SOLAR DISINFECTION OF DRINKING WATER

By

Christine Rojko

A Thesis

Submitted to the Faculty

of

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Master of Science

in

Environmental Engineering

by

_____

May 2003

APPROVED:

_____

Dr. Jeanine D. Plummer, Major Advisor

_____

Dr. Frederick L. Hart, Head of Department

ABSTRACT

Over 30% of the population in developing countries is in need of access to safe drinking water. The 875 million cases of diarrhea and 4.6 million deaths that occur each year due to a lack of a safe water supply occur primarily in these countries. It is estimated that these countries will need over $150 billion to establish full drinking water supply system coverage, a sum that they may not be able to raise within the near future. Conventional methods of drinking water disinfection, such as chemical treatment, heat pasteurization, and filtration, require facilities, materials, and fuel that may not be readily available or feasible to attain. An alternative treatment option is to utilize solar energy, which has been shown to inactivate pathogens through pasteurization and radiation effects.

This research was conducted to determine the effectiveness of solar disinfection for the inactivation of *E. coli*. Turbidity, sample volume, exposure time, and bottle size were varied. Experiments were conducted by adding *E. coli* to water samples (phosphate buffered saline with or without added montmorillonite clay or pond water) in clear drinking water test bottles. The bottles were then placed in full, direct sunlight. Samples were taken at predetermined intervals and solar intensity, weather conditions, and water temperatures were recorded during each sampling session. The viable bacterial count was enumerated using the pour plate method to determine log inactivation achieved. Laboratory experiments were also conducted to determine the effects of heating only on the inactivation of *E. coli*.

Sample volumes from 1 to 2 L and turbidity values ranging from <1 ntu to approximately 100 ntu did not significantly affect inactivation levels when samples were exposed to sunlight for at least 4 hours. In samples with 0 ntu turbidity, a minimum cumulative intensity of 20.8 J/cm^2 of wavelengths below 400 nm was required for a 7-log inactivation of *E. coli*. In samples with up to 100 ntu, a maximum fluence of 99.8 J/cm^2 was required. Temperatures up to 46.0°C did not significantly inactivate *E. coli*, therefore radiation or the synergistic effects of radiation and heating accounted for the inactivation in samples exposed to sunlight. While solar disinfection can effectively inactivate *E. coli* bacteria in water samples, waters with high levels of suspended solids and those containing spore-forming pathogens and protozoa should be tested.

ACKNOWLEDGEMENTS

I would like to sincerely thank the faculty and staff of the Civil Engineering Department at Worcester Polytechnic Institute for providing me with the opportunity to continue my education in a supportive working environment. I would like to thank my parents and Dave for their unwavering faith in me. And I would most like to express gratitude to my advisor and mentor, Professor Jeanine Plummer. She has been a constant source of moral support while providing constructive guidance throughout the year.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 Introduction

Contaminated drinking water poses a major health threat to human beings worldwide. The problem is particularly significant in developing countries and in arid areas where water sources are scarce. In developing countries, surface waters such as rivers, streams and lakes are used for multiple activities, including livestock watering, bathing, and cooking. Defecation and urination often occur near water sources as well. This water, which may be contaminated with pathogenic organisms, is also used for drinking water. People in developing countries may have no other options for drinking water because there is a lack of water distribution infrastructure and lack of funding for developing water treatment systems.

Over one billion people each year are exposed to unsafe drinking water due to poor source water quality and lack of adequate water treatment. This results in 900 million cases of diarrhea each year (Rijal and Fujioka, 2001). Five out of every 1000 of those exposed to unsafe drinking water will die from diseases carried by the contaminated water; another 2.5 will die from dehydration due to diarrhea (Burch and Thomas, 1998). Children are particularly affected; the average child in developing countries has more than two episodes of diarrhea per year. Diarrheal illness results in malnutrition, weakness, and an increase in susceptibility to diseases, and can be life threatening (Burch and Thomas, 1998). The estimated number children that die each year due to water-related diseases ranges from 2.5 million to 15 million (Burch and Thomas, 1998; Jorgensen *et al.*, 1998).

The lack of adequate drinking waters in developing countries is a continually growing problem due to population increases and increased demands on source waters. Therefore, water disinfection methods that are easily employed in developing countries are needed. Chemical disinfection options such as chlorine and iodine treatment require chemicals that must be purchased. These chemicals can be expensive and also have a limited shelf life. Physical treatment options such as boiling, UV treatment, and filtering require materials that may not be easily acquired or purchased. One alternative drinking water treatment method that has been proposed is solar disinfection, a process that is simple and easily utilized. It has been recommended by several researchers for use in countries that receive abundant sunshine, specifically those areas between latitudes 35°N and 35°S (Acra *et al.*, 1984; IDRC, 1998).

Solar disinfection is a water treatment method where a drinking water sample is exposed to solar radiation to inactivate pathogenic organisms. The method has been shown to reduce the incidence of diarrhea in children living in a Massai village in Kenya (Conroy *et al.*, 1996). Previous studies have found that solar disinfection is affected by numerous variables. These variables include the wavelengths of solar radiation, water temperature, turbidity, and container selection.

1.1 Research Objectives

The objective of this research was to test the inactivation of *E. coli* bacteria by solar disinfection. The variables tested were water matrix, turbidity, bottle size, and exposure time. First, solar disinfection experiments were conducted in which samples were

exposed to sunlight and allowed to heat. The inactivation of *E. coli* in the samples was quantified over time. Next, the effects of heating only were tested to determine the importance of temperature in the solar disinfection process. These results were compared with the results of the solar disinfection experiments.

1.2 Scope of Research

To study the effects of solar radiation and heating on the inactivation of *E. coli*, experiments were conducted from July through September 2002. Water samples were exposed to sunlight in plastic bottles. Plastic bottles were used because they are common, inexpensive containers that can be found worldwide. Phosphate buffered saline was the primary test solution, although a limited number of experiments were conducted using pond water as well. For each experiment, the test bottles were prepared and spiked with *E. coli* in the laboratory. The initial temperature and turbidity of each test bottle was recorded and samples were taken to enumerate the starting concentration of bacteria. The test bottles were then exposed to sunlight and samples were collected at predetermined intervals to determine the *E. coli* concentration. During each sampling time, air temperature, water temperature, and solar irradiance were measured. The log inactivation of *E. coli* was calculated over time.

To quantify the inactivation effects of heating only, laboratory experiments were conducted. A solution of phosphate buffered saline was spiked with *E. coli* and placed in a water bath that was heated to mimic the temperature increase observed when samples

were exposed to the sun. The results of the solar radiation and heating experiments were then analyzed and compared to the results of the heating only experiments.

1.3 Overview of Report

The following chapter is the literature review. The literature review discusses developing countries, the drinking water challenges these countries face, various water disinfection options, and previous research on solar disinfection. The methodology chapter describes the experimental design, including variables tested, test procedures used, and analytical methods employed. Experiment results are then presented and analyzed. The last two chapters provide conclusions and recommendations.

2 Literature Review

Chapter 2 discusses the worldwide problem regarding the shortage of sanitary drinking water, and the impacts of poor water quality on people in developing countries. Specific water treatment options are presented, including chemical treatment options and physical treatment options, followed by research conducted on the process of solar disinfection.

2.1 Developing Countries

The term “developing country” is broad and far-reaching. It is a common term, but one that is used to describe countries of varying degrees of wealth, infrastructure, education, agriculture, industry, and communications. As stated by Ellis (1991), there are two extremes that encompass the definition of a developing country:

> "At the one end of the scale, there are those countries with highly developed commercial and industrial sectors, well-established secondary and tertiary education systems, good communications, and a well-developed ability to improvise adequately when apparently essential equipment is not available. At the other extreme, there are countries in which the already minimal economic achievement is totally overburdened by natural or political disasters, where communications are appalling, technical and tertiary education nearly nonexistent, and the agricultural and industrial base so limited that the provision even of the simplest techniques of water supply is only achieved through external aid, and the operation and maintenance of these is a continuing nightmare." (pg 387)

Of focus in this research are countries with inadequate water treatment, inadequate or non-existent distribution systems, and poor source water quality. Although there is no universally accepted standard for defining a developing country, it will be defined here as a country in which the ingestion of water-based pathogens is of frequent concern and in which a significant portion of the population does not have access to water of acceptable

drinking water standards. The World Health Organization defines acceptable drinking water as that in which no *E. coli* or thermotolerant bacteria are detected in any 100 mL sample (WHO, 1997).

2.1.1 Drinking Water Challenges

In much of the developing world, there are no funds to develop a drinking water system infrastructure. Where treatment systems do exist, there are several issues that often preclude adequate water treatment. These include misemployment, under-employment, inoperational equipment, lack of spare parts, unavailability or cost of chemicals, inadequately trained staff, and lack of supervision (Ellis, 1991). It is estimated that $150 billion is needed for developing countries to address these issues and establish full water supply coverage (Wegelin *et al.*, 1994).

Although water disinfection is a crucial step in preventing waterborne diseases, there are several aspects of the water collection, treatment, and distribution cycle that affect whether drinking water arrives at a home in potable condition. First, source water should be carefully selected and protected to ensure it is free of contaminants. Water that receives runoff from land used for agriculture and livestock farming is likely to have pesticides, fecal matter, and other constituents that were applied to the surrounding grounds. Ellis (1991) suggests relocating cattle and other livestock from the vicinity of drinking water sources. In addition, improving the sanitation practices of the local population can reduce the potential for water supplies to be polluted. It is not considered uncommon, especially in developing countries, for defecation and urination to occur in

rivers, lakes, and other bodies of water that are also used for domestic and recreational purposes (Kloos *et al.*, 1997). These practices continue a cycle of recontamination. The second factor in preventing waterborne disease is adequate and reliable water treatment. This can be addressed by properly training water plant operators and by providing funding to ensure all necessary chemicals and equipment can be purchased. Third, distribution systems must be built and improved to prevent recontamination of treated water. Other intervention measures, such as increasing public awareness, should also be employed (Burch and Thomas, 1998; Somboonsub, 2001).

2.2 Disinfection Options

When large community-wide water treatment and distribution systems are not available, people may treat water individually or for their families. There are several water disinfection options available for small-scale use. Water disinfection methods can be divided into two categories. The first category is chemical disinfection. Chemical disinfection includes methods such as chlorination and iodine treatment. Chlorine is the most common method of drinking water treatment due to its effectiveness at inactivating several types of pathogens and its low chemical cost. Chlorinated water also retains a residual that further protects from recontamination after the water is treated (Burch and Thomas, 1998). Iodine is a second chemical treatment option and one that is commonly used by hikers and backpackers in the U.S. as an effective and transportable method of water treatment. However, iodine is not used to treat large amounts of drinking water because, weight for weight, it costs approximately 20 times more than chlorine (Ellis, 1991). Chemical costs may render such options unavailable to low-income families.

Other reasons chemical treatment is undesirable include the training needed to calculate proper chemical dosages and the unpleasant odor and taste of the drinking water. An additional disadvantage with all chemical treatment methods is that chemicals oxidize over time and therefore have limited shelf lives.

Physical treatment methods such as boiling water and UV treatment may also be used to treat drinking water. Boiling water is a simple process, but requires resources that may not be readily available. This is especially true for areas concerned with the effects of desertification and deforestation because boiling one liter of water requires approximately one kilogram of wood. The process is also time consuming and boiling water has been found to impart a disagreeable taste (Acra *et al.*, 1984; Ellis, 1991). UV radiation is the process where water is exposed to a lamp generating light at a wavelength of approximately 250 nm. This wavelength is in the middle of the germicidal band and is responsible for damaging the DNA of bacteria and viruses. However, UV treatment is only effective for low turbidity waters and therefore pretreatment such as filtering is required for poor water quality sources. Also, developing and maintaining UV radiation treatment requires the initial cost of purchasing equipment, a knowledgeable operator to properly use the equipment, and sufficient funds for maintenance. For areas that are unable to financially support such a treatment scheme, UV radiation is not a viable treatment option (Burch and Thomas, 1998).

2.3 Solar Disinfection

A potential alternative to the common disinfection methods mentioned previously is solar disinfection. Solar water disinfection is a process that entails filling a transparent bottle with water and placing it in the sun for several hours. The following sections describe the process, its potential for use, and the enhancements that can be employed to increase its effectiveness. Limitations of solar disinfection are also presented.

2.3.1 Solar Radiation as a Disinfection Mechanism

For over 4000 years, sunlight has been used as an effective disinfectant (Conroy *et al.*, 1996). When organisms are exposed to sunlight, photosensitizers absorb photons of light in the UV-A and early visible wavelength regions of 320 to 450 nm. The photosensitizers react with oxygen molecules to produce highly reactive oxygen species. In turn, these species react with DNA; this leads to strand breakage, which is fatal, and base changes, which result in mutagenic effects such as blocks to replication. For bacteria, the process is reversible as the bacteria may again become viable if conditions allow cells to be repaired (Kehoe *et al.*, 2001; McGuigan *et al.*, 1999). Viruses are unable to repair DNA damage and are therefore sensitive to optical inactivation (McGuigan *et al.*, 2001).

2.3.2 Solar Disinfection Process Variables

Previous studies have found that solar disinfection is affected by numerous variables. These variables include solar radiation wavelengths, water temperature, turbidity, and container selection. Several process enhancements have also been studied.

2.3.2.1 Solar Radiation Wavelengths

Studies have shown that visible violet and blue light have little disinfection capability. However, the other components of sunlight, UV-A, UV-B, and UV-C radiation, are able to inactivate organisms. UV-C radiation, at approximately 260 nm, has the greatest potency because it corresponds to maximum absorption by DNA. Municipal treatment plants use UV-C (at 254 nm) to disinfect drinking waters and secondary wastewater effluents because of its germicidal ability to initiate changes in nucleic acids and other structures such as enzymes and immunogenic antigens. However, near ultraviolet (UV-A) light has been found to be the most significant component of sunlight that is responsible for the inactivation of microorganisms, with an increase in effectiveness due to the synergistic effects of UV-A and violet light. This is because the UV-C component of solar radiation does not reach the earth (Wegelin *et al.*, 1994).

Acra *et al.* (1984) compared the germicidal effects of different wavelengths of light by measuring the average number of coliforms inactivated upon exposure to the varying wavelengths. They found that the most significant decrease in viable bacterial organisms occurred when they were exposed to wavelengths between 260 to 350 nm (compared to inactivation at wavelengths between 550 to 850 nm). Because wavelengths below 290

nm do not reach the earth, Acra *et al.* (1984) concluded that the most bactericidal wavelengths were between 315 to 400 nm, which corresponds to the wavelengths of the near-ultraviolet region that are not visible to the eye. The findings of Acra *et al.* (1984) are further supported by the research of others. Davies and Evison (1991) attributed half of the toxic effects of sunlight to wavelengths lower than 370 nm. Wegelin *et al.* (1994) concurred, stating that wavelengths between 300 and 370 nm have significant effects on inactivating bacteria and viruses.

Natural sunlight has been shown to have germicidal properties. Wegelin *et al.* (1994) found that a fluence of natural light of approximately 2000 kJ/m^2 or 555 Wh/m^2 resulted in a 3-log inactivation of *E. coli*. This is equivalent to 5 hours of midday summer sun as measured at Duebendorf, Switzerland. Viruses required higher fluences than bacteria for the same inactivation level: F2 coliphage, rotavirus and encephalomyocarditis virus required 9,000, 6,800, 34,300 kJ/m^2 for 3-log inactivation. Davies and Evison (1991) also found solar disinfection to be effective, with 1 log inactivation of *E. coli* in 10 hours of exposure to sunlight, and 4 log inactivation of *Salmonella typhimurium* in 4 hours of exposure.

2.3.2.2 Heating

Temperatures at or above boiling can be used to effectively pasteurize water. Liquids may also be pasteurized using lower than boiling temperatures, provided the liquids are kept at such temperatures for an extended period of time. For example, enteric viruses in water can be pasteurized in approximately 1 hour at 62°C or in 1 day at 50°C (Burch and Thomas, 1998). It is known that 10 minutes at 56°C will inactivate *Giardia lamblia, G.*

muris and *Entamoeba histolytica*. If a temperature of 50°C is attainable, amoebic cysts are inactivated (Acra *et al.*, 1984). Ciochetti and Metcalf (1984) state that milk pasteurization occurs at 62.8°C for 30 minutes or at 71.7°C for 15 seconds, and Burch and Thomas (1998) state that the typical pasteurization of any liquid is at 75°C for 10 minutes.

Pasteurization may not be ideal for some drinking water treatment situations. Effective treatment by heating requires knowledge of the water quality in order to determine the temperature the water must reach and the duration of heating that is needed. In addition, disinfection by heating may be impractical for wide scale use because pasteurization is a labor-intensive process and requires a significant amount of fuel (Burch and Thomas, 1998). However, heating may be accomplished by using sunlight, thus alleviating the problem of needing wood or other fuels for boiling.

In 1984, Ciochetti and Metcalf published the results from a study to determine the effectiveness of using a solar box cooker to pasteurize river water that had an initial *E. coli* count of 33 to 350 cfu per 100 mL. They were able to attain temperatures of 65°C in two 3.7 L jugs between mid March to mid September in California, with no coliforms detected at 60°C and 65°C. In heating tests, Ciochetti and Metcalf (1984) detected coliforms at 59°C, but none at 61°C or 63°C. Although the samples had reached pasteurization temperatures at the end of the solar pasteurization and heating tests, it is likely the samples were not held at a pasteurization temperature for the recommended

period of time. Therefore, it is possible that temperatures lower than 63°C have disinfection capabilities as well.

Conroy *et al.* (1996) exposed water samples to full sunlight in Kenya and confirmed that sunlight has a bactericidal effect on turbid water, with reductions in the initial bacterial count of over 10^3 cfu per mL. The disinfection was attributed to pasteurization effects, rather than ultraviolet light. This was confirmed with laboratory experiments by Joyce *et al.* (1996), who heated contaminated water samples to a maximum of 55°C in 7 hours and observed a 5-log inactivation of *E. coli*.

Jorgensen *et al.* (1998) tested a flow-through copper-piped system that used solar radiation to pasteurize naturally contaminated water from the Mlalakuva River near Dar es Salaam, Tanzania. They found that while fecal indicator bacteria were inactivated in water that was heated to 62°C or above, other organisms such as spore-forming bacteria were never completely inactivated, even when water temperatures of 75°C were attained. They found that temperatures of 65°C or above inactivated coliform bacteria and thermotolerant coliform bacteria, which were present in the naturally contaminated river water. Such temperatures also inactivated *Salmonella typhimurium*, *Streptococcus faecalis* and *Escherichia coli* that were cultured and added to the raw river water.

Rijal and Fujioka (2001) observed the effectiveness of heating using a modified Family Sol*Saver System (FSP). The FSP is a high-density, black polyethylene double-walled collector that was designed for liquid pasteurization. However, by exchanging the

original non-UV-transmittable plastic cover for a UV-transmittable cover, Rijal and Fujioka were able to determine the effectiveness of pasteurization versus pasteurization and solar radiation on numerous organisms, including fecal coliforms, *E. coli*, enterococci, *C. perfringens*, total heterotrophic bacteria, hydrogen sulphide producing bacteria and FRNA virus. Tests were carried out using a low turbidity (<2 ntu) water from the Manoa stream in Hawaii, diluted sewage (2.5 ntu), or seeded tap water. On the experiment conducted on a sunny day, the pasteurization only sample was able to achieve a temperature of 65°C with a corresponding inactivation of more than 3-log of *E. coli* in 3 hours. The solar radiation and pasteurization sample heated to 56°C, with the same log inactivation in 2 hours. Therefore, solar radiation and heating acted synergistically to inactivate the bacteria.

Pasteurization is an effective treatment option for liquids. However, a false sense of security may mislead one to under treat the drinking water. As detailed above, certain organisms cannot survive temperatures of 55°C while others are still viable at 75°C. Without knowing the exact composition of organisms in the water, the user may not adequately treat the drinking water before use. There is also a high capital cost associated with purchasing pasteurization equipment if the process is used for a community. However, pasteurization of liquids is independent of turbidity and pH. This, coupled with the fact that solar energy is free and solar disinfection is a simple process to employ, warrants further study for use by individuals or small families in developing countries.

2.3.2.3 Impurities

Turbidity is a significant factor in the disinfection process. The effectiveness of solar disinfection has been tested on samples with turbidities ranging from less than 10 ntu to approximately 300 ntu. Researchers have found that higher turbidity samples exposed to sunlight attained consistently higher water temperatures, which was attributed to absorption of radiation by the particulate matter (Kehoe *et al.*, 2001; McGuigan *et al.*, 1999). More turbid samples, at 300 ntu, also had less inactivation of *E. coli* compared to samples with little or no turbidity. This may be in part due to shielding of organisms by particles (Kehoe *et al.*, 2001; McGuigan *et al.*, 1999; Sommer *et al.*, 1997). Joyce *et al.* (1996) reported that less than 1% of the total incident UV light is able to penetrate beyond a water depth of 2 cm from the surface in samples with turbidities greater than 200 ntu. Therefore, it may be necessary to filter turbid waters before sun exposure.

Impurities in a water sample that cause it to be colored also have an effect on the disinfection potential for a given drinking water sample. In highly colored samples, sunlight may not have a lethal effect because the colored water may absorb wavelengths in a certain range. In these cases, it is recommended that the water sample be treated to reduce coloration before sun exposure (Acra *et al.*, 1984).

2.3.2.4 Container Selection

Container shape and color may have significant impacts on the effectiveness of solar disinfection. The bottle shape may interfere with the sun's disinfection capabilities: as the sun moves across the sky, the intensity will change and may be reduced depending on

the bottle shape. Acra *et al.* (1984) therefore recommend using round, conical bottles as opposed to square or irregularly shaped containers. However, the major limiting factor is the availability of the bottles themselves, with variables such as plastic thickness and light transmittance characteristics being difficult to assess in the field.

Acra *et al.* (1984) also noted that colorless containers allow the most transmittance of ultra-violet wavelengths and are therefore the optimal choice for use in solar disinfection. Blue and violet tinted containers also transmit radiation, yet other colors, such as orange, yellow, red and green, will absorb wavelengths with the most lethal bactericidal effects and therefore must be avoided (IDRC, 1998). With regard to pasteurization, a water sample exposed to sunlight increases in temperature due to the red and infrared components of sunlight. Blue containers would therefore absorb these components and minimize any temperature increases (Acra *et al.*, 1984). Therefore, to maximize the effects of both solar radiation and heating, colorless containers are recommended.

Container size may also be an important parameter in the solar disinfection process. Acra *et al.* (1984) specify that container size is a variable that affects solar disinfection. However, their studies do not specifically test the effect of volume size on solar disinfection. Kehoe *et al.* (2001) found no significant difference in the population dynamics of 0.5 and 1.5 L samples. In contrast, Reed *et al*. (2000) compared the time needed to achieve a 99.9% reduction in the initial fecal coliform counts of 22 L and 25 L samples and found that exposure times of 150 minutes and 290 minutes were required, respectively. A more extensive study on volume variations may be useful.

2.3.2.5 Enhancements

A number of process enhancements have been studied in order to increase the effectiveness of solar disinfection. Such efforts have included periodic agitation, using foil to increase reflectivity, and painting half the bottle black to increase achievable temperatures.

In a field experiment, Kehoe *et al.* (2001) used sterilized reagent grade water samples that they had spiked with *E. coli* and exposed to the sun. Some samples were agitated for 1 minute every 15 minutes. They found no significant difference in *E. coli* inactivation rates of the agitated versus non-agitated samples that were exposed to sunlight if the dissolved oxygen (DO) levels did not change significantly. Changes in DO levels did not occur when there were only slight increases in water temperature, such as from 32.5°C to 39°C. However, Kehoe *et al.* (2001) discovered that in samples exposed to both thermal and optical effects, increasing levels of DO did correspond to an increase in inactivation rates. In conclusion, Kehoe *et al.* (2001) recommended against agitating samples to prevent decreases in inactivation rates when significant temperature differences occur. Reed *et al.* (2000) also found that water samples with greater oxygenation had increased inactivation rates. Complete inactivation of fecal coliforms was achieved in 3 hours in an oxygenated sample, compared to the less than 1-log inactivation after 4 hours for a deoxygenated sample.

During laboratory thermal-only simulations, where sample temperature was raised from 20°C to 50°C, agitation significantly lowered the DO levels of samples. There was no

significant correlation found between the inactivation of *E. coli* in agitated versus non-agitated samples however, which implies that DO levels are not a significant factor when samples are sufficiently heated (Kehoe *et al.*, 2001).

Using sterilized reagent grade water samples spiked with *E. coli*, Kehoe *et al.* (2001) found that foil-backed samples averaged almost 1°C higher than non-foil-backed samples when exposed to sunlight for 3.5 hours. Over 6-log inactivation was reached in less than 1 hour of exposure time when aluminum foil was placed partway around sample bottles, versus more than 3 hours needed for 6-log inactivation of non-foil-backed samples.

2.3.3 Field Applications

Conroy *et al.* (1996, 1999, 2001) established the potential for field use of solar disinfection by demonstrating that this process reduced the risk of diarrhea in children. The studies were conducted in the Kajiado province of Kenya using Maasai children between 5 and 16 years of age. In 1996, the first test group consisted of 108 children that drank solar treated water. These children were given two 1.5 L plastic bottles to be filled with drinking water and put on the roof of their huts from dawn until midday. The water could then be used for drinking. The control group consisted of 98 children that were given the same directions, but rather than putting the bottles on the roof, they kept the bottles indoors. The results of this study showed that the children in the first group averaged 4.1 diarrheal episodes over a twelve-week period, versus an average of 4.5 episodes in the control group (Conroy *et al.,* 1996). In 1999, the test group was expanded to children less than six years of age. The children drinking treated water had a

two-week period diarrhea prevalence of 48.8%, versus 58.1% in the control children (Conroy *et al.*, 1999). Five years later, the researchers learned of a cholera outbreak in the test villages. They returned and found that the test families had continued to treat their drinking water with solar disinfection. However, while there was no statistical difference in the risk of contracting cholera between families using solar disinfection and those that did not, the continued use of the process by the villagers was promising as shown by the earlier successes in reducing diarrheal incidences.

2.3.4 Limitations to Solar Disinfection

There are several limitations to using solar disinfection to treat drinking water. The process of solar disinfection is best suited for regions having approximately 300 sunny days with clear skies each year, with areas between latitudes 35°N and 35°S having the optimum exposure of sunlight (Acra *et al.*, 1984; IDRC, 1998). However, any amount of cloud coverage reduces the intensity of sunlight that reaches the earth, thereby decreasing its germicidal effects. Despite this restriction, Acra *et al.* (1984) state that a longer exposure time more than compensates for the reduction in solar intensity.

Another difficulty presented with solar disinfection is that the materials needed for the process may not be readily available. Clear, cylindrical bottles are most effective at allowing solar radiation to reach the water, yet these may be difficult to obtain for large-scale use by remote communities, where plastic containers are not sold. In addition, enhancements used by various researchers, such as foil (Kehoe *et al.*, 2001), may be difficult to purchase. Devices such as solar panels, copper piping, and thermostat valves

were required to construct the solar panel described by Jorgensen *et al.* (1998) to pasteurize drinking water. Because these materials are not readily available in many less-developed areas, and knowledge of constructing a solar water heater is not widespread, this method of heating water for large-scale use is impractical in developing countries. However, small-scale individual use of plastic bottles is a treatment method that can be implemented with minimal resources and little training.

2.4 Conclusion

Solar disinfection is a process that is simple and effective. It could prove valuable for use in developing countries and in areas that need a small-scale drinking water treatment method. Studies have shown that it is effective in reducing diarrheal illness in children when implemented in field trials. However, the process does have limitations and several variables influence the effectiveness of the process such as solar intensity, temperature, turbidity, container shape, and sample volume. Therefore, this study was aimed at establishing relationships between these variables and the effectiveness of solar disinfection.

3 Methodology

Previous studies have found decreases in effectiveness of solar disinfection with increases in turbidity and sample volume and an increase in effectiveness with increased fluences and higher water temperatures. The experiments conducted for this thesis examined these variables and their impact on the inactivation of *E. coli* by solar radiation and heating. By using the same testing, sampling, and enumeration methods for each experiment, the results can be directly compared. This chapter details the experimental design, including the variables examined and the methods employed during the experiments.

3.1 Experimental Design

The objective of this research was to first test the effects of water temperature and solar radiation on artificially contaminated water samples. The first phase of experiments consisted of placing artificially contaminated sample solutions in direct sunlight and enumerating viable bacterial counts over time to quantify disinfection by solar radiation and heating. In the second phase, inactivation was quantified in samples that were heated in a water bath. These results were then compared to the solar inactivation results to determine the role of heating in solar disinfection.

3.1.1 Organism

The test organism chosen was the bacteria *Escherichia coli*. *E. coli* is currently the most specific indicator for fecal contamination of a water source; its presence in high numbers also allows for more likely determination of water contamination than if they were only

found in small numbers in water samples (Toranzos and McFeters, 1997). *E. coli* is more resistant to disinfection than other enteric bacteria and organisms such as *P. aerugenosa*, *S. flexneri, S. typhi,* and *S. enteritidis* and may also be used to determine the likely response of other pathogenic organisms to a given disinfection mechanism (Acra *et al.*, 1984). The World Health Organization has set the worldwide guideline for safe drinking water as no detectable *E. coli* or thermotolerant bacteria in any 100 mL sample (WHO, 1997).

3.1.2 Variables

Experiments were conducted to determine the effects of several variables on the solar disinfection process. The results of these experiments were then compared to the results obtained during the heating only experiments. Table 3-1 lists the variables tested for both phases of experiments.

Table 3-1: Experimental testing scheme.

Disinfection Scheme	Variable	Variable Range
Solar radiation and heating	Sample solution	Phosphate buffered saline or pond water
	Turbidity	0 ntu – 200 ntu
	Sample volume	825 mL – 1.9 L
	Exposure time	0 – 8 hours
Heating only	Sample solution	Phosphate buffered saline
	Sample volume	825 mL
	Exposure time	0 – 5 hours

3.1.2.1 Sample Solution

The primary sample solution for solar radiation and heating and heating-only experiments was phosphate buffered saline (PBS). This solution was used as a liquid medium with no

organic demand and no unknown constituents. Pond water was also used as a test solution in solar radiation and heating experiments as a comparison between using natural water versus an artificial sample solution. The natural water was collected from Salisbury Pond in Worcester, MA the day before the experiment and autoclaved before use in order to kill any indigenous organisms that were present in the sample. The pond water characteristics are shown in Table 3-2. In addition to a pond water test sample, a second sample was prepared with 50% pond water and 50% PBS. This diluted sample had an intermediate turbidity value that was compared to the pond water sample and the PBS-only sample.

Table 3-2: Salisbury Pond water characteristics.

Pond Water Parameter	**Value**
Total organic carbon	7.90 mg/L
Dissolved organic carbon	7.04 mg/L
pH	7.72
Turbidity before settling	18.7 ntu
Turbidity after 30 minutes of settling	9.68 ntu

3.1.2.2 Sample Volume

Sample volume was varied using plastic bottles with the labels removed. Depending on the size needed, 1.0 L Aquafina water bottles, 1.5 L Glaceau water bottles, and 2.0 L Price Chopper soda bottles were used. The sample volumes ranged from 825 mL to 1.9 L in order to leave space at the top of each bottle. This was necessary to prevent spilling any sample solution while passing the bottlenecks through a flame to prevent contamination both before and after removing samples. The test containers were chosen due to the worldwide availability of plastic soda bottles in varying sizes. The abundance of plastic bottles increases the likelihood of employing the solar disinfection process.

Because the bottles were likely to be made of varying thicknesses, readings were taken to determine if there was any variation in the intensity of light that passed through the plastic to the water samples. The readings are presented in the results chapter.

3.1.2.3 Turbidity

Turbidity was tested in two different testing schemes. In the first, the turbidity source was montmorillonite clay, which was added in measured quantities to PBS to achieve desired turbidity levels. In the second scheme, natural turbidity in pond water was tested. The organic matter found in the natural water may also have affected experiment results.

Turbidities of approximately 0, 20, 100, and 200 ntu were tested using montmorillonite clay. The Swiss Federal Institute for Environmental Science and Technology (EAWAG, 2003) recommends using solar disinfection to treat water with turbidities no higher than 30 ntu. To bracket this value, a sample with no turbidity added was tested along with samples that were turbid due to a significant addition of turbidity. Montmorillionite clay was added to the sample solutions to achieve these pre-determined turbidity values. It was noted that the clay tended to settle, thereby reducing the turbidity of the sample solution over time, until the sample was inverted and mixed before each sampling session. In order to test the effect of suspended solids and natural turbidity, pond water was also tested. Details of the pond water are presented in Section 3.1.2.1.

3.1.2.4 Exposure Time

The total exposure time of experiments varied from 4 to 8 hours. Sunlight is strongest from 10 am to 2 pm so initial experiments were conducted to encompass this time bracket by up to 1.5 hours before and up to 3 hours after (from 8:30 am to 4:30 pm). Results of these experiments showed that significant inactivation of *E. coli* occurred within a few hours of exposure, making the 8-hour exposure time unnecessary. Taking this into account, subsequent experiment exposure times were shortened.

3.1.2.5 Temperature

For heating-only experiments, samples were prepared using PBS with no added turbidity. An experiment was conducted to determine whether artificially contaminated samples would be inactivated by raising the temperature of the sample from a room temperature of 22.0°C to a maximum of 46.0°C. A temperature of 46.0°C was used because in the solar radiation and heating experiments, this was the maximum temperature achieved in any sample. The heating-only test was conducted in triplicate. The results of these experiments showed that there was less than 0.5 log inactivation of *E. coli* when the temperature of the test sample was raised to 46.0°C. Further studies, where turbidity and sample volume would have been varied, were therefore deemed unnecessary, as heating alone did not show significant disinfection.

3.1.3 Test Procedures: Solar Radiation and Heating Experiments

Each experiment encompassed several steps. Before each experiment, *E. coli* was grown and harvested, test solutions were prepared and autoclaved, and test bottles were sterilized. On the day of each experiment, the test solution was transferred to the test bottles, *E. coli* was spiked into the test water, and the bottles were exposed to full sunlight. Samples were taken at predetermined intervals and diluted before plating to enumerate viable colony-forming bacteria cells.

First, *E. coli* was grown in tryptic soy broth overnight. The morning of each experiment, the solution was centrifuged and the broth decanted. The pellet was resuspended in 0.01 M (1 X) phosphate buffered saline that resulted in an approximate concentration of 4×10^9 cfu/mL. See Section 3.2.1.2 for a more detailed description of the *E. coli* stock solution preparation.

The primary test solution for all experiments was phosphate buffered saline. This solution provided a liquid medium with no unknown constituents that would affect the *E. coli* bacterial population. In order to compare the results of the experiments with PBS, pond water was also tested. This water had a yellowish color, natural turbidity, and other organic matter.

All test solutions were pre-measured. For samples in which turbidity was to be measured, an additional 25 mL of test solution was prepared, to be removed at the beginning of the experiment. Once the sample volume was measured, montmorillonite

clay was added to selected samples to achieve desired turbidity levels. The solutions were then autoclaved in glass media bottles and stored in the refrigerator until the evening before the experiment. At this time, the test solutions were transferred to a 22°C incubator in order to allow sufficient time to reach room temperature.

Approximately 1.5 hours before the start of the experiment, the test solutions were transferred from the glass media bottles to the sterile plastic test bottles. Three types of bottles were prepared: test bottles, a control bottle, and a PBS only bottle. Test bottles were prepared with PBS or a solution of pond water, depending on the objective of the experiment. A control bottle was prepared with PBS to verify that the bacterial population remained constant over the duration of an experiment. A PBS only bottle was prepared, with no added *E. coli*, in order to verify the sterility of the plastic test bottles and to verify that sterile sampling conditions on the rooftop were maintained. Specific details on the preparation of the bottles are in the following sections.

3.1.3.1 Control Bottle Preparation

A control bottle was prepared for each experiment. The control bottle was the same as the test bottles; it contained a pre-measured and autoclaved test solution (PBS) with *E. coli*. However, no turbidity was added. The purpose of the control was to ensure that the bacterial population remained constant over the course of each experiment, with no influences from external factors. The bottle was therefore covered with aluminum foil and placed in a 22°C incubator. Samples were removed every hour for the duration of each experiment.

3.1.3.2 PBS Only Bottle Preparation

For each experiment, a PBS-only bottle was also tested (no turbidity and no *E. coli* were added). 1 mL of the solution from this bottle was plated in duplicate for each sampling session. The purpose of the PBS-only bottle was two fold. First, because samples were collected from this bottle in the same manner as samples were collected from the test bottles, any breach of aseptic conditions when sampling and any cross-contamination from bottle to bottle would be enumerated when plating the sample. Second, plating samples from a PBS-only bottle would verify that the interiors of the plastic test bottles were sterile.

3.1.3.3 *E. coli* Addition

For the test and control bottles, a calculated quantity of the *E. coli* stock culture was transferred to the test solutions to yield a starting concentration of 10^6 or 10^7 cfu/mL. The quantity of stock culture to be added was calculated by multiplying the desired concentration of bacteria in the test solution by the volume of the test solution. This was then divided by the approximate concentration of cells in the stock *E. coli* solution. An example calculation is presented below for an 800 mL test solution:

$$\frac{10^7 \text{ cfu}/\text{mL} * 800 \text{ mL}}{4 * 10^9 \text{ cfu}/\text{mL}} = 2 \text{ mL}$$

For each experiment, several test bottles were prepared with varying turbidities. The first bottle contained PBS with no turbidity added. The other test bottles contained either PBS

or pond water. For bottles containing PBS, an appropriate quantity of montmorillonite clay was added to achieve the desired turbidity. For test bottles containing pond water (or diluted pond water), no turbidity was added.

3.1.3.4 Pre-disinfection Procedure

After adding the test solution and *E. coli* to the test bottles, approximately 1.5 mL was removed from each bottle (test, control and PBS only bottles) to begin a dilution series for plating. This was completed in order to enumerate the starting *E. coli* concentration, before disinfection. Next, 25 mL was removed from each test bottle in order to measure turbidity. The control bottle was then wrapped in aluminum foil and placed on its side in a 22.0°C incubator. The test bottles were carried to the roof of Boynton Apartments, located on Boynton Street near WPI's Environmental Engineering Laboratory, where they were placed on their sides on a white towel. The sun intensity, weather conditions, and air temperature were recorded at the start of the experiment.

3.1.3.5 Sampling Procedures

To quantify the inactivation of bacteria in the test bottles, samples were taken at 15 minute, 30 minute, or 1 hour intervals. During each sampling session, the time, sun intensity, weather conditions, water temperatures, air temperature, and sample volumes collected were recorded. The sun intensity was recorded first while the detector was horizontal and then while the detector was held above the head and pointed directly at the sun to avoid scattered light interference. If cloud coverage was over an estimated 50%,

sun intensity readings during full sun exposure and during cloud cover were averaged. Because the test bottles are cylindrical, and therefore the test water is exposed to direct sunlight, the direct sun intensity readings are presented in Chapter 4. Intensity readings are tabulated in Appendix A.

During each sampling session, a small aliquot of 2 to 5 mL was removed from each bottle and brought to the laboratory. A dilution series was carried out and at least three appropriate dilutions were plated in order to determine the concentration of bacteria remaining in the test and control bottles, and to calculate the log inactivation of the bacteria in the test and control bottles. For the PBS only bottle, duplicate undiluted samples were plated.

3.1.4 Test Procedures: Heating Only Experiments

During the second phase of the experimental process, the effects of heating only on the inactivation of *E. coli* were tested. As shown in Chapter 4, the highest temperature achieved during the solar radiation and heating experiments was 46°C. Laboratory experiments were therefore conducted to evaluate the inactivation of *E. coli* by heating a test solution of PBS with *E. coli* to 46°C. No turbidity was added to this sample because the sun experiments showed the quickest inactivation occurred in samples with no turbidity added. Therefore, if notable inactivation did occur in this heated sample, further experiments would be conducted by varying turbidities and sample volumes.

A stock *E. coli* solution was cultured and prepared in the same manner as for solar radiation and heating experiments (see Section 3.1.3). PBS was prepared and autoclaved in glass media bottles with no addition of turbidity. A control bottle, a test bottle, and a bottle for reading temperature only were prepared by adding a calculated quantity of *E. coli* to the control bottle and the test bottle solutions. The temperature only bottle was included to prevent contaminating the test bottle solution when taking temperature readings. Approximately 3 mL was removed from each the control bottle and the test bottle in order to begin a dilution series for plating. All three bottles were then covered with aluminum foil. The control bottle was placed in a 22°C incubator and the test bottle and temperature only bottle were placed in a water bath.

The temperature of the water bath was raised from 22.0°C in increments of 3-7°C approximately every half hour in order to achieve a maximum temperature of 46°C in the test solution. The increase from 22°C to 46°C was spread out over a three- to four-hour period to mimic the temperature rise in the average experiment conducted in the sun.

Every 10 minutes, the temperature only bottle was gently inverted to mix the solution and a temperature reading was taken. If the sample was not 3°C or more higher than when the previous sample was taken, the test bottle was returned to the water bath. If the sample was 3°C or more higher than the last time a sample was taken, the test bottle was removed from the water bath, gently inverted several times, and a 3 mL sample was taken. A 3 mL sample was also then removed from the control bottle. Dilution series were carried out and the samples were plated to determine the *E. coli* concentration.

3.2 Analytical Methods

All processes were carried out using aseptic technique, including the use of 50% ethanol to sterilize workspaces and hands. All glassware, test solutions, and medias were sterilized by autoclaving at 121°C for an amount of time recommended by the autoclave manufacturer (Sterilmatic Sterilizer, Market Forge Industries Inc., Everett, MA), according to the volumes being autoclaved. Pre-sterilized pipette tips and petri dishes were used.

3.2.1 *Escherichia coli* Culture

E. coli was purchased in dehydrated form and rehydrated in the laboratory. A stock culture was prepared and used to artificially contaminate the test solutions for each experiment. The following sections detail the methods used in these processes.

3.2.1.1 Frozen *E. coli* Culture

E. coli #11775 was purchased from the American Type Culture Collection and received dehydrated. To rehydrate the culture, one test tube of 5-6 mL of tryptic soy broth (TSB) was prepared and autoclaved. Working in a sterile fume hood, the crimp was popped off the dehydrated culture vial and the stopper was removed from the vial using flamed tweezers. 1 mL of the autoclaved TSB was pipetted into the vial to rehydrate the pellet. The solution was then poured from the vial into the test tube, which was then incubated for 48 hours at 35°C. 10 mL of autoclaved 40% (by volume) glycerol was prepared.

After the 48 hour incubation period, the *E. coli* solution was removed from the incubator and 0.5 mL of the solution was pipetted into each of 10 sterile microcentrifuge tubes. 0.5 mL of the glycerol solution was then added to each microcentrifuge tube. The microcentrifuge tubes were then placed in a –70°C freezer.

3.2.1.2 Liquid Stock Culture

For each experiment, two 125 mL shaker flasks each with 50 mL of tryptic soy broth (TSB) were autoclaved and then brought to 35°C in an incubator. Sixteen to eighteen hours before the start of an experiment, the *E. coli* frozen stock culture (see Section 3.2.1.1) was removed from a -70°C freezer and brought to a laminar flow hood. The 125 mL shaker flasks containing 50 mL of TSB were inoculated each with one loopful of the frozen stock culture. The frozen stock culture was returned to the -70°C freezer and the shaker flasks were placed on a platform shaker table set at 100 rpm in a 35°C incubator. The flasks were left for 16 to 18 hours.

Approximately 1 hour prior to the beginning of each experiment, the centrifuge (Marathon 21000R, Fisher Scientific, Pittsburgh, PA) was cooled to 4°C. The *E. coli* shaker flasks (incubated for 16-18 hours) were removed from the 35°C incubator. The contents of one shaker flask were split into two Oakridge centrifuge tubes (Oakridge 50 mL centrifuge tubes 3119-0050 PPCO, Nalge Company, Rochester, NY) and centrifuged at 3650 revolutions per minute (rpm) for 20 minutes at 4°C. This formed a tight pellet in each tube. The broth of one centrifuge tube was poured off and the pellet was

resuspended in 25 mL of 0.01 M PBS. This liquid stock culture, or stock culture, contained an approximate concentration of $4x10^9$ cells/mL.

3.2.2 *E. coli* Enumeration

The concentration of viable *E. coli* bacteria was enumerated by plating samples from each test bottle. The samples were collected and a dilution series was performed; the dilutions needed for plating were estimated based on expected inactivation, with a target of 30-300 colony forming units (cfu) per plate. Expected levels of inactivation were dependent upon the observed weather conditions, the air temperature, and the water temperatures of the test bottles.

3.2.2.1 Dilution Series

For each sample, a minimum of 3 dilutions was plated with 3 replicates of each dilution. First, a dilution series was completed in order to dilute the sample to achieve a target count of 30-300 cfu per plate. The dilution series was carried out by diluting 1 mL of the sample into a test tube containing 9 mL of 0.01 M (1X) PBS. This resulted in a 10^{-1} sample. Next, 1 mL of the 10^{-1} sample was diluted into another test tube containing 9 mL of 0.01M PBS, which resulted in a 10^{-2} sample. This was continued to reach the necessary dilutions.

3.2.2.2 Pour Plate Method

The concentration of viable *E. coli* was enumerated using the pour plate method according to Method 9215B of Standard Methods (APHA *et al.*, 1998). In this method, pre-sterilized 100 mm plates (in which 100 μL to 10 mL can be plated) were used. The sample was pipetted into the center of the petri dish and 10 to 12 mL of tryptic soy agar was pipetted directly on top of the sample to ensure the sample was distributed evenly throughout the agar. The dish was mixed using a figure-eight motion, then allowed to solidify for 5 to 8 minutes. In addition, for each sample that was plated, one negative control (1 mL of PBS) was plated. The dishes were then capped, inverted, and incubated at 35 to 37°C for 24 hours. The colonies were then counted with the naked eye.

3.2.3 Media

The following sections detail the methods for preparing the tryptic soy broth used in the growth of *E. coli* bacteria, the tryptic soy agar used to enumerate the *E. coli*, and the phosphate buffered saline used as the test solution and for the dilution series.

3.2.3.1 Tryptic Soy Broth

Trypic soy broth (TSB) was prepared as the growth medium for the *E. coli* culture. The dehydrated media was purchased from DIFCO (DF0370-17-3, Becton, Dickinson and Company, Sparks, MD) and prepared according to the manufacturer's specifications. 50 g of media was placed in a beaker with 1 L of E-pure water. The broth was heated to dissolve the dehydrated media. For growing *E. coli*, 50 mL of this broth was then

measured into 125 mL shaker flasks, capped, and autoclaved at 121°C for 15 minutes. After cooling, the flasks were refrigerated for no longer than 3 weeks at 4°C.

3.2.3.2 Tryptic Soy Agar

Tryptic soy agar (TSA) was prepared for use in the pour plate method by adding 15 g/L of dehydrated agar (Bacto™ Agar 214010, Dickinson and Company, Sparks, MD) to tryptic soy broth as prepared in Section 3.2.3.1, before autoclaving. The agar was heated and stirred until slightly boiling, when it was removed from the heat and poured into glass media bottles. The agar was autoclaved at 121°C for 30 minutes then brought to 47°C in a water bath before use in the pour plate method for enumerating bacteria. Unused agar was stored in a 4°C refrigerator for no longer than 3 months. After refrigeration, autoclaved agar was re-autoclaved for 15 minutes to liquefy before use.

3.2.3.3 Phosphate Buffered Saline

Phosphate buffered saline was used as the primary test solution. A 0.1 M (10 X) PBS solution was made by adding 80 g NaCl, 2.0 g KH_2PO_4, 2.0 g KCl, and 11.56 g Na_2HPO_4 to a 1 L volumetric flask, which was then filled with E-pure water. A pH of approximately 7.2 to 7.4 was verified with an Orion model 420A pH meter (Orion Research Inc., Beverly, MA). This solution was stored for no longer than 4 weeks. The 0.1 M (10 X) solution was diluted 10-fold to create a 0.01 M (1 X) solution, which is the concentration used for experimental purposes. The 0.01 M (1 X) solution was measured and autoclaved in media bottles according to the pre-determined quantity to be used for

each experiment, and stored in the refrigerator for no longer than 4 weeks. The evening before each experiment, the appropriate quantities of autoclaved solution were placed in a 22.0°C incubator to allow the solution to reach room temperature.

3.2.4 Turbidity

Turbidity was added to specific test solutions in order to test the effects of higher turbidity levels on the inactivation of *E. coli* by solar disinfection. Calculated quantities of montmorillonite clay (#28 153-0, Aldrich Chemical Company, Inc., Milwaukee, WI), based on a calibration curve, were added to achieve pre-determined turbidity levels. The turbidity calibration curve is shown in Appendix B.

Turbidity was measured with a 2100N Turbidimeter (Hach Company, Loveland, CO) according to Method 2130B of Standard Methods (APHA *et al.*, 1998). The samples were placed in glass turbidity vials and capped. The exterior of the vials was cleaned with E-pure water and wiped with Kimwipes to remove particles and fingerprints. Turbidity readings were monitored for approximately 30-60 seconds, at which time an average reading was recorded.

3.2.5 Total and Dissolved Organic Carbon

A Total Organic Carbon Analyzer (Shimadzu TOC-5000, Shimadzu Corp., Kyoto, Japan) was used to test the pond water's non-purgeable organic carbon (NPOC) content. The

following sections describe the preparation of glassware and standards, the collection and preparation of the samples, and the operation of the analyzer.

3.2.5.1 Glassware Preparation

All glassware used for standard preparation was washed with soap and water and thoroughly rinsed. The glassware was then soaked in a 20% sulfuric acid bath overnight before rinsing with Epure water 3 times. The volumetric pipettes, volumetric flasks, and beakers were dried at 50°C and the autosampler vials were air-dried. This process ensured that the glassware was organic-free before use.

3.2.5.2 Preparation of Standards for Calibration Curve

To make a calibration curve, a stock primary standard, intermediate standard, and four working standards were prepared. To make the stock primary standard, 0.75 g of potassium hydrogen phthalate (KHP) was dried in an oven at 110°C for 30 minutes, then cooled in a desiccator for approximately 30 minutes. 0.5314 g was then measured using an analytical balance. This was added to a 250 mL volumetric flask, which was then filled to mark with Epure. This resulted in a stock primary 1000 mg/L KHP standard, where 1 mL is equal to 1 mg total organic carbon. The standard was stored in a brown glass bottle in the refrigerator for no more than 3 weeks.

The intermediate standard was prepared on the day of TOC/DOC analysis. 10 mL of the stock primary standard was pipetted into a 100 mL volumetric flask, which was then

filled to mark with Epure. This resulted in an intermediate stock solution of 100 mg/L TOC. The solution was stored in the refrigerator for no more than 2 days.

The working standards were prepared to bracket the expected sample concentrations (up to 10 mg/L). Four 100 mL volumetric flasks were half filled with Epure. 100 µL of 6 N HCl was added for NPOC analysis. The first flask had no intermediate standard added, while the second, third, and fourth flasks had 2, 5, and 10 mL of intermediate standard added, respectively. The flasks were then filled to mark with Epure. This resulted in working standards with concentrations of 0 mg/L, 2 mg/L, 5 mg/L, and 10 mg/L, respectively.

3.2.5.3 Collection and Preparation of Samples

The TOC samples were collected and preserved with 100 µL of 6 N HCl per 100 mL of sample. A pH of less than 2 was verified after acid addition. The samples were stored in the refrigerator at 4°C until analysis. The dissolved organic carbon samples were collected and filtered through a Whatman GF/C glass fiber filter with a 1.2 µm retention (Whatman International Ltd., Mardstone, England) that was prewashed with 30 mL of Epure water. The samples were preserved with HCl and stored at 4°C, as was done for the TOC samples.

3.2.5.4 Operation of the Shimadzu Analyzer

To measure the total organic carbon and dissolved organic carbon of the pond water, the TOC analyzer was warmed up for 1 hour before use. The autosampler tray was placed in the autosampler and once the machine was ready, as indicated by an "Initial Start" screen, gas flow was established from the cylinder to the analyzer. A regulator pressure of 70-85 psi was confirmed. The following variables were verified and adjusted as necessary: gas cylinder pressure above 500 psi, rinse water bottle full, carrier gas pressure gage at 4-5 kgf/cm^2, carrier gas flowmeter at 150 mL/min, IC reaction vessel bubbling, humidifier water level adequate, and dehumidifier drain container full. The furnace was then turned on. The standards and samples that had been prepared beforehand (see Sections 3.2.5.2 and 3.2.5.3) were loaded into the autosampler tray. The sample measurement conditions (sample group number, type of analysis, and vial numbers) were entered. For both standards and samples, the average concentration of 3 injections was used, with a maximum of 5 injections per sample. The allowable standard deviation between repetitive measures was set to 200 with a coefficient of variation of 2.0%. A sparge time of 3 minutes was set for NPOC analysis. The ASI conditions were set so that the autosampler rinsed the needles and flow lines to prevent cross-contamination between samples.

3.2.6 Solar Radiation

The solar irradiance was measured during each sampling session using an IL1400 Radiometer (International Light, Inc., Newburyport, MA). Measurements of solar wavelengths below 315 nm were measured with a SEL240 (#5849) detector with a

SPS300 (#24253) filter. A WBS320 (#24581) filter attached to a SEL005 (#782) detector was used to measure the intensity of solar wavelengths below 400 nm. Readings were taken with the solar detectors lying flat near the test bottles and also with the detectors held overhead, pointed directly at the sun. The direct solar intensity readings are presented in the results because they most accurately represent the intensity of sunlight affecting the test bottles. Although the test bottles were laid flat on the rooftop, they were made entirely of clear plastic and would therefore be affected by direct sunlight.

3.2.7 Bottle Sterilization

The plastic test bottles and caps were sterilized the evening before each experiment. First, the surfaces were sprayed with 50% ethanol. Next, the interiors of the bottles were washed three times, each with approximately 20 mL of 70% ethanol. The interiors were then rinsed three times with approximately 50 mL of autoclaved Epure water. Last, a mercury pen lamp (Pen-Ray 90-0004-01, UVP Inc., Upland, CA) was placed inside each bottle to expose the interior of the bottle to light at a bactericidal wavelength of 254 nm for 20 minutes. The caps were placed approximately six inches under a germicidal UV lamp (UVP Multiple-Ray 8 Watt Laboratory Lamp) with a shortwave germicidal UV-C bulb (Sankyo Denki Ultraviolet Germicidal Lamp, Sankyo Denki Co., Japan) for 20 minutes. After sterilization, the bottles were capped and left in the laminar flow hood until use the next morning.

4 Results

The impact of several variables on the inactivation of *E. coli* by solar radiation and heating were tested. Table 4-1 summarizes the experiments that were conducted. Total disinfection time was varied from four to eight hours. Turbidity values ranging from no turbidity to approximately 200 ntu were tested. The effects of sample volume were also tested using 825 mL, 1.3 L, and 1.9 L sample volumes in 1 L, 1.5 L, and 2.0 L sample bottles, respectively. When using montmorillonite clay as an artificial turbidity source, the clay particles settled between sampling sessions. A natural water source with suspended solids was therefore used to test water with natural turbidity and organic matter. The number of experiments conducted to test each variable was dependent upon weather conditions. The results of these experiments are presented in the following sections. Tabulated results are presented in Appendix A (solar intensity readings) and Appendix C (enumeration data).

Table 4-1: Experimental variables.

Disinfection Scheme	Date	Sample Solution	Turbidity (ntu)	Bottle Volume
Solar radiation and heating	6 August 2002	PBS	0.15	1 L
	12 August 2002		0.18, 20.8, 107, 219	1 L
	14 August 2002		0.95, 17.2, 112	1 L
	27 August 2002		1.13, 18.5, 112	1 L
	5 September 2002		1.05, 17.4, 121	1 L
	10 September 2002		Not recorded - No turbidity added	1 L, 1.5 L, 2 L
	25 September 2002	PBS & Pond	0.81, 4.51, 9.68	1 L
Heating	18 December 2002	PBS	Not recorded - No turbidity added	1 L
	8 January 2003			
	29 January 2003			

4.1 Transmittance of Sunlight Through Bottles

The transmittance of sunlight though the plastic bottles was tested. Three different bottle sizes were used. Measurements were taken using the detector that measures solar radiation wavelengths below 400 nm. First, solar intensity readings were taken while the detector was laid flat. Readings were then taken while the detector was held overhead and aimed directly at the sun. Next, readings were taken with the detector placed inside each bottle when the bottle was laid flat. The bottle and detector were then held overhead to obtain a direct solar intensity reading. Last, the detector was removed from the bottle and another set of horizontal and direct solar intensity readings were taken to ensure sun conditions had not significantly changed during the bottle readings. Table 4-2 summarizes the results from this test. As seen by the readings, the plastic of each bottle interfered with the passage of solar light. The plastic reduced the intensity of sunlight on average by 0.9 J/cm^2 (27%) when measured horizontally, and 1.3 J/cm^2 (31%) when measured directly. Because transmittance readings were similar for the different bottles, it is likely that plastic thickness and composition was not a significant factor in the experiments. With the exception of the experiment on 10 September 2002, the 1 L Aquafina bottles were used in all experiments.

4.2 Exposure Time

A preliminary experiment was conducted to determine an exposure time needed for the solar radiation and heating experiments. The experiment was conducted on 6 August 2002 over eight hours, from 0830 to1640 hours. A control bottle and a test bottle were

Table 4-2: Sunlight intensity passing through plastic sample bottles.

Placement of Detector	Aquafina 1 L (J/cm^2)	Glaceau 1.5 L (J/cm^2)	Price Chopper 2.0 L (J/cm^2)
H – Sun	3.43	3.43	3.44
D – Sun	4.25	4.25	4.18
H – Inside bottle	2.50	2.57	2.40
D – Inside bottle	2.86	2.91	2.94
H – Sun	3.45	3.44	3.41
D – Sun	4.25	4.21	4.26

H = Detector/bottle placed horizontally.
D = Detector/ bottle aimed directly at sun.

prepared and spiked with *E. coli* to attain a starting concentration of 10^6 cfu/mL. The control bottle was kept in the dark at room temperature throughout the duration of the experiment to verify that the *E. coli* did not multiply or die. The test bottle was placed on the rooftop. Sampling was conducted and water temperature, air temperature, and solar radiation readings were taken at least every hour for the duration of the experiment.

The inactivation results in the test and control bottles are shown in Figure 4-1. The control bottle had a consistent *E. coli* count throughout the duration of the experiment. However, the plates from the test bottle showed abnormal growths approximately two hours into the experiment. There were approximately 1 to 9 unidentifiable growths on plates with 0 to up to 111 *E. coli* cfu. The abnormal growths were irregular and rhizoid in shape, had lobate edges, and a convex, smooth, dull surface. These growths were not noticed on plates prior to the two-hour mark. It is unknown if the abnormal growths were present in the test bottle before the experiment began or if the sample became contaminated during the experiment.

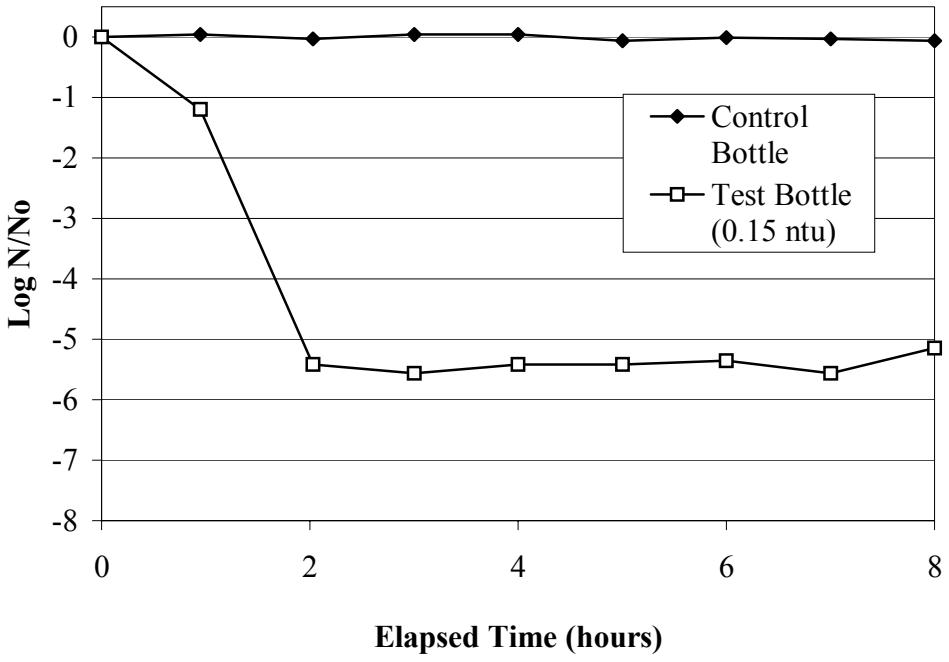


Figure 4-1: Log inactivation of *E. coli* on 6 August 2002 over an eight-hour exposure time.

Based on counts of colonies with characteristics of *E. coli,* there was a 1-log inactivation of *E. coli* bacteria after 1 hour of exposure. All plates after the two-hour mark did not have any growths that were positively identified as *E. coli* growths. Taking this into account, approximately 5 to 6 log inactivation of *E. coli* was achieved after 2 hours of exposure time. The maximum temperature achieved in the test sample was 33°C, which was reached within 5 hours of exposure time. Based on the results of this experiment, the duration of most experiments was reduced to approximately 4 hours, from 0900 to 1300 hours. For this experiment, the bottles had been sterilized by setting the bottles under the germicidal UV lamp for 15 minutes, then the interiors were rinsed 3 times with 70% ethanol and 3 times with autoclaved Epure water. Due to the presence of the abnormal

growths, the bottle sterilization process was then altered to the procedure specified in Section 3.2.7. The unidentified growths were not significant in subsequent experiments.

4.3 Turbidity Variations

Solar disinfection tests were conducted to compare its effectiveness on low turbidity samples versus high turbidity samples. Figure 4-2 shows the log inactivation of *E. coli* for a no turbidity sample and 20.8 ntu, 107 ntu, and 219 ntu samples over an eight-hour exposure time on 12 August 2002.

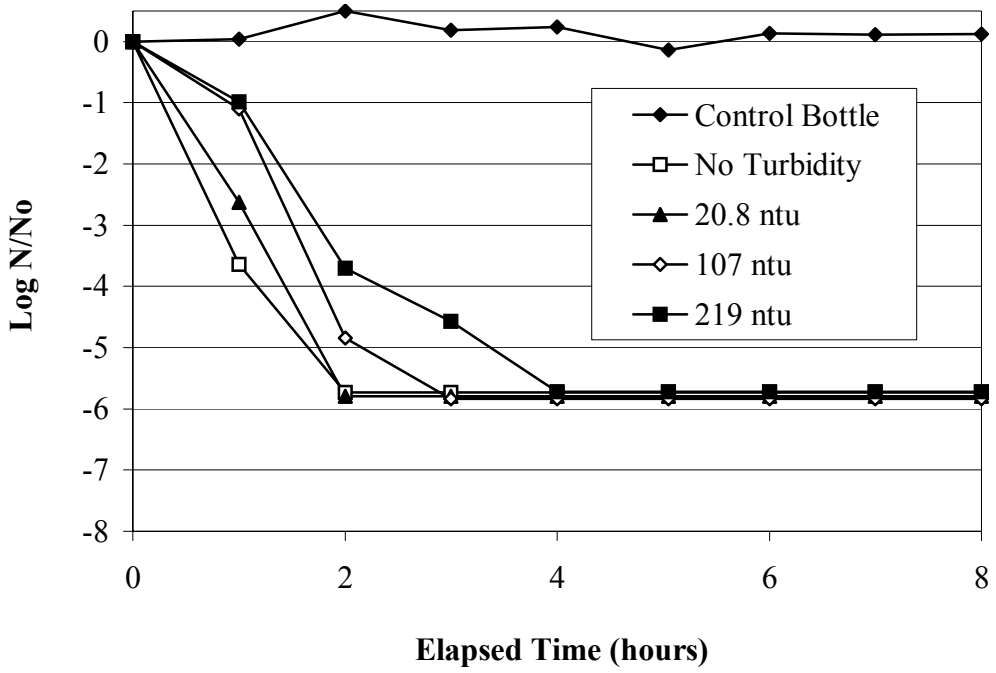


Figure 4-2: Log inactivation of *E. coli* on 12 August 2002 for varying turbidity samples versus time.

In general, it took longer for the higher turbidity samples to achieve the same log inactivation as the lower turbidity samples. After 2 hours of exposure time, the 219 ntu sample had a 3.5-log inactivation of *E. coli* while the 20.8 ntu sample had almost 6-log inactivation. However, all samples had achieved complete inactivation (no detectable counts) of *E. coli* within a 4-hour time span. Therefore, it appears that turbidity is not a significant variable when values range from approximately 0 to 200 ntu, given that the samples are exposed to sunlight for at least 4 hours. If drinking water samples are left in full sunlight from sunrise to midday, this treatment time requirement will be met.

As shown in Table 4-3, the maximum temperature achieved in the sample with no added turbidity was 45°C; the sample had been exposed to the sun for 7 hours at this time. Both the 107 ntu sample and the 219 ntu sample reached 46°C in 7 hours. The 20.8 ntu sample reached a maximum temperature of 45°C in 6 hours. The rises in water temperatures correlate to rises in air temperature, although the air temperature began to decrease after 6 hours of exposure time and the water temperatures still increased slightly. This may possibly be attributed to a release of heat by the turbidity particles. However, the differences in temperature between the different samples was 1°C or less and therefore was not considered significant.

The 20.8 ntu sample had a 5.7-log inactivation of *E. coli* after 2 hours of exposure time, when the water temperature had reached 37.0°C. The 107 ntu sample achieved the same inactivation after 3 hours of exposure (reaching 41.5°C). Complete inactivation in the 219 ntu sample was achieved after 4 hours of exposure when the sample achieved a

Table 4-3: Temperature (°C) achieved in test samples on 12 August 2002.

Elapsed Time (hours)	Sample Temperature (°C)				Air Temperature (°C)
	0.179 ntu	20.8 ntu	107 ntu	219 ntu	
0	23.5	23.5	23.5	23.5	NR
1	NR	31.5	31.5	32.0	27.5
2	36.0	37.0	38.0	38.0	33.0
3	NR	410	41.5	41.5	35.5
4	NR	44.0	44.5	44.5	36.5
5	44.0	44.5	45.0	45.5	38.0
6	NR	45.0	45.5	45.5	39.0
7	45.0	45.0	46.0	46.0	36.5
8	43.5	43.5	43.0	43.0	35.5

NR = Not Recorded.

temperature of 44.5°C. Due to the difference between temperatures of the samples when they reached complete inactivation of *E. coli*, it is likely that temperature was not a significant factor in disinfecting the samples. This conclusion is further supported by the heating-only experiments described in Section 4.7, in which less than 0.5-log inactivation was reached with temperatures up to 46.0°C.

The experiment described above shows that the 107 ntu sample and the 219 ntu sample both required more time to achieve complete inactivation than the 20.8 ntu sample. This conclusion was used to design the remaining experiments. All experiments following the 12 August 2002 experiment were conducted on samples with a maximum turbidity level of approximately 100 ntu. After the preliminary experiments testing turbidity differences (0-200 ntu) on 12 August 2002, three additional experiments were conducted on three separate days to further examine the effect of turbidity as well as to examine the effect of

varying sunlight conditions. The sample turbidities were approximately 0 ntu, 20 ntu, and 100 ntu and the experiments were conducted on 14 August, 27 August, and 5 September 2002. Figure 4-3 presents the results of one of these experiments (conducted on 14 August 2002), showing the impact of turbidity on *E. coli* inactivation over a 4-hour sunlight exposure time. After 1 hour of exposure to sunlight, there was a 4.6-log, 2.2-log, and a 1.1-log inactivation of *E. coli* in the 0.95, 17.2, and 112 ntu turbidity samples, respectively. Three hours was needed to achieve 7-log inactivation in the higher turbidity samples, compared to only 1 hour in the no turbidity sample. Similar results were found during the other two experiments conducted on 27 August and 5 September 2002 (data shown in Appendix C).

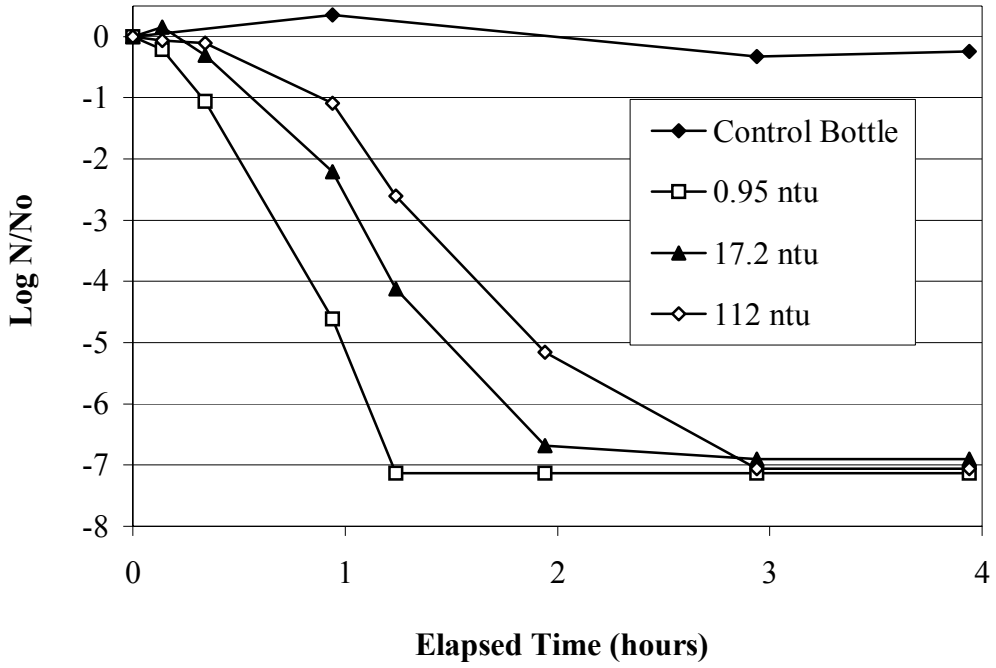


Figure 4-3: Log inactivation of *E. coli* on 14 August 2002 for varying turbidity samples versus time.

4.4 Solar Radiation Variations

The three experiments described in Section 4.3 were conducted on three separate days, which varied in sun and cloud conditions. Therefore, the data from the experiments was used to evaluate the impact of solar intensity on inactivation. The inactivation results in the samples with approximately 20 ntu turbidity are presented in Figure 4-4. For these experiments, sample conditions were kept as consistent as possible, with the only differences being in weather: sunlight intensities, air temperatures, and water temperatures. The cumulative intensity of wavelengths below 400 nm needed to achieve complete inactivation (no detectable counts) varied for the three days. The intensities were 58.4 J/cm^2 (14 August), 70.3 J/cm^2 (27 August), and 99.8 J/cm^2 (5 September).

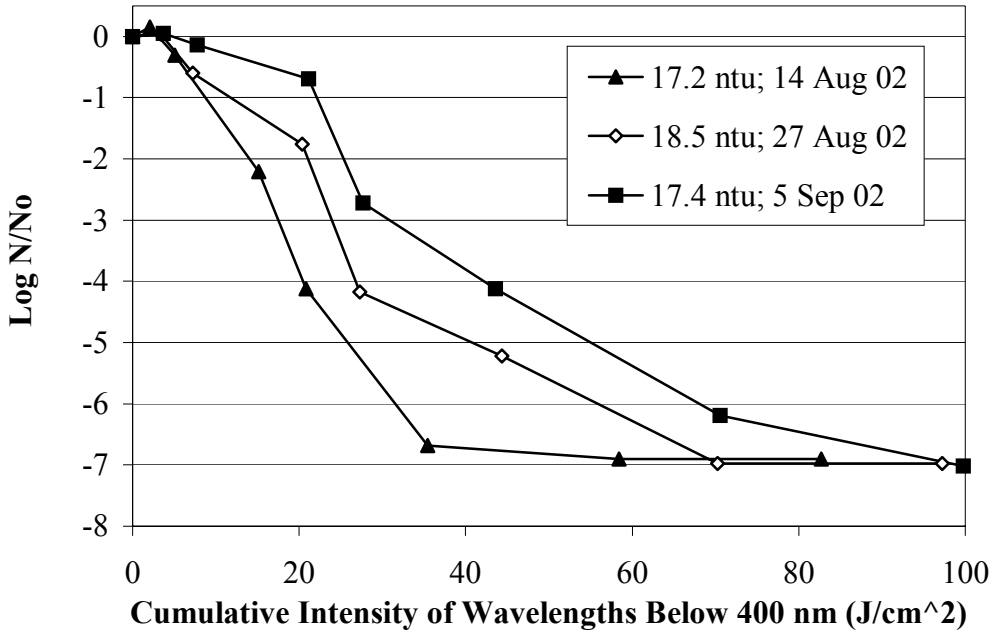


Figure 4-4: Comparison of similar turbidity samples tested on three different days.

The notable differences in the three days lay in the fact that there was lower cumulative sunlight intensity on 14 August. However, temperatures were highest on this day, and the air temperature reached 37.5°C. Figure 4-5 shows the water temperatures recorded throughout the course of each experiment. The water temperatures were the highest on 14 August, with a maximum of 46.0°C. A maximum air temperature of 31.0°C and water temperature of 38.0°C were reached on 27 August 2002. On 5 September 2002, the air temperature maximum was 25.0°C with a high water temperature of 32.0°C.

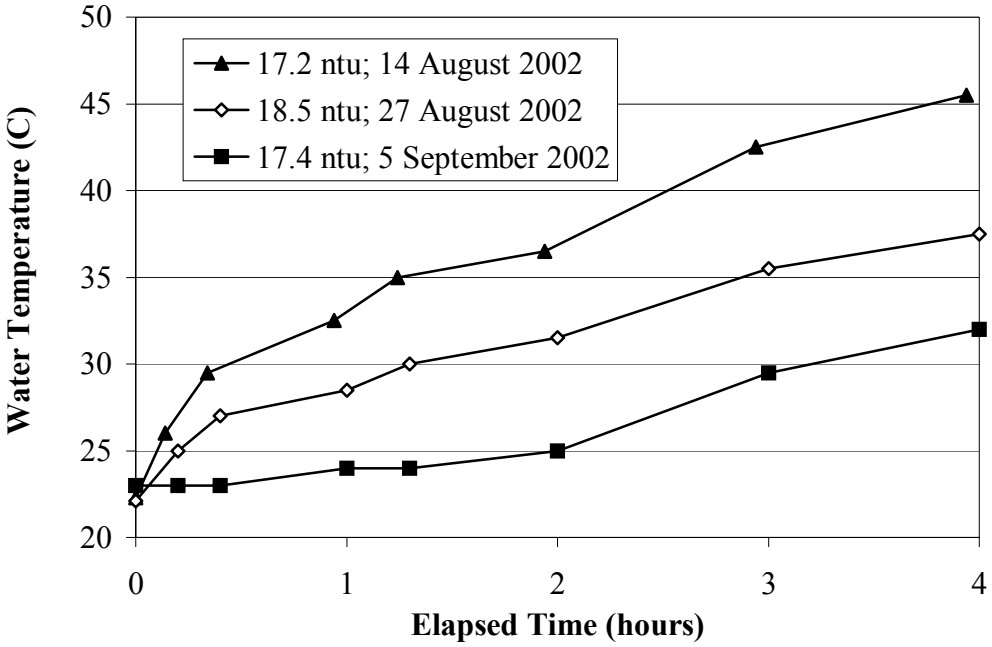


Figure 4-5: Temperature variances for similar turbidity samples on three experiment days.

In analyzing the results of the three experiments described above (see data in Appendix A), it was found that the minimum fluence of wavelengths below 400 nm required for approximately 7-log inactivation of *E. coli* in a sample with no added turbidity was 20.8, 70.3, and 70.5 J/cm^2 (on 14 August, 27 August, and 5 September 2002, respectively). Samples with approximately 20 ntu on these same days required a fluence of 58.4, 70.3, and 99.8 J/ cm^2 for 7-log inactivation; 100 ntu samples also had 7-log inactivation after fluences of 58.4, 97.2, and 99.8 J/ cm^2. The wide ranges of the minimum solar radiation doses required to disinfect the samples may be explained by the weather that characterized each day. On 14 August 2002, the sky was cloudless although very hazy. This day had the lowest recorded solar intensities, and the smallest fluences required for complete inactivation of *E. coli* in each bottle. Higher water temperatures and higher air temperatures were achieved on this day due to consistently sunny conditions during the experiment. In contrast, there was up to 40% cloud coverage on 27 August and approximately 60% cloud coverage on 5 September 2002. Average intensity readings (average of full sunshine and cloud cover) were calculated for these two days; however, actual intensities reaching the bottles could have varied throughout the day due to variations in cloud coverage.

4.5 Volume Variations

An experiment was conducted on 10 September 2002 to determine the relationship between sample volume and log inactivation. An 825 mL sample in a 1.0 L bottle, a 1.3 L sample in a 1.5 L bottle, and a 1.9 L sample in a 2.0 L bottle were tested. The sample bottles were not completely filled with solution because, in order to ensure the

test solutions did not become contaminated, the bottle openings needed to be flamed both before and after removing samples. Had the bottles been completely filled, the test solution would have spilled out while attempting to flame the openings. Previous researchers have also recommended filling the bottles only ¾ full to increase the dissolved oxygen in the water to be treated by shaking (EAWAG, 2003). Although the impact of dissolved oxygen on the solar disinfection process was not evaluated, the air space in the bottle allowed for oxygen equilibrium with the sample solution.

It was hypothesized that the larger volume samples would take longer for inactivation than the smaller sample volumes due to the larger bottle diameters and potential absorbance of solar radiation by the uppermost portion of water. The results are shown in Figure 4-6. All samples had similar log inactivation rates of *E. coli* after three hours of exposure time. However, there were differences in inactivation rates with shorter exposure times. For example, at 1 hour, the 1.5-L bottle had 3.3-log inactivation, compared to 1.3- and 2.0-log inactivation in the 1.0-L and 2.0-L bottles, respectively. The 1.5-L bottle sample had the quickest inactivation, with 7-log inactivation in 2 hours of exposure time compared to the 3 hours of exposure time required in the 1.0 and 2.0-L samples. However, only diluted samples of the 1.5 L sample bottle were plated after the 2-hour exposure time. Any bacteria that were still viable may therefore have been diluted beyond detection. Inactivation rate differences between the three sample bottles may also be explained by the differences in plastic thickness. A bottle made with thinner plastic might compensate for a larger sample volume by allowing a greater percentage of wavelengths to penetrate. However, as discussed in Section 4.1, it is not likely that the

plastic thickness had a significant impact on the disinfection process. It is more likely that the water depths were similar enough that the passage of sunlight through the samples was similar for all three bottles, given long enough exposure time.

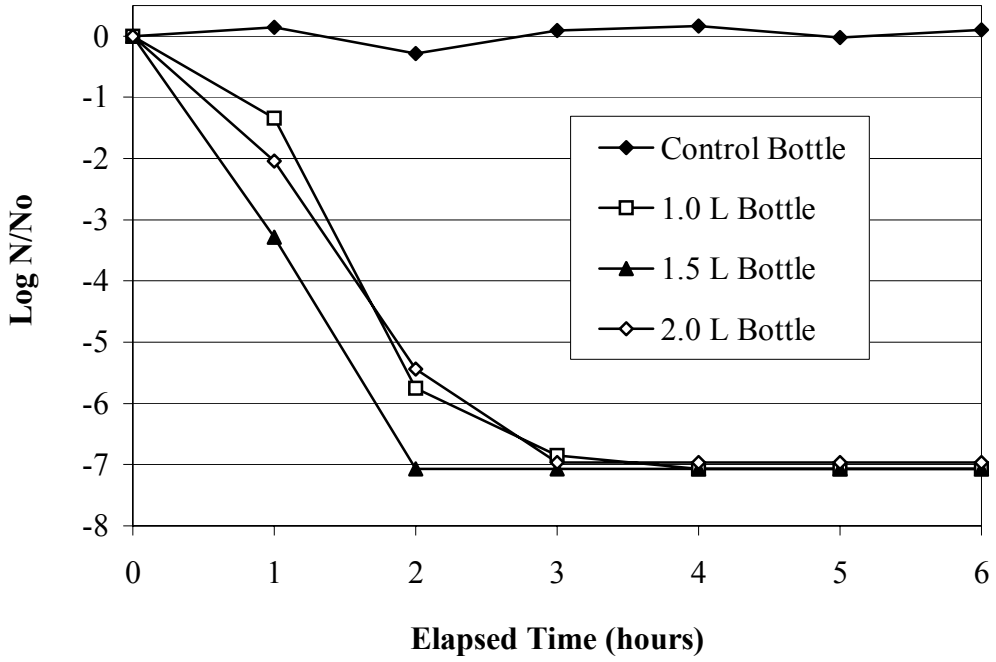


Figure 4-6: Log inactivation using 1.0-, 1.5-, and 2.0-L bottles.

4.6 Natural Water

An experiment was conducted on 25 September 2002 to compare the results of using PBS as a test solution to that of using pond water. Measurements and visual observations were made first in order to characterize the pond water. The pond water's non-purgeable organic carbon content was tested. Total organic carbon and dissolved organic carbon, important components of the carbon cycle, were measured as indicators of water quality.

Their values are influenced by vegetation and climate, among other factors, and have significant influences on biogeochemical processes, nutrient transportation, and chemical reactions. The pond water had an average dissolved organic carbon concentration of 7.04 mg/L and a total organic carbon concentration of 7.90 mg/L. The range of TOC values typically found in surface waters is 1 – 20 mg C/L (AWWA, 1999). The use of pond water in this experiment was also chosen because of the natural turbidity found in such samples. In using a natural water source, the test focus was to determine whether suspended turbidity particles interfered with the solar disinfection process.

This experiment relates the results from previous experiments, in which PBS was the test solution, to the expected results when using natural water. The experiment was conducted using 1 L sample bottles. The first sample solution was PBS; the second sample was 50% PBS and 50% pond water; and the third sample was pond water only. The temperature of the pond water only sample was slightly, though consistently, higher than both the PBS solution and the combined sample solution (see Appendix D for tabulated results). From 0 to 42.5 J/cm^2, all samples achieved increasing inactivation, yet there was up to 1.92-log difference between the samples at 26 J/cm^2. However, what is significant is that all three samples had similar results for the log inactivation of *E. coli* given exposure greater than 42.5 J/cm^2. As seen in Figure 4-7, all three samples achieved approximately 7-log inactivation of *E. coli* after exposure to a cumulative intensity of 62.2 J/cm^2 of wavelengths below 400 nm.

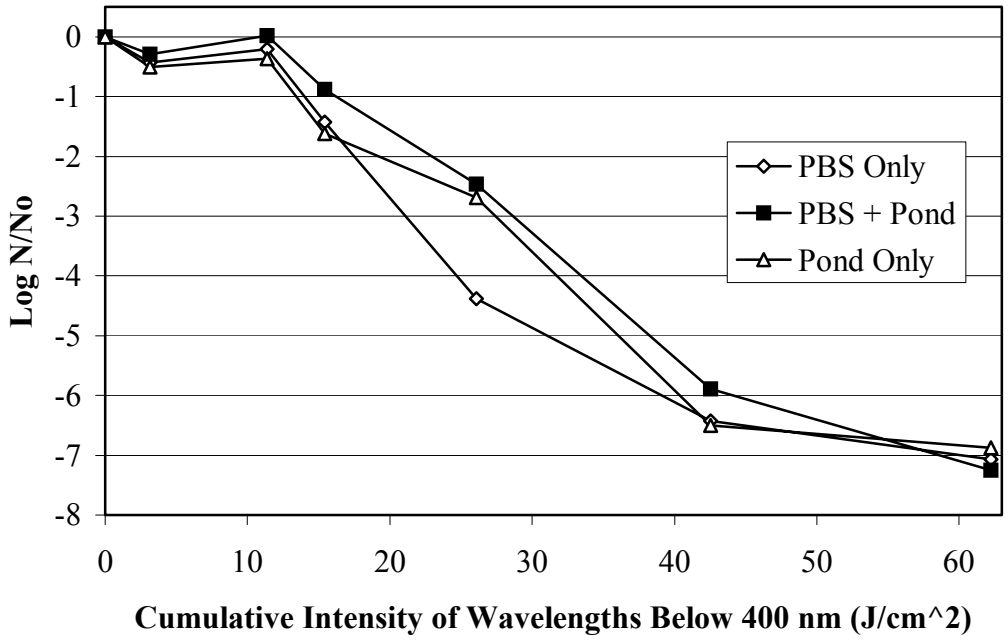


Figure 4-7: Log inactivation of samples versus cumulative sunlight intensity.

Initial turbidity readings were taken before the start of the experiment. The PBS only sample had a turbidity of less than 1 ntu; the combined sample had a turbidity of 8.42 ntu; and the pond water sample had a turbidity of 18.7 ntu. After 30 minutes of letting the samples stand, the turbidities of the combined sample and the pond water sample were read again. Each sample had an approximately 50% drop in turbidity due to a settling of particles: the combined sample was at 4.51 ntu and the pond water sample was at 9.68 ntu. The fact that the turbidity readings were less than 10 ntu, and therefore turbidity was not a significant factor in the results (EAWAG, 2003), is most likely the reason the inactivation results were similar for all three samples.

4.7 Heating Only Experiments

Three replicate experiments were conducted to determine the effects of heating on the inactivation of *E. coli*. A control bottle was prepared with PBS and spiked with *E. coli.* The bottle was kept in the dark at room temperature throughout the duration of each experiment. A test bottle was prepared using PBS spiked with *E. coli*. Light interference could possibly affect the inactivation of the bacteria and was therefore eliminated by wrapping the test bottle in aluminum foil. The temperature of the test bottle was controlled using a water bath.

Figure 4-8 displays the results of these experiments. There was less than 0.5 log inactivation or growth of *E. coli* in each experiment for any given temperature. Therefore, heating the samples to 46°C in the absence of sunlight did not reduce *E. coli* concentrations. Inactivation of *E. coli* when samples were exposed to the effects of solar radiation and heating can therefore be attributed to the bactericidal effects of solar radiation or the synergistic effects of irradiation and heating.

4.8 Conclusion

In general, sample volume was found to be an insignificant factor in sample bottles up to 2 L when samples were exposed to 3 hours of sunlight. Sample water with turbidity values from <1 ntu to slightly more than 200 ntu have similar log inactivation with 4 hours of exposure time. The following chapter provides a more specific discussion of the results obtained during these experiments.

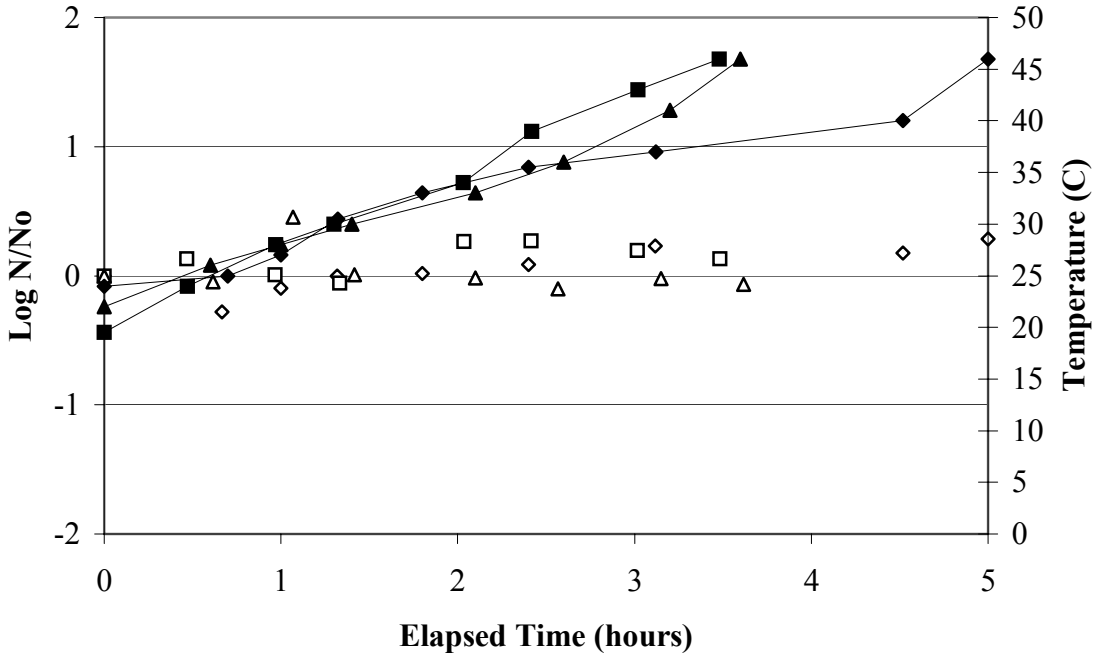


Figure 4-8: Results of heating-only experiments on *E. coli* inactivation.

(Open symbols show log-inactivation versus time; closed symbols display increases in temperature over time. Experiment was conducted in triplicate.)

5 Conclusions

This thesis research was conducted to study the effects of numerous variables on the disinfection properties of solar radiation and heating. The variables tested were turbidity, sample volume, and exposure time. The samples were exposed to sunlight during sunny or partly sunny days during the summer and early fall. Experiments were also conducted in the laboratory to quantify the effect of only heating a sample. In analyzing the results from these experiments, the following conclusions were drawn:

1. Exposure to wavelengths below 400 nm at a minimum intensity of 20.8 J/cm^2 resulted in a 7-log inactivation of *E. coli* bacteria in a sample with no turbidity on 14 August 2002. This was possible with an exposure time of approximately 2 hours. However, it is recommended that samples be exposed to direct sunlight for at least 4 hours because of all the experiments conducted on samples with no turbidity, there were no detectable counts after 4 hours of exposure time.

2. The *E. coli* bacteria were completely inactivated in samples ranging from 0 ntu to 219 ntu with 4 hours of sunlight exposure time. If drinking water samples with turbidity values below 219 ntu are exposed to sunlight during the course of an entire day, there is no significant difference in bacterial inactivation levels due to interference of turbidity particles.

3. Samples in 1.0 L, 1.5 L, and 2.0 L bottles experienced similar *E. coli* disinfection rates with exposure times of 3 hours. Water volumes from 1 to 2 L can therefore be

treated in approximately the same amount of time, given that plastic thickness does not interfere with the passage of solar radiation to the water sample.

4. The results from an experiment comparing the use of PBS, 50% PBS and 50% pond water, and pond water as test solutions showed that all sample solutions experienced similar inactivation rates. All three sample solutions had approximately 7-log inactivation of *E. coli* in 4 hours of exposure time. Therefore, source water with properties similar to the pond water used in this research can be disinfected with solar disinfection.

5. A fluence of wavelengths below 400 nm must be at least 20.8 J/cm^2 for a 7-log inactivation of *E. coli* in samples with approximately 0 ntu. Samples with up to 100 ntu require a fluence of 58.4 to 99.8 J/cm^2 for the same inactivation. A cumulative intensity of 99.8 J/cm^2 is therefore sufficient for a 7-log *E. coli* inactivation in samples ranging from no turbidity up to 100 ntu. The intensity of sunlight must be evaluated on an individual site-specific basis to ensure that the necessary treatment dose is met.

6. Temperatures up to 46°C have no significant effect on the disinfection of *E. coli* bacteria. The inactivation properties of solar disinfection are therefore due to its solar radiation component, or the synergistic effects of sunlight and heat.

6 Recommendations

Due to limited resources and time, the following section details recommendations for future research.

1. A pond water sample with natural turbidity was tested, yet the turbidity level was less than 10 ntu. Because the turbidity settled, it is recommended that water samples with higher levels of suspended particles be tested. The interference of the particles can therefore be studied more extensively.

2. *E. coli* is a common test organism and inactivation results correlate to the effects of solar disinfection on other organisms such as pathogenic bacteria. However, it is recommended that solar disinfection tests be conducted on organisms other than bacteria. For example, studies are lacking on the effects of solar disinfection on spore-forming organisms and protozoa.

3. The plates from the test bottles had abnormal growths during the initial testing phases. The organisms did not have the characteristic appearance of *E. coli* and were not positively identified. It is likely that the organisms had adhered to the interior of the test bottles and were not inactivated during the alcohol washing and UV treatments. Studies should be conducted to determine whether ingestion of these organisms is harmful and whether inadequate disinfection of the bottles before use could possibly be a downfall of the procedure.

4. The bottles used for the experiments were bought in a local grocery store in the United States. While most plastic bottles are made from similar materials, certain areas in other parts of the world may only supply plastic bottles that are colored or that are differently shaped. Before implementing this procedure for use, an evaluation must be made regarding the size and characteristics of bottles that can be found in the local area. Tests should then be conducted to determine whether these bottles are suitable for use in solar disinfection. This will also allow the researchers to test whether the local water is adequately treated with the process.

BIBLIOGRAPHY

Acra, A.; Raffoul, Z.; Karahagopian, Y. 1984. Solar disinfection of drinking water and oral rehydration solutions: guidelines for household application in developing countries. Department of Environmental Health. Beirut.

(APHA) APHA; AWWA; WEF. Standard Methods for the Examination of Water and Wastewater, 20th edition. 1998. APHA, Washington, D.C.

(AWWA) American Water Works Association. 1999. Water quality and treatment: A handbook of community water supplies, 5th ed.

Associated Press. 2001. UN promoting process to clean water. Environmental News Network.

Burch, J. and Thomas, K. 1998. Water disinfection for developing countries and potential for solar thermal pasteurization. *Solar Energy*. 64 (1-3), 87-97.

Ciochetti, D. and Metcalf, R. 1984. Pasteurization of naturally contaminated water with solar energy. *Appl Environ Microbio*. 47 (2), 223-228.

Conroy, R.; Elmore-Meegan, M.; Joyce, T.; McGuigan, K.; Barnes, J. 1996. Solar disinfection of drinking water and diarrhoea in Maasai children: a controlled field trial. *Lancet*. 348, 1695-1697.

Conroy, R.; Elmore-Meegan, M.; Joyce, T.; McGuigan, K.; Barnes, J. 1999. Solar disinfection of water reduces diarrhoeal disease: an update. *Arch Dis Child*. 81, 337-338.

Conroy, R.; Elmore-Meegan, M.; Joyce, T.; McGuigan, K.; Barnes, J. 2001. Solar disinfection of drinking water protects against cholera in children under 6 years of age. *Arch Dis Child*. 85 (4), 293-295.

Davies, C. and Evison, L. 1991. Sunlight and the survival of enteric bacteria in natural waters. *J Appl Bacteriol*. 70, 265-274.

(EAWAG) Swiss Federal Institute for Environmental Science and Technology, Department of Water and Sanitation in Developing Countries. 2003. SODIS: Solar water disinfection. Accessed from the World Wide Web, 19 March 2003: www.sodis.ch

Ellis, K. 1991. Water disinfection: a review with some consideration of the requirements of the third world. *Crit Rev Environ Control*. 20 (5, 6), 341-407.

Fujioka, R. and Siwak, E. 1985. The cidal effect of sunlight on alternative microbial indicators of water quality. *Proceedings of the AWWA Water Quality Technology Conf*, Houston, TX, December 8-11, 1985.

Gadgil, A. 1998. Drinking water in developing countries. *Annu Rev Energy Environ*. 23, 253-286.

Hockberger, P. 2000. The discovery of the damaging effect of sunlight on bacteria. *J Photochemistry and Photobiology*. 58, 185-191.

IDRC International Development Research Center—Science for Humanity. 1998. Water disinfection using solar radiation. Ottawa, Canada. 13 March 1998.

Jorgensen, A.; Nohr, K.; Sorensen, H.; Boisen, F. 1998. Decontamination of drinking water by direct heating in solar panels. *J Appl Microbiol*. 85, 441-447.

Joyce, T.; McGuigan, K.; Elmore-Meegan, M.; Conroy, R. 1996. Inactivation of fecal bacteria in drinking water by solar heating. *Appl Environ Microbiol*. 62 (2), 399-402.

Kapuscinski, R. and Mitchell, R. 1981. Solar radiation induces sublethal injury in *Escherichia coli* in seawater. *Appl Environ Microbiol*. 40 (3), 670-674.

Kehoe, S.; Joyce, T.; Ibrahim, P.; Gillespie, J.; Shahar, R.; McGuigan, K. 2001. Effect of agitation, turbidity, aluminum foil reflectors and container volume on the inactivation efficiency of batch-process solar disinfectors. *Wat Res*. 35 (4), 1061-1065.

Khammo, N.; Bartlett, A.; Clothier, R.; Whitfield, P. 2001. The attachment of *Schistosoma mansoni* cercariae to human skin cells. *Parasitology*. 124, 25-30.

McGuigan, K.; Joyce, T.; Conroy, R. 1999. Solar disinfection: use of sunlight to decontaminate drinking water in developing countries. *J Med Microbiol*. 48, 785-787.

Reed, R.; Mani, S.; Meyer, V. 2000. Solar photo-oxidative disinfection of drinking water: preliminary field observations. *Lett Appl Microbiol*. 30, 432-436.

Rijal, G. and Fujioka, R. 2001. Synergistic effect of solar radiation and solar heating to disinfect drinking water sources. *Wat Sci Technol*. 43 (12), 155-162.

Robins, M. 2000. Safe to drink. *New Scientist*. 167 (2253), 14.

Safapour, N. and Metcalf, R. 1999. Enhancement of solar water pasteurization with reflectors. *Appl Environ Microbiol*. 65 (2), 859-861.

Snyder, J. and Merson, M. 1982. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. *Bull WHO*. 60 (4), 605-613.

Sommer, B.; Marino, A.; Solarte, Y.; Salas, M.; Dierolf, C.; Valiente, C.; Mora, D.; Rechsteiner, R.; Setter, P.;Wirojanagud, W.; Aharmeh, H.; Al-hassan, A.; Wegelin, M. 1997. SODIS – An emerging water treatment process. *J Wat Suppl Res Technol – AQUA*. 46 (3), 127-137.

Somboonsub, W. 2001. Solar disinfection of rainwater in Thailand. Accessed from the World Wide Web, 18 October 2001: www.sodis.ch

Toranzos, G.A. and G.A. McFeters. 1997. Detection of indicator microorganisms in environmental freshwater and drinking water. *Manual Env. Micro.*

Wegelin, M.; Canonica, S.; Mechsner, K.; Fleischmann, T.; Pesaro, F.; Metzler, A. 1994. Solar water disinfection: scope of the process and analysis of radiation experiments. *J Water Suppl Res Technol -- AQUA*. 43 (3), 154-169.

(WHO) World Health Organization. 1997. Guidelines for drinking-water quality, 2nd ed.

Appendix A

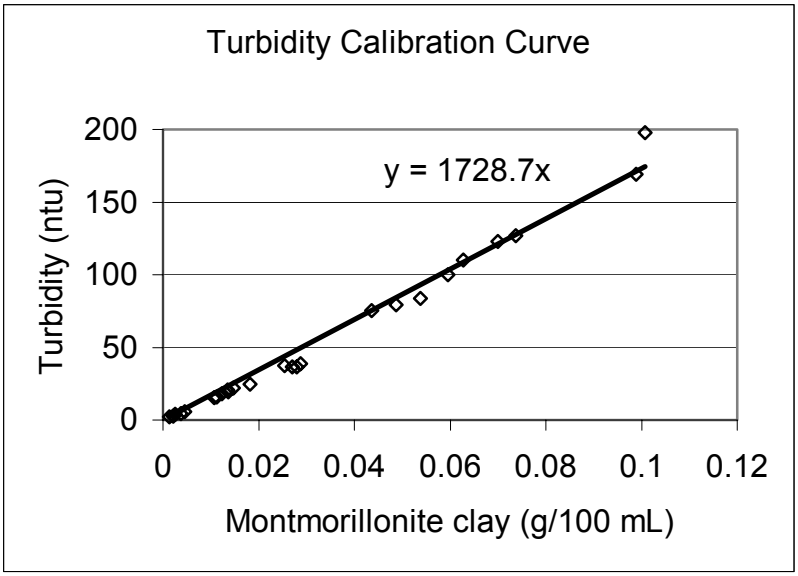
Tabulated direct solar intensity readings (mW/cm^2) for solar radiation and heating experiments. Cumulative intensity readings were calculated by multiplying average direct solar intensity readings by time (seconds) to get J/cm^2.

Date	Time	Intensity Readings of Solar Wavelengths Below 315 nm (mW/cm^2)	Cumulative Intensity of Solar Wavelengths Below 315 nm (J/cm^2)	Intensity Readings of Solar Wavelengths Below 400 nm (mW/cm^2)	Cumulative Intensity of Solar Wavelengths Below 400 nm (J/cm^2)
6-Aug-02	830	0.037	0.000	1.917	0.000
	925	0.077	0.325	2.63	12.959
	1033	0.128	0.989	3.7	33.468
	1130	0.172	1.862	4.39	57.010
	1230	0.17	2.888	4.56	83.860
	1330	0.129	3.785	4.56	111.220
	1430	0.138	4.586	4.26	137.680
	1530	0.123	5.369	3.77	161.770
	1630	0.072	5.954	2.4	180.280
12-Aug-02	840	0.048	0.000	2.044	0.000
	940	0.092	0.420	3.09	15.402
	1040	0.129	1.083	3.7	35.772
	1140	0.139	1.887	3.84	58.392
	1240	0.15	2.754	3.57	80.622
	1345	0.131	3.639	3.75	103.680
	1440	0.12	4.355	3.54	124.457
	1540	0.086	4.973	2.87	143.687
	1640	0.046	5.369	1.93	158.087
14-Aug-02	906	0.064	0.000	2.32	0.000
	920	0.07	0.056	2.53	2.037
	940	0.078	0.145	2.57	5.097
	1000	0.09	0.447	3.01	15.141
	1030	0.104	0.622	3.34	20.856
	1100	0.119	1.090	3.6	35.430
	1200	0.147	1.888	4.07	58.440
	1300	0.146	2.767	4.02	82.710
27-Aug-02	900	0.068	0.000	2.72	0.000
	920	0.078	0.088	2.90	3.372
	940	0.100	0.194	3.50	7.212
	1000	0.118	0.587	3.80	20.352
	1030	0.134	0.814	3.93	27.309
	1100	0.150	1.410	4.20	44.382
	1200	0.161	2.343	4.43	70.272
	1300	0.169	3.333	4.56	97.242

5-Sep-02	900	0.067	0.000	2.84	0.000
	920	0.085	0.091	3.26	3.660
	940	0.097	0.200	3.57	7.758
	1000	0.111	0.575	3.86	21.132
	1030	0.068	0.736	3.43	27.693
	1100	0.160	1.215	4.14	43.590
	1200	0.175	2.220	4.84	70.530
	1300	0.182	3.291	4.91	99.780
10-Sep-02	900	0.060	0.000	2.47	0.000
	1000	0.094	0.462	3.14	16.830
	1100	0.139	1.161	3.89	37.920
	1200	0.153	2.037	4.27	62.400
	1300	0.145	2.931	4.02	87.270
	1400	0.139	3.783	4.04	111.450
	1500	0.103	4.509	3.43	133.860
25-Sep-02	900	0.042	0.000	1.57	0.000
	930	0.055	0.087	1.94	3.164
	1000	0.064	0.337	1.97	11.375
	1030	0.081	0.468	2.53	15.424
	1100	0.086	0.818	2.55	26.092
	1200	0.104	1.388	2.93	42.532
	1300	0.132	2.096	3.64	62.242

Appendix B

Turbidity calibration curve using PBS and montmorillonite clay.



Montmorillonite clay (g/100 mL)	Clay Required for 800 mL Sample (g/800 mL)	Turbidity (ntu)
0.0254	0.2032	37.3
0.0046	0.0368	5.9
0.0013	0.0104	2.1
0.0026	0.0208	4.2
0.0107	0.0856	15.4
0.0022	0.0176	2.8
0.0137	0.1096	19.5
0.0038	0.0304	4.5
0.0270	0.2160	36.7
0.0135	0.1080	20.8
0.0182	0.1456	24.8
0.0280	0.2240	36.6
0.0287	0.2296	38.6
0.0147	0.1176	21.9
0.0112	0.0896	16.0
0.0123	0.0984	17.9
0.0487	0.3896	79.1
0.0700	0.5600	123.0
0.0737	0.5896	127.0
0.0988	0.7904	169.0
0.0595	0.4760	100.0
0.0538	0.4304	83.5
0.0628	0.5024	110.0
0.0436	0.3488	75.2
0.1008	0.8064	198.0

Appendix C

Results for all experiments.
Bolded numbers are inserted detection limits where no detectable counts had been found.
All counts are in cfu/mL.

Time	Elapsed Hours	A-*E. coli* Count	A-Log N/No	B-*E. coli* Count	B-Log N/No	C-*E. coli* Count	C-Log N/No	D-*E. coli* Count	D-Log N/No	E-*E. coli* Count	E-Log N/No
6 August 2002											
		Control		0 ntu							
830	0	556667	0.00	910000	0						
925	0.95	615000	0.04	57500	-1						
1033	2.03	515000	-0.03	4	-5						
1130	3	620000	0.05	3	-6						
1230	4	620000	0.05	4	-5						
1330	5	485000	-0.06	4	-5						
1430	6	550000	-0.01	4	-5						
1530	7	515000	-0.03	3	-6						
1630	8	485000	-0.06	7	-5						
12 August 2002											
		Control		0 ntu		20.8 ntu		107 ntu		219 ntu	
840	0	433333	0.00	540000	0.00	625000	0.00	690000	0.00	525000	0.00
940	1	475000	0.04	123	-3.64	1463	-2.63	54667	-1.10	54000	-0.99
1040	2	1366667	0.50	**1**	-5.73	**1**	-5.80	10	-4.84	105	-3.70
1140	3	660000	0.18	**1**	-5.73	**1**	-5.80	**1**	-5.84	14	-4.57
1240	4	745000	0.24	**1**	-5.73	**1**	-5.80	**1**	-5.84	**1**	-5.72
1345	5	313333	-0.14	**1**	-5.73	**1**	-5.80	**1**	-5.84	**1**	-5.72
1440	6	590000	0.13	**1**	-5.73	**1**	-5.80	**1**	-5.84	**1**	-5.72
1540	7	565000	0.12	**1**	-5.73	**1**	-5.80	**1**	-5.84	**1**	-5.72
1640	8	570000	0.12	**1**	-5.73	**1**	-5.80	**1**	-5.84	**1**	-5.72
14 August 2002											
		Control		0.95 ntu		17.2 ntu		112 ntu			
0906	0	10050000	0.00	13666667	0.0	8000000	0.0	11333333	0.0		
0920	0.14	omitted	omitted	8500000	-0.2	11500000	0.2	9833333	-0.1		
0940	0.34	omitted	omitted	1183333	-1.1	3933333	-0.3	8800000	-0.1		
1000	0.94	22500000	0.35	330	-4.6	49667	-2.2	923333	-1.1		
1030	1.24	omitted	omitted	**1**	-7.1	610	-4.1	27967	-2.6		
1100	1.94	omitted	-0.32	**1**	-7.1	2	-6.7	79	-5.2		
1200	2.94	4800000	-0.24	**1**	-7.1	**1**	-6.9	**1**	-7.1		
1300	3.94	5800000	0.00	**1**	-7.1	**1**	-6.9	**1**	-7.1		
27 August 2002											
		Control		1.13 ntu		18.5 ntu		112 ntu			
0900	0	9966667	0.00	6566667	0.0	9500000	0.0	8800000	0.0		
0920	0.2	omitted	omitted	8866667	0.1	10466667	0.0	7100000	-0.1		
0940	0.4	omitted	omitted	7133333	0.0	2370000	-0.6	5100000	-0.2		
1000	1	5733333	-0.24	28067	-2.4	165333	-1.8	1983333	-0.6		
1030	1.3	omitted	omitted	493	-4.1	640	-4.2	75667	-2.1		
1100	2	8300000	-0.08	16	-5.6	57	-5.2	1480	-3.8		
1200	3	2700000	-0.57	**1**	-6.8	**1**	-7.0	45	-5.3		
1300	4	8700000	-0.06	**1**	-6.8	**1**	-7.0	2	-6.7		
5 September 2002											
		Control		1.05 ntu		17.4 ntu		121 ntu			
0900	0	12900000	0.0	17766667	0.0	10366667	0.0	12066667	0.0		
0920	0.2	omitted	omitted	9666667	-0.3	11666667	0.1	9033333	-0.1		
0940	0.4	omitted	omitted	omitted	omitted	7466667	-0.1	6600000	-0.3		
1000	1	13600000	0.0	363333	-1.7	2116667	-0.7	9833333	-0.1		
1030	1.3	omitted	omitted	18800	-3.0	19767	-2.7	1273333	-1.0		
1100	2	15200000	0.1	85	-5.3	787	-4.1	omitted	-2.8		
1200	3	7700000	-0.2	3	-6.8	7	-6.2	303	-4.6		
1300	4	11833333	0.0	**1**	-7.2	**1**	-7.0	5	-6.4		
10 September 2002											
		Control		1.0 L		1.5 L		2.0 L			
0900	0	9133333	0.0	11733333	0.0	11666667	0.0	9200000	0.0		
1000	1	12833333	0.1	540000	-1.3	6067	-3.3	84667	-2.0		
1100	2	4700000	-0.3	21	-5.8	**1**	-7.1	33	-5.4		
1200	3	11366667	0.1	2	-6.8	**1**	-7.1	**1**	-7.0		
1300	4	13500000	0.2	**1**	-7.1	**1**	-7.1	**1**	-7.0		
1400	5	8666667	0.0	**1**	-7.1	**1**	-7.1	**1**	-7.0		
1500	6	11533333	0.1	**1**	-7.1	**1**	-7.1	**1**	-7.0		

25 September 2002											
		Control		PBS		PBS & Pond		Pond			
0900	0	11066667	0.0	11666667	0.00	11800000	0.00	7466667	0.00		
930	0.5	omitted	omitted	4366667	-0.43	6000000	-0.29	2300000	-0.51		
1000	1	6766667	-0.2	7333333	-0.20	12266667	0.02	3176667	-0.37		
1030	1.5	omitted	omitted	436667	-1.43	1546667	-0.88	180333	-1.62		
1100	2	7333333	-0.2	490	-4.38	40667	-2.46	15067	-2.70		
1200	3	8766667	-0.1	4	-6.43	15	-5.89	2	-6.51		
1300	4	8300000	-0.1	**1**	-7.07	1	-7.25	**1**	-6.87		
18 December 2002											
	Temperature	Control		Test Bottle							
0:00	24	9300000	0.0	8566667	0.0						
0:40	25	4900000	-0.3	8300000	0.0						
1:00	27	7466667	-0.1	8933333	0.0						
1:19	30.5	9233333	0.0	6633333	-0.1						
1:48	33	9666667	0.0	12666667	0.2						
2:24	35.5	11366667	0.1	9833333	0.1						
3:07	37	15833333	0.2	18500000	0.3						
4:31	40	13966667	0.2	10600000	0.1						
5:00	46	17933333	0.3	7933333	0.0						
8 January 2003											
	Temperature	Control		Test Bottle							
0:00	19.5	7333333	0.0	8466667	0.0						
0:28	24	9900000	0.1	9433333	0.0						
0:58	28	7466667	0.0	1900000	-0.6						
1:20	30	6433333	-0.1	11500000	0.1						
2:02	34	13433333	0.3	16700000	0.3						
2:25	39	13600000	0.3	9966667	0.1						
3:01	43	11500000	0.2	12333333	0.2						
3:29	46	9900000	0.1	8466667	0.0						
29 January 2003											
	Temperature	Control		Test Bottle							
0:00	22	11266667	0.0	11066667	0.0						
37:40	26	10100000	0.0	12900000	0.1						
1:04	28	32000000	0.5	15833333	0.2						
1:25	30	11433333	0.0	16133333	0.2						
2:06	33	10866667	0.0	10800000	0.0						
2:34	36	8866667	-0.1	12200000	0.0						
3:09	41	10733333	0.0	10300000	0.0						
3:37	46	9700000	-0.1	11400000	0.0						

Appendix D

Temperature readings during natural water experiment
(pond water, 50% pond water and 50% PBS, and PBS samples).

Time (hours)	Air Temperature (C)	PBS only	PBS & Pond	Pond only
		Water Temperature (C)		
0900	16.5	22.0	22.0	22.0
0930	18.0	21.5	22.0	22.0
1000	18.0	21.5	22.0	22.5
1030	20.0	22.0	23.0	23.5
1100	20.5	23.0	24.5	25.0
1200	23.0	25.0	26.0	27.0
1300	26.0	29.0	29.5	30.0