. Also, the availability of electricity allows for proper storage and preservation of foods and medicines and allows health facilities to remain operational. Further, improved sanitation and water supply have also shown an increase in literacy and school attendance, especially for girls, since it reduces the time required to collect water. These services lead to improved overall productivity as the time required to collect fuel or water, or to use sanitation facilities outside the home is significant. Expansions in the quality and quantity of available utilities can lower costs and make it possible for the expansion of market opportunities, thereby increasing productivity and investment, both main components of economic growth.

Conversely, an underinvestment in infrastructure has shown to reduce annual growth by 1% to 3%, according to studies done throughout Latin American and African countries (Komives, et al. 2005).

LACK OF ACCESS

Because of lack of adequate infrastructure, many people in developing countries lack access to improved water, sanitation, or electricity (Komives, et al. 2005). Regions of middle-income countries tend to have broader access to such services, than regions whose countries are predominantly lower income (Komives, et al. 2005).

> "In many countries where utility networks have been installed, the quality of service is poor. Many utility customers often have no water in the pipe, and when water is available, it is often unsafe to drink. Sanitation facilities are often inadequate, overloaded, in disrepair, or unused, and electricity service may be sporadic and of poor quality" (Komives, et al. 2005).

For people of developing communities to have access to water, either they have to install systems, which bring the water directly to them, which can be extremely costly, or they will have to spend copious amounts of time retrieving it themselves.

LACK OF RESOURCES

For people living in countries with limited resources, or limited means to pay for such resources, acquiring services such as water, electricity, or sanitation may be a luxury many can't afford (Gilman and Skillicorn 1985). Persuading villagers of developing countries that boiling water is a simple and effective

method for making water safe to drink is often very difficult, as the cost and limited availability of fuel, make it a less feasible option (Gilman and Skillicorn 1985).

For these reasons, affordable, effective, and sustainable options need to be brought to the forefront to meet the challenges facing developing countries.

Disinfection

From developed to developing nations, different methods of disinfection are employed, namely physical and chemical processes. More often than not, it is more advantageous to use a combination of both methods depending on the initial water quality, and the intended use for the treated water (Crittenden, et al. 2012). This combination is known as a physiochemical unit process (Crittenden, et al. 2012).

When emergency disinfection is necessary, disinfectants are less effective in cloudy, murky, or colored water, and therefore require filtration or settling before disinfection is employed (Emergency Disinfection of Drinking Water 2006). The utilization of these methods is limited to availability of resources, sustainability, and cost. These techniques can help to eliminate unwanted pathogens, which left untreated, could lead to disease and, to a greater extent, death.

RATE OF DISINFECTION

The main Law governing the disinfection process is known as Chick's Law, and follows a first-order reaction

$$N=N_0e^{-kt}$$

where N is the number of microorganisms, N_0 is the initial number of microorganisms, k is the rate constant, and t is the contact time (Disinfection n.d.). It was observed that for disinfection, the longer the contact time for a given concentration of disinfectant, the greater the kill (NWAIWU and LINGMU 2011). If the reaction is in fact first order, a plot of log inactivation vs. contact time will yield a straight line (NWAIWU and LINGMU 2011).

CHEMICAL

The use of chemicals for disinfection requires the addition of an oxidizing agent, such as with chlorine, chlorine compounds, or ozone, to inactivate pathogenic organisms in water (Crittenden, et al. 2012).

For many developed countries, ozonation can be used in a water treatment facility's disinfection process (Tibbetts 1995). Ozonation is a process by which ozone is passed through water for a "flash" killing effect (Tibbetts 1995). However, chlorine in smaller doses is still employed, as bacteria can be reintroduced during distribution (Tibbetts 1995). Ozonation is the most expensive alternative to chlorine for disinfection, and can cause potentially harmful byproducts such as bromate (Tibbetts 1995).

Chlorine has been employed for many decades as a disinfectant (Venkobachar, Iyengar and Rao 1976). Among its many disinfecting benefits, it is also known for its germicidal potency and persistence (Oates 2001), therefore having residual effects, even through distribution (Metcalf and Eddy 1991). However, using chlorine, as well as other disinfecting agents, could have other adverse health effects (Sedlak and von Gunten 2011). It has also been noted that the odor and taste can become unpleasant when used in useful quantities (Emergency Disinfection of Drinking Water 2006). Further, chemical disinfectants can be challenged by turbidity and some dissolved constituents (Psutka, et al. 2011).

PHYSICAL

Physical disinfection can include processes such as filtration, reverse osmosis, UV, and heating, to name a few (Crittenden, et al. 2012). Some of these processes are used in a treatment train, or a series of unit operations. (Crittenden, et al. 2012). The need and available resources determine which processes will be used. Since disinfection by heat is the focus of this research, heat and the process of pasteurization will be more thoroughly explored.

Heat

Boiling or heating with fuel is perhaps the oldest means of disinfecting water at the household level (Sobsey 2002). However, with the rising cost of

fuel, the need for sustainable methods, and the growing scarcity of fuels in different regions, boiling water is becoming a luxury not everyone can afford (Clasen*, et al. 2008). As an alternative to boiling, for over a century, pasteurization has been used to remove harmful pathogens in foods/drinks (Griffiths 2010). The idea is to use enough heat for a prescribed amount of time to make foods safe for consumption (Griffiths 2010).

The recommended holding times and temperatures for pasteurizing milk are summarized in Table 1, with the provision that if the milk is condensed, has more than 10% fat, or has added sugars, that the temperatures be raised an additional 3°C (Pasteurization: Definition and Methods 2009).

Table 1: Pasteurization temperature and required holding times for milk

Temperature	Time	Pasteurization Type
63ºC (145ºF)*	30 minutes	Vat Pasteurization
72ºC (161ºF)*	15 seconds	High temperature short time Pasteurization (HTST)
89ºC (191ºF)	1.0 second	Higher-Heat Shorter Time (HHST)
90ºC (194ºF)	0.5 seconds	Higher-Heat Shorter Time (HHST)
94ºC (201ºF)	0.1 seconds	Higher-Heat Shorter Time (HHST)
96ºC (204ºF)	0.05 seconds	Higher-Heat Shorter Time (HHST)
100ºC (212ºF)	0.01 seconds	Higher-Heat Shorter Time (HHST)
138ºC (280ºF)	2.0 seconds	Ultra Pasteurization (UP)

(Pasteurization: Definition and Methods 2009).

In applying the same principles to contaminated water, elevating the temperature to the point where pathogens can be inactivated without the need for boiling, can make it safer for drinking. It has been widely experimented and established by researchers that pathogenic microbes are inactivated at a temperature of greater than 50°C (Jagadeesh 2012). In their extensive studies, Safapour and Metcalf reported the crucial role of temperature above 50°C in the elimination of pathogens (Jagadeesh 2012), (Safapour and Metcalf 1999).

Studies in Zambia have shown that participants could visually determine when to remove their vessels from the heat source by looking for bubbles at the bottom of the vessel and when they started noticing steam (Psutka, et al. 2011). It was determined that at this point, the water is at about 70°C (Clasen*, et al. 2008), which is a sufficient pasteurizing temperature (Wegelin 1994).

Because pasteurization is intended to inactivate pathogens, any chemicals, or sediments will not be removed unless another process is used to remove them. Pasteurization is not intended for those purposes. However, boiling is the surest method to make water safe to drink and kill disease-causing microorganisms like *Giardia lamblia* and *Cryptosporidium*, which are frequently found in rivers and lakes (Emergency Disinfection of Drinking Water 2006).

Biomass to Develop Heat

More than one half of the world's population relies chiefly on wood, charcoal, and other biomass for their energy supplies (Rehfuess E 2006). The

procurement of these fuels represents a substantial commitment of time and energy, primarily for women and girls, and may detract from other productive and potentially health-promoting activities (Biran, Abbot and Mace 2004).

Boiling water using fuels such as coal or biomass as wood, crop residues, and dung (Smith 2002) can be an important cause of other health hazards, including respiratory infections, anemia, and stunting associated with poor indoor air quality and burns, especially among young children (Clasen*, et al. 2008). Further, using this type of fuel, boiling may be environmentally unsustainable and contribute to greenhouse gases (Clasen*, et al. 2008).

Electricity

Smaller applications may use electricity to supply heat by boiling water on electric stoves or portable electric kettles. These are more suitable for household disinfection techniques. Improved water supply, sanitation, and electricity services are associated with raising productivity and living standards (Komives, et al. 2005).

However, a major setback that continues to confront rural villages is the lack of access to electricity (Gilman and Skillicorn 1985), which could contribute to improved water supply and sanitation. General decreases in the availability of fuels traditionally collected by villagers and a marked increase in the cost of commercial fuels in the last decade suggest that, in the village context, boiling of

drinking-water may not be a financially viable option (Gilman and Skillicorn 1985).

SODIS

Solar Water Disinfection (SODIS) is a low cost, simple, and sustainable method to improve water quality on the household level (Graf, Togouet, et al. 2010). It works in the following way: water that may be contaminated with bacteria and viruses is poured into plastic polyethylene terephthalate (PET) bottles and then exposed to direct sunlight for a minimum of 6 hours (Graf, Meierhofer, et al. 2008). It is recommended to place the bottles on rooftops since they are often good places for maximum exposure of sunlight (see Figure 1) (Graf, Meierhofer, et al. 2008).

Figure 1: Implementation of SODIS method (SODIS: How does it work? 2011)

Both the UVA radiation (315 nm-400 nm) and slight heating are the components that are responsible for disinfecting the water (Graf, Meierhofer, et

al. 2008). Separately, heat and UV have germicidal effects in certain ranges, but the synergistic effect of both has shown to allow for disinfection at lower than pasteurization temperatures (McGuigan *et al*. 1998). “Compared to lower water temperatures, only one-third of the UV-A fluence was required to inactivate *E. coli* at synergistic threshold of 50°C” (Oates 2001), (Wegelin *et al*. 1994).

Observations have been made that indicate that water temperatures between 20 and 40°C do not affect the inactivation of *E.coli* by sunlight (Wegelin 1994); (Oates 2001). Synergistic effects have been observed, however, at a water temperature of 45°C (McGuigan 1998), (Oates 2001). If the weather is very cloudy, the exposure time is extended to 2 or more days (Meierhofer and Wegelin 2002) (Graf, Meierhofer, et al. 2008).

Further, if microbial heat resistance can be overcome, and temperatures rise past the maximum growth value, proteins have a difficult time forming their proper structures, and proteins, which have already formed, begin to unfold (Oates 2001). In the event the protein denatures, it will not function properly and could kill the organism (Brock 2000). This will help to prevent regrowth in the treated vessel.

To prevent recontamination after the water has been treated, it is recommended that the treated water not be transferred into a different storage container but consumed directly from the bottle or poured into a clean cup (Graf, Togouet, et al. 2010).

However, the SODIS method requires relatively non-turbid water, below 30 Nephelometric Turbidity Units (NTU) (SODIS News No. 3, August 1998) and requires that the depth of the water be less than 10cm, as it was found that UV radiation decreases by 50% at that depth (Sommer 1997) and why bottles no greater than 2L are recommended for the SODIS method (Oates 2001). SODIS requires that a sunlight intensity of at least 500 W/m^2 be applied for three to five hours (SODIS News No. 1, 1998), (Oates 2001). SODIS does not treat the chemical water properties, as it is serves to inactivate target organisms (Graf, Togouet, et al. 2010).

Overall, SODIS is a low-cost and simple addition to the traditional methods of treating water (e.g. boiling, chlorination) (Graf, Meierhofer, et al. 2008). Therefore, using modest resources, it is a particularly favorable way for the poorest segments of the population in the developing countries to obtain safe water (Graf, Meierhofer, et al. 2008). Uncovering the exact mechanism in this disinfection process is still underway, but many new findings have been reported.

It is suggested that even slightly irradiated cells are strongly affected in their ability to maintain essential parts of their energy metabolism, in particular of the respiratory chain (Bosshard, Bucheli, et al. 2010). Further, in increasing the oxygen content of the sample before exposing it to sunlight, photo-oxidation was improved (solar photo-oxidation disinfection) as it could impact protein oxidation.

> "Protein oxidation is known to be a key factor in cellular ageing (sic) in eukaryotes (Grune, et al. 2004) and was recently also found to be important in bacteria (Nystrom 2006). The tertiary structure of oxidized proteins is thermodynamically instable and, therefore, oxidized proteins tend to expose

hydrophobic amino acids to the outside, with the consequences of agglutination and cross-linking" ((Squier 2001); (Grune, et al. 2004); (Chiti 2006) (Bosshard, Riedel, et al. 2010)).

There have been some concerns regarding whether SODIS can cause contamination from the plastic bottles themselves (Schmid*, et al. 2008). However, tests have been conducted showing that toxicological effects are negligible (Schmid*, et al. 2008).

PULSE

To enhance the SODIS method a Fresnel lens has been added to make it an effective solution for in terms of treating larger volumes of water in a smaller amount of time. This new method has been termed PULSE—Pasteurization Using a Lens and Solar Energy.

Fresnel lenses are made of different materials, depending on the wavelength range of interest, consisting of concentric grooves which all act to either focus or collimate light (see Figure 2) (Advantages of Fresnel Lenses 2012).

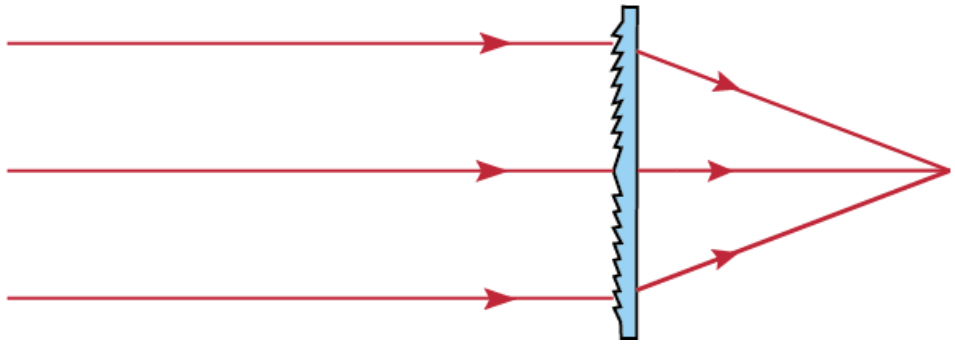

Figure 2: Fresnel lens used as a light collector (Advantages of Fresnel Lenses 2012)

The Fresnel lens is a much more compact lens, compared to conventional lenses, thereby requiring less materials to produce it, making it more easily mobile, and relatively cheap to produce (see Figure 3) (Advantages of Fresnel Lenses 2012).

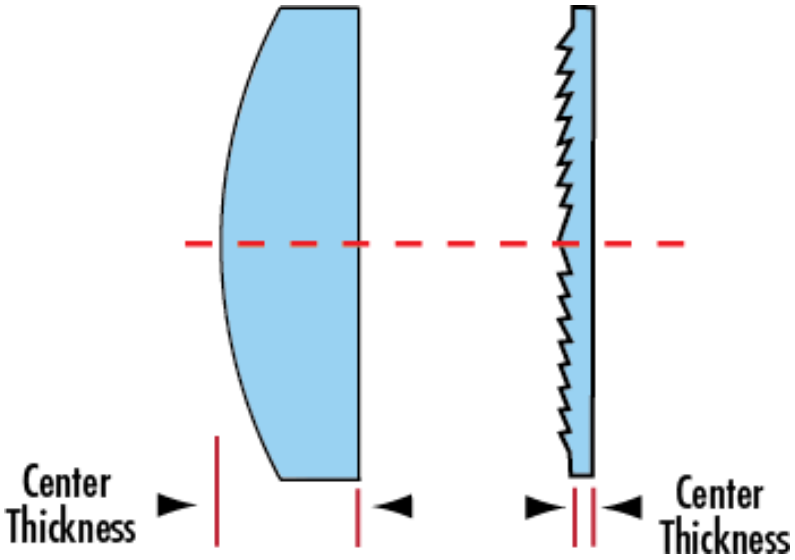

Figure 3: Comparison of a Plano-Convex lens with a Fresnel lens (Advantages of Fresnel Lenses 2012)

They can be made from many different materials depending on the application. Materials such acrylic plastic are particularly good. They allow for greater transmittance in the visible and UV portion of the spectrum (Advantages of Fresnel Lenses 2012). In adding the Fresnel lens and intensifying the light and energy transmitted to the water, considering the synergistic effects of mild heat and UV, perhaps SODIS drawbacks associated with volume, time, and even turbidity might be eliminated. If pasteurization temperatures are reached, and there is no cross contamination after treatment, the target organisms will be rendered inactive without fear of regeneration (Oates 2001). (Maniatis, Fritsch and Sambrook 1989).

For this reason, heat tests were conducted to determine whether pasteurization temperatures could be attained using a Fresnel lens, and tests to determine the decay rates of each method were also conducted to indicate how the PULSE method compared to the other methods.

3. MATERIALS AND METHODS

In this chapter, the setup and methodology for the preliminary heat tests to determine the effectiveness of the Fresnel lens in heating water and the disinfection decay rate testing for each condition (Dark, Hotplate, SODIS, and PULSE) are discussed.

HEAT TESTS

Heat tests were conducted at different periods throughout the spring and summer to determine if the Fresnel lens was able to bring the water inside the bottle to pasteurization temperatures and to determine whether the ambient temperature would greatly affect the water temperature in the bottle on which the lens was acting. Bottles were filled to 490mL of deionized (DI) water and taken to the Dean's Patio on the third floor A-wing of Bourn's Hall and placed on concrete under the Fresnel lens, which was focused on the ground before the bottle was placed. For safety purposes, as the lens can pose a human threat due to the high temperatures and intense brightness it can generate, measures were taken to help minimize human interaction with the lens. A stand was built to hold the lens throughout the procedure (see Figure 4.)

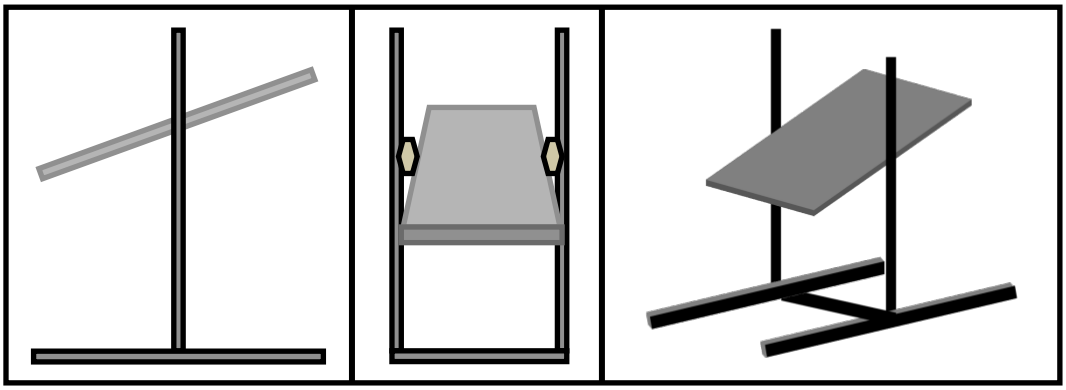

Figure 4: From left to right– (a) Side view of concept device; (b) Front view of concept device (showing knobs for adjusting lens angle); (c) Isometric view of concept device

Temperature measurements were taken with an immersion thermometer at five-minute intervals to determine whether the Fresnel lens could achieve pasteurization temperatures in the water.

DECAY RATE TESTS

Four conditions were tested during this project—Dark, Hotplate, SODIS, PULSE.

The Dark test was the background test against which all other tests would be compared. The natural die off of *E. coli* in the absence sunlight or added heat was determined in the Dark test.

The Hotplate test was used to determine the role that heat plays in disinfection, namely when pasteurization temperatures can be achieved in the absence of sunlight. This was used to determine the die off of bacteria in the presence of increasing heat (with no influence from the sun).

The SODIS test was done to model the existing method that is currently used in the developing world, using only PET (polyethylene terephthalate) bottles and solar energy.

The PULSE test was conducted as an alternative to standard heating methods, and to rival the SODIS method in its ability to provide larger volumes of water in less time, and also challenging the existing methods for heating water by demonstrating its superiority over the hotplate method, or other heating methods requiring the use of other fuels.

The tests were designed such that the set up for all conditions, (Dark, Lens, SODIS, and Hotplate) were initially the same. For all conditions, *Escherichia coli* (Migula) Castellani and Chalmers (hereafter referred to as *E. coli*) was grown on agar plates. A colony was removed from the plate under a hood using an inoculation loop, and placed in 10 mL of liquid Luria Broth (LB) medium (Maniatis, Fritsch and Sambrook 1989). The broth (pre-culture) was left to incubate for 18 hours at 37°C, until it reached its stationary phase.

After 18 hours, the broth was removed from the incubator, placed in the hood, and 2 mL of growth from the pre-culture was removed and placed in 200 mL of liquid media for further growth (culture). The culture was then placed in the incubator for an additional 18 hours.

After the final 18 hours, the culture was removed from the incubator and the cells harvested (Maniatis, Fritsch and Sambrook 1989).

The 200 mL culture was divided up into four 50 mL centrifuge tubes and centrifuged for 15 minutes at 3700 rpm (989 rcf) at 4°C. After 15 minutes of centrifugation, the centrifuge tubes were brought back to the hood, where the liquid medium was removed from each tube and was replaced with 10 mL of 1mM KCl solution, leaving only the pellet of cells on the bottom, and the fresh KCl in the tubes.

The cells were then re-suspended in the KCl solution by vortex mixing. The tubes were then re-spun in the centrifuge as aforementioned. After 15 minutes, the tubes were then brought back to the hood, where all the liquid was again removed from each tube and replaced with 10 mL of fresh 1 mM KCl. Each tube was then vortex mixed to re-suspend the cells, and then the contents of each tube were consolidated to one 50 mL centrifuge tube, with a total of 40 mL of re-suspended cells.

After the final spinning under the same conditions in the centrifuge, the cells were then re-suspended into the solution, and three 10-fold dilutions were made for OD (optical density) testing using a UV Spectrophotometer. One mL of the harvested cells was placed into a cuvette, and each of the three ten-fold dilutions was also placed into cuvettes, allowing for three cuvettes of increasing dilution to be tested against the blank solution. A cuvette with DI water was used as a blank to compare the other cuvettes for optical density. The remainder of the harvest was placed on ice until the concentration had been measured.

After the cuvettes were loaded into the spectrophotometer, a reading was taken to determine the concentration of cells in the initial harvest. An optical density measurement at 600nm of 1 is equal to a cell concentration of approximately 10^9 CFU/mL on the Spectrophotometer. Once the reading was obtained, the harvest was then used to test for different conditions.

DARK

The harvested cells were brought to the hood and one mL was drawn from the harvest and placed in a PET bottle filled to 489 mL of KCl solution. The temperature of the bottle was taken, using an immersion thermometer, and recorded.

From that PET bottle, six ten-fold dilutions were made, assuming an initial concentration of 10^9 CFU/mL per the Spectrophotometer results. A one mL sample was drawn directly from the PET bottle and plated on Petri-film (3M, Minneapolis, MN) and placed in the incubator for 24 hours at 37°C. Also, the three most dilute solutions were plated on Petri-film in triplicate and placed in the incubator for 24 hours at 37°C. The PET bottle was then removed from the hood and placed in a dark cabinet, such that there was no light interacting with the contents of the bottle.

After 24 hours, the plates were then removed from the incubator and the colonies of *E.coli* were counted and recorded. On the Petri-film, a colony of *E.*

coli will show up as a blue dot, and if there is no *E. coli* present, the film will remain its original pink color (see Figure 5).

The same procedure was used over the length of one month, taking measurements at 2, 4, 7, 14, 21, and 28 days, plating the next lower set of dilutions in triplicate every other test date. As an illustration of this method is presented in Figure 6. For example, if the initial test on day 0 measured dilutions 10^{-7}, 10^{-6}, 10^{-5} mL in triplicate, then on day 4, the dilutions plated would be 10^{-6}, 10^{-5}, 10^{-4} mL.

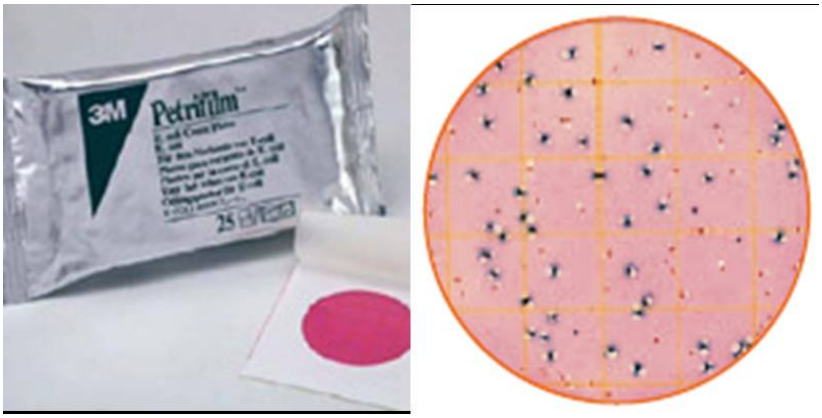

Figure 5: 3M Petri-film indicating *E.coli* growth (Wacol Lineas de Productos n.d.)

DARK

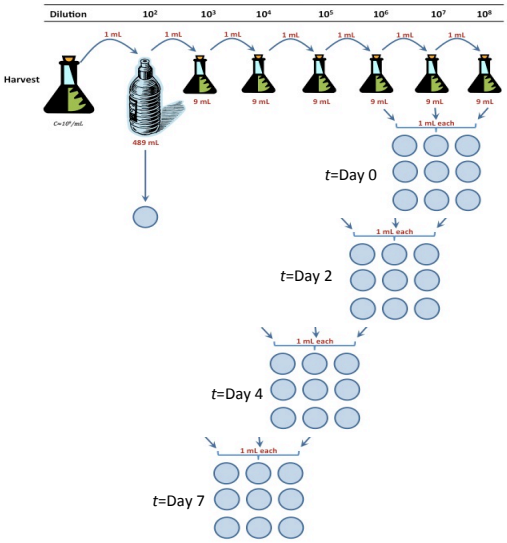

Figure 6: Plating dilutions for dark sample from *t*= Day 0 – *t*= Day 7

HOTPLATE

In preparing the Hotplate setup, 978 mL of 1 mM KCl solution were placed into a 1-L beaker; 2 mL of *E.coli* were added from the harvested culture, which was found from OD testing to have a concentration of 10^9 CFU/mL. At the initial time, at room temperature, a 1 mL sample was drawn directly from the beaker and plated on Petri film and placed in the incubator at 37°C for 24 hours.

Also, at room temperature, 10^{-7}, 10^{-6}, and 10^{-5} mL dilutions were plated in triplicate under the hood. The 1-L beaker was then placed on a hot plate (see

Figure 7), where a thermometer was held over the beaker by a clamp. A magnetic stirrer was placed in the beaker and mixing and heating were initiated. The thermometer was placed such that it was at a depth about half way of the total volume.

Figure 7: Views of hotplate set up

At temperatures, T= 35°C, 50°C, 60°C, and 70°C, one mL samples were drawn using micropipettes and were diluted and plated in triplicate. At each temperature interval, lower and lower dilutions were plated in triplicate (see Figure 8). The plates were then incubated overnight at 37°C and counted the following day.

HOTPLATE

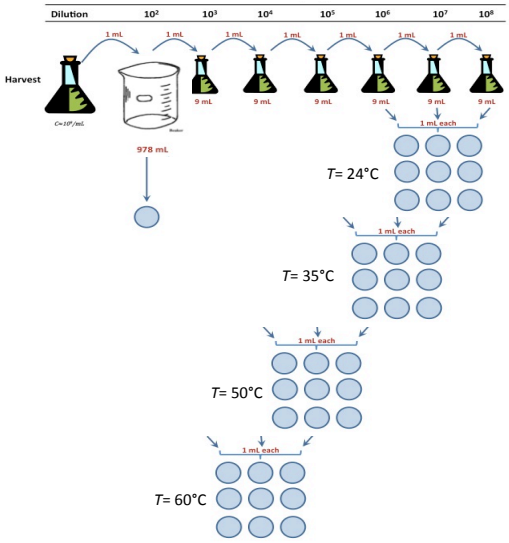

Figure 8: Plating dilutions for Hotplate sample from *T*= 24°C – *T*= 60°C

SODIS

As modeled using the existing SODIS method, a PET bottle was filled with 489 mL of 1 mM KCl, and 1 mL was drawn from the harvest and placed in the bottle after OD testing was done. From the OD, it was found that the concentration of *E. coli* was on the order of 10^9 CFU/mL in the harvest, so a concentration of roughly 10^7 CFU/mL was calculated to be in the SODIS bottle.

The initial temperature of the bottle was taken. One mL was removed directly from the bottle, plated on Petri film, and incubated at 37°C for 24 hours. Six dilutions were made, and the three most dilute solutions were plated in triplicate at time $t = 0$.

The bottle was then placed out on the Dean's Patio on the third floor on the A-wing of Bourns Hall on a metal chair in an area of full sunlight (see Figure 9). The initial time of placement, and ambient temperature outside were recorded. Samples were taken at 15-minute intervals for the first hour, then at 120 minutes, and 360 minutes. After 15-minute intervals (for the first hour), 1-mL samples were drawn and placed on ice in transport tubes, and sent to the lab for diluting and plating.

Figure 9: Set up of SODIS method on Dean's patio

At the time each sample was drawn, the ambient temperature and temperature of the bottle were also recorded. The same method was used when

collecting the samples at 120 and 360 minutes. At each 15-minute interval, the next lowest dilution was plated. For example, *t*=0 min, 10^{-7} mL, 10^{-6} mL, and 10^{-5} mL were plated. Then at *t*=15 minutes, 10^{-6} mL, 10^{-5} mL, and 10^{-4} mL were plated and so on and so forth so that by 360 minutes, samples were plated directly from the bottle with no dilution required (see Figure 10). As a measure of extra care, each time a sample was drawn and diluted from the bottle, a sample was drawn and plated directly from the bottle to ensure that complete disinfection would be caught at any stage.

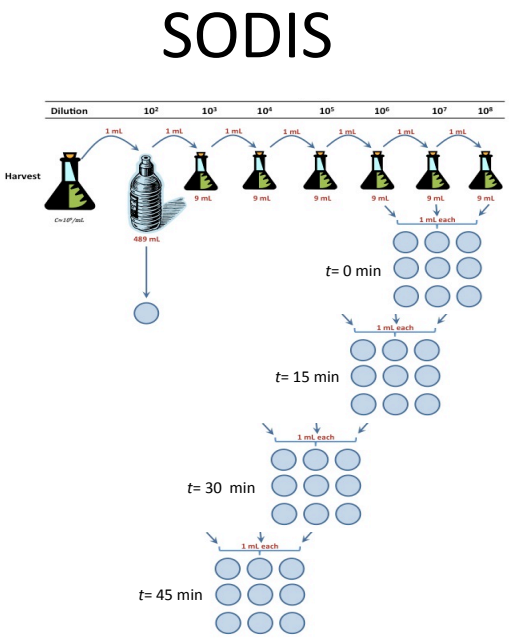

Figure 10: Plating dilutions for SODIS sample from *t*= 0 min – *t*= 45 min

PULSE

To demonstrate that a lens can enhance the SODIS method, the SODIS set-up was used, and a 3' x 2' (0.9 m x 0.6 m) Fresnel lens was used to expedite the pasteurization/disinfection process.

The set-up of the lens condition was much like the SODIS condition in that a PET bottle was filled to 489 mL of 1 mM KCl, and 1mL was drawn from the harvest and placed in the bottle after OD testing was done. From the OD, it was calculated that the concentration of *E. coli* was on the order of 10^7 CFU/mL in the PULSE bottle. The initial temperature of the bottle was taken. One mL was removed initially, plated on Petri film, and incubated overnight at 37°C. Six dilutions were made, and the three most dilute solutions were plated in triplicate at time $t = 0$. The bottle was then placed out on the Dean's Patio on the third floor on the A-wing of Bourns Hall under the lens, on the concrete ground in an area of full sunlight (see Figure 11).

Figure 11: Set up of PULSE method on Dean's patio

The initial time of placement and ambient temperature of the bottle were recorded. Samples were collected at 5, 10, 12, 15, 17, and 20 minutes, placed on ice in transport tubes, and sent to the lab for diluting and plating. At the time each sample was drawn, the ambient temperature and temperature of the bottle were also recorded.

When brought to the lab, the highest dilutions were plated at the earliest times, and as each sample time increased, lower and lower dilutions were plated (see Figure 12). The plates were incubated overnight at 37°C and counted the following day to determine concentrations.

PULSE

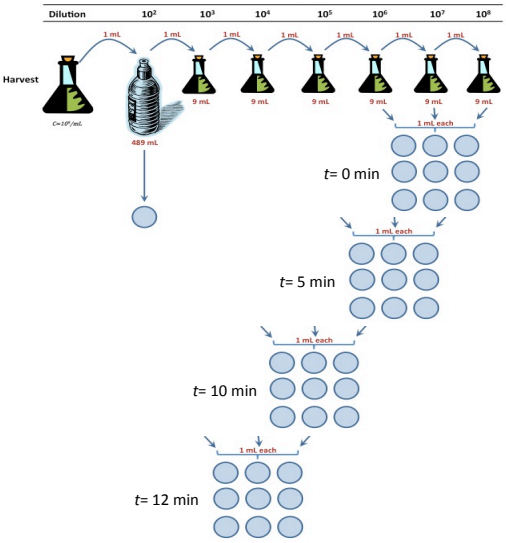

Figure 12: Plating dilutions for PULSE sample from *t*= 0 min – *t*= 12 min

4. RESULTS AND DISCUSSION

In this chapter, the data that were collected and their relevance as it pertains to achieving disinfection goals are discussed. The results of the heat tests and decay rate tests for each condition are discussed below, where the time it took to achieve a 5-log kill (if it was achieved) is emphasized. Chick's Law (first-order disinfection kinetics) was used to model the disinfection rate.

HEAT TEST RESULTS

Several tests were performed to determine whether the lens was able to achieve pasteurization temperatures. Water temperature vs. time for all of the heat test runs are shown in Figure 13. Ambient temperatures for each run are noted in the legend of Figure 13.

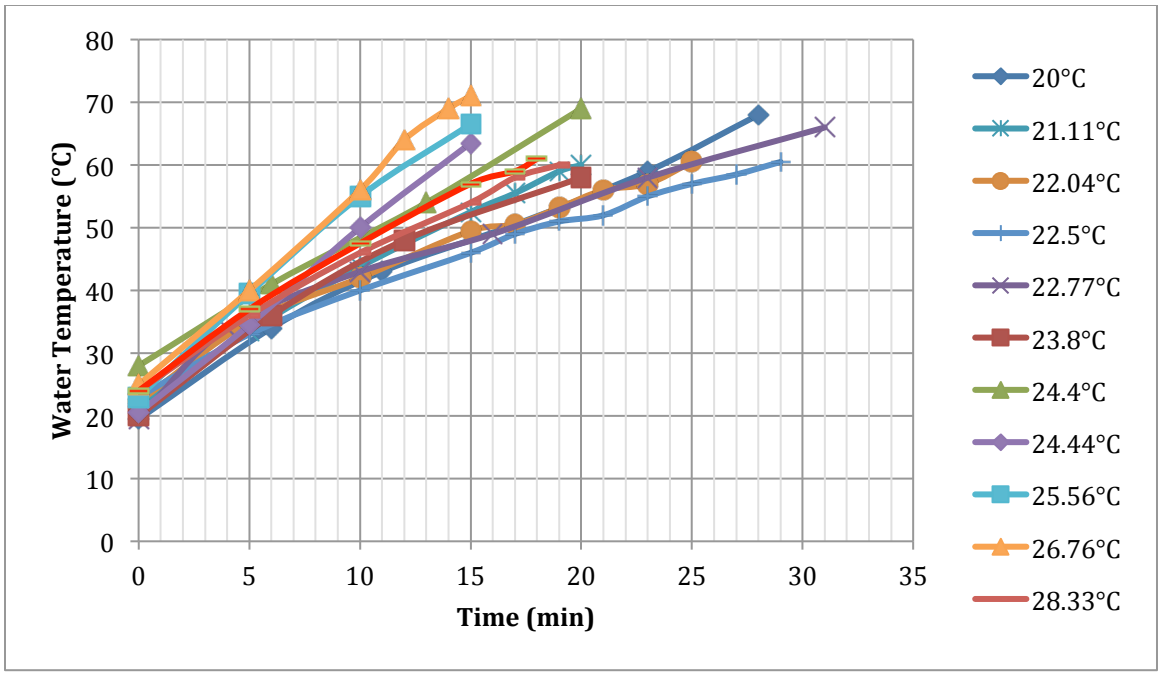

Figure 13: Heat test results; water temperature vs. time

Based on the heat test results, the lens was able to achieve pasteurization temperatures (> 50°C) within about 20 minutes, even on days where the outdoor ambient temperature was only about 20°C. However, ambient temperature wasn't the only factor that determined the temperature increase. Factors such as wind speed, cloud cover, lens focus, and UV index are considerations in correlating ambient temperature to temperature inside the bottle.

DARK RESULTS

Dark tests were performed between 3/2/12-6/9/12; they did not require any relative consistency in weather conditions since the tests were conducted indoors in a temperature controlled (22°C ± 3°C) environment. The collective

results of the Dark tests are shown in Figure 14. These include the results of three separate tests, and one in triplicate (see Table 2). The green line indicates at which point a 5-log kill would be achieved. In this case, a 5-log kill is never reached.

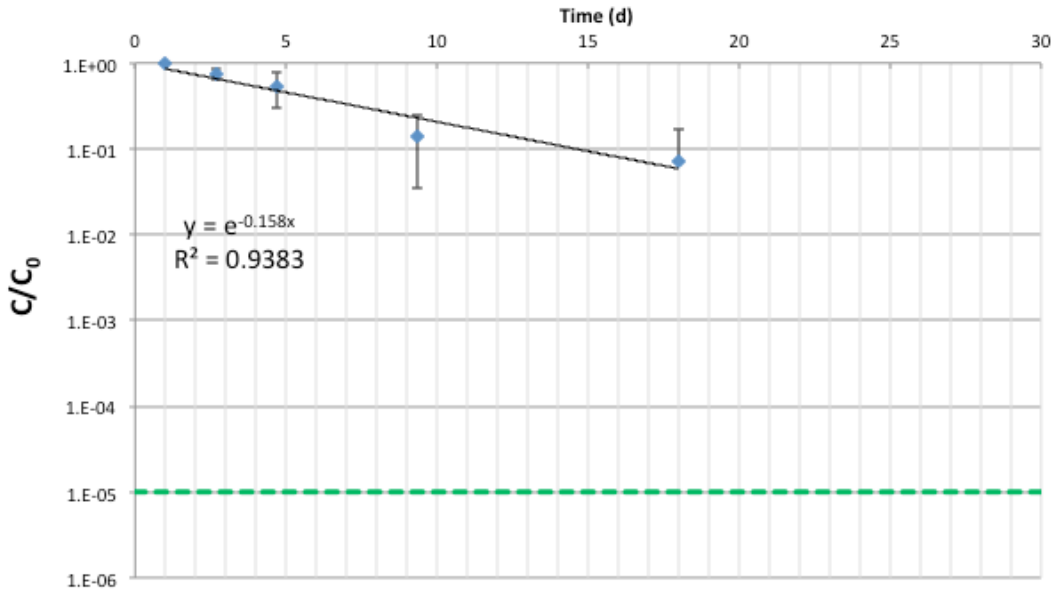

Figure 14: Average Dark Concentration vs. Time 3/2/12-6/9/12

From the Dark results, it appears that in an undisturbed environment, it takes more than two weeks to see a 2-log reduction for this particular strain of *E.coli*. For a 5-log reduction, a time frame greater than thirty days is required (see Table 2). The rate constants among the different runs are comparable, within the same order of magnitude (0.1 $days^{-1}$ to 0.3 $days^{-1}$).

To compare the rate of natural die off of the Dark test with the rate of die off for the other conditions in the same time frame, the level of disinfection achieved after two hours under Dark conditions would be minimal, less than 10%, based on the average Dark rate of decay of 0.158 d^{-1}.

Table 2: Collective Dark Data from 3/2/12-6/9/12

DARK DATA

	Run 1		Run 2A			Run 2B			Run 2C			Run 3		
Date	3.2.12 - 3.20.12		4.7.12 - 4.21.12			4.7.12 - 4.21.12			4.7.12 - 4.21.12			5.12.12 - 6.9.12		
Ambient Temp (°C)	N/A		N/A			N/A			N/A			N/A		
UV Index	N/A		N/A			N/A			N/A			N/A		
Cloud Cover	N/A		N/A			N/A			N/A			N/A		
Weather	N/A		N/A			N/A			N/A			N/A		
Volume (ml)	500		500			500			500			500		
Time (d)	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**
0	1.00E+00	22	2.96E+09	1.00E+00	22	2.91E+09	1.00E+00	22	2.90E+09	1.00E+00	22	1.79E+08	1.00E+00	22
1	N/A	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22
2	8.59E-01	22	1.53E+09	5.16E-01	22	1.96E+09	6.73E-01	22	2.17E+09	7.50E-01	22	N/A	N/A	22
3	6.67E-01	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22
4	N/A	22	8.70E+08	2.94E-01	22	1.08E+09	3.71E-01	22	3.60E+08	1.24E-01	22	1.30E+08	7.28E-01	22
7	6.24E-02	22	3.07E+08	1.04E-01	22	2.80E+08	9.61E-02	22	2.80E+08	9.66E-02	22	1.25E+08	7.00E-01	22
14	N/A	22	2.00E+07	6.76E-03	22	2.67E+07	9.15E-03	22	2.67E+07	9.20E-03	22	4.73E+07	2.64E-01	22
19	1.82E-02	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22
21	N/A	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22	3.31E+07	1.85E-01	22
28	N/A	22	N/A	N/A	22	N/A	N/A	22	N/A	N/A	22	3.19E+07	1.85E-01	22

HOTPLATE RESULTS

Hotplate tests were conducted to provide an understanding of the effect heat has on the disinfection process. The average concentration vs. time vs. temperature for the Hotplate tests is shown in Figure 15. The green dotted line indicates where a 5-log removal would take place. The individual Hotplate tests are summarized in Table 3.

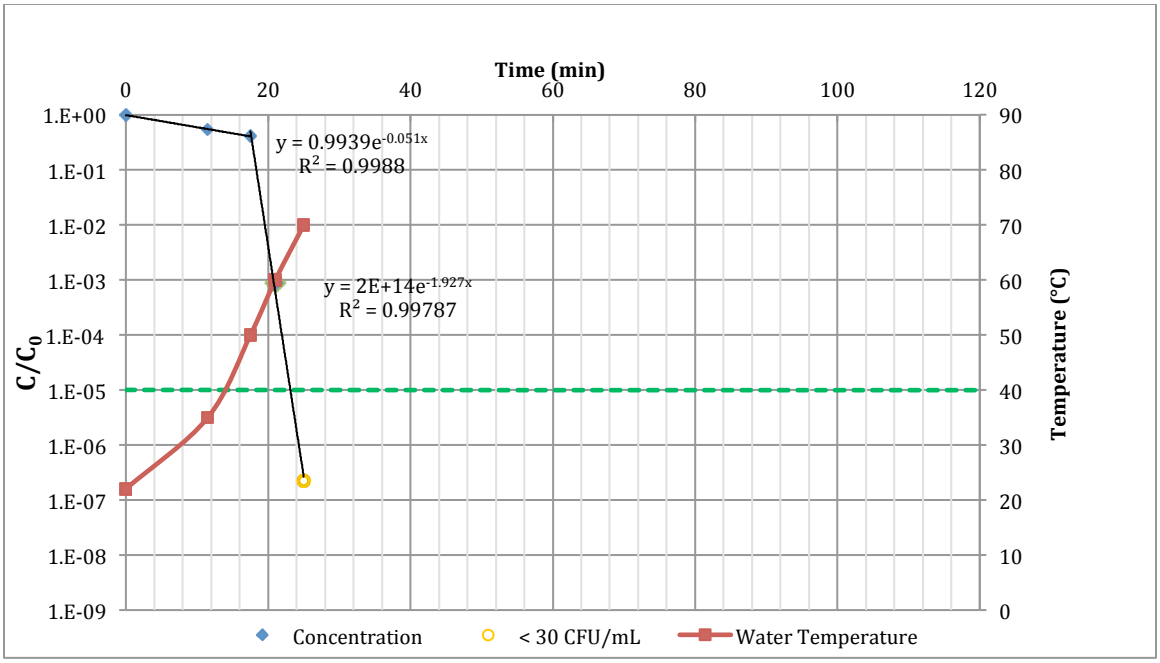

Figure 15: Average Hotplate test results concentration vs. time vs. temperature

Table 3: Collective Hotplate Data from 5/26/12-7/7/12

HOTPLATE DATA

	Run 1		Run 2		
Date	5.26.12		7.7.12		
Ambient Temp (°C)	N/A		N/A		
UV Index	N/A		N/A		
Cloud Cover	N/A		N/A		
Weather	N/A		N/A		
Volume (ml)	1000		1000		
Time (min)	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**
0	1.00E+00	20	3.20E+08	1.00E+00	20
11	N/A	N/A	3.20E+08	1.00E+00	35
12	8.73E-02	35	N/A	N/A	N/A
17	N/A	N/A	2.54E+08	7.90E-01	50
18	3.13E-02	50	N/A	N/A	N/A
21	1.73E-03	60	6.30E+03	0.00E+00	60
24	N/A	N/A	**	0.00E+00	70
26	1.34E-07	70	N/A	N/A	N/A

** Value below detectable limit of 30 cfu/ml

As shown in these results, the data are consistent with other researchers in which they found an increase in the rate of disinfection at 50°C (Safapour and Metcalf 1999).

Upon close inspection, there's a very distinct break in the rates at about 19 minutes or once 50°C is achieved; the rate of disinfection increases notably, by two orders of magnitude, going from $k = 0.051\ min^{-1}$ to $k = 1.927\ min^{-1}$ in Figure 15 A 5-log reduction is observed after 22 minutes, only three minutes after reaching 50°C.

This lag in disinfection between 20°C and 40°C can be explained by the optimum temperatures for bacterial growth; bacteria ordinarily thrive at temperatures between 35^{o}C and 39^{o}C (Oates 2001). Further, since there are no synergistic effects from exposing it to sunlight, the only inactivation mechanism is the heat, which requires higher temperatures to start achieving any disinfection.

SODIS RESULTS

Samples for the SODIS experiments were collected and tested over the summer months, resulting in relatively consistent weather conditions. The green dotted line indicates where a 5-log removal would take place (see Figure 16). An average of the all SODIS tests are shown in Figure 16. All the data from each SODIS test are summarized in Table 4.

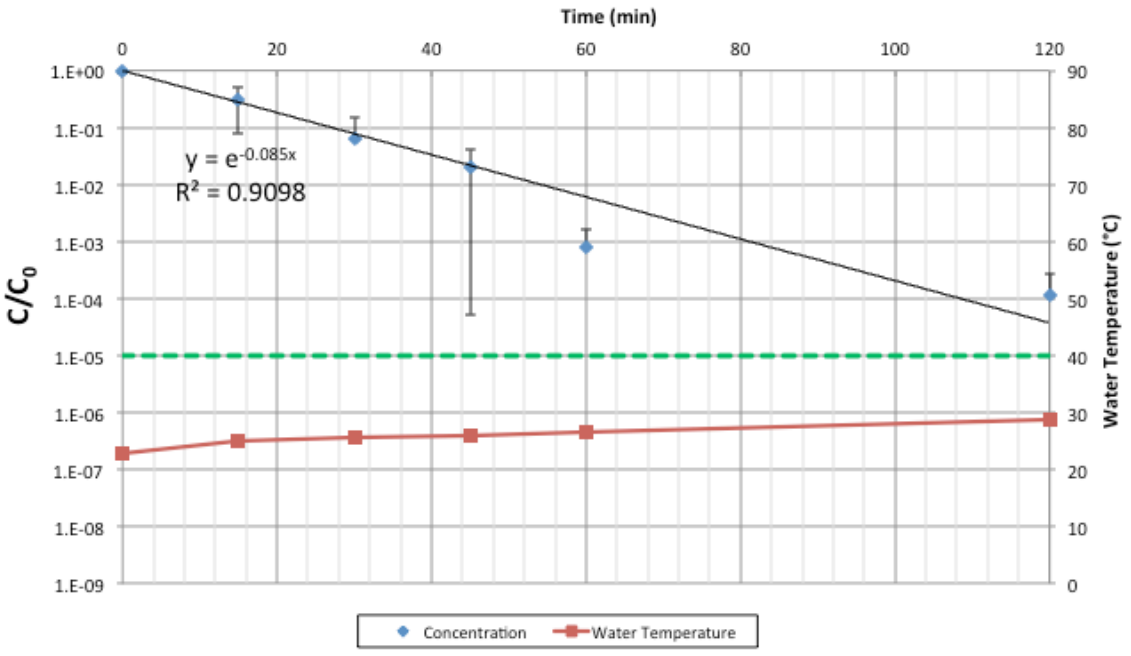

Figure 16: Averaged SODIS concentration vs. time vs. temperature from 5/12/12-5/26/12

Table 4: Collective SODIS Data from 5/5/12-5/26/12

SODIS DATA

	Run 1		Run 2A			Run 2B			Run 2C		
Date	5.5.12		5.26.12			5.26.12			5.26.12		
Ambient Temp (°C)	22 - 25		20 -23			20 -23			20 -23		
Avg UV Index	9*		5			5			5		
Cloud Cover	N/A		Cloudy			Cloudy			Cloudy		
Weather	Winds 2 - 6 mph		Winds 2 - 9 mph			Winds 2 - 9 mph			Winds 2 - 9 mph		
Volume (ml)	500		500			500			500		
Time (min)	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**
0	1.00E+00	23	3.89E+08	1.0000E+00	22.5	5.47E+08	1.00E+00	22.5	1.32E+08	1.00E+00	22.5
15	8.64E-01	26.5	2.29E+08	5.8894E-01	23.5	1.01E+08	1.85E-01	23.5	4.77E+07	3.62E-01	23.5
30	7.35E-02	28.3	2.03E+07	5.2250E-02	23	3.97E+06	7.26E-03	23	2.47E+07	1.87E-01	23
45	3.22E-03	30	3.56E+06	9.1427E-03	22	1.09E+06	1.99E-03	22	2.85E+06	2.16E-02	22
60	2.27E-04	31.4	2.48E+05	6.3652E-04	22	1.43E+05	2.62E-04	22	2.67E+05	2.03E-03	22
120	1.85E-06	34.6	3.03E+04	7.7797E-05	23	1.24E+04	2.27E-05	23	4.62E+04	3.51E-04	23
360	0.00E+00	26.6	**	2.5718E-09	20.5	**	1.83E-09	20.5	**	7.59E-09	20.5

* Denotes the average UV Index for Los Angeles
** Value below detectable limit of 30 cfu/ml

As shown in Figure 16, although complete disinfection took six hours as anticipated, disinfection begins almost immediately in contrast to the Hotplate tests. This early disinfection is believed to be due to the effects of UV in the SODIS method. The SODIS inactivation rate constant, 0.085 min^{-1}, is consistent with existing data which suggests that the inactivation rate constant is approximately 0.071 min^{-1} (Reed 1997).

Also, only minor changes in water temperature were recorded. This lower temperature (20^{o}C to 25^{o}C) could be a major reason why it takes six hours to get full disinfection. In the Hotplate tests, at 50°C, a significant increase in the rate of disinfection takes place. The SODIS method does not achieve significant temperature increase and the disinfection rate is constant over time, taking over two hours to get a 5-log reduction vs. the hotplate, which achieves a 5-log reduction in about 22 minutes.

If there was a significant change in the temperature of the water, combined with the other properties of sunlight, the SODIS method would be greatly enhanced. Since the SODIS method requires no additional fuel source, beyond solar energy, it is a very viable method for disinfection in developing countries, in contrast to the hotplate method. However, since the PULSE method extends the benefits of the SODIS and Hotplate methods, it appears the PULSE method could be the most effective in disinfecting contaminated waters in developing countries.

PULSE RESULTS

Samples for the PULSE experiments were collected and tested over the summer months, resulting in relatively consistent weather conditions. An average of the all PULSE tests in 500 mL bottles are shown in Figure 17 (500 mL bottles only) and Figure 18 (varying bottle sizes, 500 mL to 2 L). All the data from each PULSE test are summarized in Table 5.

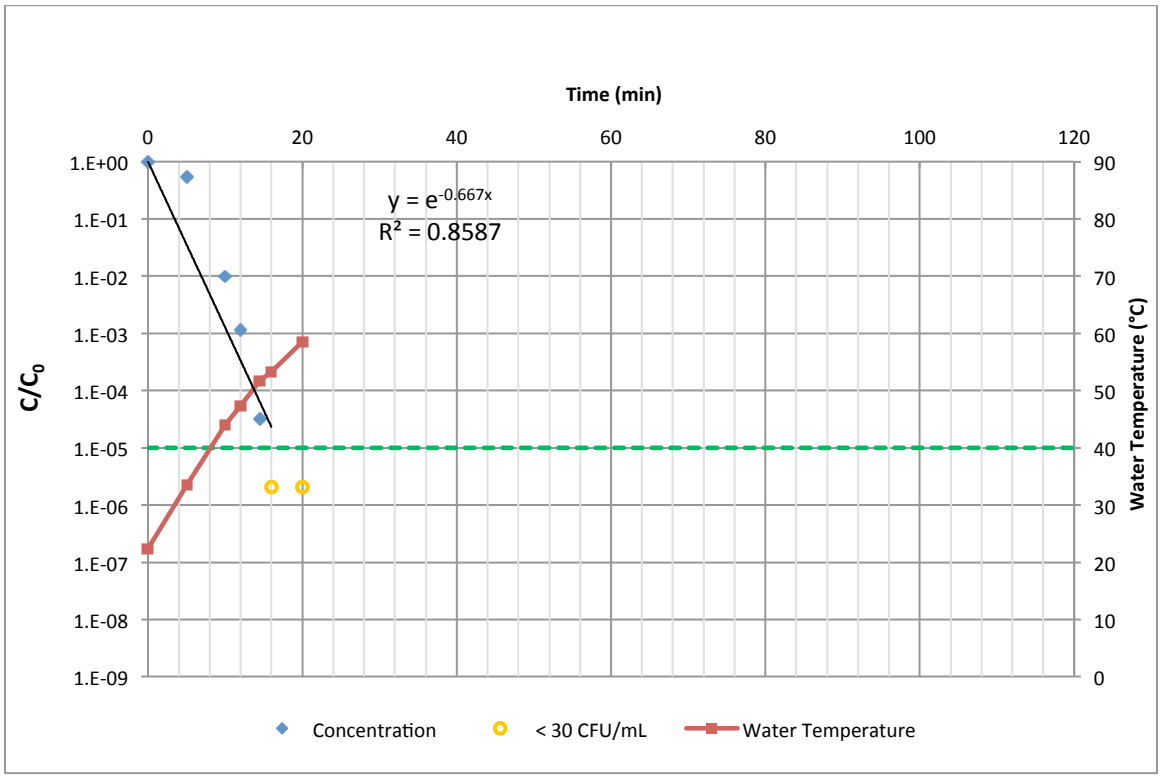

Figure 17: Averaged PULSE concentration vs. time vs. temperature in 500 mL bottles from 5/12/12-7/7/12

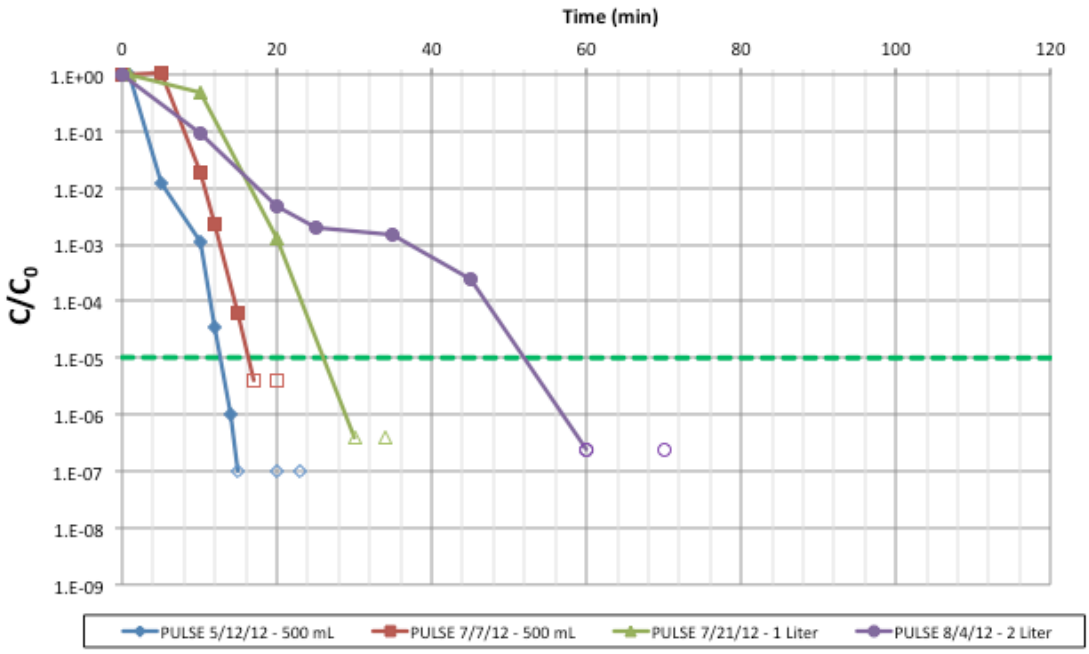

Figure 18: All PULSE tests' concentration vs. time in various volumes from 5/12/12-8/4/12

All of the PULSE tests that were conducted over the summer comparing the results of concentration vs. time for different volumes are shown in Figure 19.

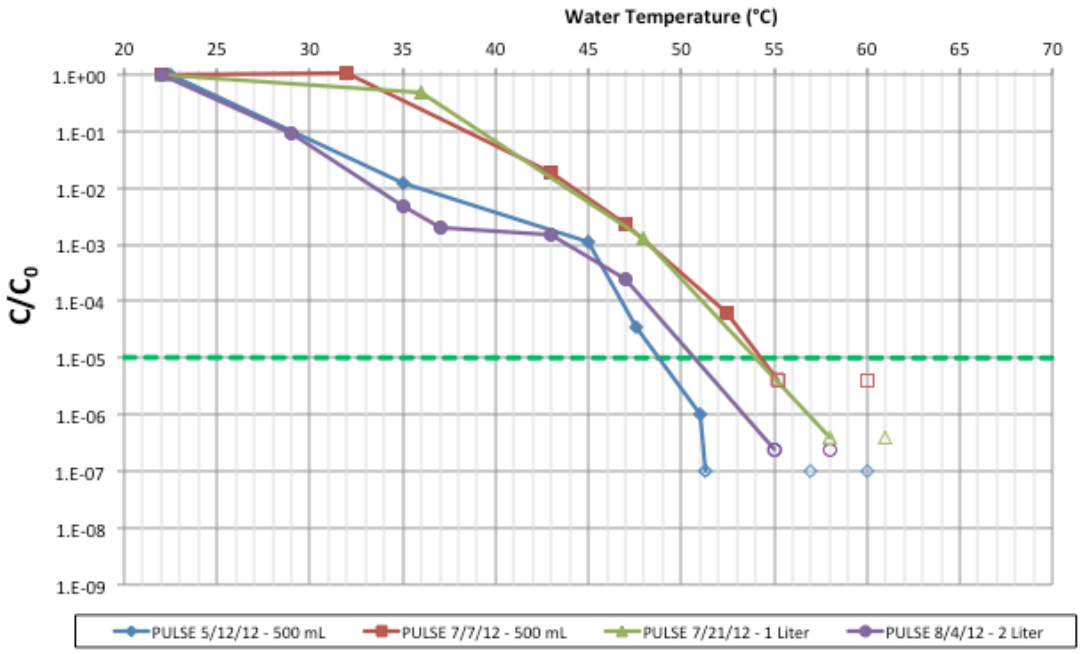

Figure 19: PULSE tests' concentration vs. temperature in various volumes from 5/12/12-8/4/12

Table 5: Collective PULSE Data from 5/12/12-8/4/12

PULSE DATA

	Run 1		Run 2			Run 3			Run 4		
Date	5.12.12		7.7.12			7.21.12			8.4.12		
Ambient Temp (°C)	27 - 28		28 - 30			32			24 - 27		
Avg UV Index	11*		10			10			8		
Cloud Cover	N/A		Sunny			Sunny			Partly Cloudy		
Weather	Winds 7-9 mph		Winds 5 - 8 mph			Winds 3 - 4 mph			Winds 2 - 6 mph		
Volume (ml)	500		500			1000			2000		
Time (min)	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**	**Conc CFU/100ml**	**C/C_0**	**Temp (°C)**
0	1.00E+00	22.5	2.47E+07	1.00E+00	22	2.60E+08	1.00E+00	22	4.08E+08	1.00000E+00	22
5	1.22E-02	35	2.60E+07	1.05E+00	32	N/A	N/A	N/A	N/A	N/A	N/A
10	1.08E-03	45	4.57E+05	1.85E-02	43	1.28E+08	4.94E-01	36	3.82E+07	9.36301E-02	29
12	3.41E-05	47.6	5.50E+04	2.23E-03	47	N/A	N/A	N/A	N/A	N/A	N/A
14	1.01E-06	51	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15	1.01E-07	51.3	1.53E+03	6.22E-05	52.5	N/A	N/A	N/A	N/A	N/A	N/A
17	N/A	N/A	**	4.05E-06	55.2	N/A	N/A	N/A	N/A	N/A	N/A
20	1.01E-07	57	**	4.05E-06	60	3.27E+05	1.26E-03	48	1.98E+06	4.85096E-03	35
23	1.01E-07	60	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	7.97E+05	1.95182E-03	37
30	N/A	N/A	N/A	N/A	N/A	**	3.85E-07	58	N/A	N/A	N/A
34	N/A	N/A	N/A	N/A	N/A	**	3.85E-07	61	N/A	N/A	N/A
35	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6.13E+05	1.50265E-03	43
45	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.00E+05	2.44998E-04	47
60	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**	2.44998E-07	55
75	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**	2.44998E-07	58

* Denotes the average UV Index for Los Angeles

** Value below detectable limit of 30 cfu/ml

As seen in the graphs, there is a nominal lag in the disinfection process once the bottle is placed under the lens. However, unlike in the Hotplate tests, disinfection still occurred at temperatures <50^{o}C as shown in Figure 17, which is consistent with research regarding synergistic effects (Oates 2001).

In the PULSE method, with an overall increase in heat, although it may be less than pasteurization temperatures, disinfection occurred much more quickly than in the SODIS method. A 5-log reduction was reached in around 23 minutes (see Figure 18) in the 1-L bottle, vs. the SODIS method, which took greater than two hours for the same percentage kill in a 500-mL bottle. The same log kill in the Hotplate method was achieved in almost the same time (22 minutes) as it did

in the PULSE method, though PULSE uses no other fuel source than what it collects from the sun. This added benefit could be due to the contribution of other properties of the sun to the disinfection process, and the fact that the PULSE method was able to achieve temperatures of at least 50°C where an increase in the disinfection rate occurs, and in this method occurs at temperatures just under 50°C (see Figure 19). It is seen in Figure 19 that as temperature continues to increase, disinfection rate also rapidly decreases, and by the time temperatures reach around 60°C, no further bacteria are detectable. Further, after complete treatment, disinfection, and proper storage of the water, no further regrowth was detected after testing for growth the following day.

COMPARISON OF METHODS

Comparing results for the Hotplate, SODIS, and PULSE methods are shown in Figure 20 (concentration vs. time) and Figure 21 (concentration vs. temperature), respectively.

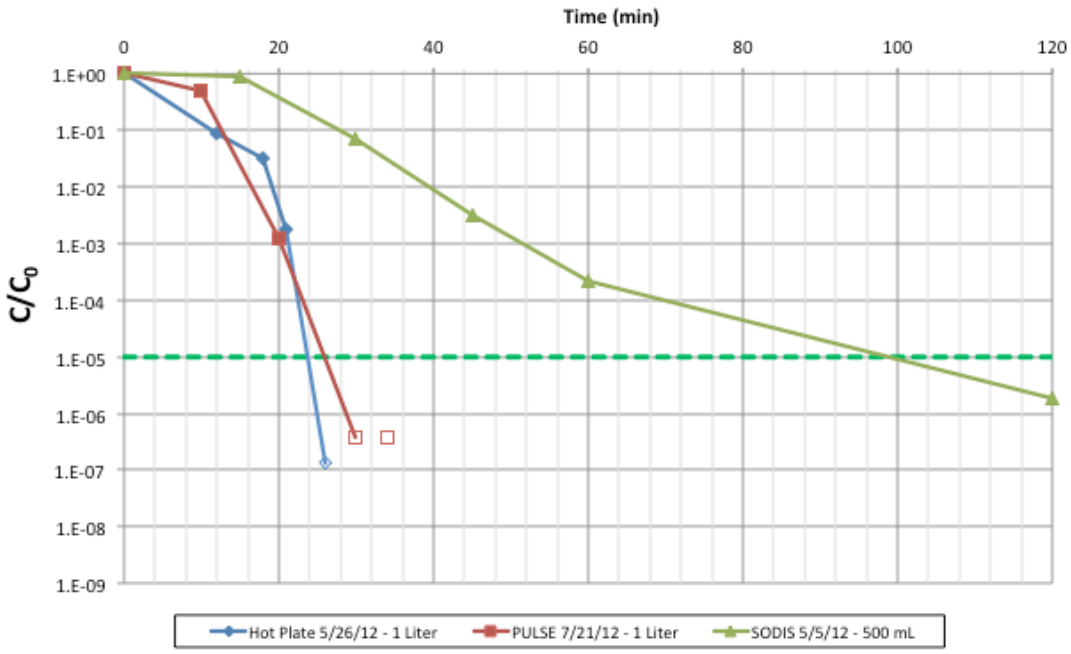

Figure 20: Concentration vs. time for Hotplate, SODIS, and PULSE tests

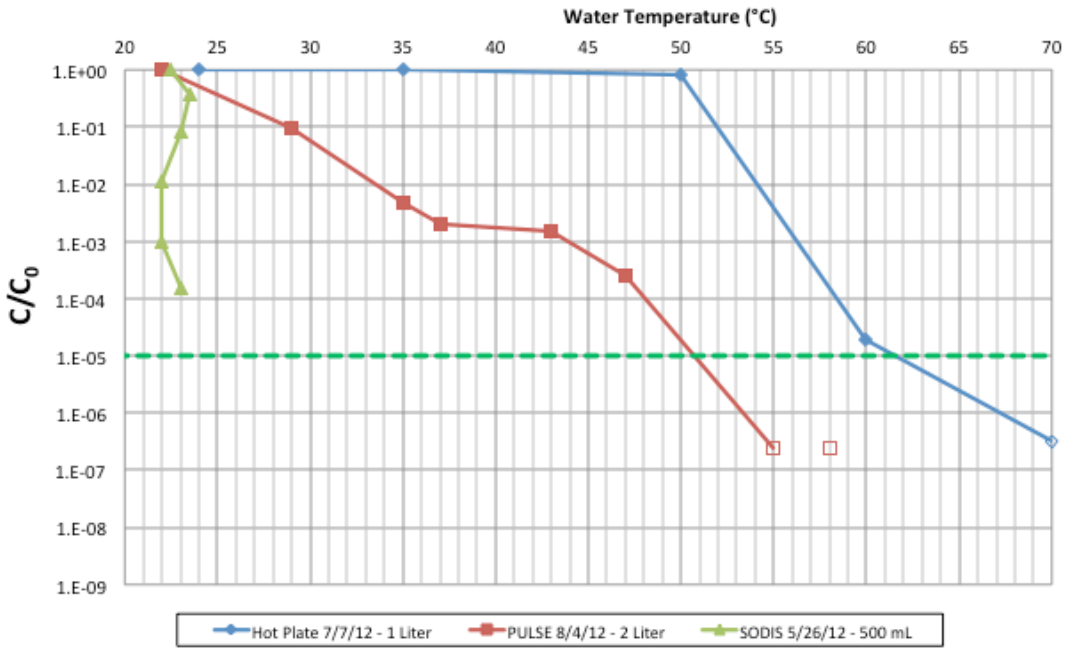

Figure 21: Concentration vs. temperature for Hotplate, SODIS, and PULSE tests

From Figure 20, it is clear that both the PULSE and Hotplate surpass the SODIS method in terms of time efficiency. The 5-log kill for PULSE and Hotplate occur within minutes of each other, both occurring at around 20 minutes when comparing 1-L vessels, whereas the SODIS method takes over two hours to achieve the same disinfection in a 500 mL bottle. When using the same volumes, the PULSE and Hotplate methods are almost identical in their disinfecting capabilities over time, except that the PULSE method requires no other source of energy beyond the free energy of the sun.

In considering the results shown in Figure 21, it is shown that the PULSE method takes full advantage of the energy from UV and heat concentrated by the lens. The initial stage of the PULSE method is dominated by UV until it reaches about 50°C, where heat effects start to overwhelm the disinfection process, and complete disinfection occurs within minutes after that point.

Since SODIS does not bring about a rapid increase in the water temperature, drastic decreases in concentration do not occur. Although heat is achieved faster in the Hotplate, there is no synergy from other properties of the sun acting on it.

However, the PULSE method seems to draw benefits from having both a rapid temperature increase, and the Sun's natural disinfecting properties.

From the data gathered, it appears the enhanced SODIS method or PULSE method, is the most effective method of those tested, to disinfect water. It takes the lens approximately 20 minutes to achieve temperatures of about 60°C, and completely disinfects the entire sample, even before reaching that temperature. Whereas, with the hotplate, even though it achieved temperatures of 60°C in less time, growth was still seen on the plates until a temperature of 70°C was achieved four minutes later, before complete disinfection was detected. This could be attributed to the fact that synergistic effects from the sun were not present and it was strictly due to thermal inactivation.

Regeneration of cells from exposure to UV has been shown before, (K. L. Mechsner 1990); (K. L. Mechsner 1991)*;* (K. L. Mechsner 1992), however, since

pasteurization temperatures had been reached during the inactivation process, this additional heat treatment could have added assurance of complete inactivation. After storing the PULSE samples and retesting the following day, there was no regrowth.

Comparing with the SODIS method, which takes six hours to show complete disinfection, the PULSE method was able to accomplish the same task in a fraction of the time. Left to its own device, as shown in the Dark test, a significant number of live coliform were still remaining after four weeks. Among all the conditions tested (PULSE, SODIS, Hotplate, and Dark), the PULSE method is the best alternative for disinfection.

5. CONCLUSION AND FUTURE WORK

Four conditions were tested against each other to determine which was the most effective method of disinfection, taking into consideration the cost, availability, and sustainability of resources. It has been shown that leaving bacteria in a water source completely untreated is the least effective way to manage water quality. Among the three other treatment options—the Hotplate, SODIS, and PULSE methods, it appears that the PULSE method is better in minimizing the time required, and increasing the volume that can be treated, via essentially the same mechanisms, while also incorporating a temperature spike.

Further, it can be argued that since using the lens requires no additional source of energy aside from the sun, that it is the more readily available/feasible method versus the hotplate method. The PULSE method has been shown to effectively inactivate bacteria in this study namely *Escherichia coli* (Migula) Castellani and Chalmers. Further, after treatment utilizing this method, given that the bottles are properly stored, no regrowth is found after testing them 24 hours later.

Based on the results obtained in this effort, other questions to address in advancing this research and determining the strength of the PULSE method include:

1. How does turbidity affect disinfection?
2. Can a plug flow reactor be designed such that the bottles are no longer necessary?

3. What is the best design that can handle the maximum temperature obtained using the lens?
4. What is the contribution of UV to the disinfection process?
5. How would alkalinity affect the results, particularly disinfection associated with UV light?
6. Is the lens as effective in the Fall/Winter months?
7. Is this process as effective against viruses?
8. How long can treated water be stored without regrowth?
9. Do different strains of bacteria respond differently to disinfection?
10. How effective is this method against parasites?

In determining answers to these other questions, the PULSE method can be optimized and used under various conditions in developing countries to combat unnecessary illness or death due to water quality problems.

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