Exploring Pretreatments for the Solar Water Disinfection (SODIS) Process

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

The use of sunlight for water disinfection has been practiced since ancient times. Only in the last three decades has solar disinfection become widely recognized as a viable means of providing safe drinking water to the disadvantaged portion of the world's population. The World Health Organization estimates that 1.6 million people die every year because of waterborne diseases.

The Swiss Federal Institute of Environmental Science and Technology and their Department of Water and Sanitation in Developing Countries have been instrumental in propagating the solar water disinfection (SODIS) process in developing countries. The reason for this technology being widely used and accepted is its ease of use and effectiveness: water is placed in clear plastic bottles and exposed to direct sunlight for approximately six hours. The microorganisms in the water absorb the sunlight and it, in turn at sufficient UV dosages, causes mutations to their genetic material, inhibiting reproduction. Although some pathogens may still be viable they are no longer infective. The result is microbiologically safe water.

Research to date has explored everything from which colour and size the SODIS containers should be to whether adding catalysts to the water before exposure improves disinfection. Apart from a few studies that examined the effect of shaking the bottles (to entrain air) before exposure, there has been limited research on pretreatments for enhancing solar disinfection.

The focus of this project was to explore two pretreatments for SODIS and determine how they affect the efficiency of the process. The first stage was to examine one of the currently used pretreatments: cleaning the water containers before use. The second stage was to develop an accessible, low-cost filtration technique to remove particles from the water before exposure to sunlight. Particles in the water disperse the light and protect the microorganisms from being inactivated, so it is important to have as few particles as possible; the recommended upper limit is 30 NTU for solar disinfection. In many instances, surface water with high turbidity (greater than 200 NTU) serves as the only source for drinking water in developing areas.

The first series of experiments in the current research evaluated if cleaning the bottles was necessary and if so, which cleaning agents would be most effective and available. The agents selected were 70% isopropyl alcohol, a soap-water mixture, and lime juice. The experiments demonstrated that cleaning with 70% isopropyl alcohol did not affect the process in any way. Cleaning with the soap-water mixture did have a slightly negative effect on the process; there was substantial microbial recovery when bottles were kept in the dark overnight. In the case of the lime juice, it actually inhibited the disinfection process. It is necessary to remove any debris that might exist within the containers before using them, but using a chemical cleaning agent or mechanically scrubbing can decrease the amount of disinfection that occurs during SODIS. Thus, it is suggested that using a chemical pretreatment is not necessary and has the potential to inhibit disinfection, especially without proper training or technical knowledge.

The second series of experiments identified the optimal design for a low-cost roughing filter that could be used to remove particles from water before exposure to sunlight. The roughing filter that was built from the same plastic pop bottles used for solar disinfection, as well as gravel and sand. It was constructed with three centimetres of gravel on the bottom of the pop bottle and then 17 cm of coarse sand was added on top to make the total filter height 20 cm. A 0.6 mm hole was made at approximately 1.5 cm from the bottom of the bottle using a standard sewing needle. Each filter run consisted of 10 L of water at approximately 200 NTU. Experimental results indicated that 95% removal of turbidity could be achieved. These roughing filters can be constructed from readily available and affordable materials in developing countries and produce an effluent water quality of less than 30 NTU when initial turbidities are greater than 200 NTU.

Finally, the third series of experiments focused on testing the newly developed roughing filter in series with SODIS to evaluate the system as a whole. The results confirmed that using the roughing filter, as a pretreatment to SODIS, is a highly effective means of improving the disinfection potential of the process. These roughing filters produce an effluent water quality of less than 30 NTU, which is required for SODIS, making them a viable pretreatment for turbid water intended for SODIS use.

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LIST OF ACRONYMS

ANOVA – Analysis of Variance

CFU – Colony Forming Unit

DO – Dissolved Oxygen

EAWAG – The Swiss Federal Institute for Environmental Science and Technology

EDTA – Ethylenediaminetetraacetic Acid

HDPE – High Density Polyethylene

INRESA – Integrated Rural Energy Systems Association

LSD – Least Significant Difference

MAC – Maximum Acceptable Concentrations

MCL – Maximum Contaminant Levels

MPN – Most Probable Number

NTU – Nephelometric Turbidity Unit

ORS – Oral Rehydration Solution

PET – Polyethylene Terephthalate

SANDEC – EAWAG's Department of Water and Sanitation in Developing Countries

SODIS – Solar Water Disinfection

UV – Ultraviolet

WHO – World Health Organization

CHAPTER 1: INTRODUCTION

"Madzi ndi moyo, the Chewa say, 'water is life', an idea reflected in key elements of Nyau practice." Museum of Anthropology, UBC, Vancouver, BC.

1.1 The Problem

A global concern is the lack of safe drinking water. The World Health Organization (WHO) has estimated that approximately 1.1 billion people do not have access to an acceptable drinking water source and that 1.6 million people die annually due to diarrhoea caused by waterborne diseases (WHO, 2004; 2007). The majority of these people are children under five years of age. They tend to live in rural areas of developing nations where few resources are available for water treatment. There is a significant difference between drinking water treatment in this context and that of North America. As such, there must be a different approach to the solution of this problem.

1.1.1 North American Drinking Water Treatment

The purpose of drinking water treatment plants is to supply water that is biologically and chemically safe to consumers at a reasonable cost (Montgomery, 1985). The biological aspect focuses on the removal or inactivation of pathogens, which can include bacteria, protozoa, viruses, and helminths. Bacteria are the simplest organisms known to humans (Droste, 1997). They are only one cell. Although they come in different shapes and sizes, they are typically about 0.1 to 10 μm in diameter (Montgomery, 1985). Protozoa are also single cells. They are larger than bacteria (10 to 50 μm) and unique in that they are able to form a cyst or barrier around themselves, providing protection from harsh conditions (Droste, 1997). This characteristic makes them very difficult to inactivate or remove from water. Viruses have genetic material that allows them to take over a host cell in order to reproduce. They are smaller than bacteria, usually about 0.05 to 0.1 μm (Montgomery, 1985). Helminths are most commonly known as worms and vary in size; eggs are on the order of 100 μm or less, while adults can be up to 60 cm long (Gamma-Dynacare Medical Laboratories, 2008). Their eggs are generally large or dense enough to be removed during conventional treatment (Montgomery, 1985).

To remove these pathogens, as well as suspended solids (e.g., soil) and dissolved solids (e.g., salt), a North American drinking water treatment plant would typically use some combination of various operations and processes, as can be seen in Figure 1.1: screens, coagulation/flocculation, sedimentation, filtration, and disinfection (Droste, 1997). The screens remove any grit or large solids that may be suspended in the source water. Coagulation is the addition of one or more chemicals to the water to bring particles together and flocculation allows those particles to grow into larger groups of particles, known as flocs. After the flocs have been formed they settle out by gravity in a clarifier or sedimentation tank. During filtration, any small particles that are left suspended in the water are removed by separating them from the water by granular media (e.g., sand, anthracite, garnet), membranes, or cake filtration. Once all the particles have been removed, the water is disinfected in order to destroy any pathogens that may remain. Ultraviolet (UV) irradiation, chlorination, chloramination, or ozonation are commonly used to achieve disinfection.

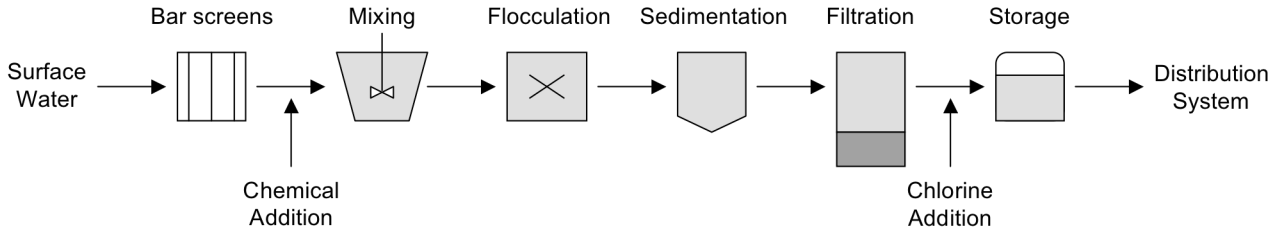

Figure 1.1: Conventional North American surface water treatment plant (adapted from Droste, 1997)

The water leaving the drinking water treatment plant must be tested to make sure it meets strict physical (e.g., turbidity), chemical (e.g., arsenic), and biological (e.g., coliforms) water quality standards. There are guidelines that outline the maximum acceptable concentrations (MAC) or maximum contaminant levels (MCL) of these properties and schedules for their sampling and testing (Health Canada, 2008; US EPA, 2008). To regularly provide high quality water to consumers, the plant must have specially trained staff, an orderly and well-maintained facility, enough capital for operation and maintenance costs, as well as resources for sampling and testing (Montgomery, 1985).

1.1.2 Drinking Water Treatment in Developing Nations

The immediate aim of drinking water treatment is to reduce the risk of disease to a tolerable level as estimated by log reductions in indicator organisms. It is a very laborious and expensive task to analyze

water for the presence and quantity of specific microorganisms, especially in developing countries (Droste, 1997). The methods currently used to identify the types of organisms present require either sophisticated equipment or a great deal of time (e.g., pour plate method). Thus, it is practical to select one or a few organisms that can warn of the presence of pathogens in the water. These indicators must meet certain criteria: be found in the digestive tract of humans and animals, easy to identify and enumerate, inexpensive to analyze, able to survive at least as long as the pathogens, high in concentration, and not pathogenic themselves (Droste, 1997).

Bacteria have been used as indicators for over a hundred years (Geldreich, 1978). More specifically, *Escherichia coli* (*E. coli*) represents the largest portion of the fecal coliforms and is typically selected as an indicator organism (Droste, 1997). "Zero *E. coli* per 100 mL is the [ultimate] goal for all water supplies" (WHO, 2006). The WHO Guidelines for Drinking Water Quality focus on health-based targets instead of minimum or maximum limits that are commonly used in North America. In developing countries only a few technologies may be available and it is not practical to use the treatment-based approach, the main idea being that a treatment that works in one area may not be available, acceptable, or even work at all in another area.

To estimate the extent to which the risk of disease has been reduced, samples are analysed to see how much *E. coli* is in a source (how many colonies would grow for every 100 mL of sample) and how much is left after treatment. The difference is represented as log reduction. For example, if a source had 10^6 CFU/mL (colony forming units per millilitre) and the treated water had 10^1 CFU/mL, then the efficiency of the treatment would be five-log reductions or 99.999% removal.

The WHO also encourages that multiple barriers (i.e., processes and/or operations to remove contaminants) be used for "drinking water safety … from catchment and source to its use by consumers" (WHO, 2006). At present there is an urgency to provide safe drinking water to the many that do not have access to it; the WHO and other organizations have recognized that alternative approaches are necessary in the meantime. These interim strategies may not be consistent with the multi-barrier approach, but they are quick, efficient, and affordable. The fact that most of the health burden is related to waterborne diseases has not been overlooked. The selected strategies focus mainly on pathogen destruction or removal at the household level and are usually adapted from point-of-use technologies that have been previously used successfully in developed countries (WHO, 2007). They

include: boiling or pasteurization, exposure to sunlight, UV irradiation, sedimentation, filtration, aeration, coagulation-flocculation or precipitation, adsorption, ion exchange, chlorination, chlorine dioxide, ozonation, iodination, acid or base treatment, silver or copper addition, and combined systems (WHO, 2007).

The WHO has compared each of these technologies using a variety of criteria (e.g., microbial regrowth potential in treated water, skill level and ease of use, sustainability). The full comparison of these technologies is available in Appendix 1. Only one technology, from this comparison, is highly effective and yet is available at no cost to its users: the solar water disinfection (SODIS) process.

1.2 Using Sunlight for Water Disinfection

A depiction of the solar water disinfection (SODIS) process can be seen in Figure 1.2. SODIS uses the light and heat from the sun to disinfect water. It requires the user to have a small glass or plastic container in the range of 0.5 to 2 L; it is assumed that users can scavenge discarded pop bottles (WHO, 2007). This technology is simple: wash the container, fill the container with water, place it in sunlight for about six hours[1] and the water is safe to drink afterwards (Solar Water Disinfection, 2002a). It is also efficient, affordable, sustainable, and becoming widely accepted.

Figure 1.2: SODIS uses the UVA radiation and thermal energy from the sun to disinfect water (Solar Water Disinfection, 2002b)

[1] Most disseminators of SODIS recommend six hours of exposure time, but research suggests five hours is sufficient (Brace Research Institute, 1988).

SODIS is ideal considering the circumstances of developing countries, but it has its limitations. The process efficiency depends on the surrounding environmental conditions, the containers, and the turbidity level of the source water. Currently, no cleaning agents are recommended for the washing step and it is not clear if this step is actually necessary. If the source water has high turbidity then another pretreatment step is necessary to ensure appropriate disinfection. Although there are different ways by which turbidity can be removed, none are specifically recommended to complement SODIS; ideally a method is needed that is as simple as SODIS and effective in providing low turbidity. Furthermore, the sun's intensity varies due to altitude, climate, and time of day. SODIS is only recommended for use between the latitudes of 35° North and South of the equator, due to the radiation being highest in this region (see Appendix 2.3). The author suspects that SODIS could be used outside of this region at least during parts of the year.

1.3 Objectives

The goals of this research are to explore pretreatments to SODIS in order to improve pathogen removal and inactivation efficiency. The specific objectives are:

- To determine which cleaning agents are available and affordable in developing countries.
- To determine whether the containers used for SODIS need to be cleaned before use.
- To evaluate which of the cleaning agents, if any, improve the SODIS process.
- To explore whether SODIS could be used outside of the recommended geographical regions.
- To construct a roughing filter pretreatment for SODIS, which produces an effluent water turbidity of less than 30 NTU.
- To test the roughing filter in series with SODIS and determine to what extent its use improves the process, if at all.

1.4 Overview

Chapter 2 is an overview of the solar water disinfection process covering its history, an explanation of the process, and current research. Chapters 3, 4, and 5 each describe an experiment carried out for this project: cleaning pretreatment, roughing filter, and filtration followed by SODIS. These chapters are organized as stand alone articles that may be refined and submitted to peer-reviewed journals. Chapter 6 summarizes conclusions and results, and Chapter 7 outlines recommendations from this work.

CHAPTER 2: BACKGROUND

Solar disinfection is a form of physical disinfection. It is important to understand its mechanics, history, and limitations before employing it for water purification.

2.1 Disinfection

There are three different mechanisms by which disinfection is achieved:

- "destruction or impairment of cellular structural organization,"
- "interference with energy-yielding metabolism," and
- "interference with biosynthesis and growth" (Montgomery, 1985).

The level of disinfection that can be achieved varies depending on the raw water characteristics such as pH, temperature, turbidity, alkalinity, and concentration of pathogens. The efficiency of drinking water technologies for developing countries is characterized by reduction in the microbial concentration or log-reduction as seen in Table 2.1.

Table 2.1: Characterization of treatment efficiency for developing countries (WHO, 2007)

Log Reduction	Percent Removal	Efficiency
< 1 log	< 90%	Low
1 to 2 log	90 to 99%	Medium
> 2 log	> 99%	High

There are two types of disinfectants: chemical and physical. Low-cost chemical disinfectants are typically categorized as free chlorine (e.g., sodium hypochlorite/"bleach") or acid/base (e.g., lime juice) (WHO, 2007). Chlorine-based disinfectants undergo hydrolysis to form hypochlorous acid, HOCl, and hypochlorite ion, OCl^-. Equation 2.1 shows the hydrolysis of chlorine. Below a pH of 7.5 the hypochlorous acid is the active form; this species "is about 80 to 100 times more effective at killing *E. coli* than is OCl^-" (Snoeyink and Jenkins, 1980).

$$Cl_2 + H_2O \Leftrightarrow HOCl + H^+ + Cl^-$$
$$HOCl \Leftrightarrow H^+ + OCl^- \qquad (2.1)$$

Equation 2.2 gives an example of how the disinfectant species is formed using "bleach". A container made from specific material is used with these chemical disinfectants to limit the formation of disinfection by-products or recontamination (WHO, 2007). The significant benefit of these disinfectants is that they provide a residual to combat against regrowth or recontamination. The disadvantage is that they are not effective against all types of pathogens and can be affected by water quality.

$$\begin{aligned} NaOCl &\Leftrightarrow Na^{+} + OCl^{-} \\ H^{+} + OCl^{-} &\Leftrightarrow HOCl \end{aligned} \qquad (2.2)$$

Physical disinfectants can be classified as thermal, optical, and ultrasound (although the latter is not yet available for use at a low cost). Thermal disinfection is the process of heating water to temperatures at which microorganisms cannot survive. Two examples of this are boiling and pasteurizing water. Boiling is a proven disinfection method used throughout the world that only requires a heat-resistant container and an energy source (e.g., wood, electricity, fuel). The WHO does not consider boiling a low-cost technology since it requires an energy source, which tends to be resource intensive (WHO, 2007). Pasteurization, on the other hand, can be achieved in a variety of ways and on any water type. It is widely acknowledged that pasteurization requires the water temperature to be raised to 62.8°C and maintained for approximately thirty minutes to kill pathogens, but lower temperatures (around 55°C) over several hours can also produce the same results (Sobsey and Leland, 2001).

Light can also damage microorganisms in a way that prevents them from being harmful; the microorganism's DNA or RNA absorbs the light and thymine dimers are formed, causing mutations that inhibit reproduction and thus infectivity (Montgomery, 1985). One application of light disinfection is ultraviolet (UV) irradiation. The UV technology requires an energy source and the purchase of UV lamps. As can be seen in Figure 2.1, UV lamps have limited application in developing countries when it is considered that 1.6 billion people do not have electricity (International Energy Agency, 2004). However, sunlight has a UV component and is a readily available resource in many parts of the world.

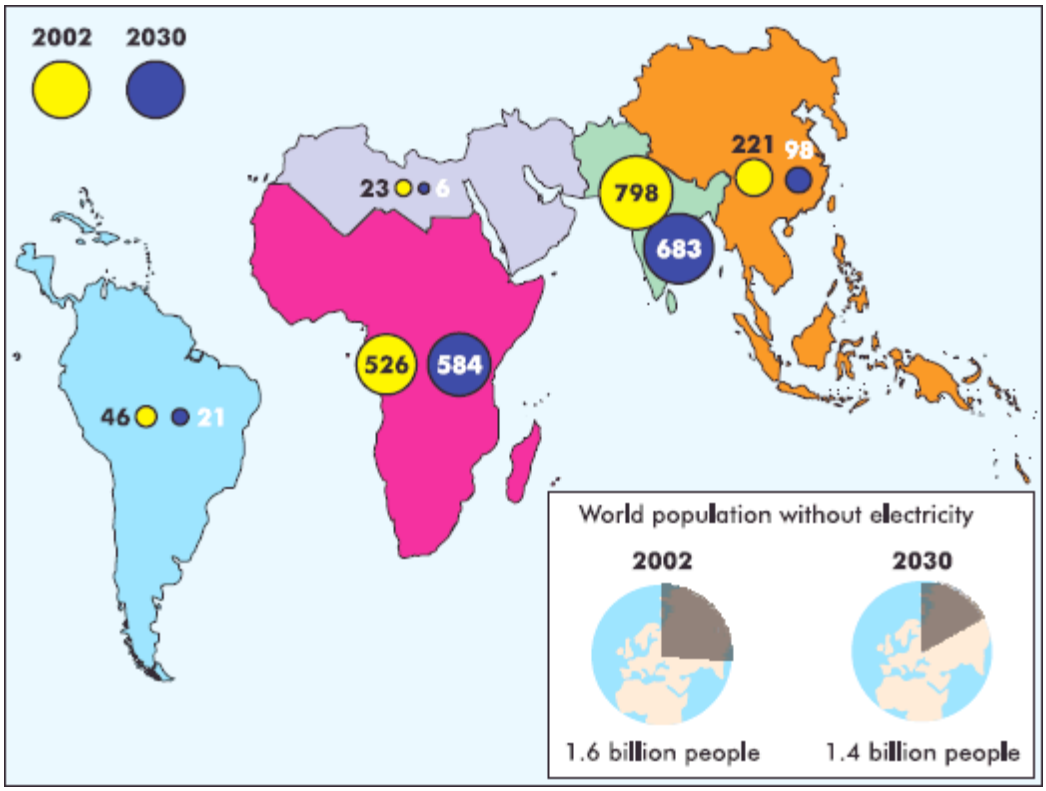

Figure 2.1: Electricity deprivation in millions of people per continent (International Energy Agency, 2004)

Sunlight is electromagnetic radiation. The total energy produced by the sun in the form of light is 3.83×10^{26} Watts (Encarta, 2008). Light reaching the top of Earth's atmosphere is 1370 W/m^2 and is referred to as the solar constant (NASA, 2007b). The energy reaching Earth's surface is much less, approximately 0.000000046% of that produced, due to the Earth's atmosphere reflecting, scattering, and absorbing many of the shorter wavelengths. Figure 2.2 is the visible light region of the electromagnetic spectrum. The small portion of light reaching Earth spans the ultraviolet (UVB and UVA, 280 to 315 nm and 315 to 340 nm, respectively), visible (340 to 760 nm), and infrared bands (760 to 3200 nm). The radiation appears as light and is felt as heat.

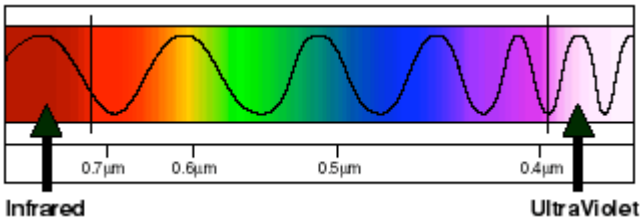

Figure 2.2: Visible light region of the electromagnetic spectrum: wavelengths in µm (NASA, 2007a)

Sunlight reaching the Earth has germicidal properties due to its heat and UV/visible components. The heat from the sun can raise water temperatures to 62.8°C, also known as the pasteurization level. The DNA or RNA of microorganisms can also absorb energy from the 200 to 300 nm wavelengths of sunlight (Bolton and Cotton, 2008). This absorbed light fuses the thymine (uracil in RNA) bases paired in DNA, creating a thymine dimer, and inducing mutations that inhibit the microorganisms from reproducing (Bolton and Cotton, 2008). Although the pathogens are still viable, they cannot replicate and therefore cannot be harmful. The disadvantage is that the process depends on the dose. The dose is referred to as fluence with respect to UV irradiation and represents the radiant energy received over a certain time period (mJ/cm^2).

Some microorganisms are more sensitive to light radiation than others (Montgomery, 1985). They also have two ways to combat UV disinfection: dark reactivation or photoreactivation. Dark reactivation mechanisms include removing the thymine dimer and making a new DNA sequence or combining parts of the DNA that were not damaged (Bolton and Cotton, 2008). This happens to some extent when the microorganisms have been removed from the inactivating light source and placed in the dark.

On the other hand, photoreactivation occurs when UVA light is absorbed (350 to 450 nm) and photolase (an enzyme) is activated to break up the thymine dimers, which restores the damaged DNA (Bolton and Cotton, 2008). This takes place when the microorganisms are re-exposed to a low dose light source, such as 5 mJ/cm^2 from a low-pressure UV lamp (Zimmer and Slawson, 2002). However, microbial recovery is not limited to reactivation; simple regrowth can occur under suitable environmental conditions (e.g. when sufficient nutrients are provided).

The intensity of sunlight reaching the Earth varies from location to location, as can be seen in Figure 2.3. Areas receiving the most intense sunlight for much of the year lie within latitudes of 40° North to 60° South. This region has limited cloud cover, rainfall, and generally, smaller angles of incidence for the sun's rays. Many developing nations also exist within these latitudes. Sunlight for water disinfection has become a viable means for providing safe drinking water where resources for treatment are scarce. Developed countries, such as the Southern USA and Australia, have little need for using sunlight for water disinfection, but their location in the plentiful solar region opens up the possibility for its use.

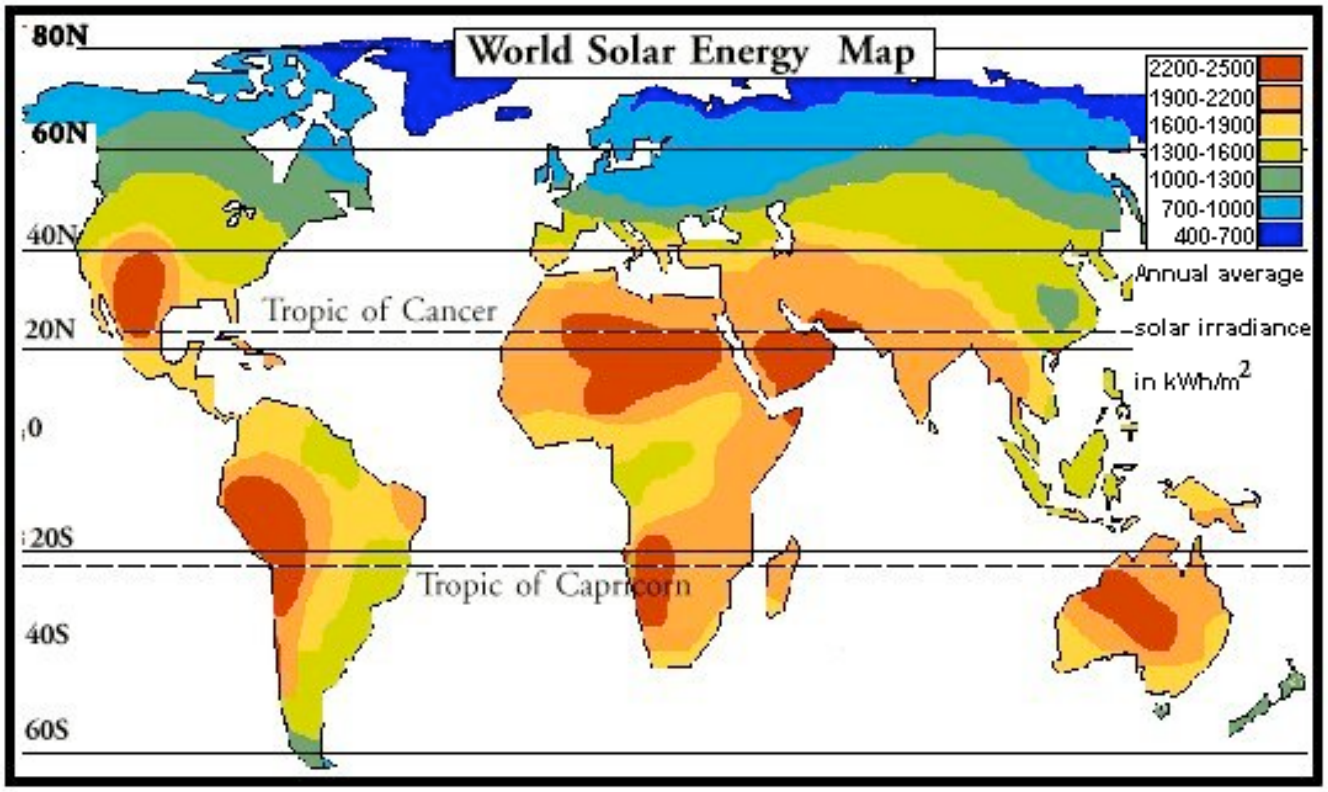

Figure 2.3: Annual average global solar irradiance in kWh/m^2 (RISE, 2006)

2.2 History of Sunlight Disinfection

Using the natural energy of the sun for water treatment is not a recent breakthrough; the Ancient Greeks were using sunlight for this purpose and other societies perhaps as early as 4000 B.C. (US EPA, 2000). There has been renewed interest in this method of disinfection over the past 30 years due in part to the environmental movement and the dire need for water treatment technologies that can be used by anyone in emergency situations.

In the late 1970s, Aftim Acra, a professor at the American University of Beirut, noticed that while the WHO was trying to control diarrhoeal disease in developing countries by using oral rehydration solutions (ORS), its efforts were being undermined when the solutions were made with microbiologically contaminated water (Acra et al., 1980). At the time, Acra's team was studying water disinfection for home use and found that sunlight killed off many pathogens, so they tried applying it to the ORS problem. They placed the contaminated water in polyethylene bags and exposed one set of bags to sunlight, one set to indoor lighting, and kept another set in the dark. The polyethylene was used

because it allowed much of the UV and visible light from the sun to pass through into the water. The results showed that the bags of water placed in sunlight had fewer viable microbes after a two-hour exposure time.

At the same time other researchers were examining the potential of sunlight disinfection on natural waters. Tyrrell (1976) was studying the effects of heat and UV light on *E. coli*. He reported that both mechanisms interfere with the microorganisms' DNA, but that the combination of the two produces even more inactivation. Cubbage's group (1979) studied the effects of light and turbidity on the rate of loss of infectivity of *poliovirus*. Their research found that both variables contributed to the loss of infectivity of *poliovirus*.

In 1981, researchers at the University of Hawaii looked at the degradation of fecal bacteria in seawater exposed to sunlight. They found that the visible portion of sunlight (380 to 770 nm) had the potential to penetrate up to 3.3 m of clear seawater (temperature between 15 and 25°C) and contributed to inducing three-log reduction of indicator organisms (Fujioka et al., 1981). Kapuscinski and Mitchell (1981) noted similar results for *E. coli* in seawater when exposed to sunlight.

Finally, a group from California State University explored the use of a homemade solar box cooker, which achieved temperatures well above 60°C, to pasteurize naturally contaminated water (Ciochetti and Metcalf, 1984). They tested for the presence of coliforms at various intervals. Initial coliform most probable numbers (MPN) ranged from 460 to 3500 per 100 mL. They found that after four hours of exposure there were no positive results for the presence of coliforms in their samples (Ciochetti and Metcalf, 1984).

Meanwhile, Acra continued to work on sunlight disinfection research for home use. In conjunction with UNICEF he published a document in 1984, summarizing his discoveries about sunlight disinfection, especially the characteristics of the containers and conditions surrounding their use (Acra et al., 1984). He suggested using containers of clear material, preferably glass or polyethylene bags or bottles, because colour, shape, and wall thickness decreased the process effectiveness. Placing these containers angled facing the equator allowed the sunlight to penetrate the water at its most direct angle and increased disinfection efficiency. The containers should also be placed in full sunlight, avoiding shade.

Acra also researched the wavelengths of sunlight responsible for disinfection. His findings were that those between 300 to 400 nm (this is the UV and near-UV portion of sunlight) were most effective at disinfection. This led to discovering that disinfection may still be achieved under cloudy conditions, but the minimum exposure time of two hours would be increased. Acra also stated that solar disinfection is effective on a variety of microorganisms.

It was not long after this time that many more joined sunlight disinfection research. In 1988 a workshop was held (at The Brace Research Institute in Montreal) to bring together researchers from all over the world that had been working on sunlight disinfection. The objectives of the workshop were to: review solar water disinfection, identify research needs, and develop a standard set of methods for testing (laboratory and field) and comparing results (Brace Research Institute, 1988). The target population for use of solar water disinfection was also defined at this workshop as those who:

> "collect their own water supplies from surface or ground water sources; are without access to treated water; and, may be interested in treating small quantities of drinking water for household requirements only" (Brace Research Institute, 1988).

Acra's findings were confirmed at the workshop. Other preliminary findings were also recorded in the proceedings. The instantaneous irradiance of 500 W/m^2 along with five hours of exposure was presented as providing the most effective disinfection (Brace Research Institute, 1988). However, this fluence did not appear to be as effective on highly contaminated waters or those with high turbidity. The turbid water decreased the process efficiency by providing a habitat in which the microorganisms could survive, making it necessary to pretreat water with high particle counts. It was also apparent that insufficient exposure could result in microorganisms reactivating. Finally, the temperature was not as important as the UV light for disinfection, at least from 12 to 40°C (Brace Research Institute, 1988).

The Integrated Rural Energy Systems Association (INRESA) also presented findings from a project they had been working on since 1985. This project included five research groups from Peru, Colombia, Egypt, Sri Lanka, and Nigeria, in addition to those at the Brace Research Institute. Their research focused primarily on the microbiological aspects of solar disinfection and the need for dark controls to be used in future studies (Brace Research Institute, 1988). They found that *E. coli*, *S. typhi*, *S. aureus*, *S. flexneri*, and *V. cholerae* can be disinfected using sunlight (Brace Research Institute, 1988).

The workshop proceedings also detailed significant findings. Hahn (1988) presented that pH remains constant during the process. Mathur and Kandpal (1988) recommended that the time for exposure be centred around noon. Their research showed that when the containers are completely filled with water, the number of interfaces through which the sunlight must travel are fewest and this minimizes reflective losses. They found that using plastic containers were a better choice than glass since the material thickness is less and plastic transmits light equally well (Mathur and Kandpal, 1988). Finally, Odeyemi's group (1988) recommended that these experiments should only be conducted in areas that lie between latitudes of 35° North and 35° South of the equator and have ample sunshine throughout the year.

After the workshop, research continued but broadened to explore other facets: flow through systems (McLoughlin et al., 2004a; 2004b), which provide a larger volume of water than the batch systems; photocatalysis to enhance the efficiency (Goswami, 1997; Vidal and Diaz, 1999; 2000; Salih, 2002; Villen et al., 2006; Duffy et al., 2004); and improvements to the existing system by using concentrators or reflectors (Safapour and Metcalf, 1999; Walker et al., 2004; Martin-Dominguez et al., 2005; Mani et al., 2006). Another aspect researched was which other microorganisms (e.g., *Salmonella typhimurium*) could be disinfected using sunlight (Smith et al., 2000).

The Swiss Federal Institute of Environmental Science and Technology and their Department of Water and Sanitation in Developing Countries (EAWAG and SANDEC) did extensive laboratory (Wegelin et al., 1994) and field tests (Conroy et al., 1996; 1999) to assess the potential of the solar water disinfection (coined SODIS) process and to develop an effective, sustainable and low-cost water treatment method (Sommer et al., 1997). Their approach consisted of three questions:

- Can sunlight be used for water disinfection?
- How should installations for the solar disinfection of water be designed and operated?
- Can solar water disinfection be socio-culturally acceptable and financially affordable?

These questions were answered in three phases: laboratory tests, field tests, and demonstrations. They revealed several important facts about the process:

- The container depth should not exceed 10 cm since the disinfection capability of sunlight decreases as depth increases (see Appendix 2.5; Kehoe et al., 2001);
- Filling the container part way, shaking it, and filling it completely before putting it in the sun increases the dissolved oxygen (DO) concentration of the water and slightly increases the effectiveness of the process (Reed 1997, 2000, and 2001; Kehoe et al., 2001); and
- Variations in weather (e.g., cloud cover) negatively affect the process (McGuigan et al., 1998).

2.3 The Solar Water Disinfection (SODIS) Process

The currently accepted version of SODIS, formalized by EAWAG and SANDEC, is depicted in **Error! Reference source not found.**2.4. SODIS is a simple technology that lends itself well for use in developing countries. Many developing countries lie between 35° North and South of the equator, receiving strong sunlight for much of the year, thus SODIS works well there (Odeyemi et al., 1988). SODIS does not require many resources (financial or material), yet provides adequate disinfection to less than 100 CFU/mL (WHO, 2006).

Figure 2.4: Depiction of the SODIS process (adapted from RCSI Research)

This technology is not perfect. The process is highly influenced by the condition of the containers, the cloudiness (turbidity) of the water, and environmental factors, such as cloud cover. A further discussion of these influences follows.

2.3.1 Containers

The condition of the containers affects the efficiency of the SODIS process. Brand new PET bottles typically have UV transmittance efficiency up to 70% (see Appendix 2.2). As the water bottles are used continuously they often become scratched, and scratches further diffract the light entering the bottles. A film can also develop on the outside of the bottles from extended interaction of the PET material and the ultraviolet light (see Appendix 2.2). Both the scratches and film decrease the level of solar radiation that is transmitted through the bottles to the water thereby reducing the amount of energy being absorbed by the microorganisms. The bottles themselves should not have a depth greater than ten centimetres (when laid on their side) since the radiation will not penetrate much beyond this depth (see Appendix 2.5).

The method of washing the bottles may also contribute to transmittance reduction. It is important to identify if the first step in the process (washing the bottles) is necessary and if so, what materials would be available, affordable, and effective for cleaning without causing adverse transmission effects.

2.3.2 Exposure

During SODIS, purification relies on exposure of the water to the sun's rays for an extended period of time, usually a fluence of 555 W-h/m^2 for approximately five or more hours, and an increase in water temperature to inactivate pathogens (see Appendix 2.6). Below 50°C, the primary mechanism of disinfection is radiation. Above 50°C, the disinfection is caused by a synergistic effect of the light and heat (see Appendix 2.6). Instead of requiring the typical five hours of exposure, when the water temperature reaches 50°C, the necessary fluence to reach disinfection is reduced to 140 W-h/m^2 for approximately one hour of exposure (see Appendix 2.6). The treatment is much less effective when there is cloud cover; 50% cloud cover results in approximately 70% available energy for disinfection (see Appendix 2.4).

Currently, SODIS is recommended in areas that lie within latitudes of 35° North and South. However, it has been reported that in mountainous regions (within these recommended latitudes) where cloud cover and lower ambient air temperatures are predominant, SODIS is not very effective (Oates et al., 2003). It is often suggested that meteorological data be gathered first to determine the amount of solar radiation a region receives before SODIS is utilized in that area. This step is not always economically feasible. The general guidelines are to follow the recommended latitudes for SODIS or refer to Figure 2.3. The solar energy map shows that there are regions outside of the recommended latitudes that have strong enough average annual irradiance for SODIS to be effective (e.g., Australia).

2.3.3 Necessity of Pretreatment

Surface water is typically the source of drinking water in developing countries and this source often has high turbidity. In other words, many particles are suspended, making the water cloudy. These particles prevent a portion of the UV light from penetrating the water and inactivating the microorganisms during SODIS (see Appendix 2.5). In fact, when the turbidity is greater than 30 nephelometric turbidity units (NTU), the only mechanism effective for microorganism inactivation is heat. The importance of checking turbidity before using SODIS has led to the development of a simple method for individuals to test the turbidity level of their water:

> "place the filled bottle [up to 2 L] on the SODIS Logo [see Figure 2.5] on top of a table in the shade (to avoid light interference) and look through the bottle from top to bottom. If you can read the letters through the water, water turbidity is less than 30 NTU. If you can still see the sun rays of the Logo, turbidity is less than 20 NTU"
> (see Appendix 2.5).

Figure 2.5: SODIS logo (see Appendix 2.5)

Turbidity-causing particles can also be physically associated with microbes and hide them from physical and/or chemical disinfectants. Such particle-association microbes must be removed via removal of the particles to which they are attached. Therefore, if the turbidity is greater than 30 NTU a pretreatment should be used (see Appendix 2.5). The raw water could be stored overnight to allow particles to settle (see Appendix 2.8), strained using cloth (WHO, 2007), or filtered (WHO, 2007). Currently, there is not a set pretreatment for SODIS. Potential options are described in Section 2.4.

2.4 Turbidity Removal for Household Treatment Systems

Particles can be removed from water by a number of methods, but in the context of developing countries sedimentation and filtration are recommended (WHO, 2007).

2.4.1 Sedimentation

Sedimentation is the process of allowing particles (sands, silts, and some microbes) to settle by gravity; it is frequently used as a pretreatment (WHO, 2007). In the developing country context, water is collected in a container and left undisturbed overnight, allowing the particles to fall to the bottom of the container. Then the clarified water can be carefully removed from the top. This technique is simple and can be used on large or small volumes of water. Unfortunately, there is no guarantee that all the solids will settle in a reasonable amount of time. Some particles are so small (clay, viruses, and bacteria) that they would take an unreasonably long time (days to months) to come out of suspension (WHO, 2007).

2.4.2 Filtration

Filtration is another process of separating solids from liquid. It can include straining, but when considering granular media filtration it is more complex than size exclusion. When particles are near the granular media, a combination of physical and chemical forces (gravitational, inertial, and diffusion) cause attachment between the two to occur (Montgomery, 1985). Interestingly, filtration is not limited to inert particles, but can be efficient at removing biological particles (microorganisms), too. The effectiveness of filtration varies widely. It is dependent on the filter media (type and size), the raw water characteristics, temperature, flow rate, chemical pretreatment, and filter depth (WHO, 2007).

There are many types of filters: precoat, ceramic, fibre (fabric and membrane), and granular media (rapid and slow). Precoat filtration consists of diatomaceous earth and water applied to a cylinder with holes called a septum (Montgomery, 1985). Diatomaceous earth is a natural material that consists of small dead marine organisms that contain silica in their cell wall. The diatom-water slurry forms a cake on the septum that strains particles from the water to be filtered; it is highly effective at removing microorganisms, but requires great pressure to push the water through the cake (WHO, 2007). Although it uses natural materials, it is not considered a low-cost technology.

Ceramic filtration requires specially manufactured clay or porous stone vessels (WHO, 2007). The water either filters from the inside out or vice versa. Like precoat filtration, ceramic filtration can remove some pathogens along with particulate matter (WHO, 2007). One disadvantage is that the filters must be cleaned frequently so that there are available absorption sites for the microorganisms and particles to absorb to the clay or stone. Although the raw materials might be available in developing countries, these filters cannot be made inexpensively since they require specific technical expertise to shape and set the materials (WHO, 2007).

Fibre filtration also works by size exclusion principles and often adsorption (particles attach to the fibre). Examples of fibre filters include paper, textiles, and polymeric membranes (WHO, 2007). The effectiveness, ease of use, and cost of fibre filters varies. For example, membrane filters are more expensive to produce than simply using cotton cloth and are therefore not considered a low-cost technology (WHO, 2007). Typically, textiles are used in developing regions; sari cloth has been shown to remove 99% of *V. cholerae* (Huq et al., 1996). However, their use for home treatment is not recommended since their pore sizes are often larger than pathogens, especially when the microbes are not attached to particles (WHO, 2007).

Granular media filtration is based on the attachment principle that the surface charge of the particles must be the opposite of the media for attachment to occur (Montgomery, 1985). These filters can remove suspended particles and microorganisms. This type of filtration is usually considered a polishing step in standard drinking water treatment (Montgomery, 1985). Common types of granular filters are slow sand, rapid sand, and roughing. Sand, anthracite or coal, and garnet are typical media used (Montgomery, 1985). For low-cost situations, sand filters are built with a base of gravel topped with sand (WHO, 2007). There may be one large filter or a series of filters through which water flows

(WHO, 2007). Roughing filters usually have larger media than sand filters and may remove 90% or more of turbidity, often as a pretreatment to sedimentation or further filtration (EAWAG, 2007).

2.5 Summary of Information Gaps

Based on the current disadvantages of SODIS, it is apparent that there is a need to explore what pretreatments are necessary for the process to work efficiently. Although washing the bottles is a recommended step, it seems that doing so could contribute to reducing the transmittance of the bottles and may not be entirely necessary. If cleaning does prove to be a necessary step, what materials should be recommended based on their availability and affordability? Furthermore, if the source water has high turbidity then there is a need for a recommended turbidity removal technique, such as a roughing filter. This filter should be as simple as SODIS and a user should be able to build one easily to decrease turbidity to less than 30 NTU. Finally, it is of interest to know whether SODIS works effectively in Canada, which is outside of the recommended geographical location for SODIS, if only during part of the year.

CHAPTER 3: CLEANING PRETREATMENT EXPERIMENTS

The currently accepted version of SODIS, formalized by EAWAG and SANDEC, consists of cleaning (type not specified) the container, filling it with water, shaking it to entrain air, and placing it in the sunlight for approximately six hours. The aim of the present study was twofold (i) to identify if chemical cleaning is actually necessary to SODIS and if so, to determine which cleaning agents would be affordable and available in developing countries and (ii) to investigate the use of SODIS in Waterloo, Ontario, which lies at a latitude of 43°28'N and well outside (the recommended region) of 35° North of the equator. Three cleaning agents were selected based on their cleaning ability and availability in developing countries: 70% isopropyl alcohol, a soap-water mixture, and lime juice. The results of these experiments indicate that cleaning with the soap-water mixture or the 70% isopropyl alcohol does not significantly improve solar disinfection. Cleaning with the lime juice, on the other hand, *inhibits* subsequent disinfection and is associated with microbial recovery.

3.1 Introduction

The solar water disinfection (SODIS) process has been and continues to be used in developing countries to provide safe drinking water. The World Health Organization (WHO) estimates that 1.1 billion people do not have access to a safe drinking water source and that 1.6 million people die every year due to water-borne diseases (WHO, 2004; 2007). SODIS is an ideal technology for relieving this burden because it uses minimal resources: nothing more than the abundant light and heat from the sun and a clear glass or plastic container (0.5 to 2 L). In most cases, users collect discarded pop bottles as their containers.

Sunlight that reaches Earth's surface spans the ultraviolet (UVB and UVA), visible, and infrared bands (280 to 3200 nm). The UV and visible components and heat from sunlight have germicidal properties (Wegelin et al., 1994). The DNA or RNA of organisms in the water absorbs the 200 to 300 nm wavelengths (Bolton and Cotton, 2008). This absorbed energy creates thymine dimers, which induce mutations that inhibit the microorganisms from reproducing (Montgomery, 1985). Although the pathogens are still viable, they cannot replicate and are no longer infective. Sunlight also raises the temperature of the water inside the containers. Heat alone causes pasteurization at 62.8°C. However, water temperatures of 50°C and greater cause a synergistic effect with the light energy and disinfection

occurs more rapidly (Wegelin et al., 1994). For example, when the water temperature is less than 50°C, the required fluence is 555 W-h/m^2 for five or more hours. When the water temperature reaches 50°C, the required fluence decreases to 140 W-h/m^2 for one hour (see Appendix 2.6).

The currently accepted version of SODIS, formalized by The Swiss Federal Institute of Environmental Science and Technology and their Department of Water and Sanitation in Developing Countries (EAWAG and SANDEC), consists of cleaning the container, filling it with water, shaking it to entrain air, and placing it in the sunlight for about six hours (Solar Water Disinfection, 2002a). It has been shown that the condition of the containers and environmental factors affect the efficiency of the process (see Appendix 2.1; 2.2; 2.3; 2.4). In particular, new polyethylene terephthalate (PET) containers have a UV transmittance of up to 70%, but continuous use reduces their transmittance from scratches and a film developing on the outside due to the interaction of the PET and UV light. Furthermore, the recommended geographic region for SODIS is between latitudes of 35° North and South of the equator due to the high solar irradiance experienced there.

The aim of this study was to (i) identify if the cleaning step is actually necessary to SODIS and if so, to determine which cleaning agents would be affordable and available in developing countries and (ii) to investigate the use of SODIS in Waterloo, Ontario, which lies at a latitude of 43°28'N and well outside of the recommended region. The results of these experiments are reported in Section 3.3.

3.2 Materials and Methods

Discarded PET water bottles were collected, rinsed with chemical cleaning agents, and then filled with source water contaminated with *E. coli* (initial concentration approximately 10^6 CFU/mL). The *E. coli* concentration in each bottle was measured over five hours of sunlight exposure.

3.2.1 Bottle and Chemical Preparation

To determine what type of containers to use for SODIS, availability in developing countries must be taken into consideration. Both clear glass and plastic pop bottles are prevalent in developing countries (see Appendix 3). Volume depth should not exceed ten centimetres, which approximately corresponds to the height of a two litre pop bottle laid on its side (see Appendix 2.5). The selection of used 500 mL

clear PET water bottles was based on this information and to limit the amount of source water that would have to be prepared for each experiment (see Appendix 2.1; 2.8). These bottles were collected and their labels removed prior to experiments. The UV transmittance of the bottles was not measured because it would not be easy to do this without proper equipment in a developing country and it was assumed that bottles manufactured in North America would have consistent values due to quality control standards.

Next, cleaning agents were selected. The WHO supports the use of 70% isopropyl alcohol and a bar soap-water solution for cleaning purposes in clinics in developing countries (WHO, 1998). A paper in the Journal of Food Protection indicated that lime juice disinfected water against *V. cholerae* (Dalsgaard et al., 1997). Individuals who were either living or had recently lived in the Philippines, Australia, Indonesia, Thailand, and Peru were asked what types of bar soap could be found and purchased by locals. Many different brands were available, but the composition of each was very similar: sodium tallowate and/or sodium palmate, water, sodium cocoate and/or sodium palm kernelate, glycerin, sodium chloride, fragrance or parfum, acid (coconut, palm kernel, tallow, or palm), and ethylenediaminetetraacetic acid or EDTA (Sparks, 2007; Campbell, 2007). These sources also confirmed that 70% isopropyl alcohol was common and so were limes. Thus, these three cleaning agents were selected.

Lime juice was prepared for experiments by manually squeezing juice from limes purchased at a local grocery store (the limes were imported from Mexico). The volume and pH were recorded for the juice of each lime. Once all the limes had been squeezed, the juices were combined. Again the volume and pH were recorded. The juice was stored at 4°C.

Isopropyl alcohol (70%) was purchased at a local grocery store, along with Ivory bar soap. Ivory or soaps of similar composition can be found in developing countries (Sparks, 2007; Campbell 2007). A piece of the bar was cut with dimensions 2.5 cm x 2.5 cm x 2.5 cm (WHO, 1998). The cube was placed in a 20 L PET container with four litres of distilled water. The container was capped and shaken by hand until the soap had dissolved (approximately five minutes). The mixture was kept at room temperature.

3.2.2 Source Water Synthesis

Synthetic source water was prepared for these experiments. Natural source water was avoided since its complexity would have produced further variability in successive experiments. Tap water was selected because it contained elements of natural water, such as minerals (Table 3.1), but required dechlorination of its monochloramine.

Table 3.1: Inorganic species concentrations for tap water in the City of Waterloo (adapted from Region of Waterloo, 2007)

Inorganic Species	Concentration Range
Antimony	< 0.003 mg/L
Arsenic	< 0.0025 mg/L
Barium	0.078 to 0.157 mg/L
Boron	< 0.01 to 0.05 mg/L
Cadmium	< 0.2 μg/L
Chromium	< 0.001 mg/L
Mercury	< 0.05 μg/L
Selenium	< 5.0 μg/L
Sodium	7.82 to 169 mg/L
Uranium	< 0.01 mg/L

Typical dechlorination methods are chemical addition (e.g., sodium thiosulfate, sulphur dioxide), using granular activated carbon (GAC), or passive dissipation (White, 1999). Although chemical addition dechlorination is quick (e.g., less than ten seconds when sulphur dioxide is used), it was avoided due to the necessity of neutralizing the chemicals afterward (White, 1999). GAC was not considered since post-filtration would have been necessary to remove the carbon fines (Sobon, 2007). Passive dechlorination allows water to sit in a basin while the chlorine dissipates over time due to passive aeration and surface interaction (Ganesh et al., 2006). Although slow (typical decay takes days), this method was selected because it does not require chemical addition and sufficiently removes the monochloramine (Ganesh et al., 2006).

Dechlorination was performed by running tubing (Masterflex 6402-17) from a peristaltic pump (Cole-Parmer Instrument Co. Model No. 7553-70, 6 to 600 rpm) into a container (Reliance Aqua Lux 26 L)

with 20 L of tap water and pumping air (between 600 to 700 mL/min) into the water. Complete dechlorination took seven days of continual pumping. The water's total chlorine content was measured using a HACH DR/2000 spectrophotometer and HACH Method 8167 (HACH Company, 1995). The turbidity, temperature, and pH were also measured: turbidity with a HACH 2100P Portable Turbidimeter (HACH 4650000), temperature using a Long-Stem Thermometer (Traceable, 4352), and pH with an Orion pH meter (Model 720A) standardized with pH 7 buffer (VWR, 34179-148).

3.2.3 Bacterial Preparation and Enumeration

E. coli was used as the indicator organism in this study due to its frequent use as a fecal indicator in drinking water treatment. A portion of the *E. coli* stock culture (ATCC, 11229)[2] was thawed and centrifuged at 10 000 rpm for ten minutes to isolate the cells from the broth. The cells were washed three times with sterile 0.1% peptone water, made from BD Bacto Peptone (BD, 90000-382), to remove the nutrients and centrifuged between each washing (same speed and time). Then the pellet was resuspended in 15 mL sterile 0.1% peptone water to produce a total initial viable count of 10^9 colony forming units per millilitre (CFU/mL).

The desired *E. coli* concentration for the experiments was 10^6 CFU/mL. Using Equation 3.1, the total volume of the *E. coli* solution to add to the 15 L of dechlorinated source water (Section 3.2.2) to achieve this concentration was 15 mL.

$$C_1V_1 = C_2V_2 \qquad (3.1)$$

$$C_1 = 10^9 \text{ CFU/mL}, V_1 = 15 \text{ mL}, C_2 = 10^6 \text{ CFU/mL}, V_2 = 15 \text{ L}$$

To confirm that the original suspension was 10^9 CFU/mL, one millilitre of the suspension was diluted in a series of dilutions (dilution factors from 10^1 to 10^7). One millilitre was taken from the 10^6-dilution and added to 15 mL of Nutrient Agar (BD, 213000)[3], mixed, and poured onto a sterile nine-centimetre Petri plate (Fisher Scientific, 08-757-9B). This was repeated for the 10^7-dilution. Plates were incubated at 37°C for 24 hours and colonies were counted the following day. The colonies were checked for

[2] The ATCC species was selected due to its commercial availability and use in other laboratory studies.

[3] Nutrient Agar was used because it was available and being used for other studies in the laboratory. Although not specific for *E. coli*, since only *E. coli* were spiked into test water and it grows well on Nutrient Agar, colonies that were detected on test culture plates could reasonably be attributed to *E. coli* as long as colony morphology remained consistent with that known for *E. coli* cultures.

uniformity of size and shape, and the total colony forming units were reported per millilitre of the original sample.

3.2.4 Bottle Cleaning Experiments

Discarded 500 mL PET water bottles were collected for these experiments. Of these, 15 similar bottles were selected based on their condition (i.e., no scratches or dents). Three sets of bottles were used to test the effectiveness of each of three cleaning agents (alcohol, soap, lime juice) and there were two sets of control bottles. Positive control bottles had no cleaning agent, but contained *E. coli*-spiked dechlorinated water to confirm the viability of the *E. coli* culture. Negative control bottles had no cleaning agents, nor any *E. coli*-spiked water (just dechlorinated tap water) to check for possible contamination.

As can be seen in Table 3.2, there were five sets of three bottles. Within each set, one bottle was placed inside a windowless cupboard as a dark control, while the other two bottles were placed on a concrete rooftop[4] in complete sunlight. One of these sunlight-exposed bottles was used for sampling and the other for measuring temperature.

For each of the three negative control bottles, 175 mL non-spiked water was used to rinse the bottles. Then these bottles were filled with 460 mL and shaken by hand for one minute to aerate the water[5]. Finally, the containers were topped up to a final volume of 515 mL with only headspace in the neck portion of the bottle (i.e., five millilitres) for ease of sampling. The same procedure was applied to the three positive control bottles using spiked water.

[4] See Appendix 2.8; the bottles were not painted with a black stripe nor placed on a corrugated metal sheet so as to enable the procedure followed in this work to represent the most conservative approach where sunlight and temperature effects are not optimized.

[5] See Appendix 2.8; aerated water was used, but the DO was not measured because no guideline is specified for SODIS.

Table 3.2: Experimental setup for cleaning pretreatment experiments

Cleaning Agent	**Test Water Quality**	**Bottle Location**
70% Isopropyl Alcohol	Spiked ~ 10^6 CFU/mL	Sunlight (Temperature) Sunlight Dark
Lime Juice	Spiked ~ 10^6 CFU/mL	Sunlight (Temperature) Sunlight Dark
Soap - Water Mixture	Spiked ~ 10^6 CFU/mL	Sunlight (Temperature) Sunlight Dark
None (Positive Control)	Spiked ~ 10^6 CFU/mL	Sunlight (Temperature) Sunlight Dark
None (Negative Control)	Not Spiked	Sunlight (Temperature) Sunlight Dark

The remaining bottles were pre-rinsed with the cleaning agent to be tested (five millilitres, the volume held by one bottle cap) and 270 mL of spiked water. For example, three bottles were pre-rinsed with five millilitres of alcohol and 270 mL of spiked water in each, then capped and shaken by hand for one minute to “clean” the interior of the bottle. The contents were discarded and the bottles refilled with 460 mL of spiked test water. These bottles were shaken by hand for one minute to aerate the water and topped up to a final volume of 515 mL, similar to those bottles described above. Figure 3.1 shows the bottles being exposed to sunlight.

Figure 3.1: SODIS being used in Waterloo, Ontario (latitude of 43°28'N)

3.2.5 Microbial Sampling and Analysis

At the beginning of each experiment, initial samples were drawn from the bottles to be exposed to sunlight ("Light Bottles") and those to be kept in the dark ("Dark Control Bottles"); the bottles to be exposed to sunlight for measuring the temperature ("Temperature Bottles") were not sampled for microbial analysis to avoid contamination. Samples were taken using a BD 3 mL Sterile Medical Syringe with Slip Tip (BD, BD309586). The temperature of the water was measured from the Temperature Bottles using a Traceable Long-Stem Thermometer (Traceable, 4352). The pH of the water was measured using an Orion pH meter (Model 720A) standardized with pH 7 buffer (VWR, 34179-148). The Light and Temperature Bottles were placed in full sunlight for five hours[6]. The ambient temperature[7] and irradiance values were obtained from the University of Waterloo Weather Station during each experiment (UW Weather Station).

Successive samples were taken from the Light Bottles at various times (0.5, 1, 1.5, 2, 2.5, 3.5, and 5 hours). Samples were taken from the Dark Control Bottles at 2.5 and 5 hours. At each sampling point for the Light Bottles, the temperature was also recorded from the Temperature Bottles using the digital thermometer. The Dark Control Bottles were kept at room temperature. After the five-hour samples had been taken, the Light and Temperature Bottles were removed from the sunlight. They were placed with

[6] See Appendix 2.8; early in the experiments it was shown that the water temperature would not reach 50°C, so the five-hour exposure time was selected based on SODIS guidelines. This also facilitated completion of one experiment within a single workday.

[7] The ambient temperature was also measured at the test surface and found to be consistent to within ± 2°C with the weather station values.

the Dark Control Bottles overnight. Another sample was drawn at 24 hours from the Light and Dark Control Bottles to check for microbial recovery. The temperature was checked to see if the exposed bottles had returned to room temperature. The pH was checked to see if any change had taken place.

A one-millilitre portion from each sample was diluted in a series of dilutions (dilution factors from 10^1 to 10^4) using standard sterile techniques. One millilitre was taken from a single dilution and added to 15 mL of Nutrient Agar (BD, 213000), mixed, and poured onto a sterile nine-centimetre Petri plate (Fisher Scientific, 08-757-9B). Duplicate analyses of the dilutions were performed. Plates were incubated at 37°C for 24 hours and colonies were counted the following day. The total colony forming units (CFU) were reported per mL of the original sample.

3.3 Results

Some assumptions were made for the analysis of the data resulting from these experiments. The first assumption was that the data from replicate samples follow a normal distribution. Although the population from which samples were taken in these experiments may not be normally distributed, the sample distribution was observed to tend toward the normal distribution. Secondly, only two samples were drawn for each sample-point so it was not possible to calculate variance; as such, homoscedasticity (same variance) has been assumed. This then also implies that the residuals are uniform. This assumption typically provides good estimation, but may lead to underestimating the correlation of variables (Box et al., 2005).

3.3.1 Initial Water Characteristics

The results of the dechlorination process used to prepare tap water for the experiments are shown in Figure 3.2. To avoid use of chemical dechlorination agents, which would interfere with the sunlight disinfection and complicate the analysis, aeration was the preferred method. The removal rate (k) was observed to be -0.56 d^{-1} as estimated by first order kinetics.

The turbidity, temperature, and pH of the bulk dechlorinated source water were recorded for each day of the experiments. The negative control water, not spiked with *E. coli*, had an average turbidity of 0.26 nephelometric turbidity units (NTU) ± 0.17 NTU. The positive control water, spiked with *E. coli*, had

0.32 NTU ± 0.13 NTU. The average water temperature was 23°C ± 1°C. The average pH of the negative control water was 8.00 ± 0.09 and that of the positive control water was 7.94 ± 0.30.

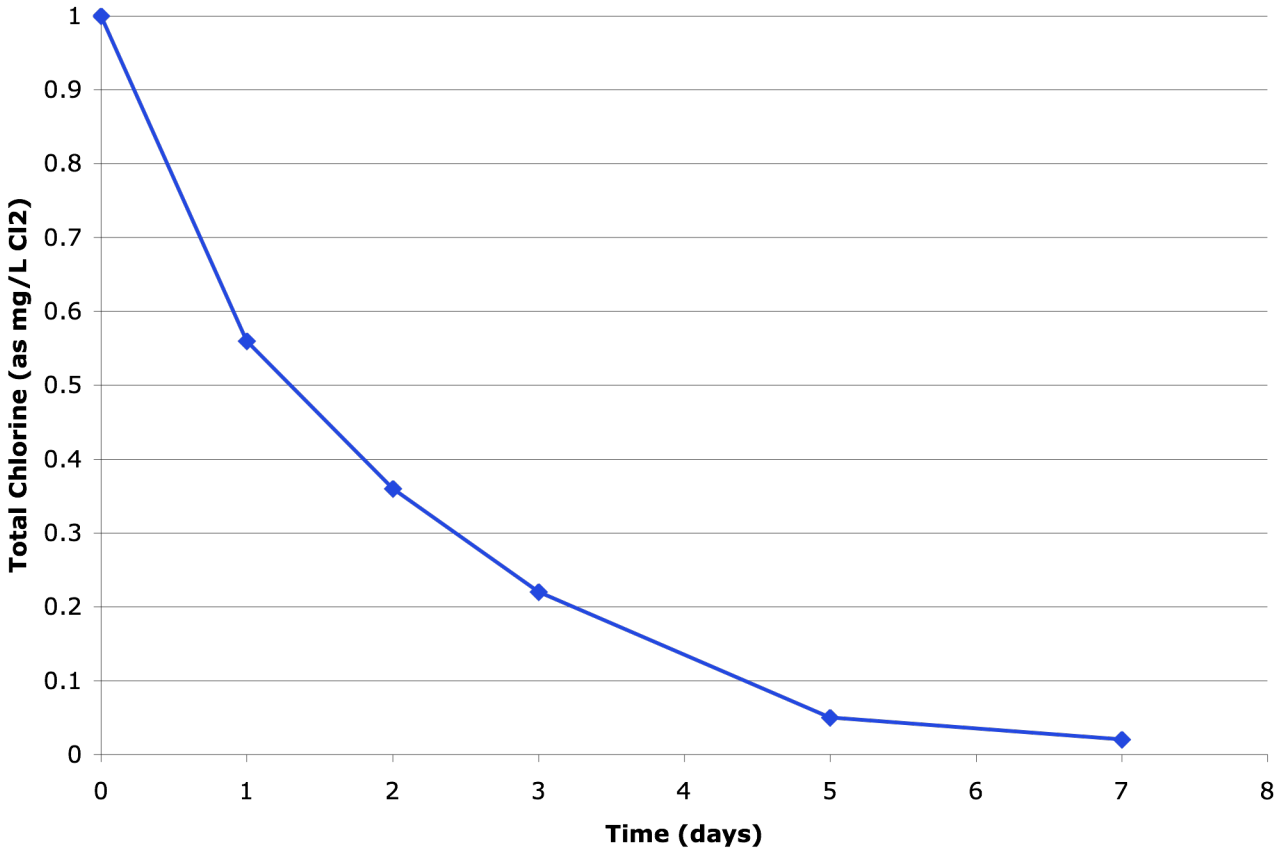

Figure 3.2: Dechlorination of tap water by aeration (initial conc. = 1.0 mg/L)

3.3.2 Cleaning Agent Solution Stability

The pH of each cleaning agent stock solution was measured routinely to determine if any significant changes would occur over the course of the experiments. The soap-water mixture and alcohol were stored at room temperature, but the lime juice was kept refrigerated at 4°C. The temperature of the lime juice was allowed to come to room temperature before measuring pH. It was expected that all the cleaning agents would have a constant pH over the course of their storage. The average pH of the soap-water mixture, alcohol solution, and lime juice were 9.29 ± 0.51, 8.17 ± 0.63, and 2.66 ± 0.14, respectively. Figure 3.3 shows that the pH of the soap-water mixture tended to increase slightly with storage time whereas the pH of the alcohol fluctuated. The pH of the lime juice was the most constant.

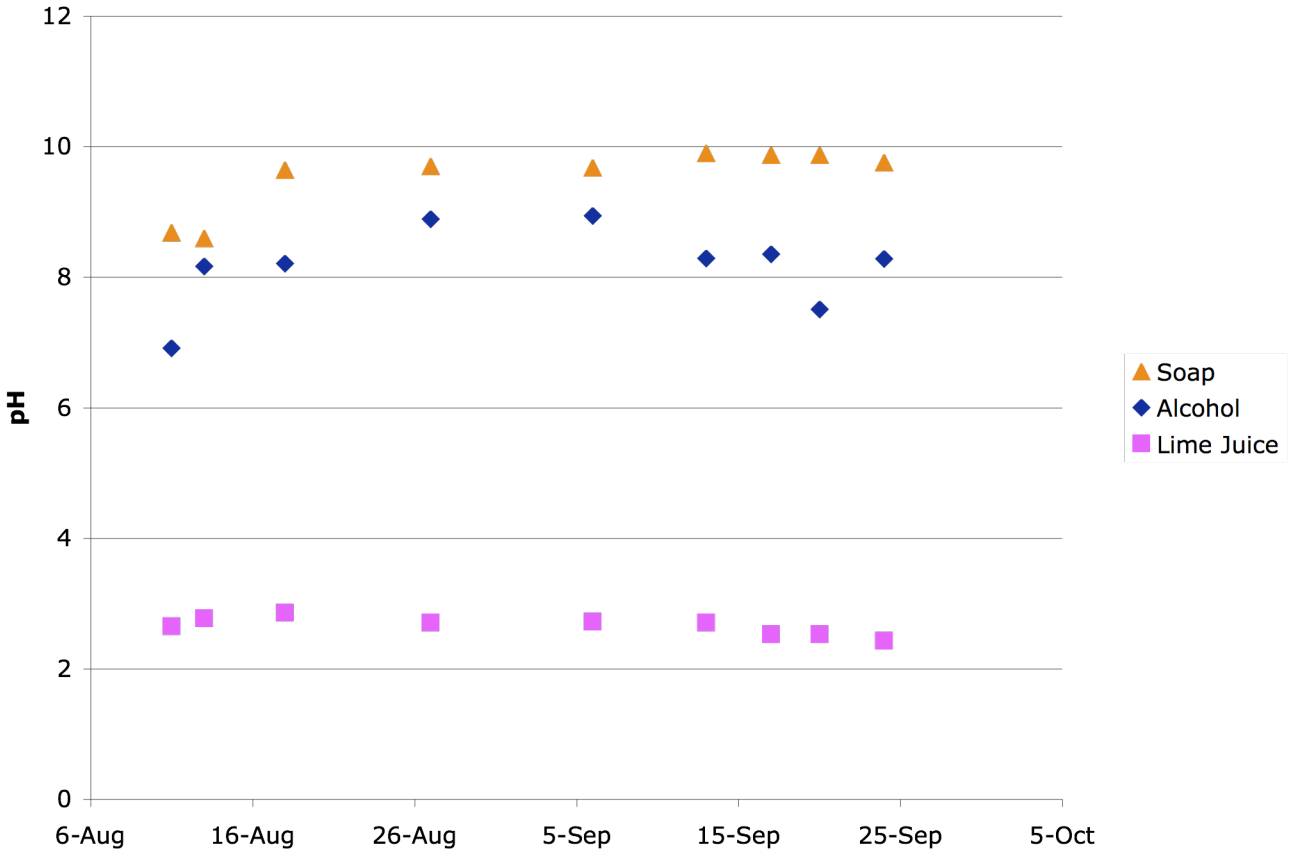

Figure 3.3: Variation of cleaning agent pH with storage time

3.3.3 Dark Control Bottles

No statistically significant variation was found in the Dark Control Bottles' temperatures over the course of the experiments. This is consistent with small changes observed in the room temperature, which fluctuated between 22°C and 24°C. The pH did not change over the course of the experiments, but initial pH values varied between the bottles, as can be seen in Table 3.3.

Table 3.3: Average pH of Dark Control Bottles

Cleaning Agent	Alcohol	Soap	Lime Juice	Positive Control
Average pH	8.01	7.99	7.31	7.98
Standard Deviation	0.07	0.05	0.09	0.02

The concentration of *E. coli* within the Dark Control Bottles was determined at the following times: 0, 2.5, 5, and 24 hours. Figure 3.4 illustrates the change of *E. coli* concentrations in the Dark Control Bottles over the course of the experiments.

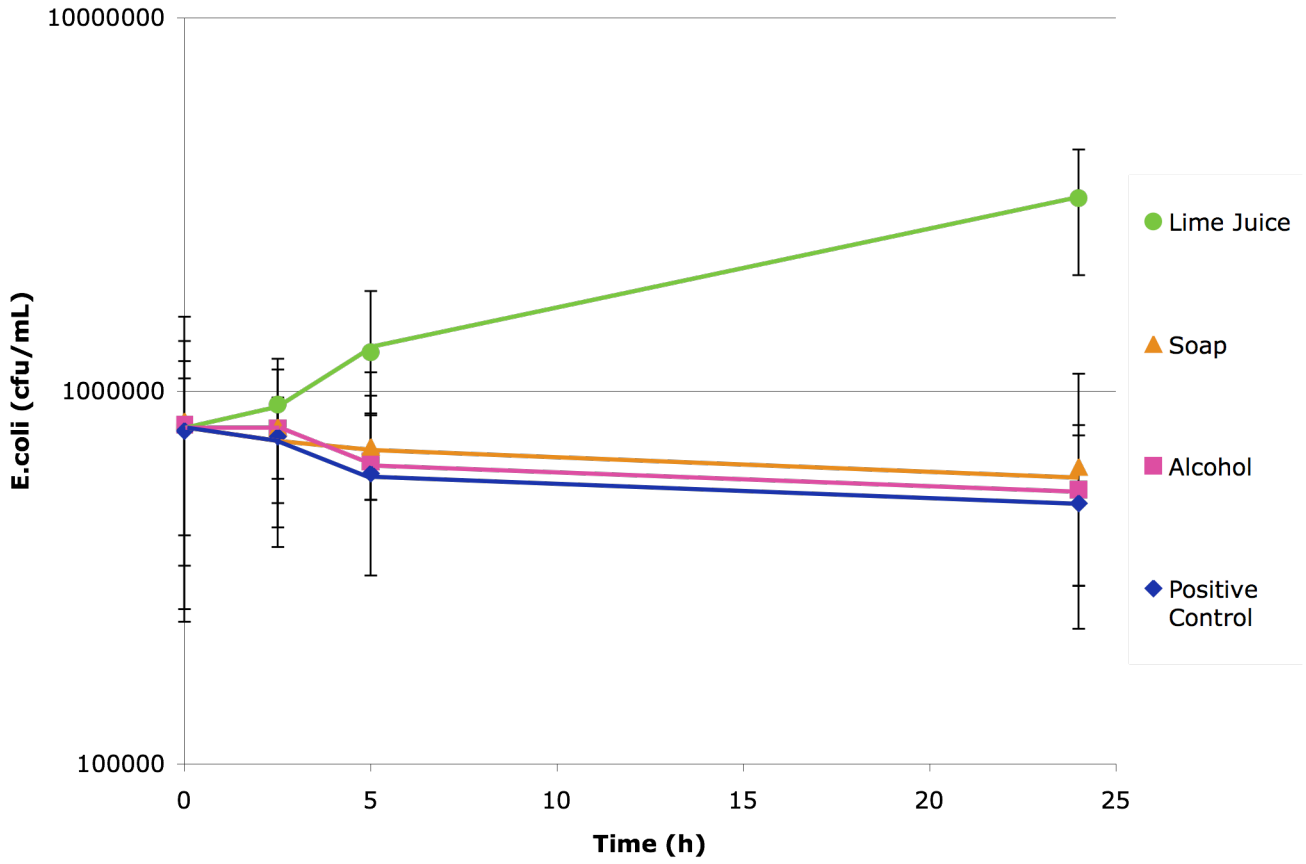

Figure 3.4: Dark Control Bottles' average *E. coli* concentration changes over 24 hours for the cleaning pretreatment experiments (error bars represent the variation of concentration at sample points)

There was a statistically significant difference in *E. coli* concentrations found between samples from the bottles cleaned with lime juice relative to the other bottles at times 2.5, 5, and 24 hours. The following tables and figures detail how the statistical analysis was carried out for these experiments using data from the Dark Control Bottles obtained at the 5-hour sample time. Table 3.4 shows the *E. coli* concentrations for the five-hour sample time, while Table 3.5 shows the analysis of variance (ANOVA) for this data. From the ANOVA table it is clear that the null hypothesis must be rejected for the statement "all treatments are the same"; the F_{obs} (calculated F) value is 36.06, which is much greater than the F_{table} (F-statistic) value, 2.79.

Table 3.4: *E. coli* concentrations (CFU/mL) in the Dark Control Bottles after five hours

Alcohol	Soap	Lime Juice	Positive Control
590000	520000	870000	560000
580000	640000	1210000	480000
650000	740000	1320000	320000
670000	680000	1210000	670000
730000	1120000	1850000	620000
650000	890000	1640000	590000
670000	510000	1500000	810000
740000	730000	1470000	720000
540000	710000	1050000	470000
860000	510000	1140000	610000
640000	860000	1290000	590000
610000	870000	980000	970000
600000	550000	970000	520000
510000	590000		490000

Table 3.5: ANOVA for Dark Control Bottles after five hours

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	3.86×10^{12}	1.29×10^{12}	36.06	2.79
Within Cleaning Agents	51	1.82×10^{12}	3.57×10^{10}		
Total	54	5.69×10^{12}			

Following the ANOVA, a normal probability plot was made to determine if the residuals were normally distributed. Figure 3.5 shows that the residuals lie in a straight line; therefore, the residuals are normally distributed.

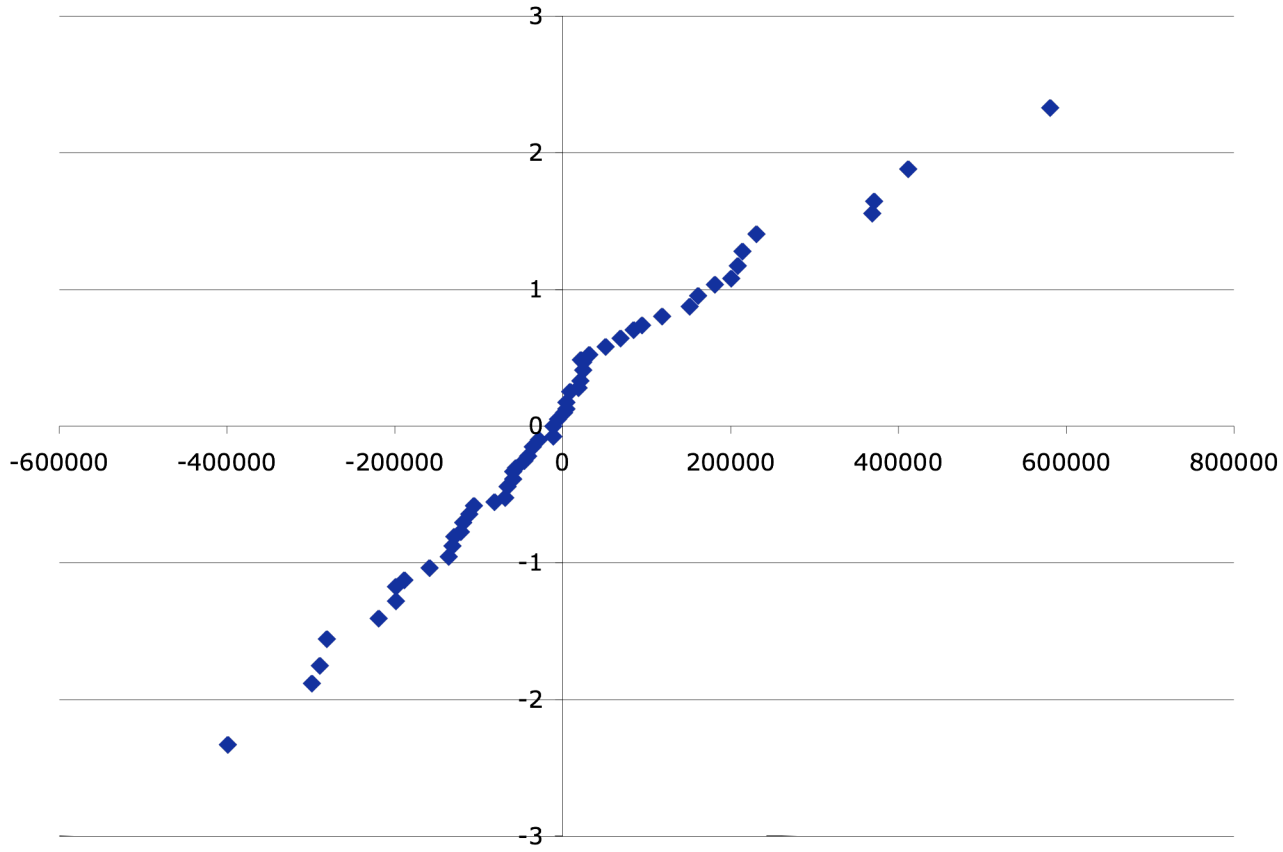

Figure 3.5: Normalized residuals for *E. coli* concentrations in Dark Control Bottles after five hours

The least significant difference (LSD) was also calculated (see Equation 3.2).

$$LSD = s.e.(t_{52,0.005}) = 191\ 000 \qquad (3.2)$$

Table 3.6 outlines the variables used in its calculation. The number of different cleaning agents used for the experiments (k) was four. The number of required experiments (c) was six. To achieve an overall confidence interval of 95% (b), the experiments were performed at a significance level of 99% (α). To calculate the LSD, the standard error of the difference between two means (*s.e.*) and the t-statistic were determined.

Table 3.6: Required values for calculating LSD for Dark Control Bottles after five hours

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	3.57×10^{10}
Average number of sample size	$\overline{n}$	14
Standard error for two means	$s.e. = \sqrt{2(MSE)/\overline{n}}$	71 400
t-statistic	$t_{df,\alpha}$	2.68

The average *E. coli* concentrations for the Dark Control Bottles are found in Table 3.7. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (191 000). Hence, the lime juice treatment is the only statistically different treatment. (See Appendix 4 for ANOVA at each sample point.)

Table 3.7: Average *E. coli* concentrations for Dark Control Bottles over all cleaning pretreatment experiments

Dark Control Bottles	Average Conc. (CFU/mL)
Alcohol	646 000
Lime Juice	1 330 000
Soap	709 000
Positive Control	601 000

3.3.4 Sunlight-Exposed Bottles

Table 3.8, shows the average fluence received by the bottles on each day of experiments. August 27, September 6, and September 13 are the only days on which the fluence dipped below 555 W-h/m^2 (see Appendix 2.6), but on average the observed fluence was still higher.

Table 3.8: Average fluence for each cleaning pretreatment experiment

Experiment	Fluence (W-h/m^2)	Standard Deviation
August 18	730	153
August 27	675	127
September 6	639	124
September 13	556	70
September 17	710	63
September 20	687	56
September 24	686	59

As can be seen in Figure 3.6, the minimum instantaneous irradiance reaching the surface of the bottles was 321 W/m^2 and the maximum instantaneous irradiance was 946 W/m^2. The variation that can be observed during experiments carried out on August 18, August 27, September 6, and September 13 was due to transitory, scattered clouds. There was a positive correlation between the log-reduction of *E. coli* concentrations in the Light Bottles and the irradiance they received between time zero and two hours of exposure (not shown).

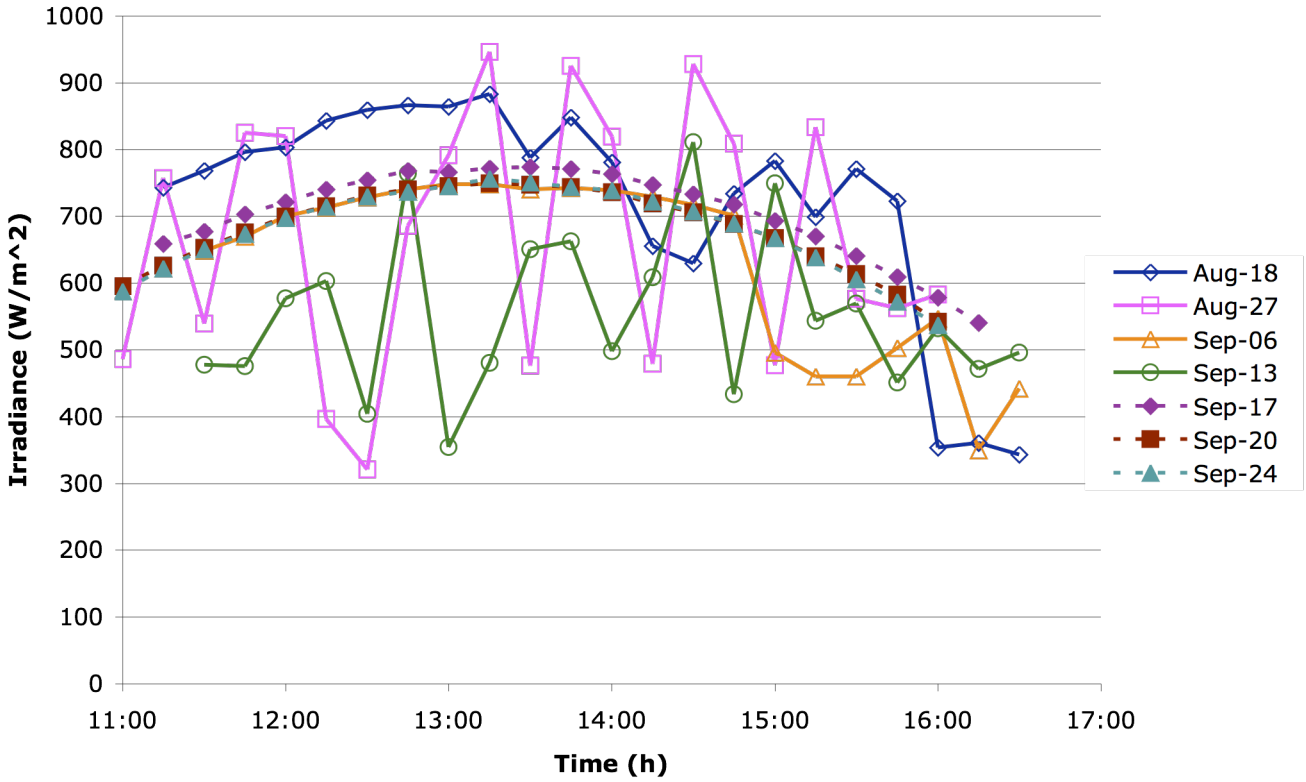

Figure 3.6: Irradiance for each day of cleaning pretreatment experiments (full lines for cloudy days and broken lines for clear days)

Figure 3.7 shows that the minimum ambient temperature experienced was 16°C and the maximum ambient temperature was 32°C. There was a positive correlation between the temperature in the bottles and the ambient temperature (not shown).

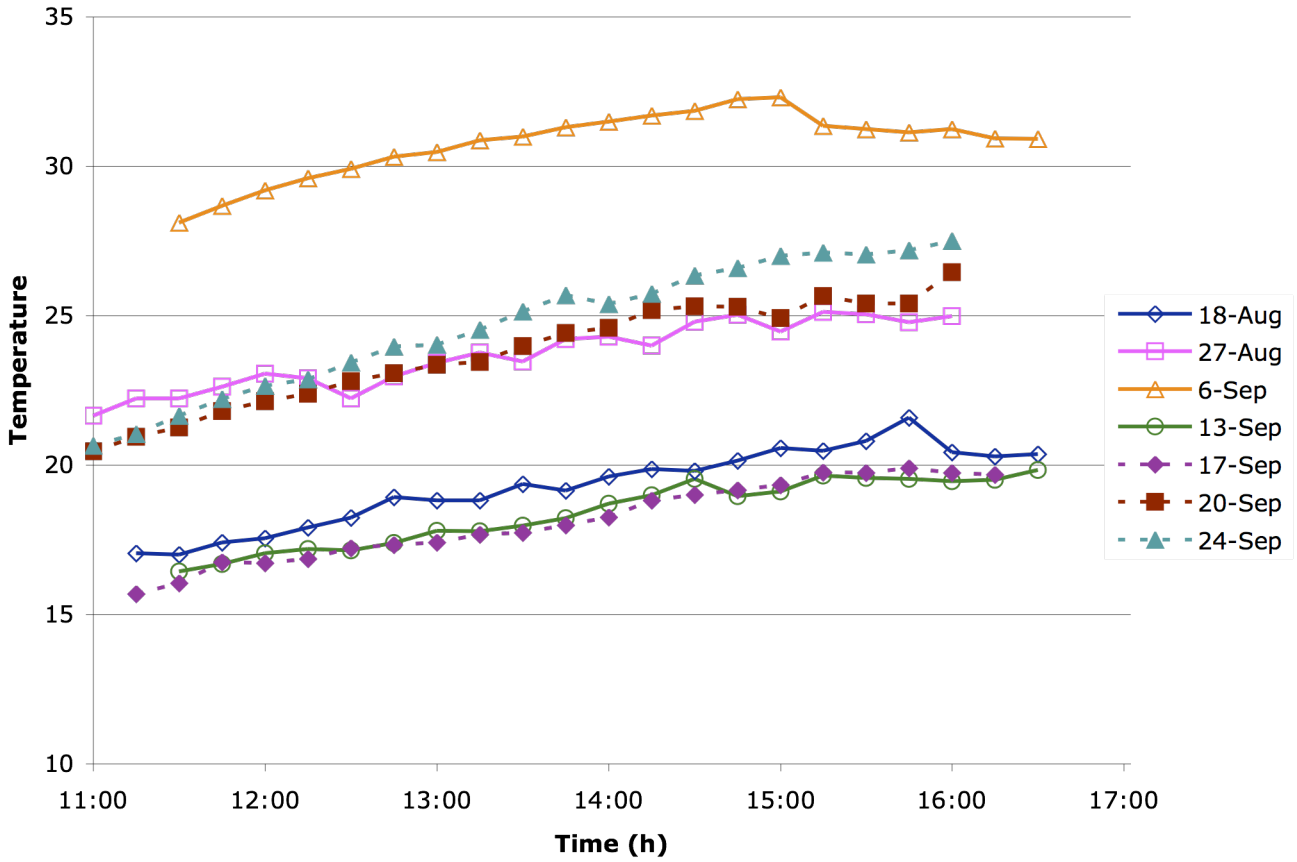

Figure 3.7: Ambient temperature for each day of cleaning pretreatment experiments (full lines for cloudy days, broken lines for clear days)

The change in temperature experienced by the Temperature Bottles can be seen in Figure 3.8. The minimum water temperature was 20°C (at the beginning of an experiment) and the maximum was 41°C (measured after five hours of exposure). Thus, disinfection was primarily attributed to irradiation.

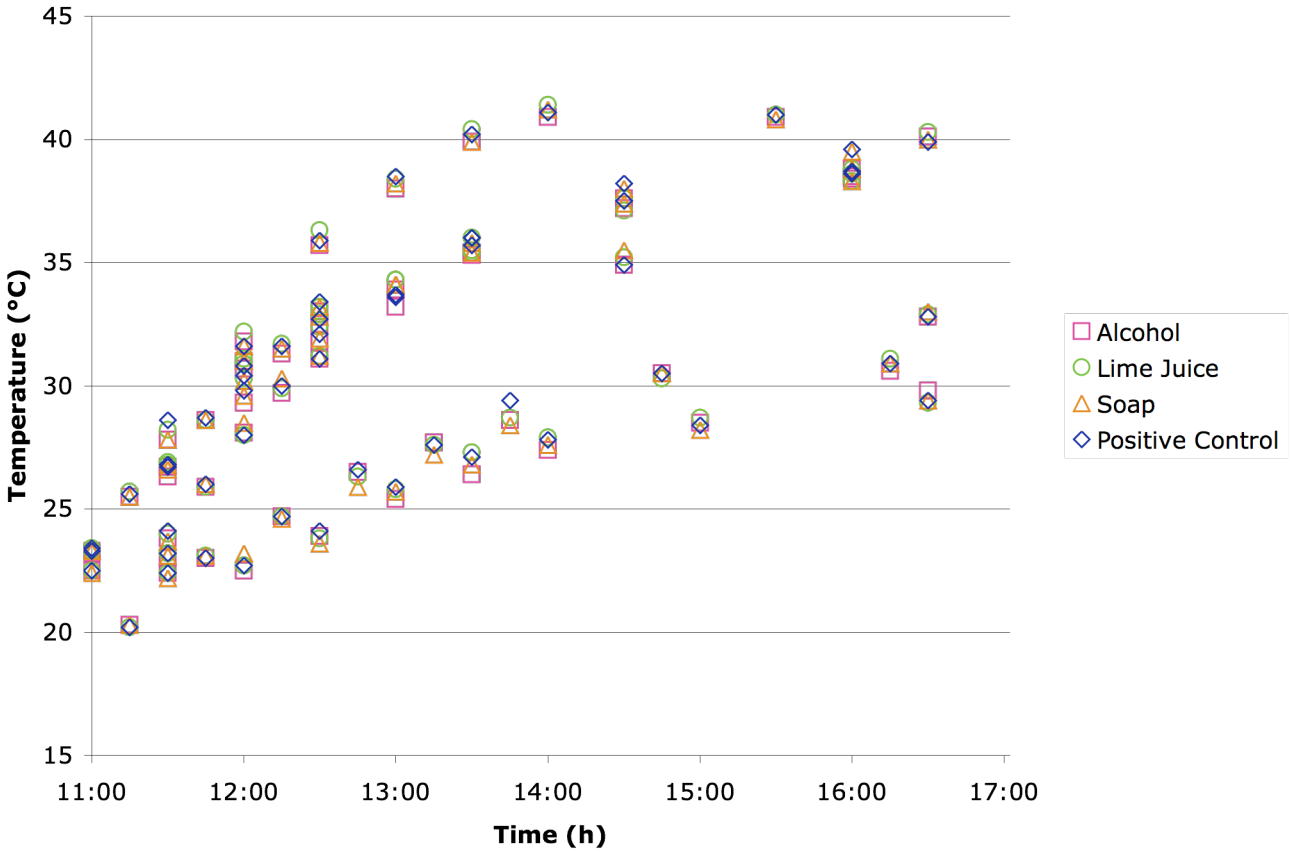

Figure 3.8: Water temperature inside the Temperature Bottles for each cleaning pretreatment experiment (all experiments together)

The pH was measured in the Light Bottles immediately before sunlight exposure. The average initial pH for each bottle after the pre-cleaning and refilling steps is shown in Table 3.9. The bottles rinsed with alcohol and soap had variations of ± 0.07 and ± 0.05, respectively. Those rinsed with lime juice had a variation of ± 0.09. The pH was also periodically checked during experiments and found to vary by ± 0.02.

Table 3.9: Average pH of Light Bottles before sunlight exposure

Cleaning Agent	Alcohol	Soap	Lime Juice	Positive Control
Average pH	8.01	7.99	7.31	7.99
Standard Deviation	0.07	0.05	0.09	0.01

There was no statistically significant difference in initial microbial density or disinfection efficiency at sample times 0 or 0.5 hours (Figure 3.9). Times 1, 1.5, 2, 2.5, and 5 hours had a statistically significant

difference of the disinfection efficiency between the bottles rinsed with lime juice and the other bottles. It should be noted that the data for each sample point were analysed in their raw form and a check of normality was carried out. In cases where the residuals were not normally distributed, due to the data not following the assumptions of normality and homoscedasticity, the data were transformed using a log transformation before the analysis of variance was carried out again for that sample point (Osborne, 2002; McDonald, 2007).

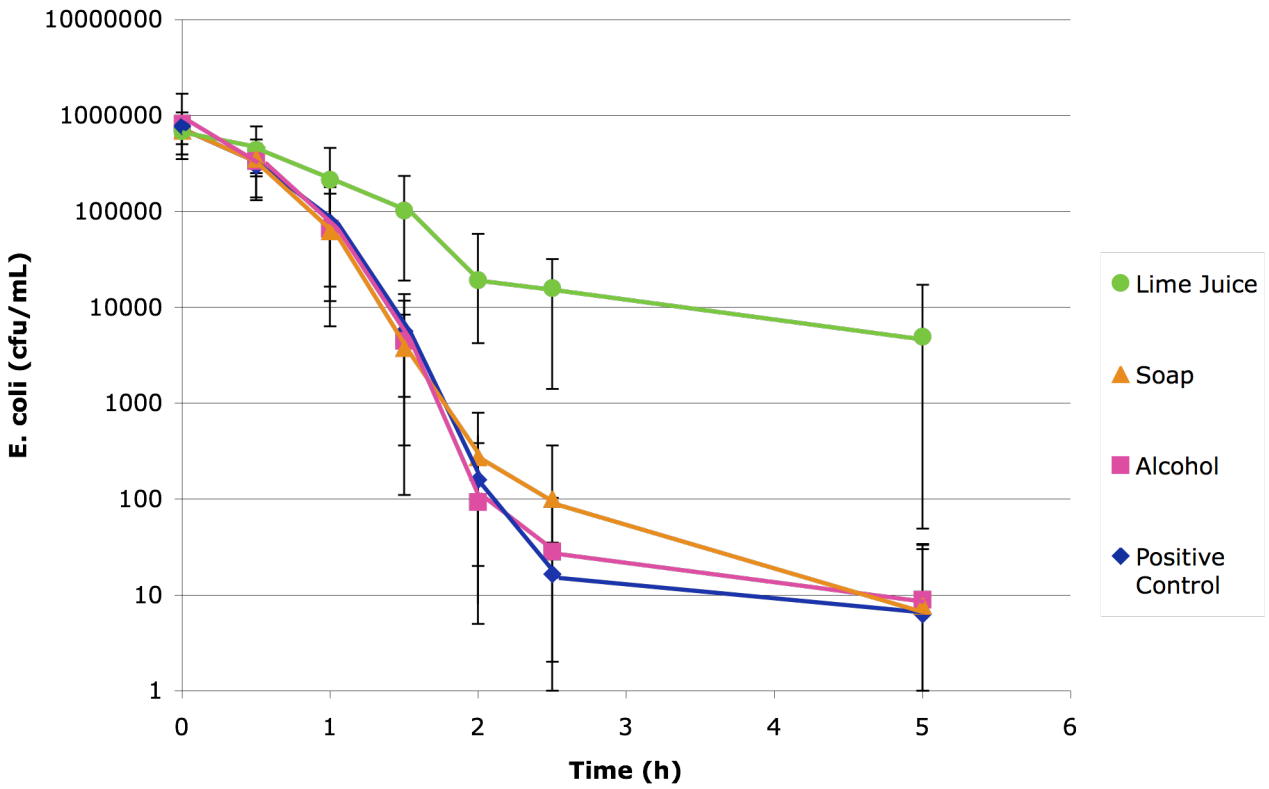

Figure 3.9: Light Bottles' average *E. coli* concentration changes over five hours for the cleaning pretreatment experiments (error bars represent the maximum and minimum concentrations at sample points)

Reductions in *E. coli* numbers (log reductions) were calculated for comparison between the treatments. The two to five hour time period was selected since *E. coli* concentrations of less than 100 CFU/mL were found in some bottles during this period. As can be seen in Table 3.10, the positive control bottles and those rinsed with alcohol and soap-water had minimum log-reductions greater than 2-log which qualify the efficiency of SODIS as high (see Table 2.1). On the other hand, the lime juice-rinsed bottles

experienced smaller reductions. However, there is some overlap of the ranges for each cleaning agent. Figure 3.9 shows that the *E. coli* concentration in the bottles rinsed with lime juice frequently did not reach the acceptable limit of 100 CFU/mL, recommended by the WHO for drinking water.

Table 3.10: Log reductions experienced from two to five hours during cleaning pretreatment experiments

Log reduction	Positive Control	70% isopropyl alcohol	Soap-water mixture	Lime Juice
Minimum	3.39	3.49	3.08	1.23
Maximum	5.73	6.10	5.87	4.65
Mean	4.86	4.92	4.43	2.47
Median	5.13	4.90	4.42	2.07

To demonstrate how the statistical analysis was carried out for these experiments, a sample time (five hours) reflecting all the required analysis has been selected. Table 3.11 shows the ANOVA for the five-hour sample point. It is clear that the null hypothesis must be rejected for the statement "all cleaning agent treatments are the same"; the F_{obs} (calculated F value) value is 13.46, which is much greater than the F_{table} (F-statistic value) value, 2.88.

Table 3.11: ANOVA for the Light Bottles after five hours

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	25.89	8.63	13.46	2.88
Within Cleaning Agents	33	21.16	0.64		
Total	36	47.05			

A normal probability plot was used to determine if the residuals were normally distributed. Figure 3.10 shows that the residuals lie in a straight line; therefore the residuals are normally distributed.

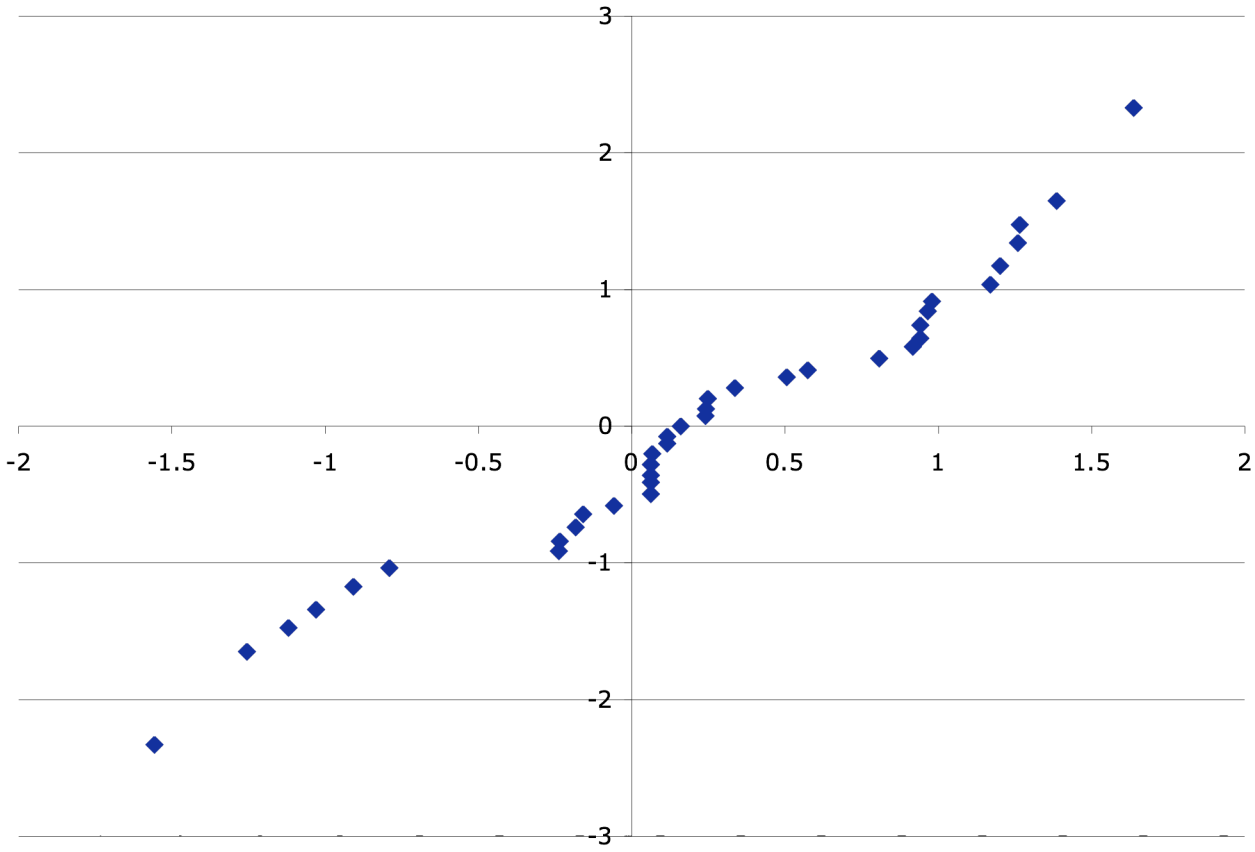

Figure 3.10: Normalized residuals for *E. coli* concentrations in Light Bottles after five hours

The least significant difference was also calculated (see Equation 3.3).

$$\text{LSD} = s.e.(t_{33,0.005}) = 1.01 \qquad (3.3)$$

Table 3.12 outlines the variables used in its calculation. The number of different cleaning agents used for the experiments (k) was four. The number of required experiments (c) was six. To achieve an overall confidence interval of 95% (b), the experiments were performed at a significance level of 99% (α). To calculate the LSD, the standard error of the difference between two means (*s.e.*) and the t-statistic were determined.

Table 3.12: Required values for calculating LSD for Light Bottles after five hours

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	0.64
Average number of sample size	$\bar{n}$	9.25
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	0.37
t-statistic	$t_{df,\alpha}$	2.72

The average log-transformed *E. coli* concentrations for the Light Bottles are found in Table 3.13. The difference between the average concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (1.01). Hence, the lime juice treatment is the only statistically different treatment. (See Appendix 5 for ANOVA at each sample point.)

Table 3.13: Average log-transformed *E. coli* concentrations (CFU/mL) for Light Bottles over all cleaning pretreatment experiments after five hours

Light Bottles	Average log CFU/mL
Alcohol	0.54
Lime Juice	2.60
Soap	0.54
Positive Control	0.36

Twenty-four hours following their initial exposure to sunlight, the Light Bottles pre-cleaned with soap and with lime juice showed a notable difference of disinfection efficiency compared to the alcohol-rinsed bottles and the positive control. As can be seen in Figure 3.11, there was substantial microbial recovery, after overnight storage in the dark, in the lime juice- and soap-treated bottles. At the 24-hour sample time, the lime juice-rinsed bottles had concentrations one-log less than their original, indicating that at least some of the damage caused by the sunlight to the microorganisms was irreversible. The soap-rinsed bottles had concentrations within one-log more than the 100 CFU/mL limit. These bottles appeared to have a film on the inside after conducting experiments. This film may have contributed to

the *E. coli* recovery. The observed effect is similar to that reported for extended use of the bottles, decreased light transmission (see Appendix 2.2).

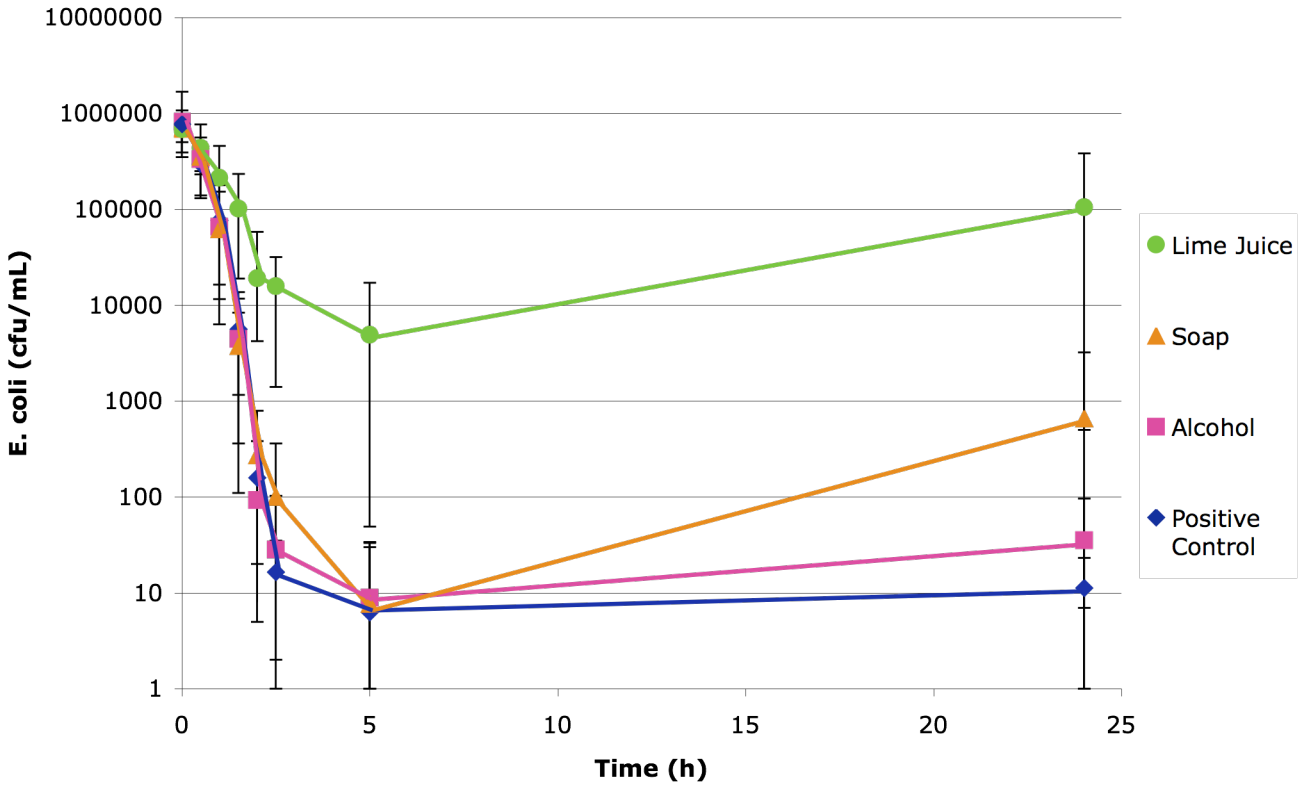

Figure 3.11: Light Bottles' average *E. coli* concentration changes over 24 hours during cleaning pretreatment experiments (error bars represent the maximum and minimum concentrations at sample points)

There was also *E. coli* recovery in the dark control bottles that were pre-cleaned with lime juice. Original *E. coli* concentrations (time zero) were approximately 7.5×10^5 CFU/mL. After 24 hours, concentrations for the dark control set of lime juice-treated bottles were an average of 3.3×10^6 CFU/mL and those that had been exposed to sunlight were an average of 8.9×10^4 CFU/mL. Thus, five hours of light exposure, followed by overnight storage, still produced a considerable reduction in *E. coli* concentrations (almost two-log).

3.4 Discussion

The temperature in the exposed bottles did not approach a constant temperature over the duration of an experiment. The temperature consistently rose at each sample point and may have continued to rise further if experiments had not been concluded after five hours of exposure. The maximum temperature that was reached was 41°C (see Figure 3.8), which is below 50°C, the lower limit for heat and light disinfection mechanisms to work together (McGuigan et al., 1998). The implication for these experiments is that disinfection was primarily attributable to light.

The lower bottle temperatures can be attributed to the low ambient air temperature (see Figure 3.7) and cloud cover (see Figure 3.6) experienced on some experiment days. It is noteworthy that the irradiances experienced in Waterloo, Ontario, were similar to those in countries that lie within the latitudes 35° North and South (see Figure 2.3), the recommended region for SODIS (Odeyemi et al., 1988). Waterloo lies at a latitude of 43°28'N and the highest irradiance recorded during experiments was 946 W/m^2. The fluence only dipped below 555 $W\text{-}h/m^2$ (see Appendix 2.6) during three experiments (see Table 3.8).

Despite the differences in pH of the stock cleaning agent solutions (see Figure 3.3), the initial pH of the water in the Light and Dark Control Bottles were similar. These initial pH values were also very close to neutral. The average initial pH value of the Dark Control Bottles was 7.83 ± 0.30 and the average initial pH of the Light Bottles was 7.83 ± 0.31.

Cleaning with the soap-water mixture and the 70% isopropyl alcohol did not improve the solar disinfection within the bottles as shown in Figure 3.9. Cleaning with lime juice actually *inhibited* subsequent disinfection and was associated with recovery after overnight storage (see Figure 3.11). The data suggest that the lime juice either fostered an environment that allowed the *E. coli* to resist disinfection and administer light-repair after exposure or provided nutrients for the microbes that escaped disinfection to repopulate (Mesquita, 2007). The soap treatment also showed evidence of contributing to recovery of the *E. coli* (see Figure 3.11), although not as greatly as the lime juice treatment and only after light exposure.

The results obtained following rinsing with lime juice can be compared to a recent study (Fisher et al., 2007) that looked at using chemical additives to hasten the solar disinfection process. In one part of the Fischer study, a small volume of ascorbate and copper sulphate were added to water immediately before sunlight exposure. The result was a protective effect similar to that experienced in this project (see Figure 3.11). However, further tests with lemon juice and sweet lime juice (as sources of ascorbate) without copper resulted in increased efficiency of disinfection.

Although the findings of Fisher et al. (2007) may appear to be in direct opposition to those of this project, this is not the case. The Fisher study used *E. coli* K12 MG1655 and *E. coli* K12 NCM3722 whereas this research used *E. coli* ATCC 11229. Discrepancies such as this may result from the use of different strains of bacteria that have different growth rates, which are responsible for sensitivity to certain factors such as pH (Berney et al., 2006). It has also been suggested that acid resistance may not be consistent within populations and that sub-populations may have increased resistance:

> "The existence of [such populations] has important implications for the safety of acid foods [and water], and the causes of this phenomenon need to be clarified" (Benito et al., 1999).

Another difference was the pH of the source water. In the Fisher study, enough lemon or lime juice was added to decrease the pH to less than 5. Dalsgaard's group (1997) also reported the reduction of *V. cholerae* increased corresponding to lower water pH (after the addition of lime juice). However, in this project, the pH of the water exposed to sunlight and used for controls was between 7 and 8 (see Table 3.3; 3.8). Fisher et al. (2007) hypothesized that the lower pH acts as a stress on the cells, which allows for easier inactivation during sunlight exposure.

3.5 Conclusions

The major conclusion for SODIS disseminators would be to clarify that the bottles do not need to be cleaned with any chemical agent. In the case of lime juice, if not added in the proper volume, the efficiency of the process can actually be hindered. In situations where a soap solution might be used, its effect of creating a film appears similar to that of extended use and it should therefore be avoided. A further recommendation would be that the bottles be rinsed with water only, immediately before first use to remove any debris or organic material (that might facilitate microbial recovery) from inside.

Another finding from this research was that SODIS might have potential for use outside of the recommended region. Although Waterloo, is approximately 8° of latitude outside the SODIS region, it was still possible to employ the process during part of the year and achieve appropriate disinfection (i.e., *E. coli* concentrations below 100 CFU/mL). SODIS could be used from at least mid-April to mid-October in Waterloo based on historic irradiation data (providing the fluence is at least 555 W-h/m^2). The implication is that the utility of SODIS can be expanded outside of the suggested geographic range.

The characterization of the dechlorinated tap water was minimal for these experiments (pH, turbidity, and temperature). Subsequent studies may want to conduct an in depth characterization of the source water (DO, UV transmittance, etc.) and treated water for comparison purposes. Furthermore, the transmittance of the PET bottles should be measured in future laboratory studies as a check on the consistency of the material between bottles.

CHAPTER 4: ROUGHING FILTER EXPERIMENTS

Surface water is typically the source of drinking water in developing countries and often has high turbidity. These particles prevent a portion of the UV light in solar radiation from inactivating microorganisms during SODIS. Currently, there is not a set pretreatment for SODIS, although a simple method has been devised for checking the turbidity beforehand. If the turbidity is greater than 30 NTU, when SODIS is being used, disinfection will be primarily attributable to heat. The objective of this research was to construct a roughing filter that would produce an effluent water turbidity of less than 30 NTU. The filter was constructed of widely available materials. As SODIS already involves clear PET bottles (0.5 to 2 L), the 2 L version was selected as the housing for the filter. To determine the proper filter height, numerous filters were constructed and tested. From these experiments, the optimal filter media height was determined to be 20 cm, which consisted of three centimetres of gravel (6 to 20 mm grain size) on the bottom of the bottle topped up by approximately 17 cm of coarse sand (1 to 6 mm grain size). Various hole diameters were tested to find the optimal flow rate. The hole size that provided reasonable filter time and sufficient contact time for attachment of particles to the media was 0.6 mm (i.e., the diameter of a common sewing needle). Under these conditions, the flow rate was 2.7 L/h. The filter provided an average of 95% turbidity removal when the source water was 200 NTU with between 0.32- and 0.64-log reductions of *E. coli*.

4.1 Introduction

The solar water disinfection (SODIS) process continues to be a technology of choice for drinking water treatment in developing countries. It is recommended as a point-of-use treatment by the World Health Organization (WHO) for relieving the burden of water-borne diseases due to its ease of use and minimal required resources. The process consists of exposing collected water in clear polyethylene terephthalate (PET) containers, typically 0.5 to 2 L scavenged pop bottles, to full sunlight for approximately six hours (Solar Water Disinfection, 2002a).

Often the drinking water source in developing countries is surface water. Surface water usually has high turbidity (i.e., particles suspended in it, making it cloudy). These particles prevent a portion of the UV light in solar radiation from penetrating the water and inactivating the microorganisms during SODIS (see Appendix 2.5). In fact, when the turbidity is greater than 30 nephelometric turbidity units (NTU), the mechanism of inactivation of the microorganisms is heat (see Appendix 2.7).

These turbidity-causing particles can also be associated with microorganisms; the microorganisms attach to the particles and are protected from physical and/or chemical disinfectants. The only way to remove these microorganisms is to remove the particles to which they are attached. Of the various methods that exist for removing turbidity at the household (point-of-use) level, sedimentation and filtration are recommended for developing countries (WHO, 2007).

Sedimentation consists of particles (sands, silts, and some microbes) settling by gravity. In the developing country context, water is collected in a container and left undisturbed overnight for the particles to settle to the bottom of the container. Then the water on top, the clarified water, is carefully removed by slowly pouring it into a vessel for storage or use. It is important not to disturb the settled particles or they may be resuspended in the clarified water. This technique can be used on any volume of water. The limitation is that not all particles will settle in a reasonable amount of time; small particles like clay or viruses can take up to months to come out of suspension (WHO, 2007).

Filtration is another process of separating solids from a liquid by attachment principles. There are many types of filters, but granular media filters are the most recommended for use in developing countries (WHO, 2007). Common types of granular media filters are slow sand, rapid sand, and roughing filters. Sand, anthracite or coal, and garnet are the typical media used for filtration. In low-cost situations, these filters are built with a base of gravel with sand on top (WHO, 2007). There may be one filter or a series of filters through which the water flows. Roughing filters differ from the other types in terms of the size of media used in them. These filters have larger sized media (greater than one millimetre grain size) for removing up to 90% of the turbidity as a pretreatment to sedimentation or further filtration (EAWAG, 2007).

Much of the recent research using biosand filters (or arsenic biosand filters) supports using gravel with a grain size from 6 to 20 mm (Ngai and Walewijk, 2003; Hamoda et al., 2004). The coarse sand used in these filters ranges in grain size from 1 to 6 mm (Ngai and Walewijk, 2003). The gravel is used as an underdrain (typically between 5 and 10 cm deep), providing support for the filter (Hamoda et al., 2004; Stauber et al., 2006; Pandey, 2004). The total filter depth is usually in excess of 40 cm of sand (Ngai and Walewijk, 2003; Pandey, 2004; Hamoda et al., 2004).

The media can either be collected from a local source, such as a river, or purchased from a local supplier, such as a rock-crushing operation (Ngai and Walewijk, 2003). Since standard sieves are not normally available in developing countries, another means of characterizing the media has been devised: a metal bowl with 0.5 cm holes drilled in it to separate the gravel from sand and a mosquito net folded three times to separate coarse from fine material (Hurd, 2001). All the media is then rinsed with water until the rinse water appears clear.

The type of container to use for filter housing is often determined based on availability. Since plastic pop bottles are prevalent in developing countries (Harder, 2007; Higgins, 2007) and clear bottles are currently used for SODIS[8], this type of container was a natural selection for the roughing filters in this study. The two litre PET pop bottles are approximately 30 cm in height discounting the narrow neck portion.

The objectives of this study are to: build roughing filters with pop bottles, gravel, and coarse sand; determine the most appropriate flow rate for turbidity removal and home use; and identify the optimal height of sand in the filter for turbidity and *E. coli* removal. The results of these experiments are reported in Section 4.3.

4.2 Materials and Methods

Roughing filters were constructed with used two litre PET pop bottles and granular media from a local supplier. The media were separated by sieving, then characterized, and finally reassembled into required proportions. The various filters were tested with synthetic source water of high turbidity (greater than 200 NTU) and contaminated with *E. coli* (initial concentrations were approximately 10^6 CFU/mL).

4.2.1 Media Preparation

Granular media (a mixture of sand and gravel that is commonly used for asphalt and concrete mixes) was obtained from Dufferin Aggregates (Kitchener, Ontario). Such media is available in developing

[8] See Appendix 2.8; 1 to 2 L PET bottles are currently used for the SODIS process, indicating they would also be readily available for use as the filter housing.

countries from a local source (e.g. a river) or a rock crushing operation (Ngai and Walewijk, 2003). The stock media was sieved using a Gilson Sieve Shaker TS-1 (Gilson, Ohio) to obtain coarse sand (1 to 6 mm) and gravel (6 to 20 mm). The sieves used were ¾", ½", ⅜", #4, #8, #16, #30, and #50 (Gilson, Ohio). After sieving the coarse sand, the mass retained on the ¾", ½", ⅜", #4, #8, and #16 sieves was used for this project. (See Appendix 6 for the sieve analysis.) The media was then rinsed with tap water approximately five times until the water runoff was clear. This was done to remove fine particles. The media was dried on hotplates at about 70°C, but just as easily could have been dried in the sun.

Standard sieves are not widely available in developing countries, so a simple sieving technique using a metal colander with 0.5 cm holes was also tested. The retained portion represented the gravel. The portion passing through the holes was sieved on a mosquito net (folded three times) to separate coarse and fine sand. The amount retained on the net represented the coarse sand (Hurd, 2001).

The standard sieving technique was first carried out on the stock media to determine the appropriate grain sizes for building the filters. Afterward, the gravel and coarse sand that had been separated by the standard sieving technique were sieved again using the simple sieving technique. This technique was extremely precise, with a statistically insignificant mass of the media being lost through the colander (a loss of 0.1 kg of gravel from 25 kg) and the mosquito net (a loss of 0.8 kg of sand from 87 kg).

4.2.2 Turbid Source Water Synthesis

Synthetic source water was prepared for these experiments. Natural source water was not used since its complexity (varying parameters) would have introduced variability into the experiments. Tap water was selected because its quality is relatively constant and it contains elements of natural water (e.g., minerals). The turbidity of the tap water was increased using kaolin powder. Specifically, ten grams of food grade kaolin powder (J.T. Baker Chemical Co., 2242-01) was added to two litres of deionized water, mixed by hand, and the suspension was allowed to settle overnight (Sobon, 2007). The suspended portion was removed carefully and added to tap water in a 26 L high density polyethylene (HDPE) container (Reliance Aqua Lux). The turbidity of this water was measured using a HACH 2100P Portable Turbidimeter (HACH, 4650000). More tap water was added, and manually mixed, until 200 NTU was obtained and there was a final volume of ten litres.

4.2.3 Bacterial Preparation and Enumeration

E. coli was used as the indicator organism in this study due to its frequent use as a fecal indicator in drinking water treatment. A 15 mL portion of the *E. coli* stock culture (ATCC 11229)[9] was thawed and centrifuged at 10 000 rpm for ten minutes to isolate the cells from the broth. The cells were washed three times with 15 mL sterile 0.1% peptone water, made from BD Bacto Peptone (BD, 90000-382), to remove the nutrients and centrifuged between each washing (same speed and time). Then the pellet was resuspended in 15 mL sterile 0.1% peptone water to produce a total initial viable count of 10^9 colony forming units per millilitre (CFU/mL).

Using Equation 4.1, the total volume of the *E. coli* suspension to add to the ten litres of turbid source water (Section 4.2.2) to make the desired bacterial concentration of 10^6 CFU/mL was ten millilitres.

$$C_1V_1 = C_2V_2 \qquad (4.1)$$

$$C_1 = 10^9 \text{ CFU/mL},\ V_1 = 10 \text{ mL},\ C_2 = 10^6 \text{ CFU/mL},\ V_2 = 10 \text{ L}$$

Initial *E. coli* concentrations were confirmed to be approximately 10^6 CFU/mL for each experiment as described in Section 4.2.5. To confirm that the original suspension was 10^9 CFU/mL, one millilitre of the suspension was diluted in a series of dilutions (dilution factors from 10^1 to 10^7). One millilitre was taken from the 10^6-dilution and added to 15 mL of Nutrient Agar (BD, 213000)[10], mixed, and poured onto a sterile nine-centimetre Petri plate (Fisher Scientific, 08-757-9B). This was repeated for the 10^7-dilution. Plates were incubated at 37°C for 24 hours and colonies were counted the following day. The total colony forming units were counted and reported per mL of the original sample.

Before adding the *E. coli* to the synthetic source water, the water had to be dechlorinated (see Section 3.2.2 for details) and 10 mM $NaH_2PO_4 \bullet H_2O$ was added as a buffer. The turbid water was too basic (pH greater than 8.5) for the *E. coli* to survive due to the kaolinite. The buffer brought the pH back to neutral. The pH of the water was measured using an Orion pH Meter (Model 720A) standardized with a pH 7 buffer (VWR, 34179-148).

[9] The ATCC species was selected due to its commercial availability and use in other laboratory studies.

[10] Nutrient Agar was used because it was available and being used for other studies in the laboratory. Although not specific for *E. coli*, since only *E. coli* were spiked into test water and it grows well on Nutrient Agar, colonies that were detected on test culture plates could reasonably be attributed to *E. coli* as long as colony morphology remained consistent with that known for *E. coli* cultures.

4.2.4 Filter Construction and Experiments

Three sets of experiments were conducted using the filters constructed with the prepared media (Section 4.2.1) and two litre pop bottles: one set exploring the effect of flow rate ("Flow Rate Experiments"), another set investigating different media configurations ("Filtration Experiments"), and the third comparing simple settling to filtration ("Settling Experiment").

At the bottom of a two litre pop bottle (filter housing), gravel was placed to a depth of three centimetres with varying heights of coarse sand on top. (The neck portion of the bottle was removed to facilitate construction.) For the Flow Rate Experiments, coarse sand was added until it reached a height of 20 cm. A hole was made approximately 1.5 cm from the bottom of the bottle (to facilitate effluent collection). There were five bottles, each with a different hole diameter: 0.5, 1.0, 2.0, and 3.0 mm. Turbid water (20 L) was pumped into each filter using a peristaltic pump (Cole-Parmer Instrument Co., Model No. 7553-70). The pump speed was set to match the filter flow rate in order to achieve a constant head of water above the filter media. The flow rate was measured using a 10 mL graduated cylinder and stopwatch. A turbidity measurement was taken as water entered the filter. The effluent was collected in one-litre glass beakers with 15 mL poured into the turbidity measurement vial for measuring effluent turbidity. The source water was stirred by hand at regular intervals (after 500 mL of water had been filtered) to keep its turbidity constant.

The Filtration Experiments tested five filters with various depths of media: 5, 10, 15, 20 (Figure 4.1), and 25 cm. Each had a hole of 0.6 mm diameter (determined from the Flow Rate Experiments). For the first phase of these experiments, turbid water (10 L) was pumped into each filter (as described above). During the second phase, *E. coli* was added to the same volume of turbid source water (approximate initial concentration of 10^6 CFU/mL). The flow rate and turbidity were measured using the procedures mentioned above. The effluent for the second phase was collected in one-litre glass bottles for biological safety purposes. (The filled bottles were autoclaved at 121°C for 15 minutes to sterilize the water before discarding it.)

Figure 4.1: The 20 cm roughing filter

To determine if the roughing filter was a viable, quick, and compatible means of reducing turbidity before SODIS, settling was carried out using similar source water (i.e., 200 NTU, 10 L) for comparison. The filter used in this experiment was the 20 cm filter, shown to be the optimal design by the previous Filtration Experiments. The water was filtered under the same conditions as previous experiments (i.e., pumped into the filter, flow rate of 2.7 L/h, collected in one-litre glass beakers).

For the settling part of this experiment, the ten litres of source water was placed in a 26 L HDPE container (Reliance Aqua Lux). The water was mixed by hand at the beginning of the experiment (time zero) and 15 mL was then removed to measure turbidity. The turbidity was measured again every hour for eight hours. The 15 mL sample was drawn from the lower portion of the settling water.

4.2.5 Microbial Sampling and Analysis

Two millilitres were taken from the one-litre glass bottles at each sampling point during the second phase of Filtration Experiments. Each millilitre was diluted in a series of dilutions (dilution factors of 10^2 to 10^4) using standard sterile techniques. One millilitre was taken from a single dilution and added to 15 mL of Nutrient Agar (BD, 213000), mixed gently by hand, and poured onto a sterile nine-centimetre Petri plate (Fisher Scientific, 08-757-9B). Plates were incubated at 37°C for 24 hours and counted the following day. The totals were reported as CFU/mL of the original sample.

4.3 Results

Some assumptions were made for the analysis of the data resulting from these experiments. The first assumption was that the data from samples follow a normal distribution. Although the population from which samples were taken may not be normally distributed, the sample distribution was observed to tend toward the normal distribution. Secondly, only two samples were drawn for each sample-point so it was not possible to calculate variance; as such, homoscedasticity (same variance) has been assumed. This then also implies that the residuals are uniform. This assumption typically provides good estimation, but may lead to underestimating the correlation of variables (Box et al., 2005).

4.3.1 Flow Rate Experiments

Figure 4.2 shows the effect that the hole diameter had on turbidity removal efficiency and Figure 4.3 the corresponding flow rate. A complete replication of this experiment was carried out. The largest average removal was 94% and was obtained using a hole size of 0.5 mm. The flow rate for this filter was very slow: 1.16 L/h. It was assumed that users would want to have the highest possible flow rate achievable, yet still maintain sufficient turbidity removal for SODIS. For a source water of 200 NTU, and keeping in mind the 30 NTU limit for SODIS, the removal efficiency would need to be at least 85%. Extrapolating from Figure 4.2, 85% removal efficiency corresponds to a hole diameter of 0.6 mm. (Simply inserting a standard sewing needle into the bottle makes a hole of 0.6 mm diameter.)

Figure 4.3 shows that with a 0.6 mm hole diameter the flow rate increased to 2.84 L/h. (The flow rate was expected to increase with increasing hole size, following a linear relationship, but in fact followed a cubic relationship.) For an average family (four people), their minimum daily drinking water requirement would be 20 L (Howard and Bartram, 2003). The filter flow rate would provide users the option of filtering their water immediately after collection or allowing their water to filter overnight (approximately eight hours to filter 20 L) before using it for SODIS.

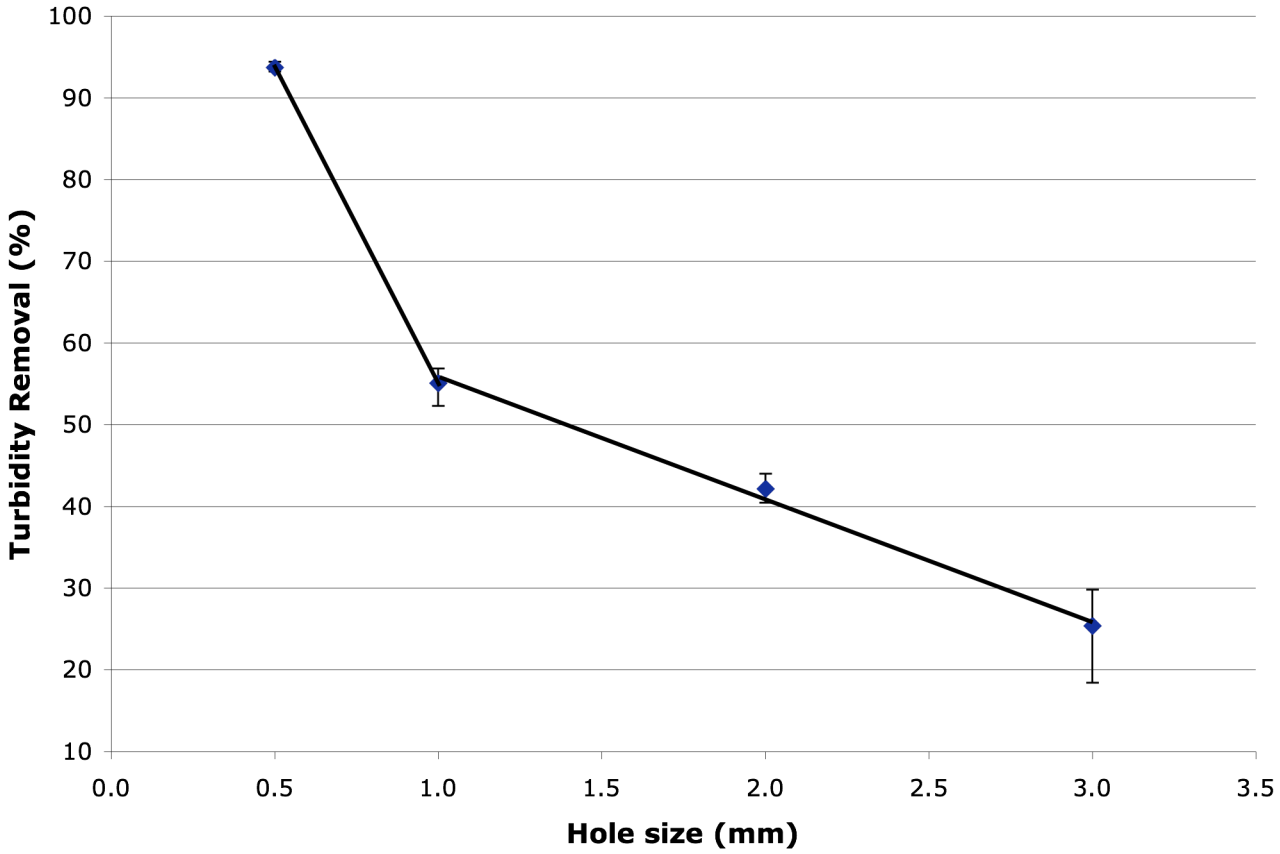

Figure 4.2: Effect of hole size on turbidity removal efficiency (error bars represent maximum and minimum turbidity reduction)

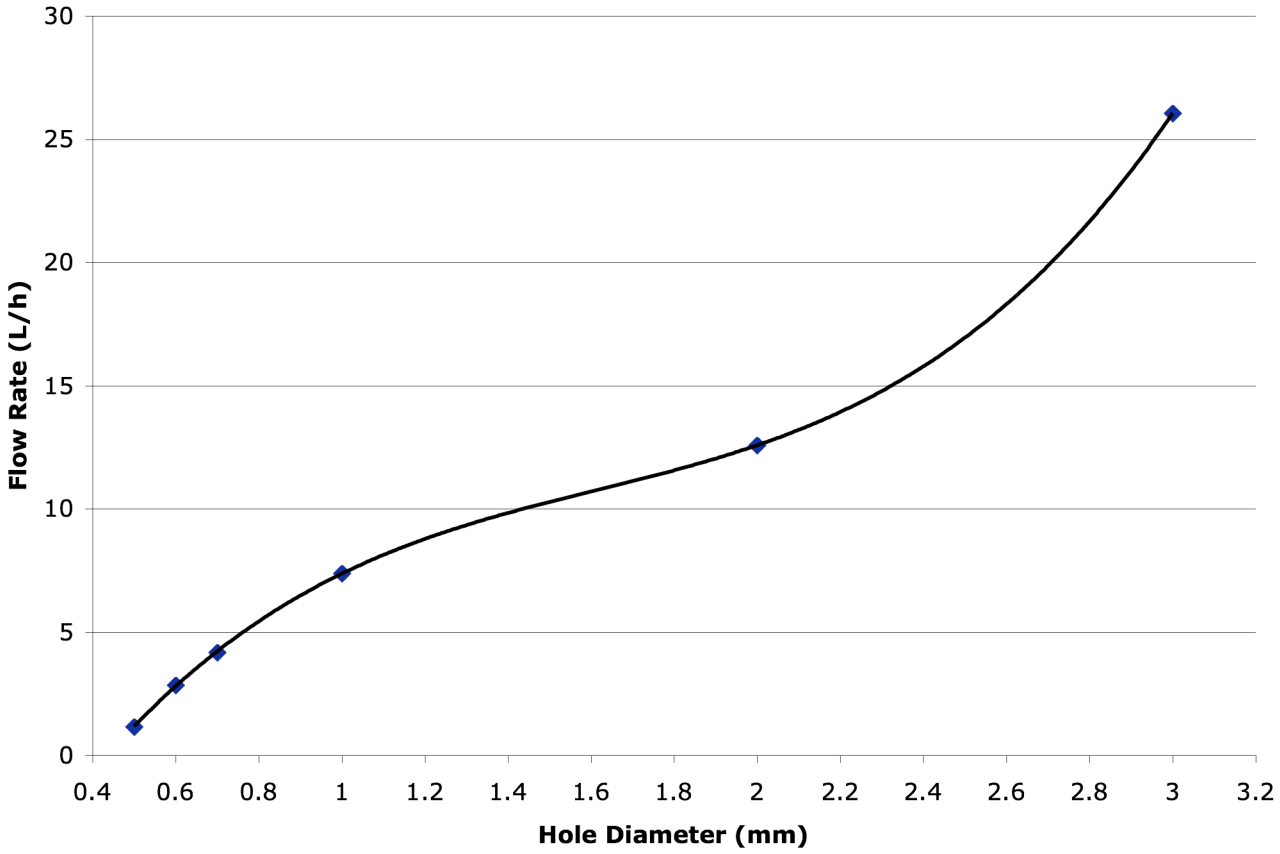

Figure 4.3: Effect of hole size on flow rate

4.3.2 Filtration Experiments

Different depths of coarse sand were placed in five filters to see which ones removed the most turbidity. Each filter had a hole diameter of 0.6 mm and was operated with a constant head. For these experiments, the turbidity of the source water was measured before and after the *E. coli* was added and there was no difference in the measured values. Figure 4.4 shows the effluent turbidity for each of the five filters of different depths (described in Section 4.2.4). As can be seen from this figure, the ripening phase occurred during the first two litres for the 15 and 20 cm deep filters, while the other filters took longer to reach steady state. The turbidity measured in the first litre from the 15 and 20 cm filters was consistently above 30 NTU and would not be recommended for SODIS unless it is retreated.

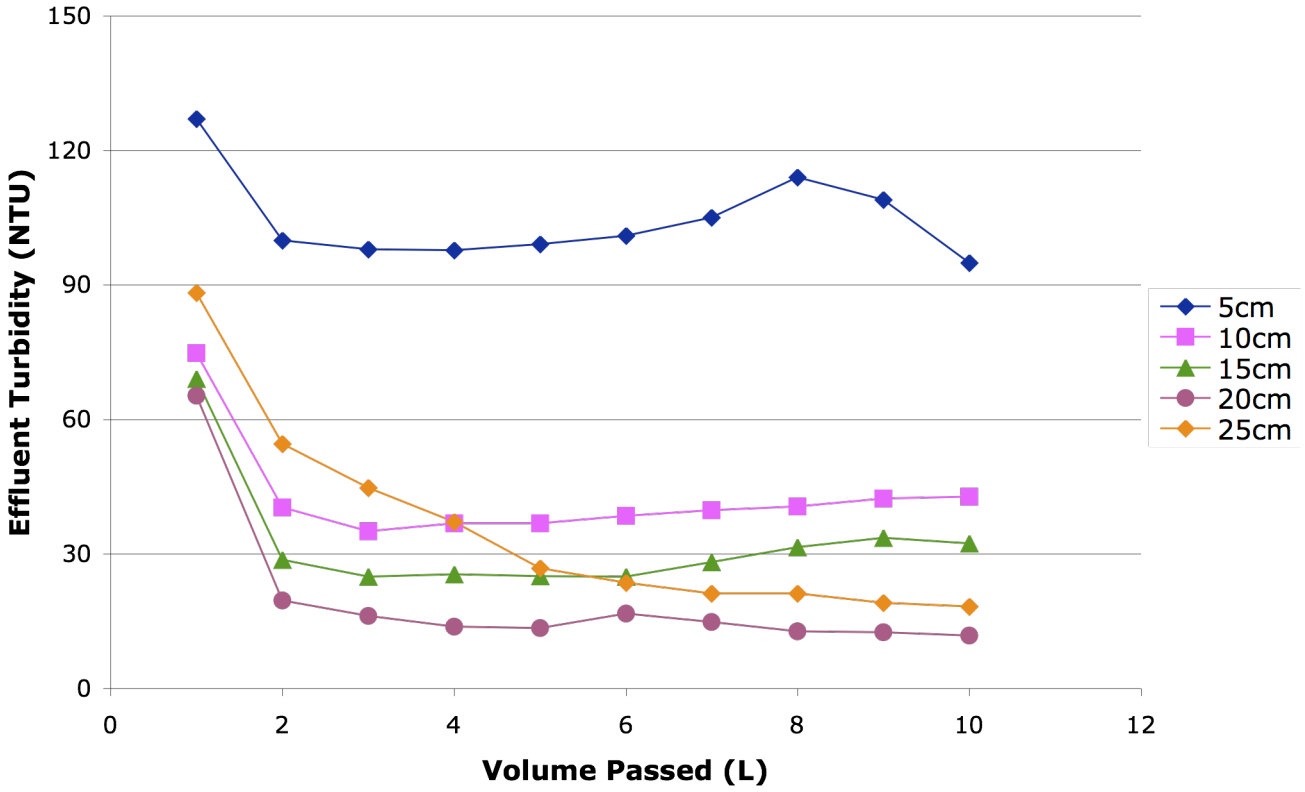

Figure 4.4: First phase effluent turbidity from the various filters

Table 4.1 shows the turbidity removals of the various filters at steady state during the first phase of experiments. The smallest average steady state removal efficiency was 50% by the 5 cm filter. The largest average removal efficiency was 93% (which corresponds to 14 NTU) by the 20 cm filter. The 25 cm filter did not perform as well as the 20 cm filter in this experiment. The 25 cm filter did not have more than a few centimetres headspace and needed constant attention so that water did not overflow the bottle. Each of the 15, 20, and 25 cm filters had more than 85% turbidity removal (at steady state) during these experiments.

Table 4.1: Turbidity removal at steady state for first phase of experiments

Turbidity Removal (%)	**Total Filter Media Height**				
at Steady State	**5 cm**	**10 cm**	**15 cm**	**20 cm**	**25 cm**
Mean	50	80	86	93	89
Median	51	80	86	93	89
Maximum	54	82	88	94	91
Minimum	44	78	83	90	87

Figure 4.5 shows the effluent turbidity from these same filters when *E. coli* was present in the source water. Figure 4.5 illustrates that ripening typically happened during the first two litres, save for the 5 cm deep filter. The turbidity measured in the first litre was consistently above 30 NTU and it is not recommended for SODIS unless it is passed through the filter again.

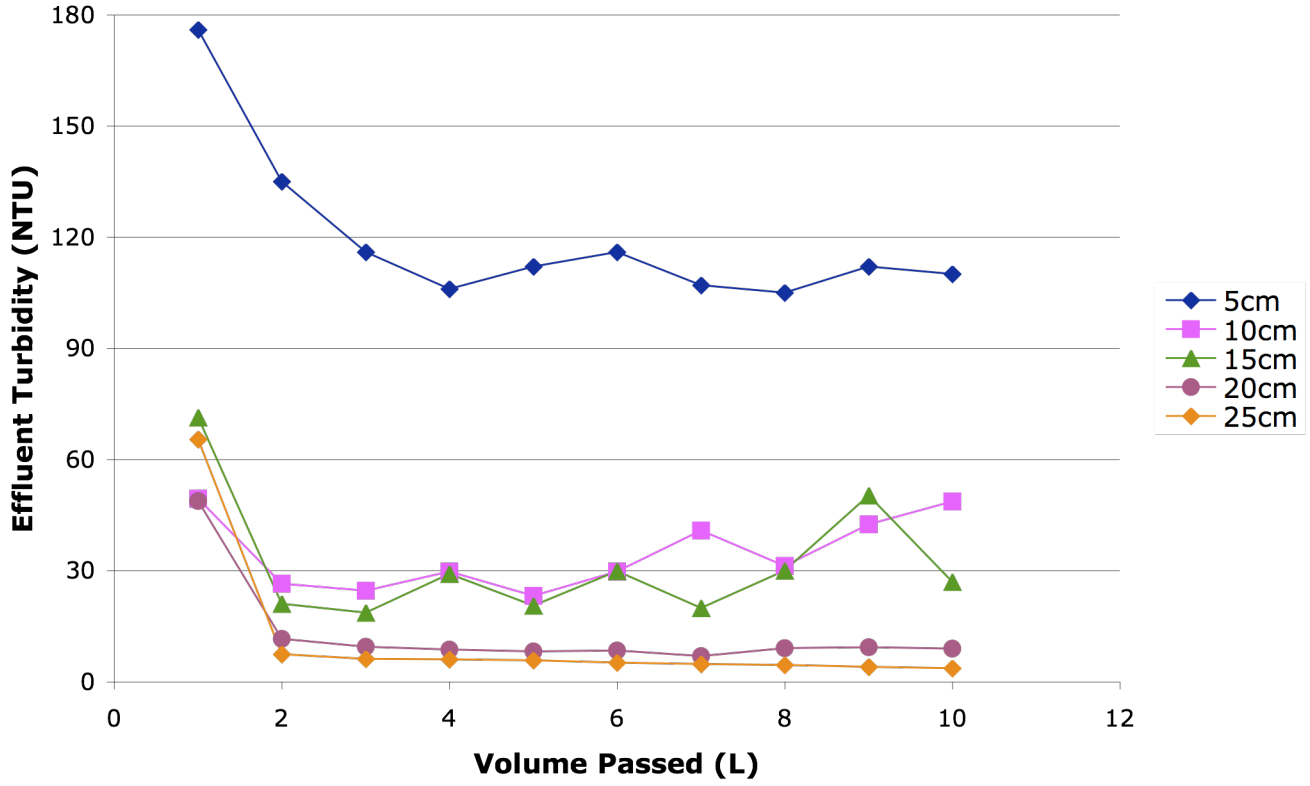

Figure 4.5: Second phase effluent turbidity from the various filters with *E. coli*-spiked water (initial conc. = 10^6 CFU/mL)

Table 4.2 shows the turbidity removals of the various filters at steady state during the second phase of experiments. When the experiment was repeated with *E. coli* added to the source water, the smallest average steady state removal efficiency was 47% by the 5 cm filter and the largest average removal efficiency was 97% (which corresponds to 6 NTU) by the 25 cm filter. The 25 cm filter performed better during this phase of experiments, following the trend present in Figure 4.5 (increased removal with increased filter bed depth). This suggests that the more sand, or deeper the filter, the longer the overall ripening phase. Although the effluent turbidity from each of the deeper filters was below the 30 NTU limit at steady state (in each phase of experiments), only one design has been selected for the roughing filter pretreatment to SODIS. The 20 cm filter provides more removal than required and still has enough headspace to not require constant monitoring.

Table 4.2: Turbidity removal at steady state for second phase of experiments (*E. coli* present in source water)

Turbidity Removal (%)	**Total Filter Media Height**				
at Steady State	**5 cm**	**10 cm**	**15 cm**	**20 cm**	**25 cm**
Mean	47	85	89	96	97
Median	47	86	90	96	97
Maximum	50	90	92	97	98
Minimum	41	78	86	94	96

Reductions in *E. coli* concentrations (log reductions) were calculated for comparison between filters. The average steady state reduction was approximately 0.5-log as can be seen in Table 4.3. This is not a substantial decrease of bacterial concentrations, but may possibly facilitate quicker disinfection during SODIS by removing solids that prevent light penetration into the water. There is some overlap of the ranges for each filter, but it is evident that the 5 cm filter did not perform as well as the others.

Table 4.3: Log reductions of *E. coli* at various filter depths

	Total Filter Media Height				
Log reduction	**5 cm**	**10 cm**	**15 cm**	**20cm**	**25 cm**
Minimum	0.15	0.43	0.49	0.32	0.43
Maximum	0.55	0.88	0.61	0.64	0.73
Mean	0.34	0.57	0.55	0.52	0.57
Median	0.41	0.48	0.54	0.54	0.58

4.3.3 Settling Experiment

The turbidity removal by settling is shown in Figure 4.6. It took six hours for ten litres to reach an average water quality of less than 30 NTU, the SODIS guideline. At the end of eight hours the average quality was 21 NTU.

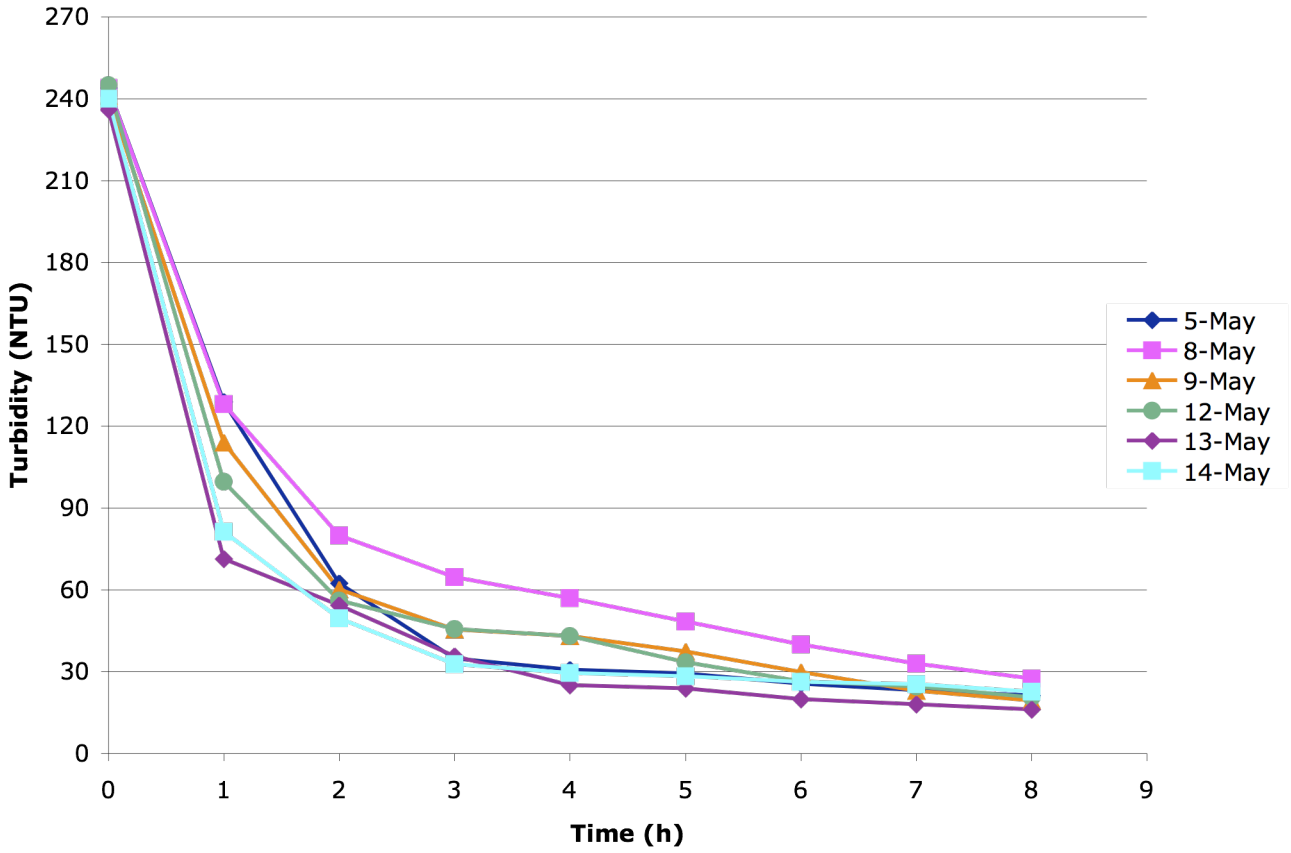

Figure 4.6: Turbidity removal by settling

The 20 cm roughing filter produced an effluent turbidity of less than 10 NTU after one hour of filtration (hole diameter was 0.6 mm, flow rate of 2.7 L/h). The ten litres were completely filtered in a total of five hours and the effluent turbidity remained less than 10 NTU. Therefore, using the roughing filter provided an effluent with less turbidity and required less time to do so than using settling.

4.3.4 Breakthrough

Breakthrough in the 20 cm roughing filter was achieved after approximately 35 L had been filtered. Figure 4.7 illustrates the progression of filter performance with successive experiments. Breakthrough happened during the seventh run of the filter on June 6; the effluent turbidity was consistently above 30 NTU during this run. The first litre of each run was discarded since it was part of the ripening phase; however, this volume could be retreated if used with SODIS.

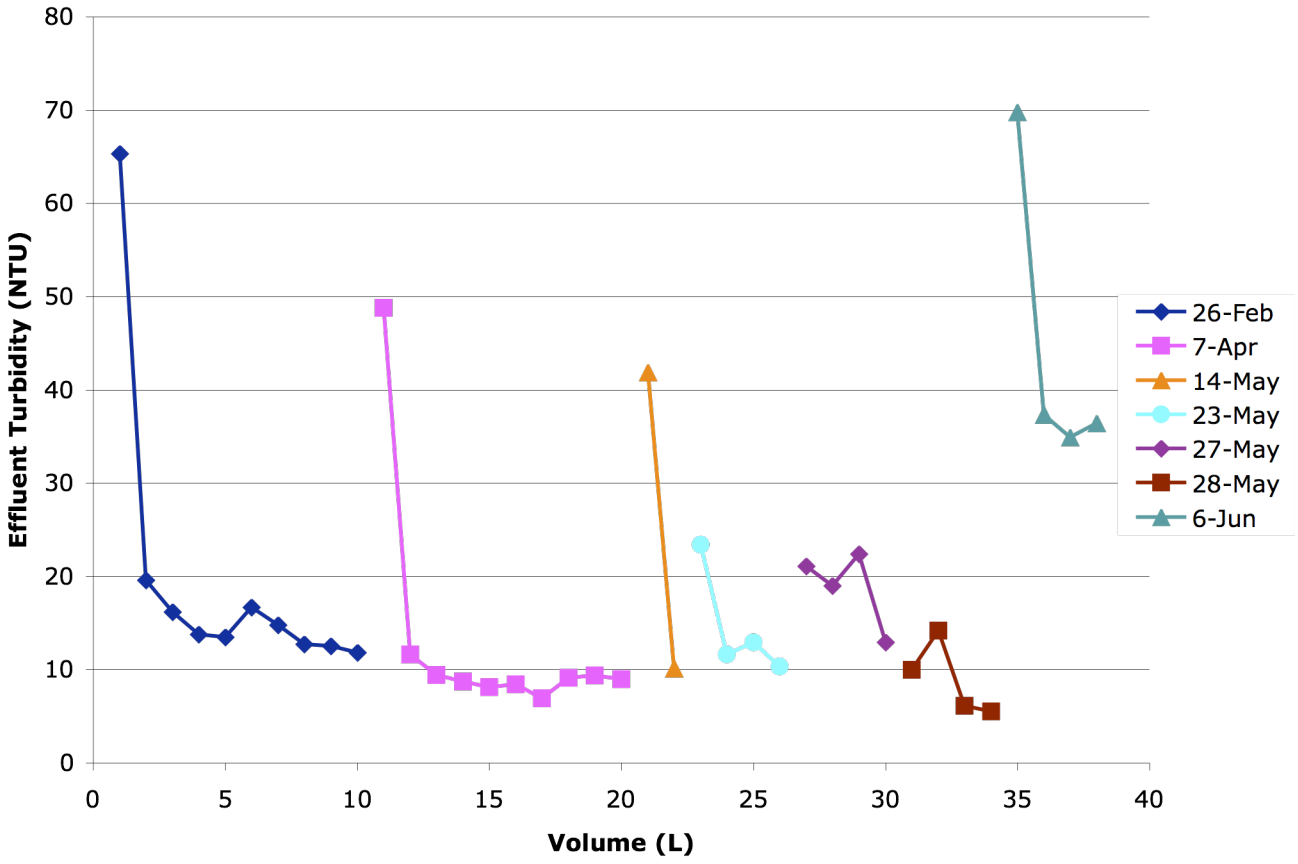

Figure 4.7: Progression of filter performance with successive experiments (20 cm roughing filter)

Table 4.4 shows that the maximum source water turbidity entering the filter was 437 NTU, while the minimum was 167 NTU. The average across all experiments was 232 NTU. The maximum effluent turbidity was 70 NTU (during ripening), with the minimum at 5.5 NTU, and the average was 19 NTU.

Table 4.4: Turbid source water and effluent values across all roughing filter experiments

Values	Source Turbidity (NTU)	Effluent Turbidity (NTU)
Maximum	437	70
Minimum	167	5.5
Mean	232	19
Median	203	13

4.4 Discussion

The simple sieving technique described by Hurd (2001) was validated in this study (see Section 4.2.1). Comparison of this technique to standard sieving confirmed no significant difference between the two. The simple sieving technique should be considered an appropriate method for use in developing countries.

Using a hole diameter of 0.6 mm allowed for a quick flow rate between 2.7 and 2.8 L/h and still provided sufficient contact time between the particles and granular media in the filter for adequate turbidity removal (see Section 4.3.1). These flow rates correspond to a constant head of water above the filter. This may be the optimal operating conditions for these filters, but may not be how they would actually be used.

The 15, 20, and 25 cm filters exceeded the goal of less than 30 NTU in their effluent (see Figures 4.4 and 4.5). The 25 cm filter did not perform as well as the 20 cm filter in the first phase of the Filtration Experiments. This was unusual given that the trend for the other filters was an increase in turbidity removal with an increase in filter depth. The 25 cm filter performed better during the second phase of Filtration Experiments, following the expected trend. This suggests that the more sand, or deeper the filter, the longer the overall ripening phase of the filter.

The 25 cm filter did not have more than a few centimetres headspace and needed constant attention so that water did not overflow the bottle. Although it provided effluent quality less than 30 NTU, it would not be practical for the average SODIS user. Thus, the recommended filter design is the 20 cm filter, with 3 cm of gravel underdrain, topped up with coarse sand. This filter does not require constant attention and less sand needs to be sieved for its construction. The flow rate for this filter is approximately 2.8 L/h when the diameter of the hole is 0.6 mm.

The 20 cm filter removed more than 90% turbidity for turbidities up to approximately 250 NTU; it provided an effluent with at most 20 NTU (see Tables 4.1 and 4.2). The target turbidity for SODIS water is 30 NTU, so this filter met the guideline and would allow users to quickly filter their water. The easiest way to use this filter would be to allow water to flow by gravity into it from a container with a similar hole diameter, collect the effluent in PET bottles, and expose the effluent water to sunlight.

The roughing filters had an average *E. coli* reduction of 0.5-log (see Table 4.3). This was lower than expected. It has been recorded that roughing filters achieve 1- to 2-log reductions for fecal coliforms (EAWAG, 2007). This difference may be due to a lack of developed biological growth on the media. In slow sand filtration much of the microorganism removal occurs in the schmutzedecke, the thick microbial layer at the top of the filter (Droste, 1997). The lack of *E. coli* removal may also be related to a lack of dissolved or suspended organic matter in the synthetic source water, which prevented the formation of the schmutzedecke (Droste, 1997). It would be expected that microbial removal in the filter would increase along with organic matter in the source water.

Comparing the roughing filter to simple settling showed that the roughing filter was substantially quicker for turbidity removal (see Section 4.3.3). The roughing filter provided effluent turbidities of less than 10 NTU after one hour and could provide a total of ten litres of the same quality water in five hours. On the other hand, settling required six hours to produce ten litres of water with an average quality of 30 NTU, the SODIS limit (see Figure 4.6). This comparison supports the idea that if a user collects water in the morning, filters are a more appropriate pretreatment for SODIS since the turbidity could be removed quickly and exposure could take place immediately following filtration. In the situation where users collect their water in the evening for use the following day, settling might be an appropriate turbidity removal technique since the user is not concerned with exposing the water immediately to the sunlight; however, filtration would still provide effluent with less turbidity.

The 20 cm filter consistently produced effluent water qualities below 30 NTU until a total volume of 35 L had been filtered (see Figure 4.7). The source water turbidity varied between 167 and 437 NTU. This is a typical range of surface water quality and as such, the filter would perform satisfactorily on such water in a developing country. Depending on the size of the family using the roughing filter (the volume required to filter per day) and the source water quality (the turbidity), a single filter might reach breakthrough (greater than 30 NTU effluent) within a few days of use. The relationship between the breakthrough volume at higher flow rates (i.e. that is a larger hole diameter) and turbidity removal should be examined; in this study turbidity values less than 30 NTU were favoured over the 30 NTU limit. As well, simple methods of re-establishing the filter's capacity should be explored. The volume of water of specific turbidity (e.g., 200 NTU) that could be filtered before reaching the 30 NTU limit should also be determined.

4.5 Conclusions

The major finding from this research is that the 20 cm roughing filter is capable of pretreating water intended for SODIS use. The filter was constructed of the same two litre bottles used for SODIS, as well as coarse sand and gravel. A requirement of SODIS is that water must have turbidity less than 30 NTU in order for sunlight to penetrate the water and disinfect the microorganisms in it. The 20 cm filter developed in this study, consistently provided effluent water turbidities less than 30 NTU at steady state. The ripening phase was typically the first two litres, with the first litre being greater than 30 NTU; this first litre should be refiltered before using it for SODIS. This filter also reduced *E. coli* concentrations of the water by 0.5-log. Although this is not a substantial decrease of bacterial concentrations, it may possibly facilitate quicker disinfection during SODIS.

Another finding was that the roughing filter provided an effluent water quality with lower turbidity than simple settling and in a much quicker time. This suggests that when users want to pretreat their water and immediately expose it to sunlight, the filter is the preferred pretreatment option. Otherwise, when the water is not directly used for SODIS, settling may be selected as the pretreatment; however, the filter will provide water with less turbidity even after eight hours of settling.

CHAPTER 5: ROUGHING FILTER AND SUNLIGHT EXPERIMENTS

The currently accepted version of SODIS, formalized by EAWAG and SANDEC, consists of cleaning a container, filling it with water, shaking it to entrain air, and placing it in the sunlight for approximately six hours. Surface water is typically the source for drinking water in developing countries and often has high turbidity. Turbidity-causing particles prevent a portion of UV light in solar radiation from inactivating microorganisms during SODIS. Although a simple method has been devised to check turbidity beforehand, there is no set pretreatment for SODIS that will bring high turbidity down to recommended levels. The aim of this research was to use the previously developed roughing filter (described in Chapter 4) in series with SODIS to determine its effect on the process. This filter was constructed with 3 cm of gravel (6 to 20 mm grain size) placed on the bottom of a 2 L PET bottle with coarse sand (1 to 6 mm grain size) on top. The total filter depth was 20 cm. A 0.6 mm diameter hole was made, using a common sewing needle, near the bottom of the pop bottle to achieve an average flow rate of 2.85 L/h. The average turbidity removal was 93% for approximately 240 NTU and the average *E. coli* reduction in the filter was 0.35-log. The results of these experiments showed that filtering increased the solar disinfection efficiency; non-filtered water experienced an average of 2.7-log reduction, while filtered water had an average of 4.1-log reduction after five hours of sunlight exposure.

5.1 Introduction

Using sunlight to disinfect water is not a new idea; the Greeks were using this method as early as 4000 B.C. (US EPA, 2000). Today, approximately one billion people do not have access to safe drinking water and 1.6 million die every year due to water-borne diseases (WHO, 2004; 2007). There is a great need for simple drinking water treatment technologies that can relieve this burden. This problem has, in part, led us back to using sunlight for water disinfection.

The solar water disinfection (SODIS) process is ideal for drinking water treatment in developing countries and emergency situations because of its ease of use and minimal required resources. The process consists of exposing water in clear PET containers, typically 0.5 to 2 L discarded pop bottles, to full sunlight for approximately six hours (Solar Water Disinfection, 2002a). The DNA or RNA of

microorganisms absorbs some of this energy, creating thymine dimers, which produce mutations that inhibit the organisms from reproducing and being pathogenic (Montgomery, 1985).

Typically, the drinking water source in developing nations and emergency situations is surface water. Surface water characteristically has many particles, making it cloudy. These particles are detrimental to SODIS because they prevent sunlight from penetrating the water and inactivating the microorganisms (see Appendix 2.5). These turbidity-causing particles can also be associated with microorganisms, which attach themselves to the particles to gain protection from physical or chemical disinfectants. In this case, the only way to remove the organisms is to remove the particles to which they are attached. Thus, it is necessary to have a pretreatment for SODIS. Different methods exist for removing turbidity at the point-of-use level, but only two are recommended for use in developing countries: sedimentation and filtration (WHO, 2007). Sedimentation has already been shown to be somewhat ineffective at separating solids from liquid in a reasonable amount of time (Section 4.3.3); the smallest particles like clay and viruses can take up to months to come out of suspension (WHO, 2007).

On the other hand, filtration, especially granular media filtration, is widely recommended for use in developing countries (WHO, 2007). When considering the point-of-use (home) application of SODIS, slow sand and rapid sand filters are not likely candidates for pretreatment because they require a great deal of space and media; they are more viable options for community-wide treatment. Roughing filters are typically only used as a pretreatment to sedimentation or further filtration and may remove up to 90% of turbidity (EAWAG, 2007). The roughing filters do not require the careful selection of fine media like slow sand or rapid sand filters, since their required grain sizes are greater than one millimetre (EAWAG, 2007).

The construction of roughing filters in developing countries, as a pretreatment to SODIS, must be as simple and cost effective as SODIS itself. The type of container to use as the filter housing is based on availability. Plastic pop bottles are prevalent in developing countries (see Appendix 3) and clear bottles are already used for SODIS (see Appendix 2.8), making this type of container a natural choice. Current research for biosand filtration has shown that media can often be obtained from a local source, such as a river, or purchased from a local supplier, such as a rock-crushing operation (Ngai and Walewijk, 2003). The gravel used for the underdrain (typically between 5 and 10 cm in height) should have grain sizes in the range of 6 to 20 mm (Ngai and Walewijk, 2003; Hamoda et al., 2004; Stauber et al., 2006).

The coarse sand should have grain sizes in the range of 1 to 6 mm (Ngai and Walewijk, 2003). Biosand filters usually have a bed depth in excess of 40 cm of sand. The roughing filters (described in Chapter 4) are restricted to less than 30 cm due to the height of the pop bottle; two litre pop bottles are 30 cm tall, but the filter also requires headspace.

Roughing filters have previously been designed and lab tested (Chapter 4). The optimal configuration was shown to be a filter with depth of 20 cm and hole diameter of 0.6 mm. The objective of this study was to determine the effect that pretreatment using this type of roughing filter has on the SODIS process.

5.2 Materials and Methods

The 20 cm roughing filter was constructed using a two-litre PET pop bottle and granular media from a local source. The media were separated by sieving, then characterized, and finally reassembled into required proportions. The filter was tested with synthetic source water with high turbidity (greater than 200 NTU) and spiked with *E. coli* (initial concentrations were approximately 10^6 CFU/mL). A portion of the source water and the effluent from the filter were added to 500 mL PET water bottles, which were exposed to sunlight for five hours.

5.2.1 Media Preparation for 20 cm Roughing Filter

Granular media (a mixture of sand and gravel that is commonly used for asphalt and concrete mixes) was obtained from Dufferin Aggregates (Kitchener, Ontario). Such media is available in developing countries from a local source (e.g. a river) or a rock crushing operation (Ngai and Walewijk, 2003). The stock media was sieved using a Gilson Sieve Shaker TS-1 (Gilson, Ohio) to obtain coarse sand (1 to 6 mm) and gravel (6 to 20 mm). The sieves used were ¾", ½", ⅜", #4, #8, #16, #30, and #50. After sieving, the mass retained on the ¾", ½", ⅜", #4, #8, and #16 sieves was used for constructing the roughing filter. (See Appendix 6 for the sieve analysis.) The media was then rinsed with tap water approximately five times to remove fine particles. The media was dried on hotplates at about 70°C, but could just as easily have been dried in the sun.

5.2.2 Turbid Source Water Synthesis

Synthetic source water was prepared for these experiments. Natural source water was not used because its varying parameters would have introduced complexity into the analysis. Tap water was selected due to its quality being relatively constant and containing elements of natural water (e.g., minerals, turbidity). The turbidity of the tap water was increased using kaolin powder. Specifically, ten grams of food grade kaolin powder (J.T. Baker Chemical Co., 2242-01) was added to two litres of deionized water, mixed by hand, and the suspension was allowed to settle overnight (Sobon, 2007). The suspended portion was removed carefully and added to dechlorinated tap water (dechlorination is explained in Section 3.2.2) in a 26 L HDPE container (Reliance Aqua Lux). The turbidity of this water was measured using a HACH 2100P Portable Turbidimeter (HACH, 4650000). More dechlorinated tap water was added, and manually mixed, until 200 NTU was obtained. The source water was autoclaved for 15 minutes at 121°C to prevent contamination.

5.2.3 Bacterial Preparation and Enumeration

E. coli was used as the indicator organism in this study due to its frequent use as a fecal indicator in drinking water treatment. A portion of the *E. coli* stock culture (ATCC 11229)[11] was thawed and centrifuged at 10 000 rpm for ten minutes to isolate the cells from the broth. The cells were washed three times with sterile 0.1% peptone water, made from BD Bacto Peptone (BD, 90000-382), to remove the nutrients and centrifuged between each washing (same speed and time). Then the pellet was resuspended in 15 mL sterile 0.1% peptone water to produce a total initial viable count of 10^9 colony forming units per millilitre (CFU/mL).

Using Equation 5.1, the total volume of the *E. coli* solution to add to the five litres of turbid source water (Section 5.2.2) to make the desired bacterial concentration of 10^6 CFU/mL was five millilitres.

$$C_1V_1 = C_2V_2 \tag{5.1}$$

$$C_1 = 10^9 \text{ CFU/mL}, V_1 = 5 \text{ mL}, C_2 = 10^6 \text{ CFU/mL}, V_2 = 5 \text{ L}$$

To confirm that the original suspension was 10^9 CFU/mL, one millilitre of the suspension was diluted in a series of dilutions (dilution factors from 10^1 to 10^7). One millilitre was taken from the 10^6-dilution

[11] The ATCC species was selected due to its commercial availability and use in other laboratory studies.

and added to 15 mL of Nutrient Agar (BD, 213000)[12], mixed, and poured onto a sterile nine-centimetre Petri plate (Fisher Scientific, 08-757-9B). This was repeated for the 10^7-dilution. Plates were incubated at 37°C for 24 hours and colonies were counted the following day. The totals were reported as CFU/mL of the original sample.

Before adding the *E. coli* to the source water, the water had to be dechlorinated (see Section 3.2.2) and 10 mM $NaH_2PO_4 \bullet H_2O$ was added as a buffer. The turbid water was too basic (pH greater than 8.5) for the *E. coli* to survive due to the kaolinite. The buffer brought the pH back to neutral. The pH of the water was measured using an Orion pH Meter (Model 720A) standardized with a pH 7 buffer (VWR, 34179-148).

5.2.4 Roughing Filter Construction

The roughing filter was constructed by placing three centimetres of gravel at the bottom of a two litre pop bottle and adding coarse sand on top of the gravel to a height of 20 cm. (The neck portion of the pop bottle was removed to facilitate construction.) A 0.6 mm diameter hole was made at approximately 1.5 cm from the bottom of the bottle.

5.2.5 Roughing Filter and Sunlight Experiments

There were a total of five litres of turbid *E. coli* contaminated source water for each experiment. The flow from the spigot of the 26 L HDPE container (Reliance Aqua Lux) was adjusted to match the filter flow rate to achieve a constant head of water above the filter; the flow rate was measured using a 10 mL graduated cylinder and stopwatch. A turbidity measurement was taken as the water entered the filter. The first litre of effluent was collected in a glass bottle with 15 mL removed to measure the turbidity. The first litre of effluent was not used in the experiments since its turbidity was above 30 NTU; the first two litres represented the ripening phase of the filter (see Section 4.3.2; 4.3.4).

[12] Nutrient Agar was used because it was available and being used for other studies in the laboratory. Although not specific for *E. coli*, since only *E. coli* were spiked into test water and it grows well on Nutrient Agar, colonies that were detected on test culture plates could reasonably be attributed to *E. coli* as long as colony morphology remained consistent with that known for *E. coli* cultures.

Following the filtration of the first litre, another 1.5 L was filtered with the effluent being collected in 500 mL PET water bottles[13]. After filtration was complete, 1.5 L of the source water was placed in three other 500 mL PET water bottles. As summarized in Table 5.1, there were two sets of three bottles for each experiment: one for filtered and one for non-filtered water.

Table 5.1: Experimental setup for roughing filter and sunlight experiments

Bottle Set	**Test Water Quality**	**Bottle Location**
Filtered Effluent	Turbidity < 30 NTU	Sunlight (Temperature)
	~ 10^5 to 10^6 CFU/mL	Sunlight
		Dark
Unfiltered (Positive Control)	Turbidity ~ 200 NTU	Sunlight (Temperature)
	~ 10^6 CFU/mL	Sunlight
		Dark

For each set of bottles, one bottle was to be kept inside a windowless cupboard as a dark control and two were to be exposed to sunlight on a concrete rooftop[14]. One bottle from each set was used to measure temperature, while the other was used for sampling. These samples were analyzed for changes in *E. coli* concentrations over the course of the experiment.

Each bottle was rinsed with the source water to remove any debris that might be inside before being filled; this deviates from the currently accepted version of SODIS, but is consistent with the recommendation provided in Section 3.5. The filled bottles were shaken by hand for approximately one minute to aerate the water[15]. There was a final volume of 515 mL in each bottle; there was only enough headspace in the neck of the bottle to provide ease of sampling. Figure 5.1 shows the two sets of bottles being exposed to sunlight.

[13] See Section 3.2.1, Appendix 2.1, and Appendix 2.8.
[14] See Appendix 2.8; the bottles were not painted with a black stripe nor placed on a corrugated metal sheet so as to enable the procedure followed in this work to represent the most conservative approach where temperature and sunlight effects are not optimized.
[15] See Appendix 2.8; aerated water was used, but the DO was not measured because no guideline is specified for SODIS.

Figure 5.1: SODIS in action; bottles on the left were pretreated using the roughing filter, while bottles on the right contained unfiltered water (Waterloo, Ontario)

5.2.6 Microbial Sampling and Analysis

At the beginning of these experiments, initial samples were drawn from the bottles to be exposed to sunlight ("Light Bottles") and those to be kept in the dark ("Dark Control Bottles"); the bottles to be exposed to sunlight for measuring the temperature ("Temperature Bottles") were not sampled for microbial analysis to avoid contamination. Samples were extracted with a BD 3 mL Sterile Medical Syringe with Slip Tip (BD, BD309586). The temperature of the water was measured from the Temperature Bottles using a Traceable Long-Stem Thermometer (Traceable, 4352). The Light and

Temperature Bottles were placed in full sunlight for five hours[16]. The ambient temperature[17] and irradiance values were obtained from the University of Waterloo Weather Station during each experiment (UW Weather Station).

Successive samples were taken from the Light Bottles at times 0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 hours. Samples were extracted from the Dark Control Bottles at times 2.5 and 5 hours. At each sampling point for the Light Bottles, the temperature was also recorded from the Temperature Bottles using the digital thermometer. The Dark Control Bottles were kept at room temperature. After five hours of exposure, the Light and Temperature Bottles were removed from the sunlight. They were placed with the Dark Control Bottles overnight. Another sample was drawn at 24 hours from the Light and Dark Control Bottles to check for microbial recovery. The temperature was also checked to see if the exposed bottles had returned to room temperature.

One millilitre from each sample was diluted in a series of dilutions (dilution factors from 10^1 to 10^4) using standard sterile techniques. One millilitre was taken from a single dilution and added to 15 mL of Nutrient Agar (BD, 213000), mixed gently by hand, and poured onto a sterile nine-centimetre Petri plate (Fisher Scientific, 08-757-9B). Duplicate analyses of the dilutions were performed. Plates were incubated at 37°C for 24 hours and counted the following day. The totals were reported as CFU/mL of the original sample.

5.3 Results

Some assumptions were made for the analysis of the data resulting from these experiments. First, the data from replicate samples was assumed to follow a normal distribution. Although the population from which samples were taken may not be normally distributed, the sample distribution was observed to tend toward the normal distribution. Second, only two samples were drawn for each sample-point so it was not possible to calculate variance; as such, homoscedasticity (same variance) has been assumed. This then also implies that the residuals are uniform. This assumption typically provides good

[16] See Appendix 2.8; early in the experiments, it was shown that the water temperature would not reach 50°C, so the five-hour exposure time was selected as per SODIS guidelines. This also facilitated completion of one experiment in a single workday.

[17] The ambient temperature was also measured at the test surface and found to be consistent to within ± 2°C of the weather station values.

estimation, but may lead to underestimating the correlation of variables (Box et al., 2005). It should also be noted that the data for each sample point (Dark Control Bottles and Light Bottles) were analysed in their raw form and a check of normality was carried out. In cases where the residuals were not normally distributed, due to the data not meeting the assumption of normality or homoscedasticity, the data were transformed before the analysis of variance was carried out again for that sample point (Osborne, 2002; McDonald, 2007).

5.3.1 Initial Water Characteristics

The turbidity and pH of the bulk source water were recorded for each day of experiments. The initial water characteristics can be seen in Table 5.2. The source water had an average turbidity of 240 NTU with a standard deviation of 55 NTU. The average pH of the source water was 7.19 ± 0.19.

Table 5.2: Initial source water characteristics for roughing filter and sunlight experiments

Initial Water Characteristics	**Turbidity (NTU)**	**pH**
Mean	240	7.19
Median	230	7.26
Maximum	334	7.34
Minimum	174	6.86

5.3.2 Filtration Pretreatment

The 20 cm roughing filter was used in these experiments because it had been shown in Chapter 4 to perform better than the other filter configurations. It provided an average flow rate of 2.85 L/h with a standard deviation of 0.12 L/h. Figure 5.2 shows the effluent turbidity for each day of experiments. Ripening occurred during the first 1.5 L, save for on June 6. The turbidity of this volume did not always exceed 30 NTU, but the first litre was discarded and not used in the sunlight portion of these experiments; however, it could have been refiltered and used. The average steady state turbidity removal efficiency was 93%.

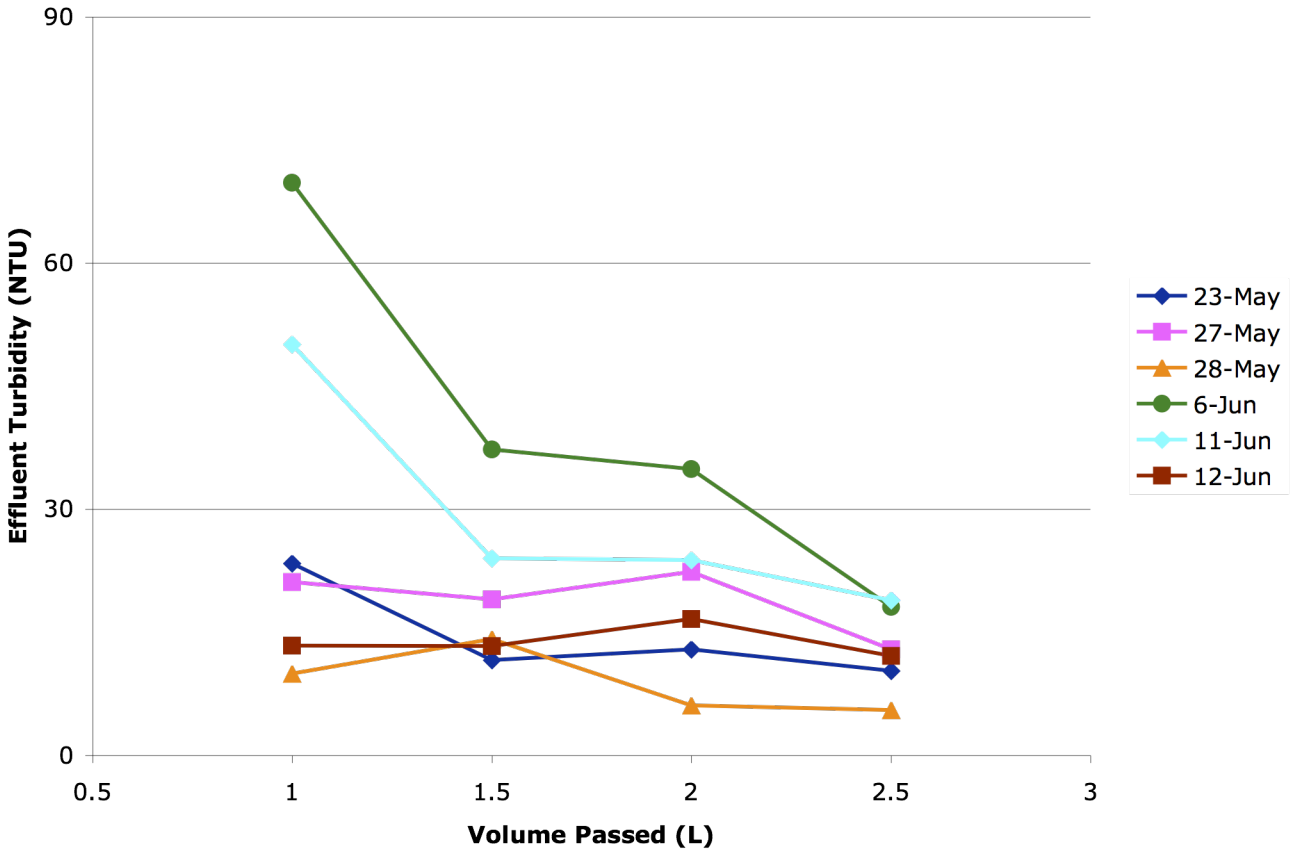

Figure 5.2: Effluent turbidity on each day of experiments for the roughing filter and sunlight experiments

Table 5.3 summarizes reductions in *E. coli* concentrations over all experiments. The average reduction was 0.35-log.

Table 5.3: Reduction of *E. coli* concentrations during filtration pretreatment

Values	***E. coli* Log Reduction**
Mean	0.35
Median	0.33
Maximum	0.46
Minimum	0.28

5.3.3 Dark Control Bottles

No statistically significant variation was found in the Dark Control Bottles' temperature over the course of the experiments. It was consistent with the changes in room temperature, which fluctuated between 21°C and 26°C.

The concentration of *E. coli* within the two Dark Control Bottles (one with non-filtered and one with filtered water) was sampled at 0, 2.5, 5, and 24 hours. Figure 5.3 illustrates the change of *E. coli* concentration in the Dark Control Bottles over the course of the experiments. There was a statistically significant difference in *E. coli* concentrations found between samples from the unfiltered and filtered waters.

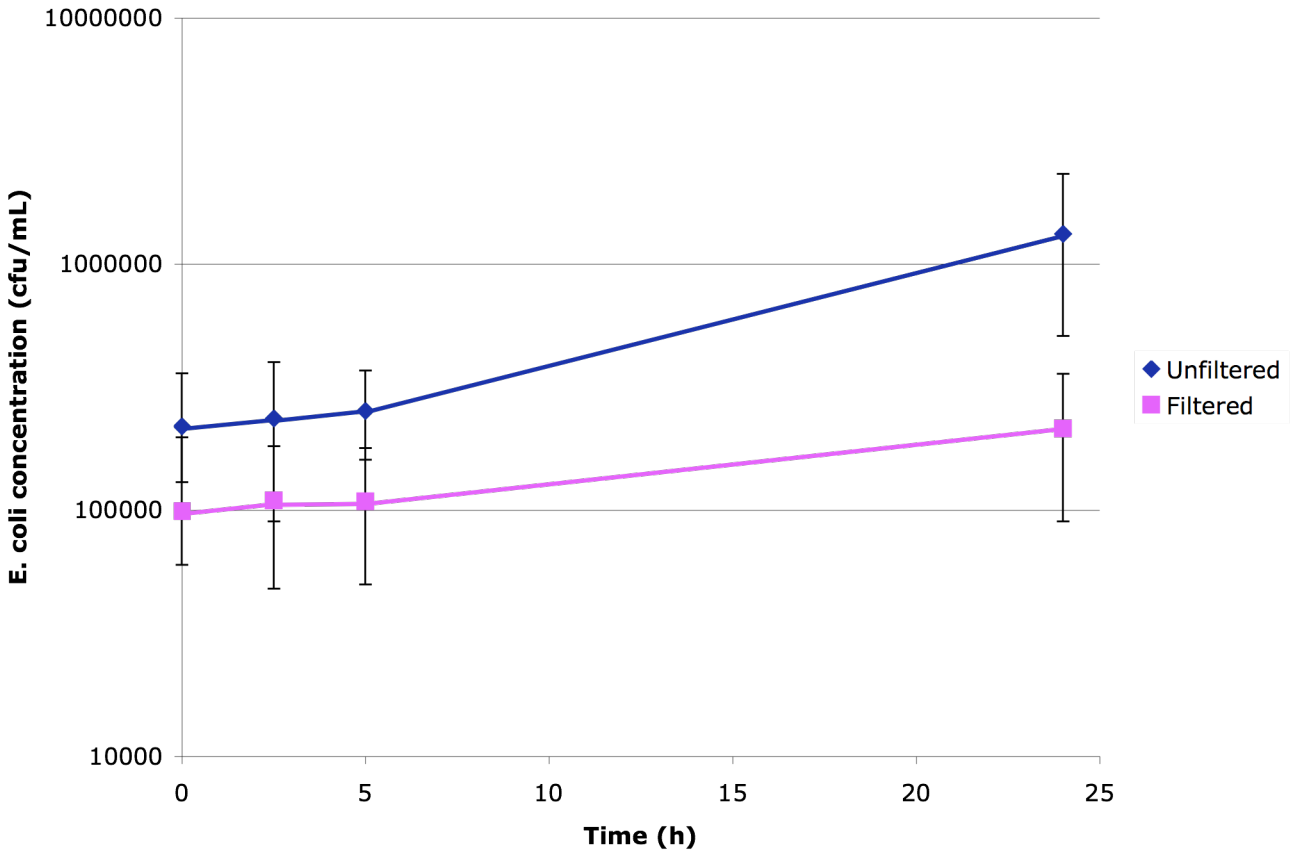

Figure 5.3: Change in average *E. coli* concentration in the Dark Control Bottles during the roughing filter and sunlight experiments (error bars represent the maximum and minimum concentrations at sample points)

The following tables and figures demonstrate how the statistical analysis was carried out for these experiments, using data from the Dark Control Bottles obtained at the 24-hour sample time. Table 5.4 shows the *E. coli* concentrations for the 24-hour sample time, while Table 5.5 shows the analysis of variance (ANOVA) for this data; the data were transformed using y^{λ}, where λ was 0.5. From the ANOVA table it is clear that the null hypothesis must be rejected for the statement "all bottles are the same"; the F_{obs} (calculated F) value is 56.55, which is much greater than the F_{table} (F-statistic) value, 4.35.

Table 5.4: *E. coli* concentrations in Dark Control Bottles after 24 hours

Unfiltered Water	**Filtered Water**
670000	109000
510000	123000
1470000	250000
1190000	267000
1650000	94000
2010000	90000
1820000	358000
1530000	232000
700000	218000
750000	335000
2320000	290000

Table 5.5: ANOVA for Dark Control Bottles after 24 hours

Source	**df**	**Sum of Squares**	**Mean Square**	$\mathbf{F_{obs}}$	$\mathbf{F_{table}}$
Between Bottles	1	2.48×10^6	2.48×10^6	56.55	4.35
Within Bottles	20	8.76×10^5	4.38×10^4		
Total	21	3.35×10^6			

Following the ANOVA, a normal probability plot was made to determine if the residuals were normally distributed. Figure 5.4 shows that the residuals lie in a relatively straight line; hence the residuals are normally distributed. (See Appendix 7 for ANOVA at each sampling point.)

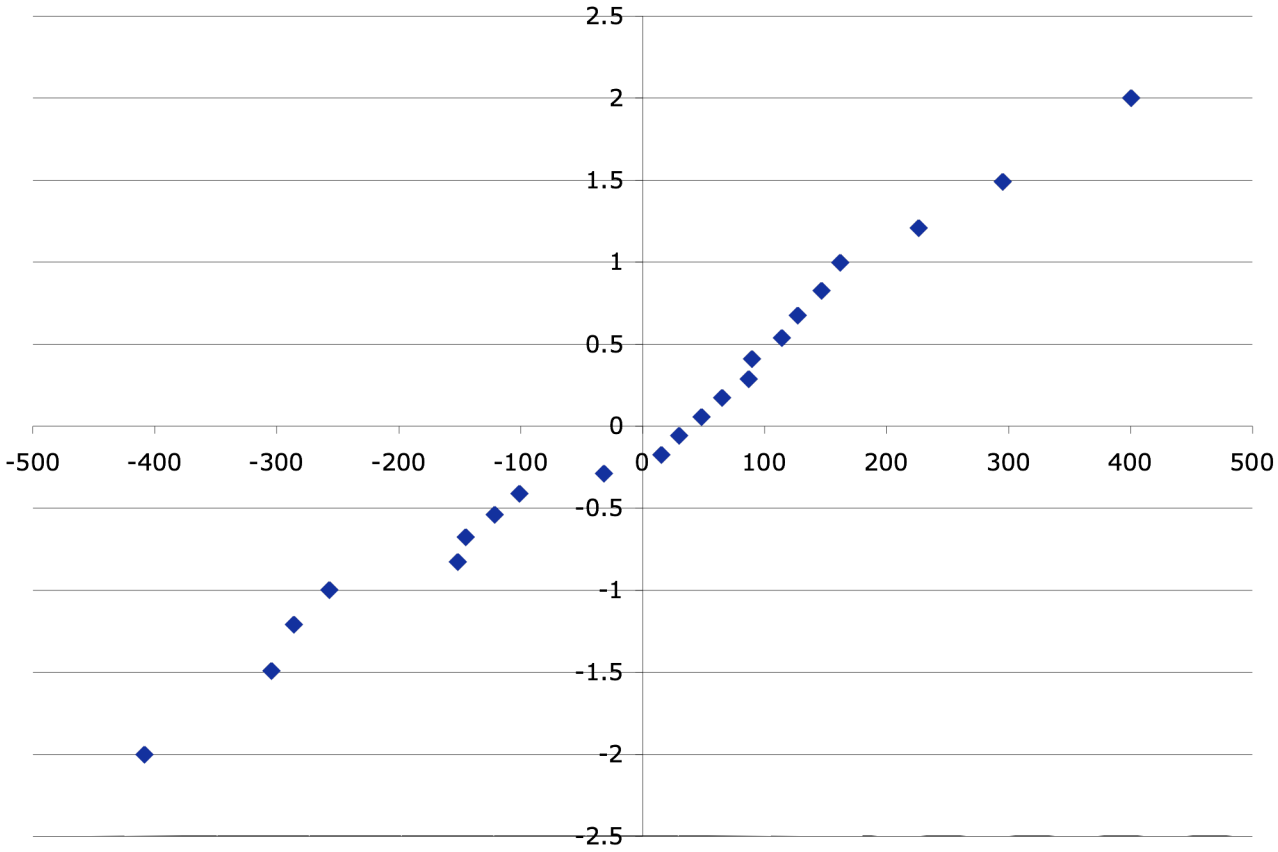

Figure 5.4: Normalized residuals for *E. coli* concentrations in the Dark Control Bottles after 24 hours

5.3.4 Sunlight-Exposed Bottles

Figure 5.5 displays the irradiance measured for each day of experiments. The minimum irradiance reaching the surface of the bottles was 322 W/m^2 and the maximum irradiance was 1065 W/m^2. The variation that was observed during experiments carried out on May 23, June 6, and June 12 was due to transitory, scattered clouds. The variation observed on June 11 was due to total cloud cover during the first three hours of the experiment. There was a positive correlation between log-reductions of *E. coli* concentrations in the Light Bottles and the irradiance they received (not shown).

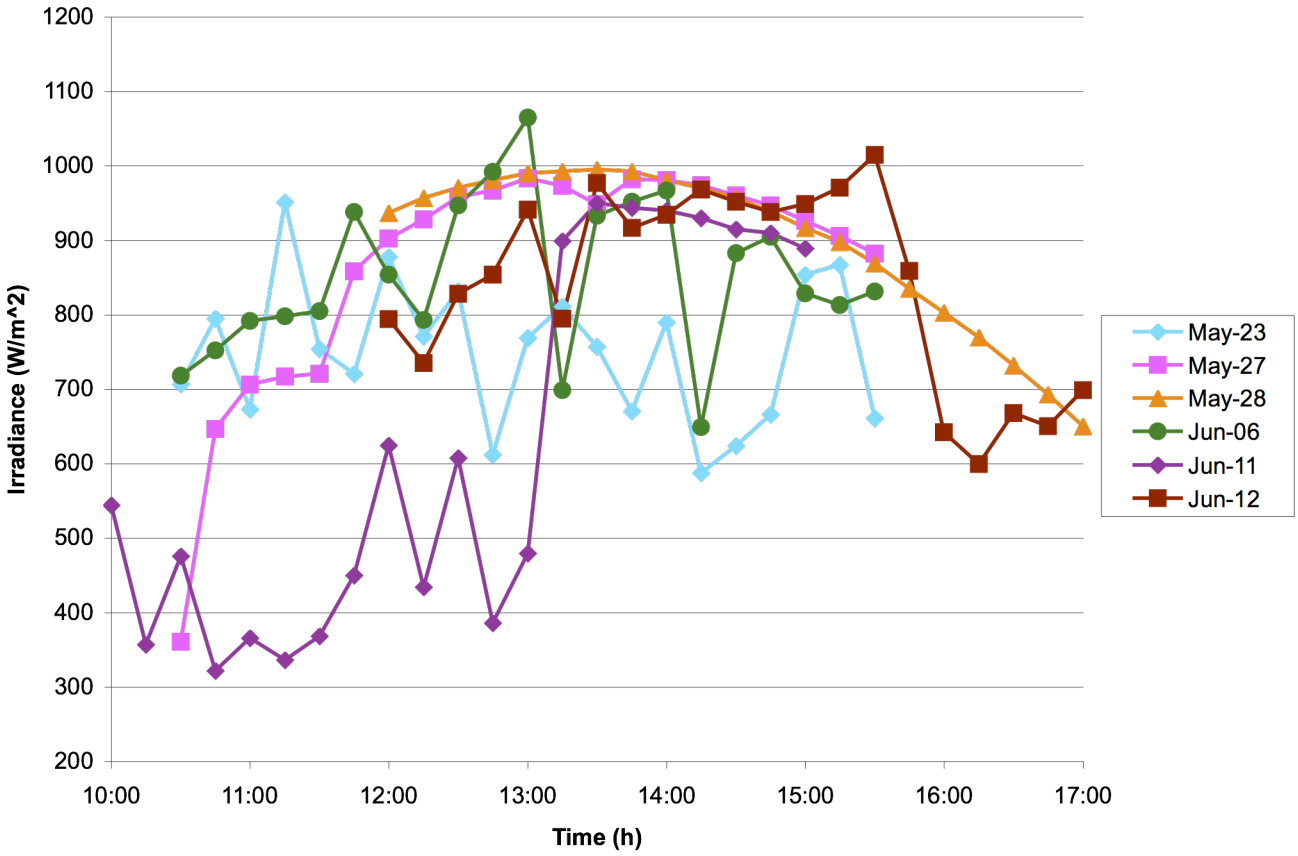

Figure 5.5: Irradiance for each day of roughing filter and sunlight experiments

Table 5.6 summarizes the average fluence and its variation for each experiment. The fluence only dipped below 555 W-h/m^2 (see Appendix 2.6) during the June 11 experiment, but on average the observed fluence was still higher.

Table 5.6: Average fluence for each roughing filter and sunlight experiment

Experiment	Fluence (W-h/m^2)	Standard Deviation
May 23	753	66
May 27	880	131
May 28	902	102
June 6	857	76
June 11	621	243
June 12	847	123

Figure 5.6 shows ambient temperature for each day of experiments. The minimum ambient temperature was 6°C and the maximum ambient temperature was 30°C. There was a positive correlation between the temperature in the bottles and the ambient temperature (not shown).

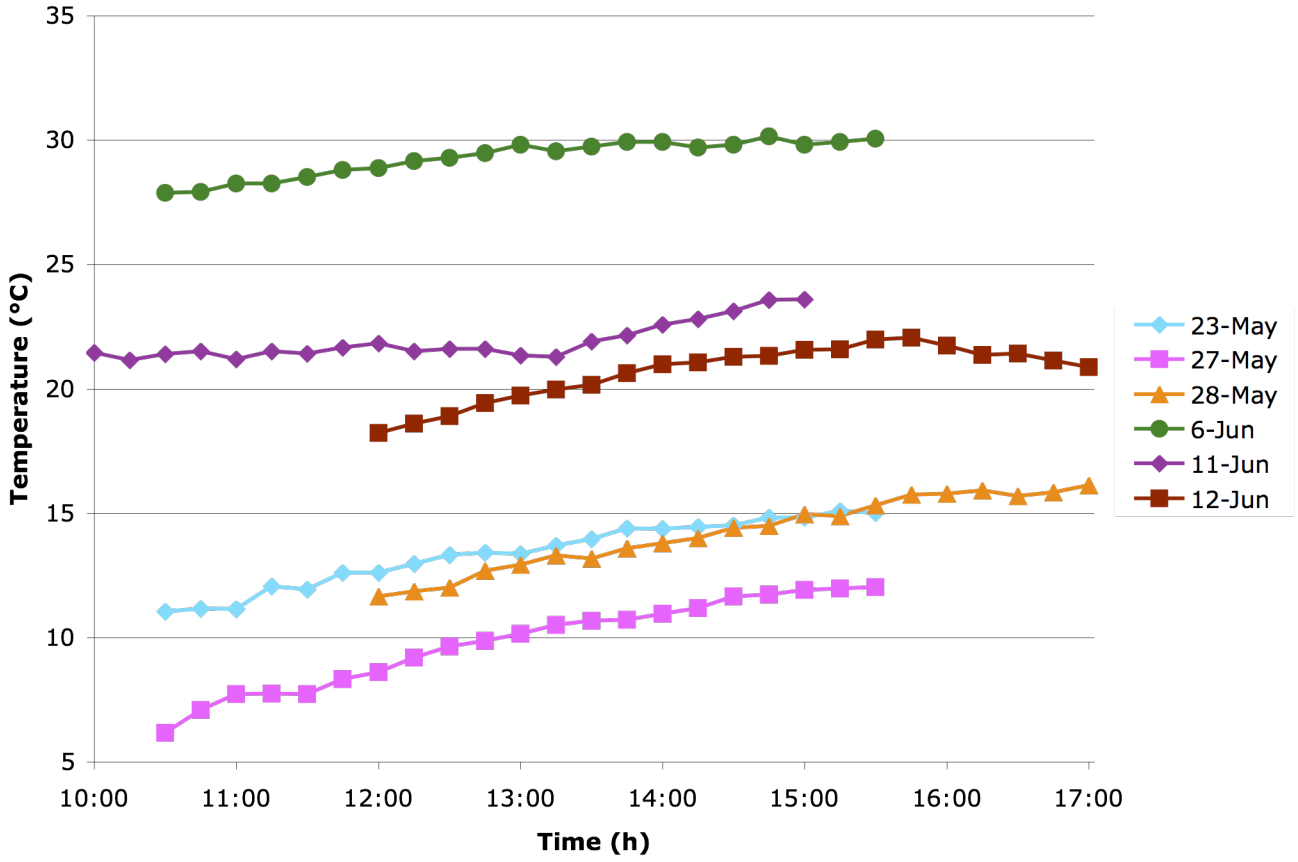

Figure 5.6: Ambient temperature for each day of roughing filter and sunlight experiments

The change in temperature experienced by the Temperature Bottles can be seen in Figure 5.7. The minimum water temperature was 21°C (while the ambient temperature was low) and the maximum was 44°C (measured after five hours of sunlight exposure). Thus, disinfection is primarily attributed to irradiation.

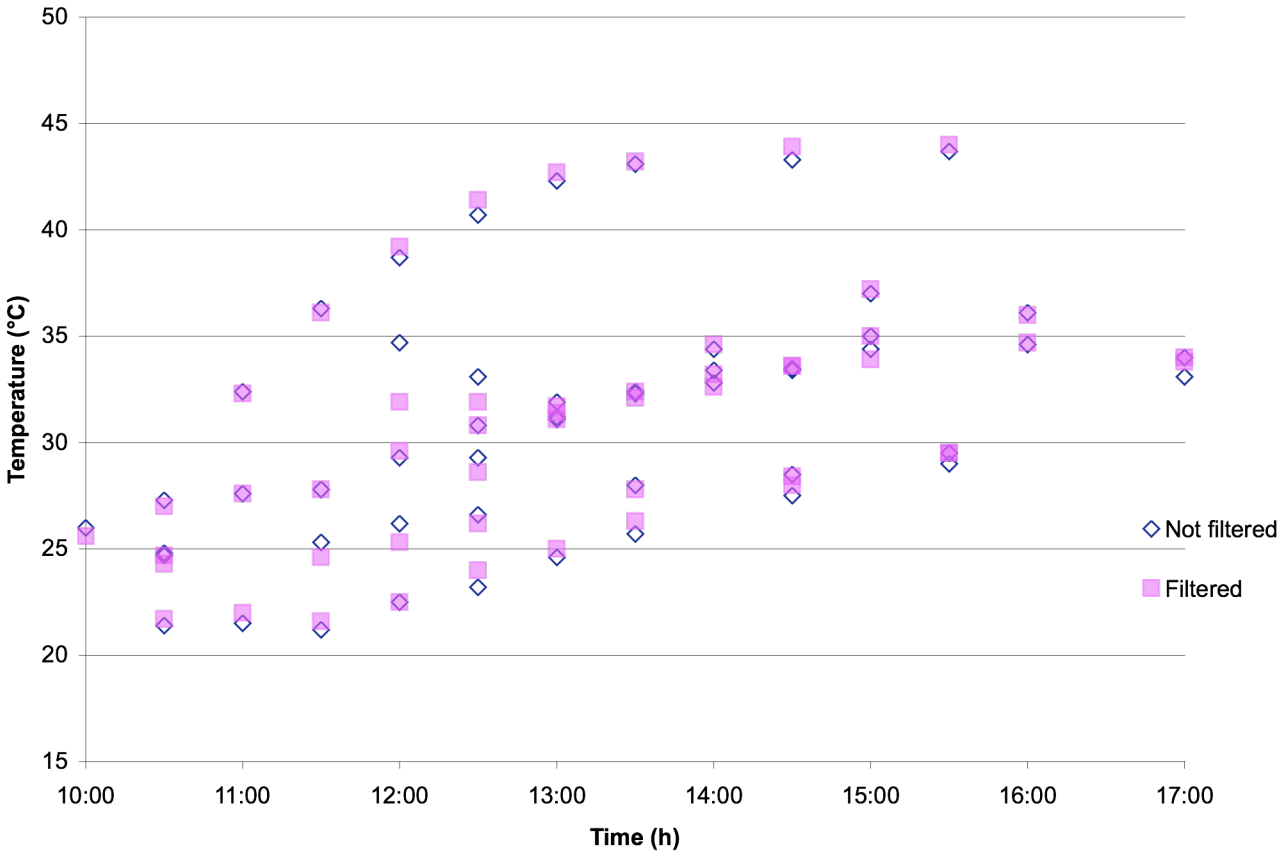

Figure 5.7: Water temperature inside the Temperature Bottles for each roughing filter and sunlight experiment (filtered and non-filtered water; all experiments together)

There was a statistically significant difference in *E. coli* concentration between the exposed bottles at all sample times, except 0.5 hours (Figure 5.8). At time zero the difference was attributed to the microbial removal during filtration. At the successive sample times (save 0.5 hours), the statistically significant difference between the disinfection efficiency of the bottle with filtered water and the bottle with non-filtered water was caused by the turbidity; the turbidity shielded the microorganisms from disinfection.

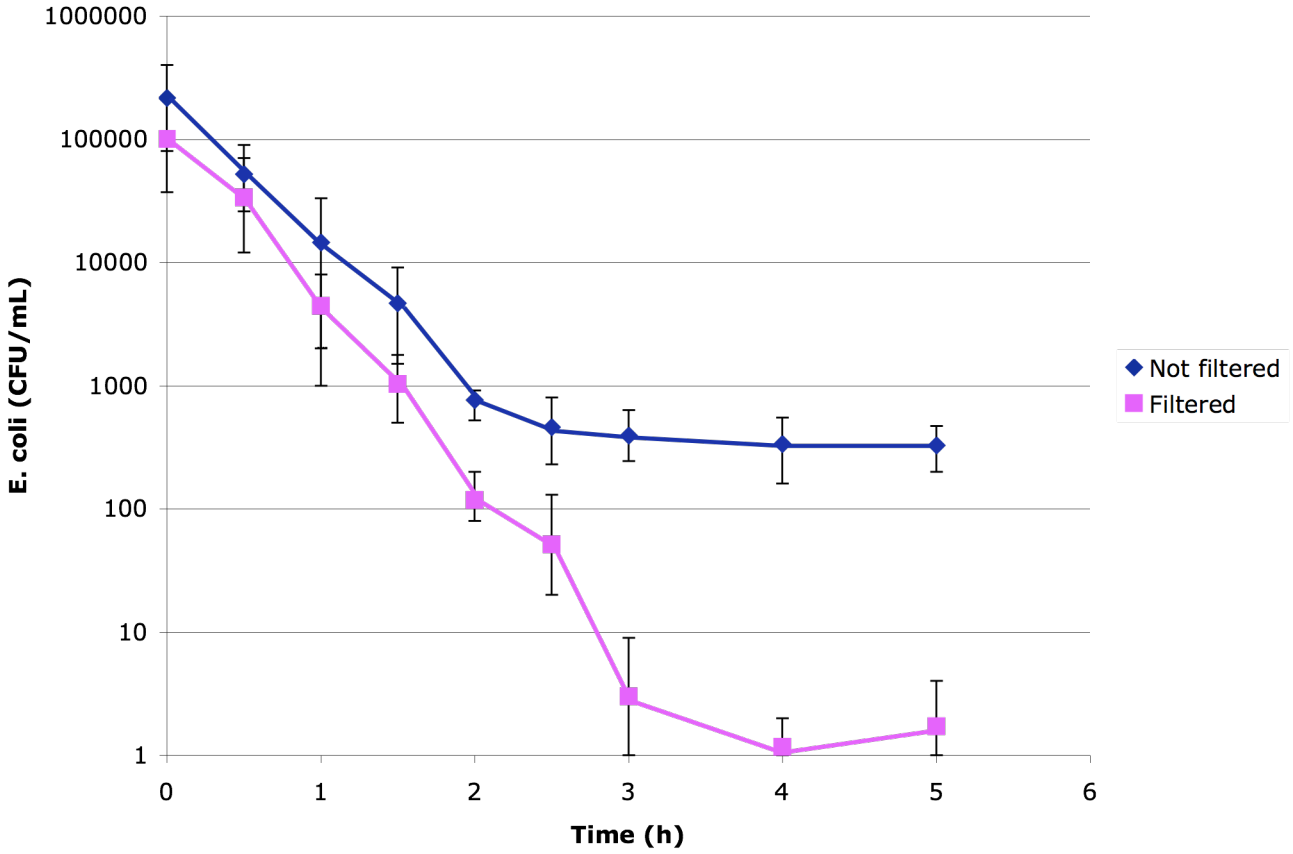

Figure 5.8: Light Bottles' average *E. coli* concentration changes over five hours for all roughing filter and sunlight experiments (error bars represent the maximum and minimum concentrations at each point)

The following tables and figures demonstrate how the statistical analysis was carried out for the Light Bottles, using data obtained for the 24-hour sample point. The data for the 24-hour sample point have been transformed using y^{λ}, where λ was 0.15. Table 5.7 shows the ANOVA for this data. It is clear that the null hypothesis must be rejected for the statement "all bottles are the same"; the F_{obs} (calculated F) value is 65.18, which is much greater than the F_{table} (F-statistic) value, 4.54.

Table 5.7: ANOVA for the Light Bottles after 24 hours

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Between Bottles	1	70.85	70.85	65.18	4.54
Within Bottles	11	11.96	1.09		
Total	12	82.81			

Following the ANOVA, a normal probability plot was used to determine if the residuals were normally distributed. Figure 5.9 shows that the residuals lie in a straight line; hence the residuals are normally distributed. (See Appendix 8 for ANOVA at each sampling point.)

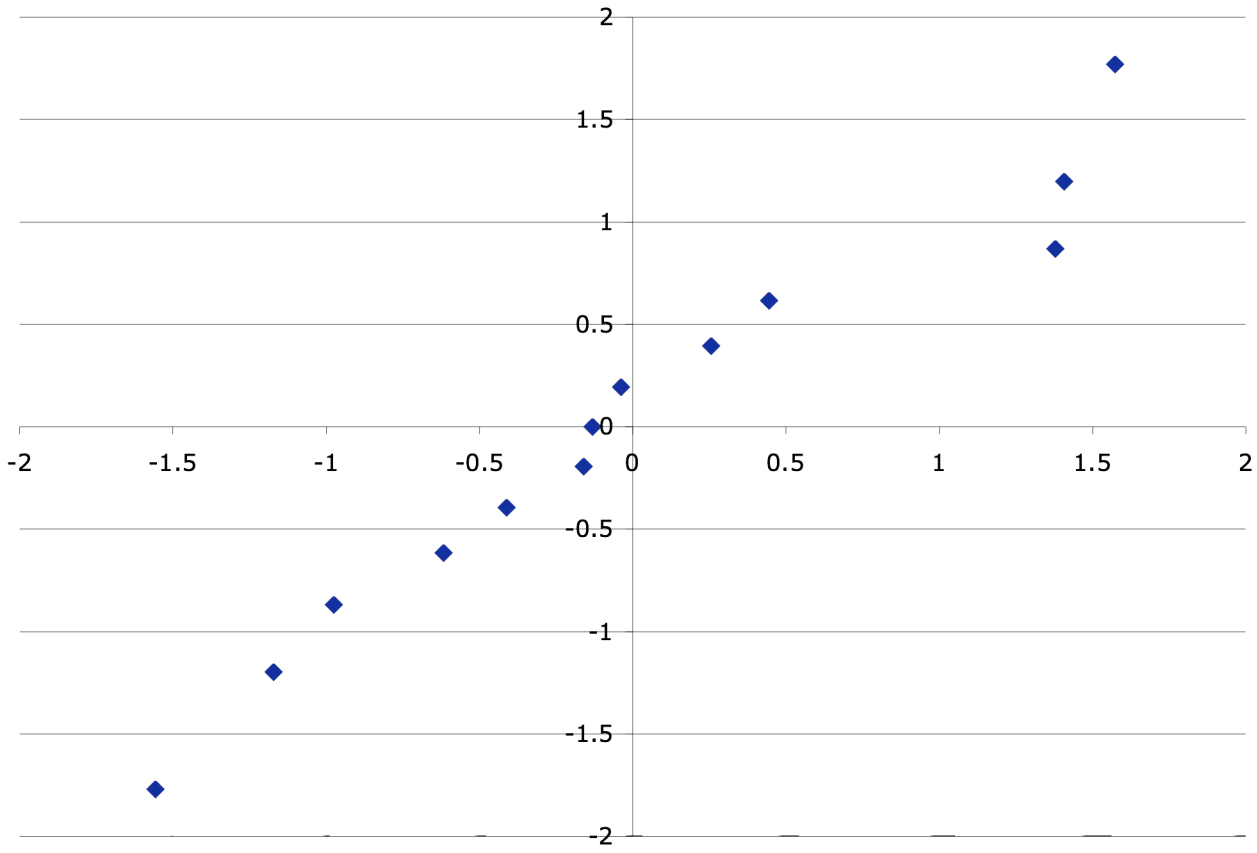

Figure 5.9: Normalized residuals for *E. coli* concentration in the Light Bottles after 24 hours

Reductions in *E. coli* concentrations (log reductions) were calculated for comparison between the treatments. The two to five hour time period was selected because *E. coli* concentrations of less than 100 CFU/mL were found during this period; concentrations of less than 100 CFU/mL are required for drinking water by the WHO. As can be seen in Table 5.8, the filtered and unfiltered water had minimum log-reductions of 2.70 and 2.21, respectively, qualifying the efficiency of SODIS as high (see Table 2.1). However, the average reduction of *E. coli* concentrations in the unfiltered water was substantially less than the reduction in the filtered water. Referring back to Figure 5.8, it shows that the *E. coli* concentrations in the unfiltered water bottles never reached the acceptable limit of 100 CFU/mL.

Table 5.8: Log reductions experienced from two to five hours during the roughing filter and sunlight experiments

Log reduction	Not Filtered	Filtered
Minimum	2.21	2.70
Maximum	3.16	5.28
Mean	2.70	4.11
Median	2.75	4.58

As can be seen in Figure 5.10, there was substantial recovery of *E. coli* after overnight dark storage for the Light Bottles. Twenty-four hours following their initial exposure to sunlight, the Light Bottles with unfiltered water still showed a notable difference in *E. coli* concentrations compared to those with filtered water.

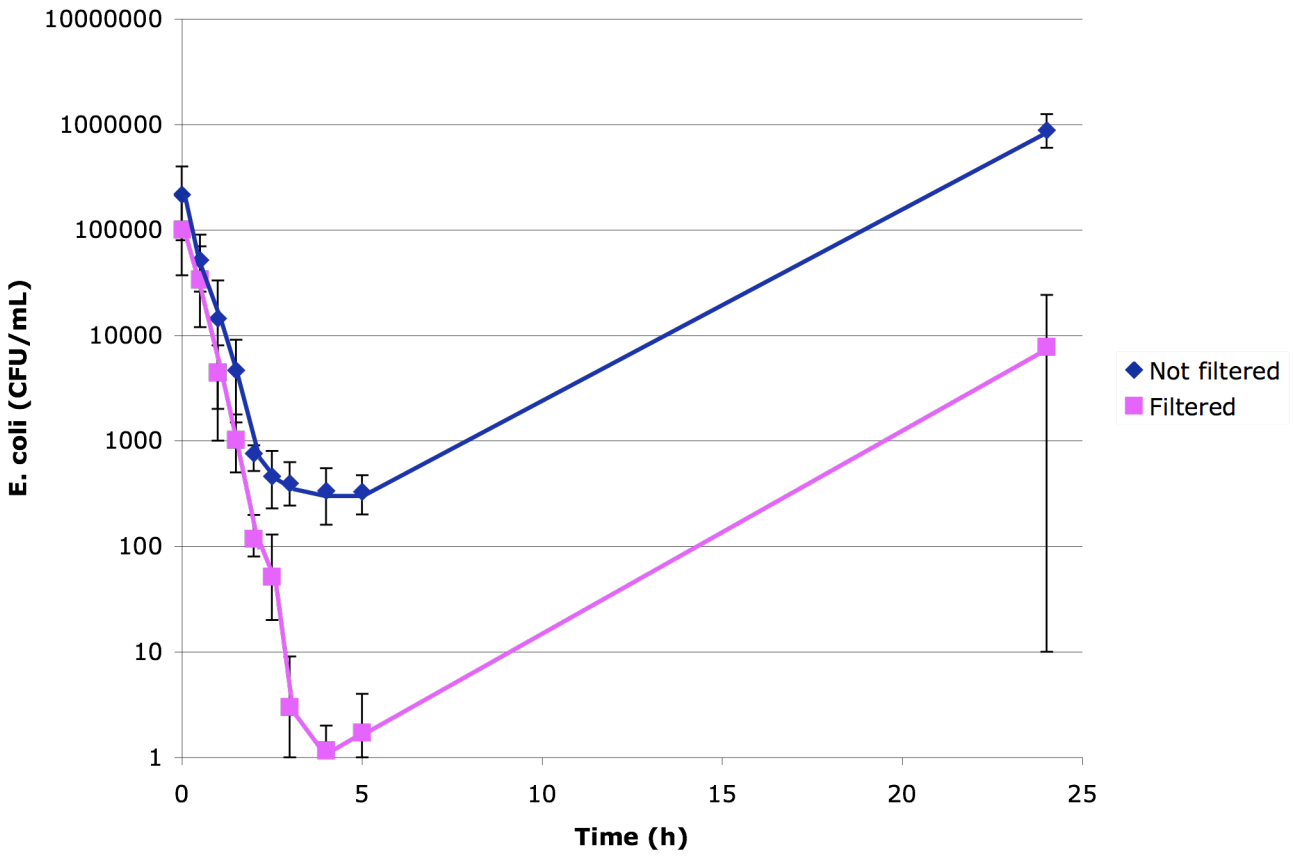

Figure 5.10: Average *E. coli* concentration changes after overnight storage in the dark for the roughing filter and sunlight experiments (error bars represent the maximum and minimum concentrations at each sample point)

The 24-hour samples showed that the unfiltered water bottles had concentrations approximately 0.71-log more than their original, indicating that the damage caused to the microorganisms during sunlight exposure was completely reversible. The filtered water bottles had concentrations within two-log less than their original; at least some of the damage caused in this case was irreversible. The May 27 and May 28 experiments were the only experiments that had 24-hour concentrations below the 100 CFU/mL limit. The turbidity of these effluent waters was less than 10 NTU.

5.4 Discussion

The initial synthetic source water turbidity was approximately 240 NTU, similar to some surface waters in developing countries. The temperature in the exposed unfiltered water bottles during this research did not exhibit a statistically significant difference over the temperature in the exposed filtered water bottles (see Figure 5.7). The maximum temperature reached was 44°C and was measured in one of the filtered water bottles after five hours of exposure.

It is noteworthy that these experiments were carried out in Waterloo, Ontario. The irradiances recorded during these experiments were similar to those of countries within latitudes of 35° of the equator, the recommended region for SODIS (Odeyemi et al., 1988). Waterloo lies at a latitude of 43°28'N and the highest irradiance recorded during these experiments was 1065 W/m^2. Only during one experiment (see Table 5.5) was the fluence below 555 $W\text{-}h/m^2$ (see Appendix 2.5).

The roughing filter produced an effluent water quality of less than 30 NTU, making it suitable for SODIS. The filter flow rate was approximately 2.85 L/h, which allowed sufficient contact time for particle removal. The average turbidity removal efficiency was 93%, which corresponded to an effluent of 17 NTU when the source was 240 NTU. The average *E. coli* removal efficiency was 0.35-log. This was not as large of a microbial reduction as expected and may be due to a lack of organic matter in the source water or a lack of biofilm in the filter. However, the difference in the initial *E. coli* concentrations for the unfiltered and filtered water bottles was statistically significant and has been attributed to filtration.

Filtration improved solar disinfection, as shown in Figure 5.8. On average, there was a 4.11-log reduction in *E. coli* concentrations during the five hours of sunlight exposure for the filtered water. Specifically, *E. coli* concentrations of less than 100 CFU/mL were obtained between 2 and 2.5 hours of exposure; this was consistent with previous experiments carried out in Waterloo where the turbidity was less than 1 NTU (see Figure 3.9). However, the remaining turbidity was associated with substantial microbial recovery (see Table 5.7). After overnight storage, 24 hours from initial exposure, the unfiltered water bottles had *E. coli* concentrations in excess of their initial level, while the filtered water bottles had concentrations within two-log less of their original; this trend can also be seen in the Dark Control Bottles (see Figure 5.3). The filtered water bottles that had turbidities less than 10 NTU did not show signs of *E. coli* recovery in excess of 100 CFU/mL. This suggests that if the water to be used for SODIS has substantial turbidity (i.e., greater than 10 NTU), even after filtration, it should be used immediately after exposure to sunlight and not stored for future use.

5.5 Conclusions

The major conclusion from this study is that the roughing filter, developed in Chapter 4, improves the SODIS process efficiency through pretreatment of water exceeding 30 NTU (limit for SODIS). The filter was capable of removing an average of 93% turbidity from highly turbid (average 240 NTU) source water and slightly decreasing *E. coli* concentrations (by 0.35-log). The filtered water then experienced more disinfection than unfiltered water: 4.11-log reduction and 2.70-log reduction, respectively.

Once the necessary exposure for SODIS has been completed it would be imperative that the water be used immediately if it has turbidity greater than 10 NTU. As was seen in these experiments, remaining turbidity in the water provided shelter for the microorganisms during sunlight exposure, so that some of the organisms were not inactivated. Overnight storage allowed for microbial recovery (possibly from the nutrients provided by the buffer added to the source water and dead cells, as well as reactivation) wherein concentrations rebounded to within two-log of their original concentration for filtered water.

CHAPTER 6: CONCLUSIONS

This thesis consisted of three experiments involving cleaning pretreatment, roughing filter pretreatment, and roughing filter pretreatment followed by SODIS. The following sections summarize the key findings from each study.

6.1 Cleaning Pretreatment

The objectives of this research were: (i) to determine if cleaning SODIS containers before use was necessary and if so, which cleaning agents would be affordable and available in developing countries and (ii) to investigate employing SODIS in Waterloo, Ontario, which lies outside the recommended geographic region (within 35° of latitude of the equator) for SODIS use. The cleaning agents that were selected were 70% isopropyl alcohol, a soap-water mixture, and lime juice.

The key findings were as follows:

- Although Waterloo, Ontario, located at a latitude of 43°28'N, is approximately 8° of latitude outside the recommended region for SODIS, it was still possible to employ the process during August and September and achieve appropriate disinfection (i.e., *E. coli* concentrations below 100 CFU/mL).

- The maximum irradiance recorded during these experiments was 946 W/m^2, which is comparable to those found within the favourable geographic region.

- The maximum water temperature reached was 41°C, which is below the 50°C threshold for synergistic light and heat mechanisms. Thus, disinfection was primarily attributable to light.

- Cleaning with the 70% isopropyl alcohol and the soap-water mixture did not improve solar disinfection.

- Cleaning with the lime juice *inhibited* subsequent solar disinfection and resulted in substantial recovery of *E. coli* after overnight storage, possibly due to the nutrients provided in the juice, and/or reactivation.

In conclusion, it was possible to successfully use SODIS in Waterloo (outside of the recommended geographic region) and the cleaning pretreatment with chemical agents was not necessary for improving the process.

6.2 Roughing Filter Pretreatment

The purpose of this series of experiments was to construct a roughing filter with materials available in developing countries and determine if it could produce an effluent water quality with less than 30 NTU (in order to be used for SODIS). The source for drinking water in developing countries is typically surface water that has high turbidity (greater than 200 NTU). Turbidity-causing particles block sunlight from penetrating the water and protect microorganisms from the light, thus it is necessary to remove turbidity in excess of 30 NTU before SODIS is used.

Under the conditions tested the key findings were as follows:

- The optimal roughing filter design consisted of a 2 L PET bottle (similar to those already in use for SODIS) with a gravel underdrain (3 cm), covered with 17 cm coarse sand to give a total filter depth of 20 cm. A hole diameter of 0.6 mm was made using a standard sewing needle near the bottom of the bottle, which produced a flow rate between 2.7 and 2.8 L/h.

- The optimal roughing filter removed more than 90% turbidity for turbidities up to 250 NTU; the effluent was at most 20 NTU. This filter also reduced *E. coli* concentrations by 0.5-log (initial concentrations were approximately 10^6 CFU/mL).

- The first two litres of filtered water represented the ripening phase of the filter. The turbidity associated with the first litre was consistently greater than 30 NTU and was not used in these experiments. This volume should be refiltered before using it for SODIS.

- The 20 cm roughing filter provided effluent of less than 10 NTU after one hour of filtration and provided a total of ten litres of the same quality water in five hours.

- A comparison to simple settling showed that settling required eight hours to produce a total of ten litres of water with an effluent quality of 20 NTU.

The 20 cm roughing filter appears to be an appropriate pretreatment for water intended for SODIS. It consistently provided effluent water turbidity of less than 30 NTU, meeting the SODIS guideline. This filter also reduced the *E. coli* concentration of the water by 0.5-log. Its effluent water quality had lower turbidity than simple settling and provided it in a much quicker time.

6.3 Roughing Filter Pretreatment Followed by SODIS

The aim of this study was to use the previously developed roughing filter in series with SODIS to evaluate its effect on the overall treatment process. The synthetic source water created for these experiments was approximately 240 NTU to simulate surface water in developing countries.

The key findings were as follows:

- Again this research was carried out in Waterloo, Ontario, located at a latitude of 43°28'N, approximately 8° of latitude outside the recommended region for SODIS. It was still possible to employ the process during May and June and achieve appropriate disinfection (i.e., *E. coli* concentrations below 100 CFU/mL). The maximum irradiance recorded during these experiments was 1065 W/m^2, which is greater than that recorded during the cleaning pretreatment experiments and comparable to those found in regions recommended for SODIS.

- The average turbidity removal using the roughing filter was 93%, which corresponded to 17 NTU when the influent quality was 240 NTU. The filter had the same ripening phase (two litres) as the previous experiments and the first litre was discarded because it had turbidity greater than 30 NTU. However, the effluent having turbidity greater than 30 NTU could have been retreated and used.

- Effluent turbidities greater than 10 NTU corresponded to substantial recovery of *E. coli* after overnight storage (concentrations above 100 CFU/mL).

- The maximum water temperature reached within the bottles was 44°C and was recorded for a bottle with filtered water. Turbidity-causing particles inhibit light disinfection; however, the likelihood of disinfection by heat is greater. The maximum-recorded water temperature was below the 50°C threshold for synergistic light and heat mechanisms, thus disinfection was primarily attributed to irradiation.

- The average *E. coli* removal efficiency of the filter was 0.35-log. On average, there was a 4.11-log reduction of *E. coli* concentrations measured in the filtered water and a 2.70-log reduction in the unfiltered water after five hours of sunlight exposure. Thus, the roughing filter did improve the efficiency of solar disinfection.

The roughing filter successfully pretreated the water to less than 30 NTU, the limit for SODIS (see Appendix 2.5). The filter removed an average of 93% turbidity from the highly turbid (average 240 NTU) source water and slightly decreased the initial *E. coli* concentration. On average, there was a 4.11-log reduction in *E. coli* concentrations measured in the filtered water during the five hours of sunlight exposure; the non-filtered water did not attain *E. coli* concentrations below 100 CFU/mL, as recommended for drinking water by the WHO.

6.4 Summary

The implications of this research are as follows:

- The utility of SODIS can be expanded outside of the suggested geographic region at least during parts of the year (confirmed for May through September in Waterloo, Ontario).

- Using a chemical cleaning pretreatment is not necessary and has the potential to inhibit disinfection, especially without proper training and/or technical knowledge.

- A roughing filter can be constructed from readily available and affordable materials in a developing country.

- This roughing filter can produce an effluent water quality of less than 30 NTU, which is required for SODIS, provided the first litre is refiltered.

- Furthermore, the filter is a viable pretreatment for turbid water intended for SODIS use.

- Water with turbidity greater than 10 NTU that has been exposed to sunlight should be used immediately.

CHAPTER 7: RECOMMENDATIONS

The major recommendations from this research to SODIS disseminators are:

- Clarify that the bottles do not need to be cleaned with any chemical agent. In the case of lime juice, if not added in the proper quantity then the efficiency of the process can be hindered. In situations when a soap solution might be used, its effect of creating a film would be similar to that of extended use of the bottle. A further recommendation would be that the bottles be rinsed with water only, immediately before use, just to remove any debris that might be inside.

- When users need to pretreat their water (i.e., if its turbidity is greater than 30 NTU) and immediately expose it to sunlight, the 20 cm roughing filter described in this research is the preferred pretreatment option. Otherwise, settling may be used; however, the filter will still provide water with less turbidity (approximately 10 NTU compared to 20 NTU, respectively). Users should also check the turbidity of the first litre of effluent; it should be refiltered if the turbidity is greater than 30 NTU. This is part of the ripening stage of the filter.

- After sunlight exposure, it is imperative to use the water immediately if it has turbidity greater than 10 NTU. The turbidity in the water provides shelter for microorganisms during sunlight exposure, so that some of the organisms may not be inactivated. When water that has been exposed to sunlight is stored for a long period of time afterward (e.g., overnight), then microbial recovery is possible and concentrations may rebound to their original level.

Recommendations for future work are many. With respect to the difference in results obtained in this research and by Fisher and Dalsgaard's groups (1997; 2007) there remains a need to determine whether lime juice addition has a considerably positive or negative effect, or both, on the disinfection efficiency of *E. coli*. It would appear that the volume of lime juice added to the water must be substantial in order to decrease the pH below a threshold in order to increase solar disinfection. Thus, this threshold must be found. Fisher's group used a pH of 5, but is this the exact threshold? The required volume of lime juice that corresponds to the necessary decrease in pH must be explored. Furthermore, as was suggested by Benito et al. (1999), the water-borne pathogens that have a sensitivity specific to lime juice or pH must be determined.

In regards to the roughing filter, it will be necessary to introduce it to users to measure its true simplicity and value. If users cannot easily obtain the materials at a low cost or cannot build the filter themselves, its utility will be low. Field trials will also help gauge how the filter performs with natural water instead of synthetic water. The effect of natural organic matter on filter performance should be determined and the difference in turbidity removals for ground water versus surface water should be explored. It will also be necessary to determine a simple way for renewing the filter media once breakthrough occurs.

Some general areas related to SODIS that should be studied include: its utility in regions of the world that are outside of the recommended region; a simple method for alerting users when appropriate disinfection has been achieved; whether high turbidity waters actually increase the potential for disinfection to occur by heat; and the relationship between turbidity and microbial recovery after overnight storage (i.e., how much turbidity causes concentrations greater than 100 CFU/mL after 24 hours).

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APPENDIX 1: COMPARISON OF RECOMMENDED TECHNOLOGIES FOR HOUSEHOLD WATER TREATMENT

The following table presents a comparison of technologies recommended for household drinking water treatment (WHO, 2007).

Criterion	Boiling with fuel	Solar disinfection with UV + heat (SODIS or SOLAIR)	Solar disinfection with heat only (opaque vessels and solar panels)	UV disinfection with lamps	Free chlorine and storage in improved vessels	Chemical coagulation-filtration + chlorine disinfection
Microbial reductions	Yes, extensive	Yes, extensive for most pathogens	Yes, extensive for most pathogens	Yes, extensive for most pathogens	Yes, extensive* for most pathogens	Yes, extensive
Diarrhoeal disease reductions	Yes	Yes, 9-26%; two studies	None reported from studies, but expected due to high temperature (55°C)	None reported from studies, but expected due to germicidal effects	Yes, 15-48%; many studies	None reported from studies yet, but expected due to multiple treatments
Disinfectant residual	No	No	No	No	Yes	Yes
Quality requirements of water	None	Low turbidity (<30 NTU) for effective use; pre-treat turbid water	None	Low turbidity (<30 NTU) and low in UV-absorbing solutes, such as NOM, iron and sulphites	Low turbidity (<30 NTU) and low chlorine demand for effective use; pre-treat turbid water	None; applicable to poor quality source water
Chemical changes in water	No, usually except deoxygenating and chemical precipitation	None or not significant	None or not significant	None or very little	Yes; may cause taste and odour and disinfection by-products	Yes, may cause taste and odour and disinfection by-products
Microbial regrowth potential in treated water	Yes, with storage beyond 1-2 days	Yes, with storage beyond 1-2 days	Yes, with storage beyond 1-2 days	Yes, with storage beyond 1-2 days	None to low if chlorine residual maintained	None to low if chlorine residual maintained

Skill level and ease of use	Low skill, easy use	Low skill; very easy use	Low skill; easy use with training	Moderate skill, training needed for maintenance cleaning and lamp replacement	Low skill; easy use with training	Moderate, training needed in adding chemicals, mixing, decanting and filtering
Availability of needed materials	Requires a source of fuel	Requires plastic (PET) bottles and dark surface (on one side of vessel or on surface where vessel is placed	Requires black bottles of cook vessels and a solar reflector or solar cooker	Requires UV units and replacement lamps and a reliable source of electricity (power)	Requires source of free chlorine or chlorine generator and source of safe storage vessels	Requires a source of the chemical mixture (coagulants and chlorine disinfectant); may limit availability
Limits to water volume treated	Yes, difficult to scale up above usual cooking volumes	Yes, treats 1-1.5 litres per bottle; can simultaneously treat multiple bottles	Yes, treats 1-4 litres per container; can simultaneously treat multiple vessels with multiple solar panels or solar cookers	No, units can treat several litres per minute and much, depending on lamp size and number and reactor volume	No, easily scaled up	Yes, chemical mixture treats fixed volumes of 10-20 litres; repeated treatment of additional volumes
Performance verification requirements	Observe water for a rolling boil	Measure that target temperature is reached (thermometer or wax indicator	Measure that target temperature is reached (thermometer or wax indicator)	Must verify lamp output; may be a limitation if unit lacks a UV sensor	Measure chlorine residual or microbial quality (indicators) or both	Observe (measure) turbidity reduction and measure chlorine residual
Acceptability*	High	High to Moderate	High to Moderate	High	High to Moderate	High to moderate
Sustainability	High, unless fuel is scarce	High, probably	High, probably	High, probably	High	High, probably; limited data
Length of treatment time	Minutes to tens of minutes	Hours (full sun), days (clouds), not effective if no sun	Hours (full sun), days (part sun), not effective if no sun	Seconds to minutes, depending on water volume treated and reactor design	Tens of minutes	Tens of minutes

*High is >75%; moderate is 50-75%

APPENDIX 2: SODIS TECHNICAL NOTES

The following sections contain technical notes from SANDEC.

2.1 SODIS Technical Note #2

SODIS **Technical Note # 2**

Materials:
Plastic versus Glass Bottles

SUMMARY

Plastic bottles made from PET are recommended for SODIS use as they should not contain substances hazardous to health. Good transmittance of UV-A light is required when glass bottles are to be used for SODIS.

BACKGROUND INFORMATION

Plastic: Preference for PET

Plastic mineral-water and soft-drinks bottles are gradually replacing glass. Plastic bottles are made of either PET (polyethylene terephtalate), or PVC (polyvinyl chloride), both containing additives like UV-stabilisators to increase their stability or to protect them and their content from oxidation and UV radiation. Additives are large molecules which hardly imigrate through the PET material. Still, they are a potential health risk. In PET, additives are much less used than in PVC (less than 1% for PET), making PET the preferred material for SODIS treatment. Various types of transparent plastic materials are good transmitters in the UV and visible range of solar spectrum.

PET or PVC? A simple Test

There are several simple methods to determine wether a bottle is made of PVC or PET. One is the appearance. Bottles made of PVC have often a bluish gleam. This bluish hue is especially marked at the edges of a piece of bottle material that has been cut out. If PVC is burnt, the smell of the smoke is pungent, whereas the smell of PET is sweet. PET burns more easily than PVC.

Glass: UV-A Transmission

The transmission for ultraviolet radiation is largely determined by the content of iron oxide in the glass. Ordinary window glass in thicknesses of 2 mm or more is practically opaque to UV-radiation. Certain specific glasses (Pyrex, Corex, Vycor, Quartz Glasses) transmit significantly more ultraviolet radiation than the ordinary window glass. However, for an appropriate technology like SODIS large scale utilization of these special glasses may not be very attractive due to their high costs and rare availability in the developing areas of the world.

The advantages of PET are

- ☺ Low weight
- ☺ Relatively unbreakable
- ☺ Transparent
- ☺ Taste-neutral
- ☺ Chemical stable

The disadvantages of PET are

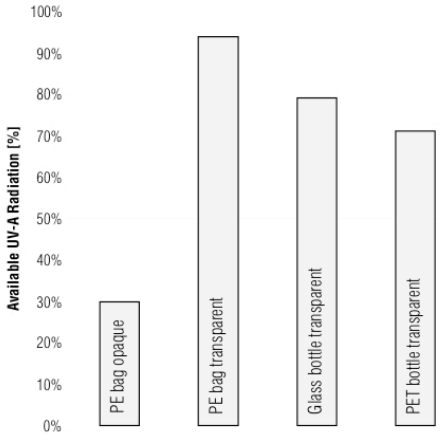
- ☹ Limited heat resistance (deformations above 65 °C)
- ☹ Scratches and other ageing effects

Advantages of Glass

- ☺ No scratches
- ☺ No photoproducts
- ☺ Heat resistance

Disadvantages of Glass

- ☹ Easily smashed
- ☹ High costs
- ☹ Weight



UV-transmission of PE, Glass and PET (examples)

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2.2 SODIS Technical Note #3

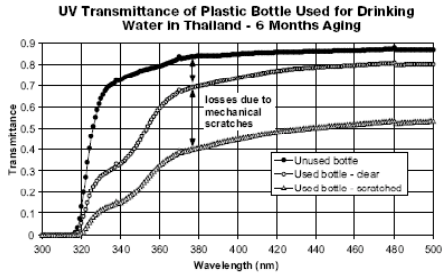
SUMMARY

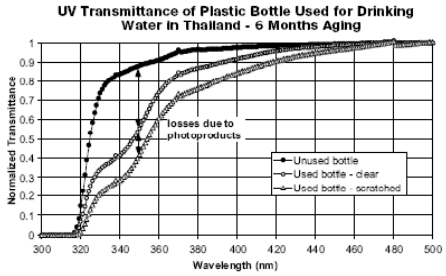
SODIS bottles are used daily and for a long period of time. Ageing of the PET-bottles leads to a reduction of UV-transmittance which, in turn, can result in a less efficient inactivation of microorganisms. The additives in the PET material, which are used to protect it from degradation by sunlight, have no influence on the water quality, since at the inside of the bottle no photoproducts are generated.

BACKGROUND INFORMATION

Transmittance Losses

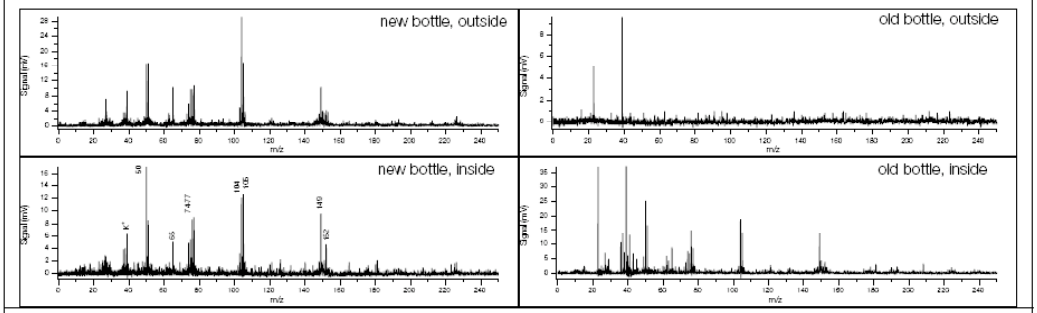
Ageing of the bottles leads to a reduction of UV-transmittance which, in turn, can result in a less efficient inactivation of microorganisms. The figure below illustrates the UV transmittance for used and unused bottles. The figure on the left shows the transmittance losses due to mechanical scratches whereas the figure on the right illustrates the losses due to photoproducts. Smooth and careful cleaning is necessary to avoid mechanical scratches.





Photoproducts

PET, like all polymeric materials, undergoes reactions with oxygen or degradation under sunlight. The UV A and B components of sunlight in the 290-400 nm wavelength range lead to photochemical reactions resulting in optical and mechanical property changes. To improve their stability, additives are widely used to protect them from oxidation, UV radiation effects, weathering etc. In the course of the polymer's life, the additives will be depleted from the host material by photochemical reaction or diffusion. This can greatly influence the properties of the material. The figure below illustrates the difference between new and old bottles exposed to sunlight for 6 months. The outer surface of the bottles clearly indicates the difference between a new and an old bottle. Hardly a difference is, however, visible between the inner surface of the old and the new bottle in the mass spectrum. Since the inner surface of the bottle does not seem to be affected by UV radiation, it is very unlikely that photoproducts of polymer additives will pollute the treated drinking water and cause health problems.



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2.3 SODIS Technical Note #5

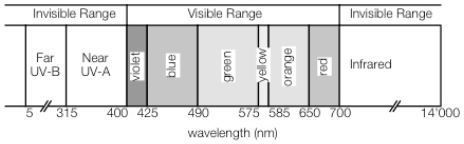
SUMMARY

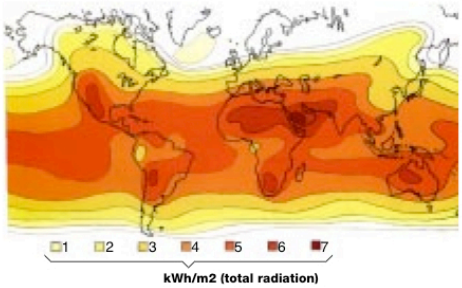
Solar radiation reaching earth is composed of UV-B, UV-A, visible and infrared light. High radiation intensities are generally available in most developing countries, especially in those around the equator. UV-A is the most important spectrum for SODIS.

BACKGROUND INFORMATION

Solar radiation spectrum

The sun continuously radiates enormous amounts of solar energy at wavelengths that cover the ultraviolet, visible, and infrared bands. Not all of the solar radiation received at the periphery of the atmosphere reaches the surfaces of the earth. This is because the earth atmosphere plays an important role in selectively controlling the passage towards the earth's surface of the various components of solar radiation. Radiations with short wavelengths are selectively scattered much more extensively than those with longer wavelengths by atmospheric gases or particles that are smaller in dimension than the wavelength of a particular radiation. Most of the radiation with a range of wavelengths from 200 to 300 nm is absorbed by the ozone (O_3) layer in the upper atmosphere.





Global Solar Energy Distribution

Solar radiation is unevenly distributed and varies in intensity from one geographic location to another depending upon the latitutde, season, and time of day.

The most favourable region for SODIS lies between latitudes 15°N and 35°N and embraces the regions that are naturally endowed with the most favourable conditions for solar energy applications. These semi-arid regions are characterized by having the greatest amount of solar radiation, more than 90% of which comes as direct radiation because of the limited cloud coverage and rainfall (less than 250 mm per year and usually more than 3000 hours of sunshine per year).

The second most favourable region lies between the equator and latitude 15°N. Because humidity is high and cloud cover is frequent, the proportion of scattered radiation is quite high. There is a total of about 2500 hours of sunshine per year.

It is important to note that the majority of developing countries fall within the more favourable regions between latitudes 35°N and 35°S. For this reason they can count on solar radiation as a steadfast source of energy that can be readily exploited cheaply by both rural and urban households for a multitude of purposes, including solar disinfection of drinking water.

UV-A is important for SODIS

The inactivation rate of micro-organisms increases with decreasing wavelength: Visible light → UV-A → UV-B → UV-C (260 nm). The maximum DNA absorbtion corresponds to the wavelength of UV-C. Comparing UV-A radiation and with visible light for example, more than the double amount of light is needed when using visible light only for the inactivation of microorganisms.

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2.4 SODIS Technical Note #6

SUMMARY

Solar radiation intensity varies over time and geographical location. During completely overcasted days the UV-A radiation intensity is reduced to one third of that recorded during a cloudless day.

BACKGROUND INFORMATION

Solar radiation received at ground level has been measured at meteorological stations for many years in most western countries. This has not been the case in the developing world, where the potential and need for the development of sunlight as an alternative source of energy are even greater.

Seasonal variation

Solar UV-A intensity shows both seasonal (because of changes in the earth's angle of tilt) and daily variation. This variation is depens on the latitude and is mainly responsible for the climate in that region. Regions near the aequator encounter lower variance of light intensity during the year than those in the northern or southern hemisphere. In Beirut for example (Latitude: 56°N), a horizontal surface (Figure 1), the intensity reaches a peak level of some 18 W/m^2 in June and decreases to its lowest level close to 5 W/m^2 in December. The difference between these two levels (13 W/m^2) is appreciable and important.

Daily variation (weather changes)

Figure 2 below shows the variation in received solar UV-A radiation intensity throughout the day under clear and cloudy weather conditions in Beirut (April and October 1985). With increasing cloudiness, less radiation energy is available. The reduction is depending on the wavelength as shown in Figure 3.

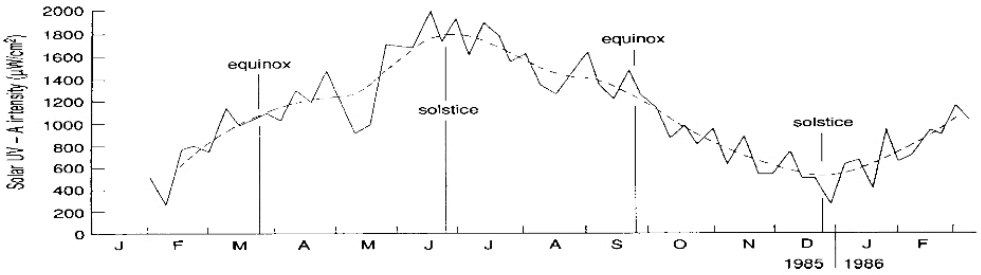

Figure 1: Mean weekly values (solid line) and moving averages (broken line) for solar UVA-A radiation intensity (horizontal surface)

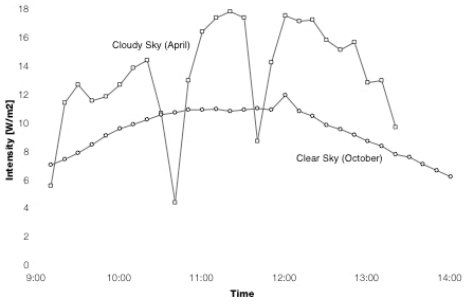

Figure 2: Variation of UV-A intensity during daytime under different weather conditions

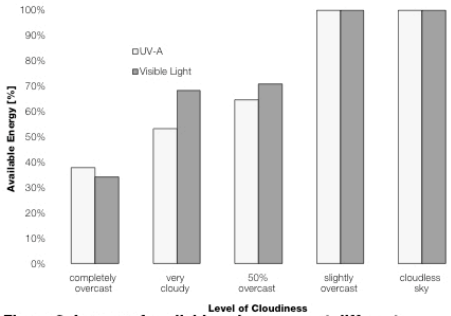

Figure 3: Losses of available solar energy at different weather conditions

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2.5 SODIS Technical Note #7

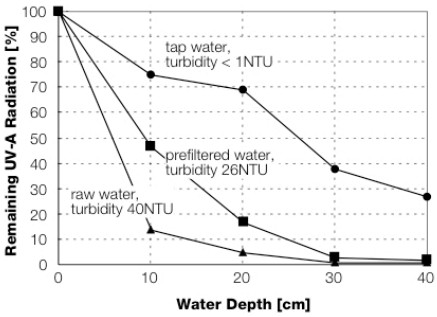
SUMMARY

Radiation intensity is reduced by increasing turbidity and water depth. Raw water of low turbidity (< 30 NTU) should be used for SODIS. Similarly, the water depth should be small and not exceed 10 cm in order to allow sufficient radiation of the water.

BACKGROUND INFORMATION

Water Turbidity

Turbidity is used as a parameter to characterise the optical properties of liquids containing absorbers and scatterers; i.e. suspended particles. As shown in Figure1, high turbidity substantially reduces the light penetration in water and therefore reduces the disinfection efficiency of the SODIS treatment process. To ensure safe water disinfection, the raw water should have a low turbidity (less than 30 NTU=Nephelometric Turbidity Units).



Water depth

UV-radiation is reduced by increasing water depth. At a water depth of 10 cm and moderate turbidity level of 26 NTU, UV-A radiation is reduced to 50%. The black lower surface of SODIS bags and bottles induces a temperature gradient which causes the water to circulate within the container thereby improving the inactivation efficiency. In any case, containers used for SODIS should be as flat as possible, with a water depth less than 10 cm.

Water Turbidity Test

To decide whether the water needs filtering, place the filled bottle on the SODIS Logo (see Figure below) on top of a table in the shade (to avoid light interference) and look through the bottle from top to bottom. If you can read the letters through the water, water turbidity is less than 30 NTU. If you can still see the sun rays of the Logo, turbidity is less than 20 NTU.
If water turbidity is higher than 30 NTU, coarse and settleable solids can be separated by storing the raw water for one day, and turbidity can be reduced possibly by flocculation / sedimentation (using alum sulphate or crushed Moringa oleifera seeds) or by filtration.

SODIS Logo for Turbidity Test. If one can read the letters, the turbidity is less than 30 NTU. If one can see the sun rays of the Logo, turbidity is less than 20 NTU.

REFERENCES

Wegelin, M. et al. (1994). Solar water disinfection: scope of the process and analysis of radiation experiments. *J Water SRT-Aqua*, 1994, **43**, No. 3, 154-169. [P1]
SODIS News No. 3, August 1998

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2.6 SODIS Technical Note #9

SUMMARY

SODIS uses two components of sunlight: The UV-A light for irradiation of the microorganisms and the infrared light for water heating. This combined use has synergetic effects which enhances the inactivation efficiency of the process. A 3-log reduction of E. coli requires a fluence of 555 W·h/m² (dose of solar radiation integrated in the 350-450 nm wavelength range), which correspond to ~ 5h of mid-latitude midday summer sunshine. At a threshold water temperature of 50 °C the required fluence is reduced to 140 W·h/m² and hence, requires an exposure time of approx. 1 hour only.

BACKGROUND INFORMATION

Radiation effects

Radiation an short wavelengths induces lethal effects in bacteria and viruses. The shorther the wavelength, the more efficient microorgansims are eliminated. The radiation affects DNA, nucleic acids and enzymes. Table 1 shows the UV-A resistance of some microorganisms.

Table 1: UV-A resistance of some microorganisms (Acra, 1989)

Test organism	Fluence (W·h/m2) required to inactivate:		
	90%	99%	99.90%
Streptococcus faecalis	8.90	17.80	26.72
Coliforms	8.24	16.59	24.74
Escherichia coli	6.36	12.72	19.08

Temperature effects

Microorganisms are heat sensitive. Table 2 lists up the required temperature to eliminate microorganisms within 1, 6 or 60 minutes. It can be seen that it is not required to boil the water in order to kill 99.9% of the microorganisms. Heating up water to 50-60 °C for one hour has the same effect.

Table 2: Thermoresistance of microorganisms

Microorganisms	Temperature for 100 % Destruction		
	1 Min.	6 Min.	60 Min.
Enteroviruses			62 °C
Rotaviruses			63 °C for 30 Min.
Faecal Coliforms	at 80 °C complete destruction		
Salmonellae		62 °C	58 °C
Shigella		61 °C	54 °C
Vibrio Cholera			45 °C
Entamoeba Histolytica Cysts	57 °C	54 °C	50 °C
Giardia Cysts	57 °C	54 °C	50 °C
Hookworm Eggs and Larvae		62 °C	51 °C
Ascaris Eggs	68 °C	62 °C	57 °C
Schistosomas Eggs	60 °C	55 °C	50 °C
Taenia Eggs	65 °C	57 °C	51 °C

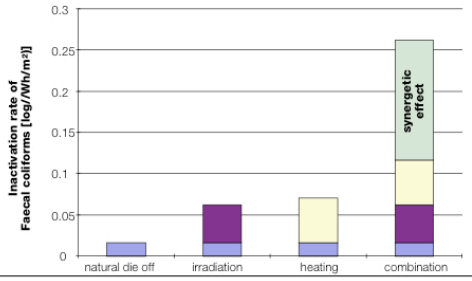
SODIS process: Synergetic effect

The SODIS treatment process is based on the synergetic effect of radiation and temperature. The die rates of Faecal coliforms exposed to irradiation heating increases substantially, when both stress factors occur. Table 3 and Figure 1 both show the synergetic effect of UV-radiation and water temperature on viruses and bacteria.

Table 3: Inactivation by artificial sunlight of coliphage f2 and the animal viruses EMCV and rotavirus. Time and fluence required for a 99.9% reduction at different temperatures are shown.

f2	Time (h)	Fluence (Wh/m²)
20 °C	3.3	2502
50 °C	1.3	973
EMCV		
20 °C	12.5	9535
50 °C	1.8	1390
Rotavirus		
20 °C	2.5	1890
40 °C	0.7	528

Figure 1: Synergetic effect of UV-radiation and temperature on Faecal coliforms in raw water.



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2.7 SODIS Technical Note #10

SUMMARY

Turbid water protects the microorganisms from being irradiated. The microorganisms will therefore only be exposed to thermal effects. Hence, the raw water used for SODIS application should be as clear as possible and not exceed a turbidity of 30 NTU.

BACKGROUND INFORMATION

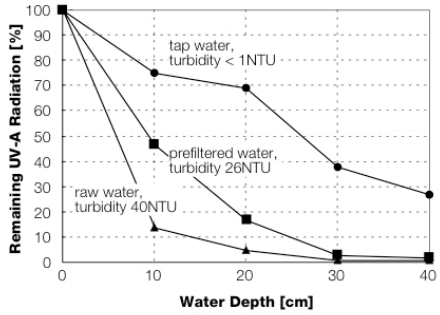
Turbidity effects

Suspended particles in water cause radiation scattering by deflection from their surfaces in all directions. Turbidity is used as a parameter to characterise the optical properties of liquids containing absorbers and scatterers.

In short, turbidity

- reduces solar radiation intensity (Figure 1)
- protects microorganisms from being irradiated (being either under floating solids or in settleable solids)
- reduces the efficiency of the SODIS process (Figure 2)

Figure 1: Reduction of UV-A radiation as a function of water depth and turbidity



Influence of temperature

UV-A radiation intensity is more reduced in turbid than in clear water, therefore reducing the SODIS-efficiency. However, the water temperature almost reaches the same level as in non-turbid water. The microorganisms are therefore inactivated by the temperature rather than by UV-A radiation. Table 1 shows some test results with bottles and bags at different levels of turbidity. With very high turbidity, not all pathogens could be eliminated in the bottle, because of the influence of the water depth (8 cm with bottle compared to 4 cm with bag).

Nevertheless turbidity has only a moderate effect on the efficiency of SODIS, the raw water used should be as clear as possible and not exceed a turbidity of 30 NTU.

Figure 2: Inactivation of Faecal coliforms in 15 minutes under different test conditions

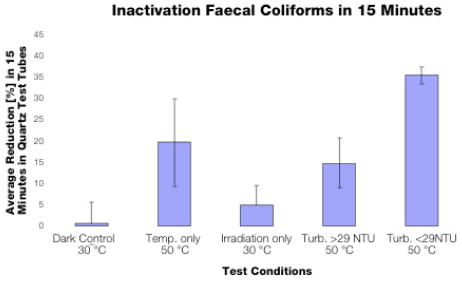


Table 1: Inactivation of Faecal coliforms in bags and bottles at different turbidity levels

	Faecal coliforms [CFU/100ml]		
Turbidity (NTU)	Begin	End (Bag)	End (Bottle)
5-10	290	0	0
10-20	16	0	0
30-40	1950	0	0
250	9050	0	116

REFERENCES

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2.8 SODIS Technical Note #13

SUMMARY

SODIS works with the synergetic effect of water temperature and UV-radiation. The prescription given here mainly have the effect that sunlight and temperature are optimized. High water temperature is reached by using black surfaces or black paint and not too large volumes of water per exposure area. Radiation dose is depending on the choice of the material for the container, location and orientation of the container, water depth, turbidity and exposure time.

BACKGROUND INFORMATION

Containers

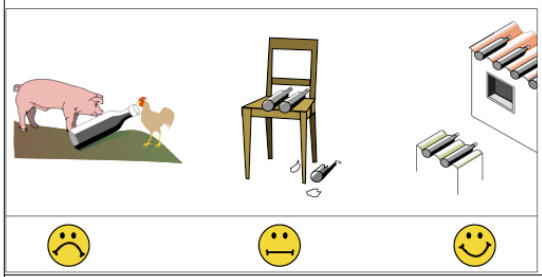
- Collect plastic bottles of 1-2 litre volume (preferably PET bottles) generally available as soft drink bottles
- Measure the light transmittance of the material with a photospectormeter (important is a good transmittance of the UV-A light, 320 - 400 nm spectrum)
- Check the water tightness of the bottles inclusive condition of the screw cap
- Clean the bottles thoroughly in- and outside
- Paint the bottles half-side black when black paint is available

Exposing Procedure

- Fill the bottles completely with raw water
- Screw the plug tightly
- Expose the bottles in the morning hours to sunlight on a place which is irradiated the full day
- Place the bottles in horizontal position on a firm blackened support, preferably on a corrugated iron sheet/roof or on a tile roof
- Collect the bottles in the late afternoon and bring them to a safe place for cooling
- Consume the treated water directly from the bottle using a clean glass or a cup, store it possibly overnight for additional cooling

Additional Prescriptions

- Use clean water free of settleable solids and of a low turbidity (max. turbidity > 30 NTU, see Technical Note #10, Influence of Turbidity, for more details). Separate coarse and settleable solids by storing the raw water for one day and reduce turbidity possibly by flocculation / sedimentation using alum sulphate or crushed Moringa oleifera seeds or by filtration.
- Use aerated water. Standing water with a low dissolved oxygen concentration should be aerated by shaking the containers or swirling the water with a stick before filling the containers.
- Observe a minimum exposure time to sunlight of one hour once the water temperature has reached 50 °C. At high ambient temperatures and intensive sun radiation one might be able to use the containers two times a day.
- Expose the water for five hours during a sunny day in case the water temperature has not reached the required 50 °C. Should the sky be covered with clouds expose the water for two consecutive days before consuming it.
- Collect rain water from a clean area (e.g. from a corrugated or tile roof) during rainy days to cover your drinking water demand.



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APPENDIX 3: POP BOTTLES AVAILABLE IN VARIOUS COUNTRIES

The table below shows various pop bottles, their characteristics important to SODIS, and their availability throughout the world (Harder, 2007; Higgins, 2007).

Brand Name	Availability	Material	Colour
Coca-Cola	Worldwide	Glass/PET	Clear, green
Fanta	North & South America, Europe	Glass/PET	Clear
7-Up	N/A	Glass/PET	Clear, green
Sprite	North America, Ecuador	Glass/PET	Clear, green
Kola Real (Big Cola)	Mexico, Central & South America	N/A	Clear
Inca Cola	Peru, Latin America	Glass/PET	Clear
Pepsi-Cola	Worldwide	Glass/PET	Clear
Thums Up	India	Glass	Clear
Tropicola	Cuba	N/A	Clear
Mecca Cola	Europe, Middle East, Asia, Africa	PET	Clear
Cola Turka	Turkey	PET	Clear
Zam Zam Cola	Iran, Middle East, Europe, Asia	Glass/PET	Clear
Parsi Cola	Iran, Middle East	Glass/PET	Clear
Evoca Cola	United Kingdom, South Africa	PET	Clear
Flora vanti	Ecuador	PET	Clear
Schwepps	Australia, North America	PET	Clear, green

APPENDIX 4: ANOVA FOR DARK CONTROL BOTTLES DURING CLEANING PRETREATMENT EXPERIMENTS

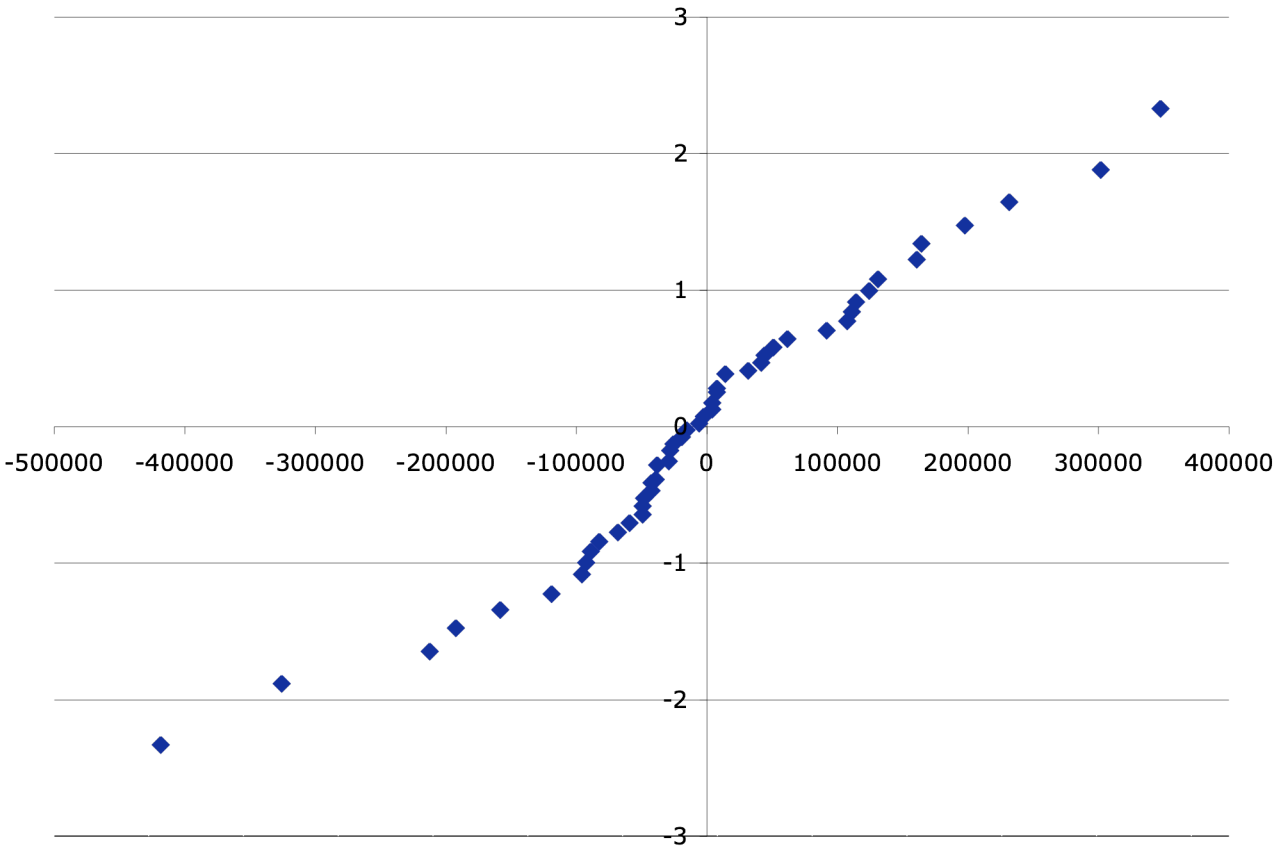
The table below shows the analysis of variance (ANOVA) for time zero. From the ANOVA table it is clear that the null hypothesis cannot be rejected for the statement "all treatments are the same"; the F_{obs} value is 0.075, which is less than the F_{table} value, 2.80.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	1.64×10^{10}	5.47×10^{09}	0.075	2.80
Within Cleaning Agents	48	3.51×10^{12}	7.32×10^{10}		
Total	51	3.53×10^{12}			

The next table shows the ANOVA for the 2.5-hour sample time. From the ANOVA table it is clear that the null hypothesis must be rejected; the F_{obs} value is 2.90, which is slightly greater than the F_{table} value, 2.82.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	1.79×10^{11}	5.97×10^{10}	2.90	2.82
Within Cleaning Agents	44	9.07×10^{11}	2.06×10^{10}		
Total	47	1.09×10^{12}			

Following the ANOVA, a normal probability plot (below) was made to determine if the residuals were normally distributed. The points lie on a straight line and this shows that the residuals are normally distributed.



The least significant difference (LSD) was also calculated. The LSD is shown below, while the following table outlines the variables used in its calculation. The number of cleaning agents (k) was four. The number of required experiments (c) was six. To achieve an overall confidence interval of 95% (b), the experiments were performed at a significance level of 99% (α). To calculate the LSD, the standard error of the difference between two means (s.e.) and the t-statistic were determined.

$$\text{LSD} = s.e.(t_{52,0.005}) = 158\ 000$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	2.06×10^{10}
Average number of sample size	$\bar{n}$	12
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	58 600
t-statistic	$t_{df,\alpha}$	2.69

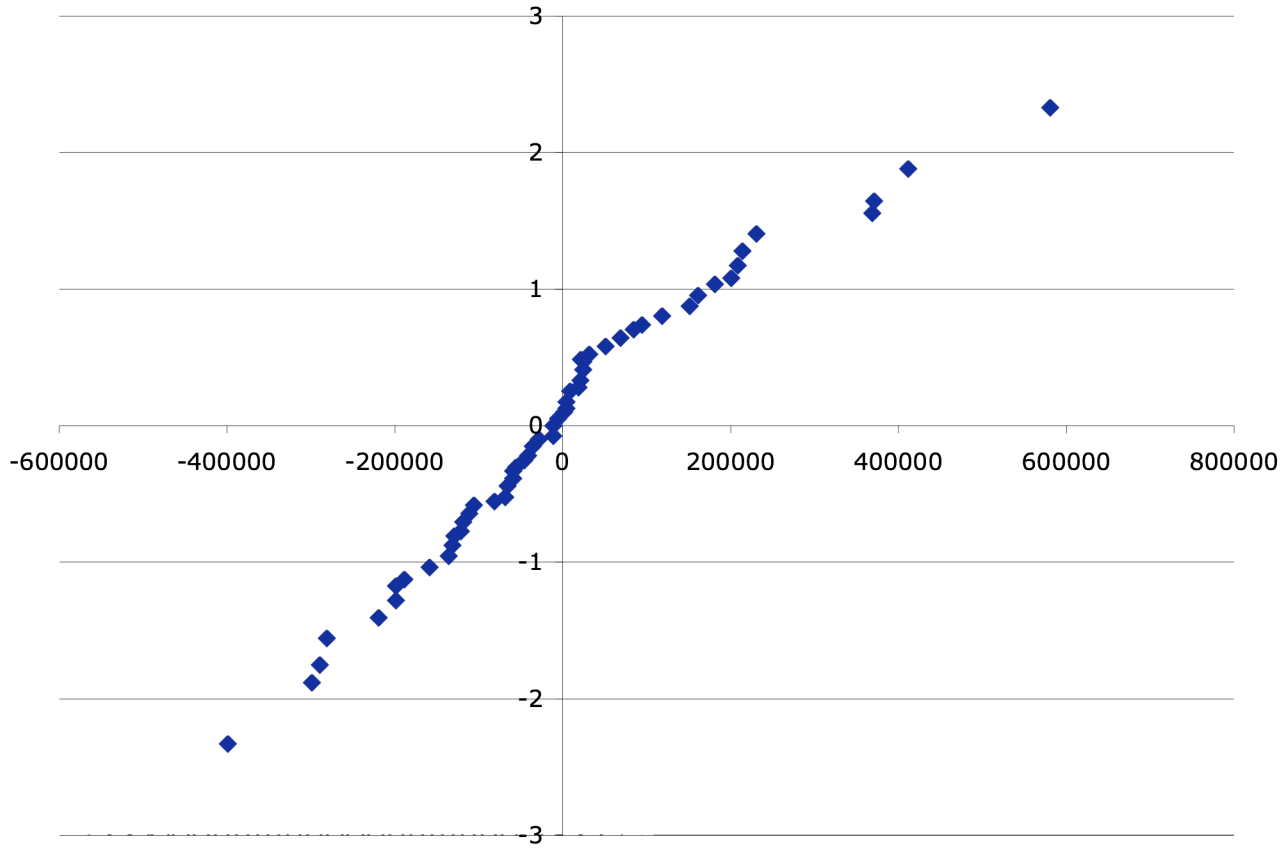
The average *E. coli* concentrations for the Dark Bottles are found in the table below. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (158 000). Hence, the lime juice treatment is the only statistically different treatment.

Dark Control Bottles	Average CFU/mL
Alcohol	792 000
Lime Juice	918 000
Soap	799 000
Positive Control	756 000

The following table shows the ANOVA for the sample taken after 5 hours. From the ANOVA table it is clear that the null hypothesis must be rejected for the statement "all treatments are the same"; the F_{obs} value is 36.06, which is greater than the F_{table} value, 2.79.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	3.86×10^{12}	1.29×10^{12}	36.06	2.79
Within Cleaning Agents	51	1.82×10^{12}	3.57×10^{10}		
Total	54	5.69×10^{12}			

The following figure is the normalized residuals for the samples taken after five hours. The points lie on a straight line and indicate that the residuals are normally distributed.



The LSD is shown below, while the following table outlines the variables used in its calculation.

$$\text{LSD} = s.e.(t_{52,0.005}) = 191\ 000$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	3.57×10^{10}
Average number of sample size	$\bar{n}$	14
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	71 400
t-statistic	$t_{df,\alpha}$	2.68

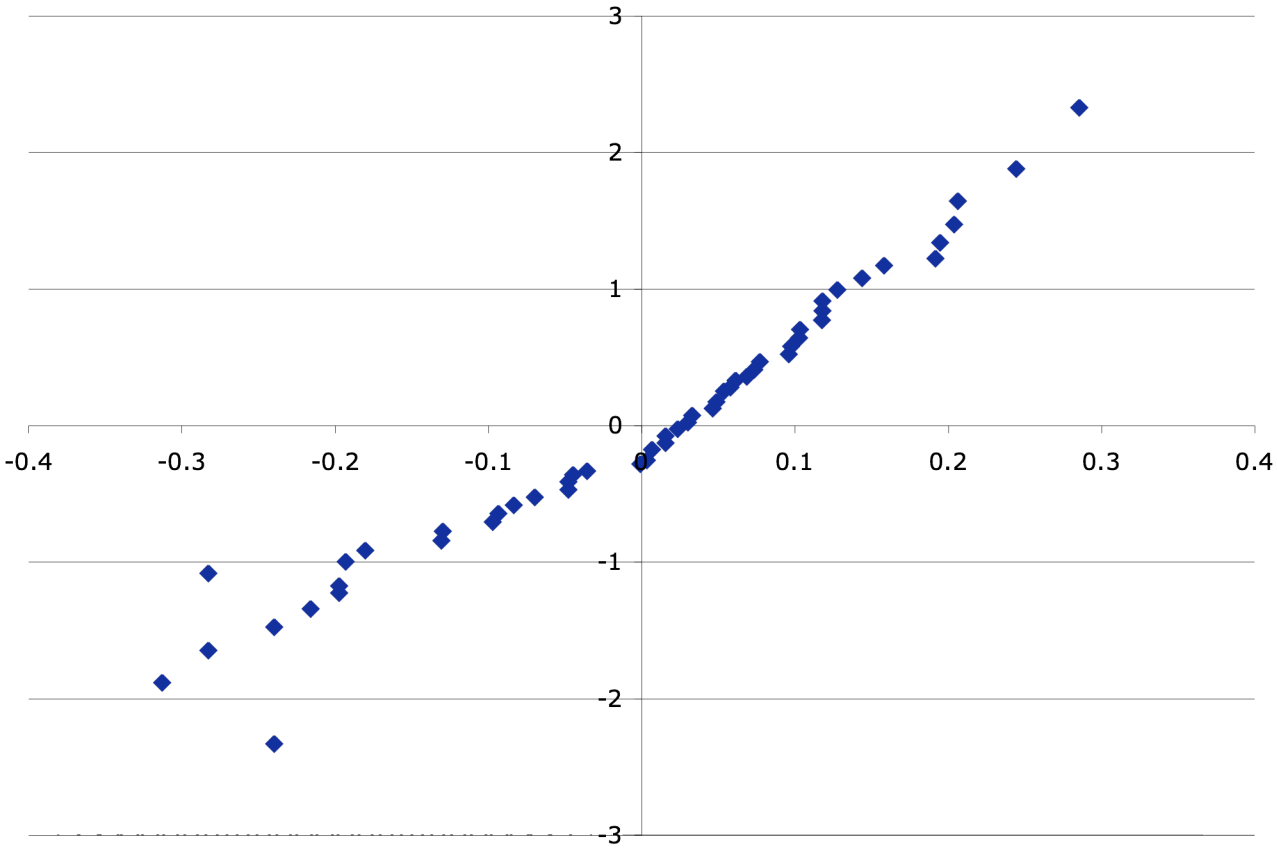
The average *E. coli* concentrations for the Dark Bottles after five hours are found in the table below. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (191 000). Hence, the lime juice treatment is the only statistically different treatment.

Dark Control Bottles	Average CFU/mL
Alcohol	646 000
Lime Juice	1 334 000
Soap	709 000
Positive Control	601 000

The next table below shows the ANOVA for the 24-hour sample time. The data was transformed using the log transformation before the analysis was carried out. This transformation was used since the data from the lime juice-rinsed bottles was much greater than that from the other bottles. The transformation allowed an appropriate comparison of the data. From the table it is clear that the null hypothesis must be rejected; the F_{obs} value is 82.59, which is greater than the F_{table} value, 2.79.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	5.76	1.92	82.59	2.79
Within Cleaning Agents	48	1.12	2.32×10^{-02}		
Total	51	6.88			

The next figure shows the normalized residuals for the samples taken after 24 hours. Since the points lie on a straight line, they are normally distributed.



The LSD is shown below, while the following table outlines the variables used in its calculation.

$$\text{LSD} = s.e.(t_{50,0.005}) = 0.16$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	2.32×10^{-02}
Average number of sample size	$\bar{n}$	13.5
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	0.059
t-statistic	$t_{df,\alpha}$	2.68

The average *E. coli* concentrations for the Dark Control Bottles after 24 hours are in the table below. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (0.16). Hence, the lime juice treatment is the only statistically different treatment.

Dark Bottles	Average CFU/mL
Alcohol	5.72
Lime Juice	6.50
Soap	5.76
Positive Control	5.67

APPENDIX 5: ANOVA FOR LIGHT BOTTLES DURING CLEANING PRETREATMENT EXPERIMENTS

The table below shows the analysis of variance (ANOVA) for time zero. From the ANOVA table it is clear that the null hypothesis cannot be rejected for the statement "all treatments are the same"; the F_{obs} value is 0.53, which is less than the F_{table} value, 2.78.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	7.05×10^{10}	2.35×10^{10}	0.53	2.78
Within Cleaning Agents	52	2.31×10^{12}	4.45×10^{10}		
Total	55	2.38×10^{12}			

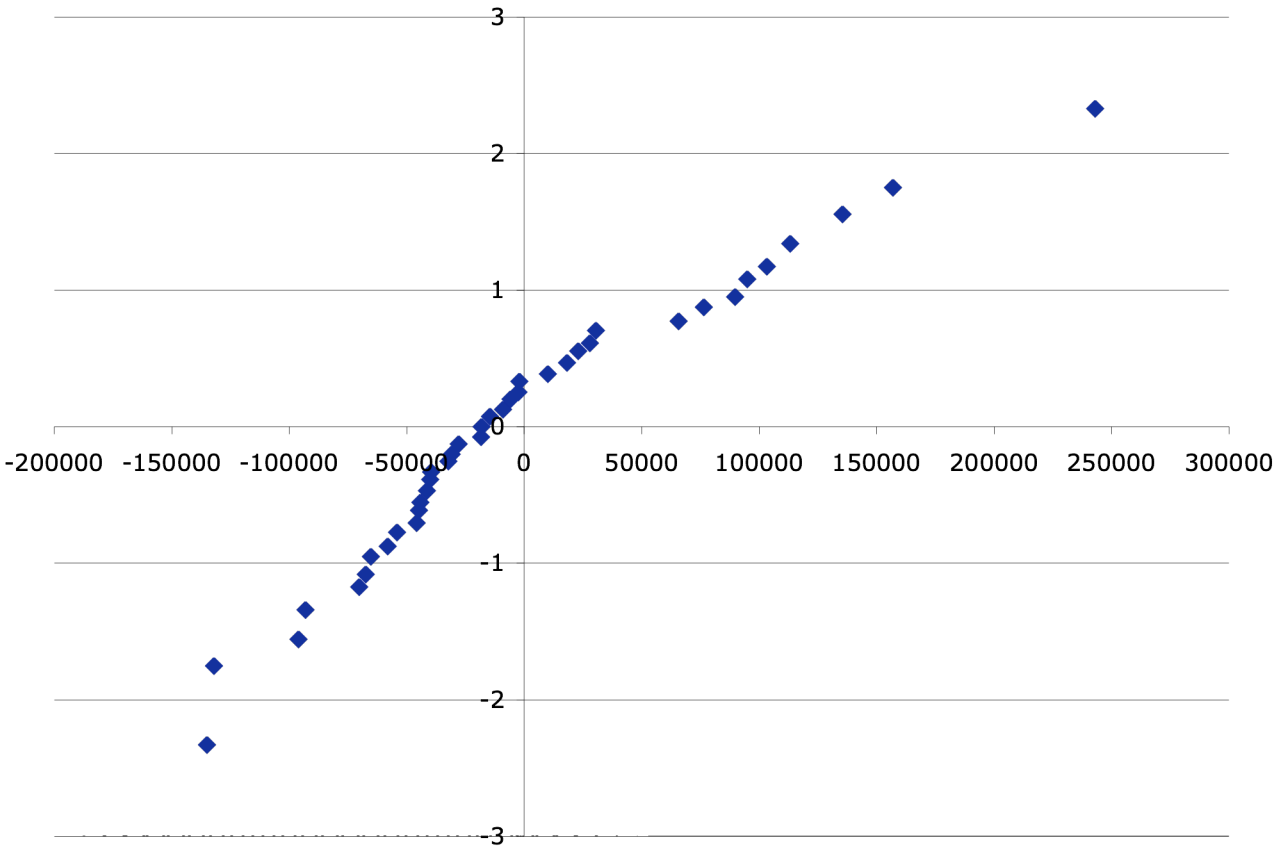
The next table shows the ANOVA for the 0.5-hour sample time. The null hypothesis again cannot be rejected since the F_{obs} value is 1.92, which is less than the F_{table} value of 2.82.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	8.82×10^{10}	2.94×10^{10}	1.92	2.82
Within Cleaning Agents	44	6.75×10^{11}	1.53×10^{10}		
Total	47	7.63×10^{11}			

The following table shows the ANOVA for the samples taken after one hour. The null hypothesis must be rejected since the F_{obs} value is 7.49, which is greater than the F_{table} value of 2.88.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	1.57×10^{11}	5.22×10^{10}	7.49	2.88
Within Cleaning Agents	35	2.44×10^{11}	6.96×10^{09}		
Total	38	4.00×10^{11}			

The figure below shows the normalized residuals for the one-hour sample time. The residuals lie on a straight line indicating they are normally distributed.



The least significant difference (LSD) was also calculated. The LSD is shown below, while the following table outlines the variables used in its calculation. The number of cleaning agents (k) was four. The number of required experiments (c) was six. To achieve an overall confidence interval of 95% (b), the experiments were performed at a significance level of 99% (α). To calculate the LSD, the standard error of the difference between two means (s.e.) and the t-statistic were determined.

$$\text{LSD} = s.e.(t_{35,0.005}) = 103\ 000$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	6.96×10^{09}
Average number of sample size	$\bar{n}$	9.75
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	37 800
t-statistic	$t_{df,\alpha}$	2.725

The average *E. coli* concentrations for the Light Bottles after one hour are shown below. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (103 000). Hence, the lime juice treatment is the only statistically different treatment.

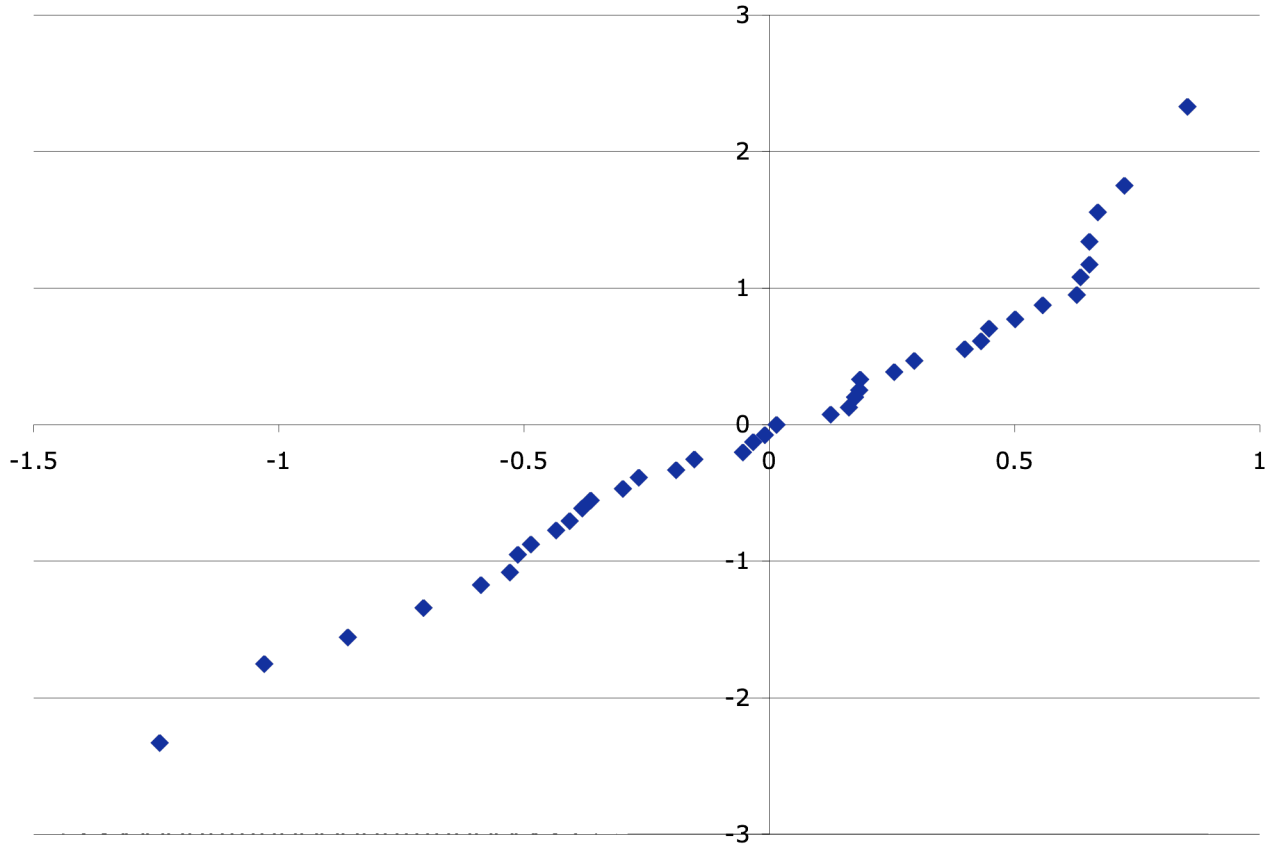
Light Bottles	Average CFU/mL
Alcohol	66 000
Lime Juice	62 000
Soap	213 000
Positive Control	77 000

It should be noted that the data from the 1.5-, 2-, 2.5-, 5-, and 24-hour sample times were transformed using the log-transformation before the analysis of variance was carried out. This transformation was used since the *E. coli* concentrations from some of the bottles were much greater than those in the other bottles. The transformation allowed an appropriate comparison of the data.

The ANOVA below is for the sample taken after 1.5 hours. The null hypothesis must be rejected since the F_{obs} value is 17.89, which is greater than the F_{table} value of 2.88.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	16.29	5.43	17.89	2.88
Within Cleaning Agents	35	10.57	0.30		
Total	38	26.85			

The next figure shows the normalized residuals. Since the points lie on a straight line, the residuals are normally distributed.



The LSD was also calculated (shown below).

$$\text{LSD} = s.e.(t_{35,0.005}) = 0.68$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	0.30
Average number of sample size	$\bar{n}$	9.75
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	0.25
t-statistic	$t_{df,\alpha}$	2.725

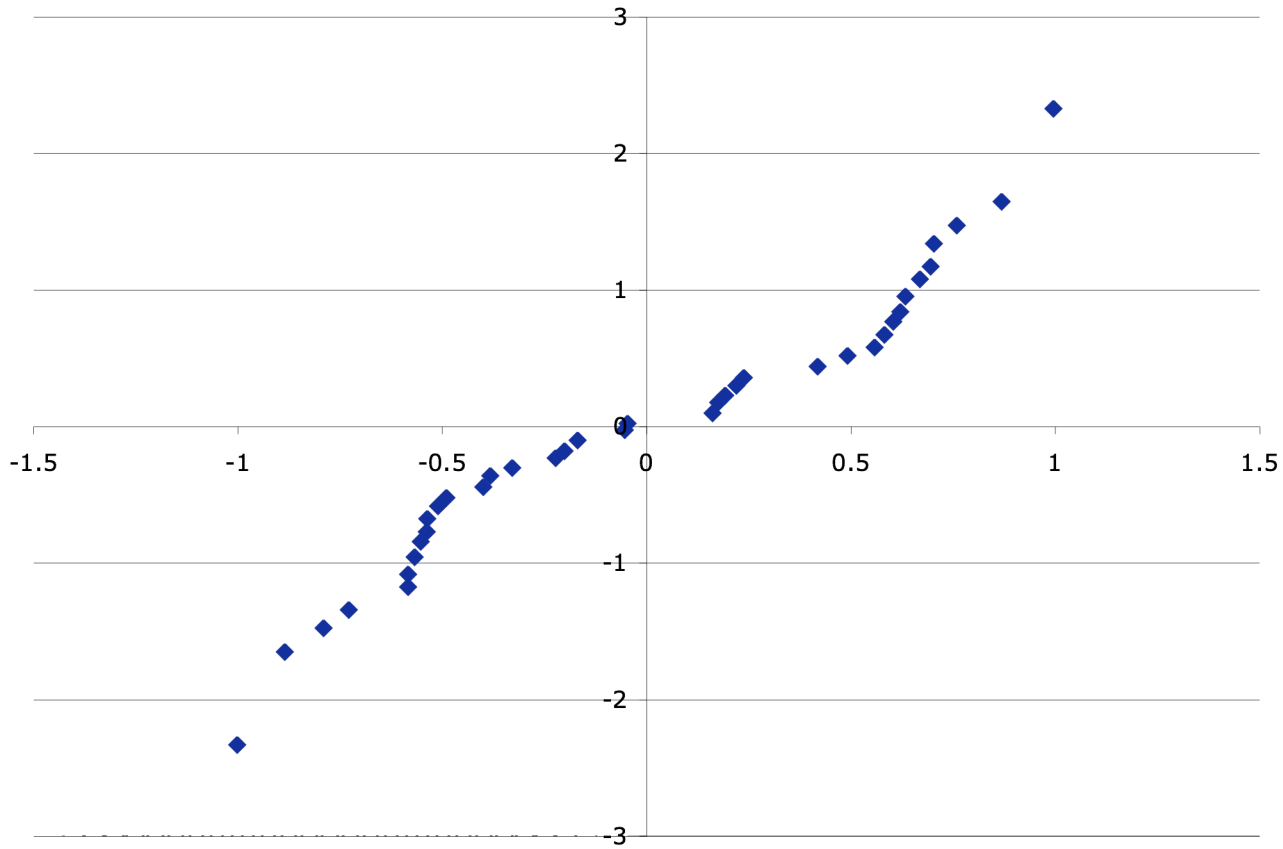
The average *E. coli* concentrations for the Light Bottles after 1.5 hours are shown in the table below. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (0.68). Hence, the lime juice treatment is the only statistically different treatment.

Light Bottles	Average CFU/mL
Alcohol	3.42
Lime Juice	4.87
Soap	3.47
Positive Control	3.28

The next table is the ANOVA for the 2-hour sample time. The null hypothesis must be rejected since the F_{obs} value is 36.03, which is greater than the F_{table} value of 2.88.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	38.23	12.74	36.03	2.88
Within Cleaning Agents	34	12.02	0.35		
Total	37	50.25			

The following figure depicts the normalized residuals for the two-hour sample time. As can be seen, the points lie on a straight line; this feature suggests that the residuals are normally distributed.



The LSD for the samples taken after 2 hours is shown next.

$$\text{LSD} = s.e.(t_{34,0.005}) = 0.74$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	0.35
Average number of sample size	$\bar{n}$	9.5
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	0.27
t-statistic	$t_{df,\alpha}$	2.72

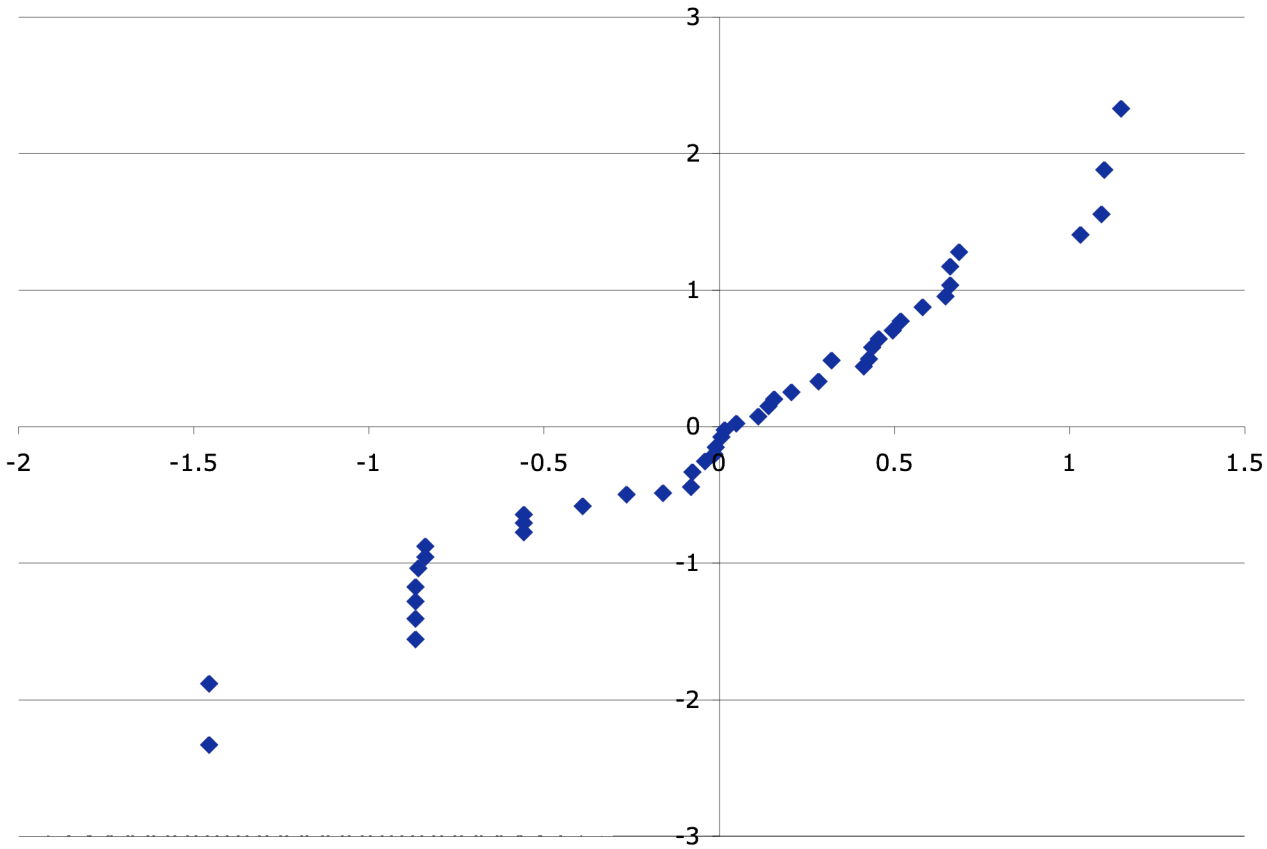
The average *E. coli* concentrations for the Light Bottles after two hours are shown. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (0.74). Hence, the lime juice treatment is the only statistically different treatment.

Light Bottles	Average CFU/mL
Alcohol	1.58
Lime Juice	4.16
Soap	2.03
Positive Control	1.90

The ANOVA for the 2.5-hour sample is shown next. The null hypothesis must be rejected since the F_{obs} value is 41.24, which is greater than the F_{table} value of 2.84.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	58.78	19.59	41.24	2.84
Within Cleaning Agents	40	19.00	0.48		
Total	43	77.78			

The figure below shows the normalized residuals. The points lie on a relatively straight line; this indicates that the residuals are normally distributed.



The LSD was calculated for the samples taken after 2.5 hours.

$$\text{LSD} = s.e.(t_{40,0.005}) = 0.79$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	0.48
Average number of sample size	$\bar{n}$	11
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	0.29
t-statistic	$t_{df,\alpha}$	2.70

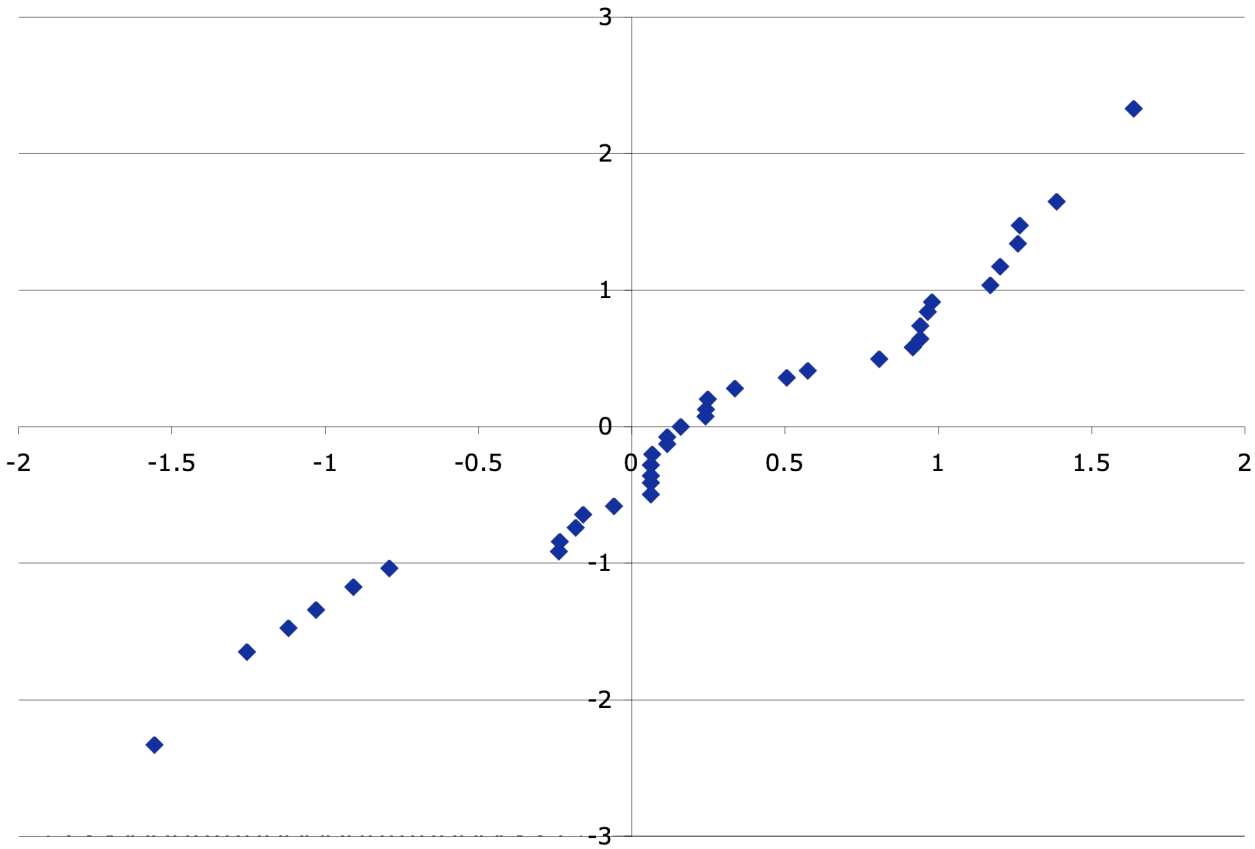
The average *E. coli* concentrations for the Light Bottles after 2.5 hours are shown in the following table. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (0.79). Hence, the lime juice treatment is the only statistically different treatment.

Light Bottles	Average CFU/mL
Alcohol	0.87
Lime Juice	3.98
Soap	1.46
Positive Control	0.86

The next table shows the ANOVA for samples taken after five hours. The null hypothesis must be rejected since the F_{obs} value is 13.46, which is greater than the F_{table} value of 2.88.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	25.89	8.63	13.46	2.88
Within Cleaning Agents	33	21.16	0.64		
Total	36	47.05			

The figure below is a normalized residual plot. As shown, the points lie on a straight line. This indicates that the residuals are normally distributed.



The LSD for the 5-hour sample time is below.

$$\text{LSD} = s.e.(t_{33,0.005}) = 1.01$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	0.64
Average number of sample size	$\bar{n}$	9.25
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	0.37
t-statistic	$t_{df,\alpha}$	2.72

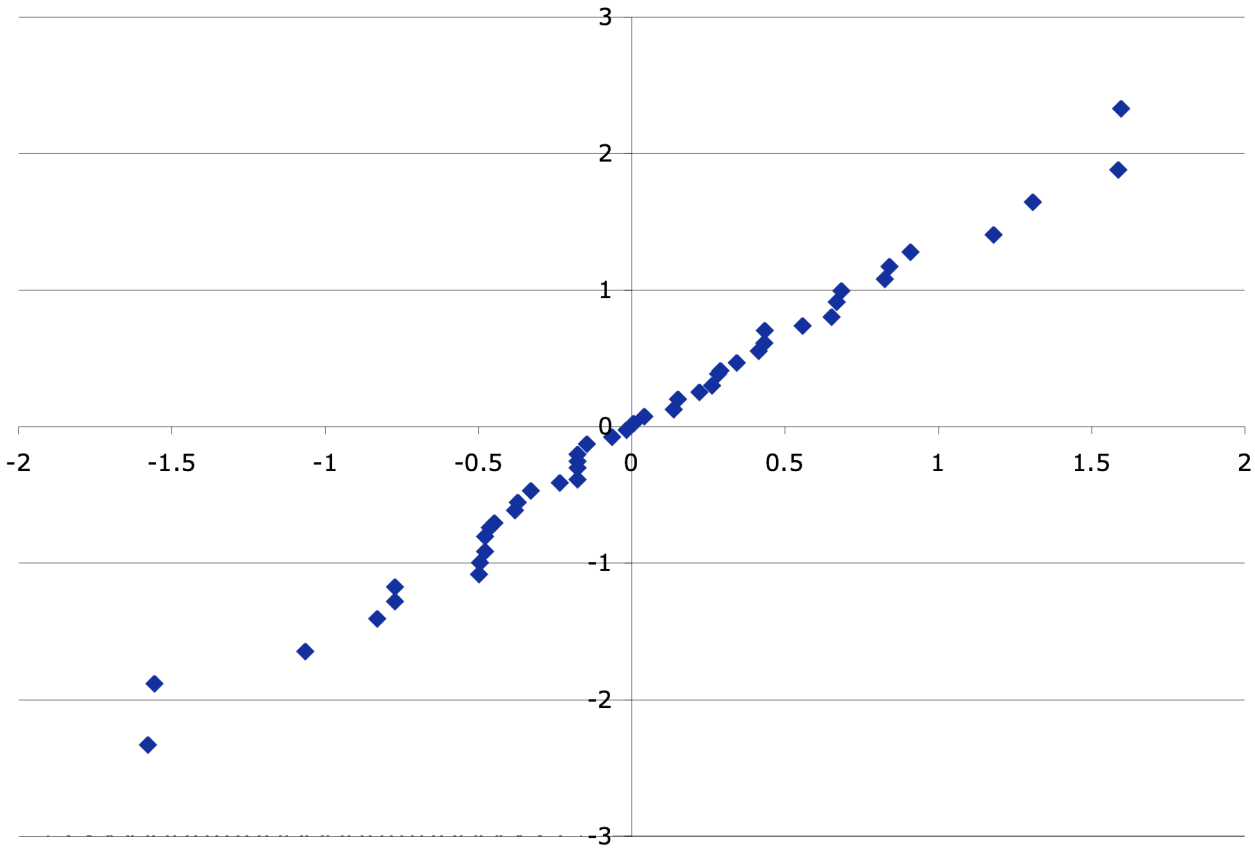
The average *E. coli* concentrations for the Light Bottles after five hours are shown in the following table. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the other treatment averages is larger than the LSD (1.01). Hence, the lime juice treatment is the only statistically different treatment.

Light Bottles	Average CFU/mL
Alcohol	0.54
Lime Juice	2.60
Soap	0.54
Positive Control	0.36

Finally, the next table shows the ANOVA for the 24-hour sample time. The null hypothesis must be rejected since the F_{obs} value is 41.71, which is greater than the F_{table} value of 2.82.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	3	66.90	22.30	41.71	2.82
Within Cleaning Agents	42	22.46	0.53		
Total	45	89.35			

The figure below shows the normalized residuals. The points lie on a straight line, showing that the residuals are normally distributed.



The LSD was also calculated for the samples taken after 24 hours.

$$\text{LSD} = s.e.(t_{42,0.005}) = 0.82$$

Term	Constant	Value
Number of treatments	k	4
Required number of tests	$c = k(k - 1)/2$	6
Overall level of significance	b	0.05
Level of significance	$\alpha = b/c$	0.01
Mean square error	MSE	0.53
Average number of sample size	$\bar{n}$	11.5
Standard error for two means	$s.e. = \sqrt{2(MSE)/\bar{n}}$	0.30
t-statistic	$t_{df,\alpha}$	2.70

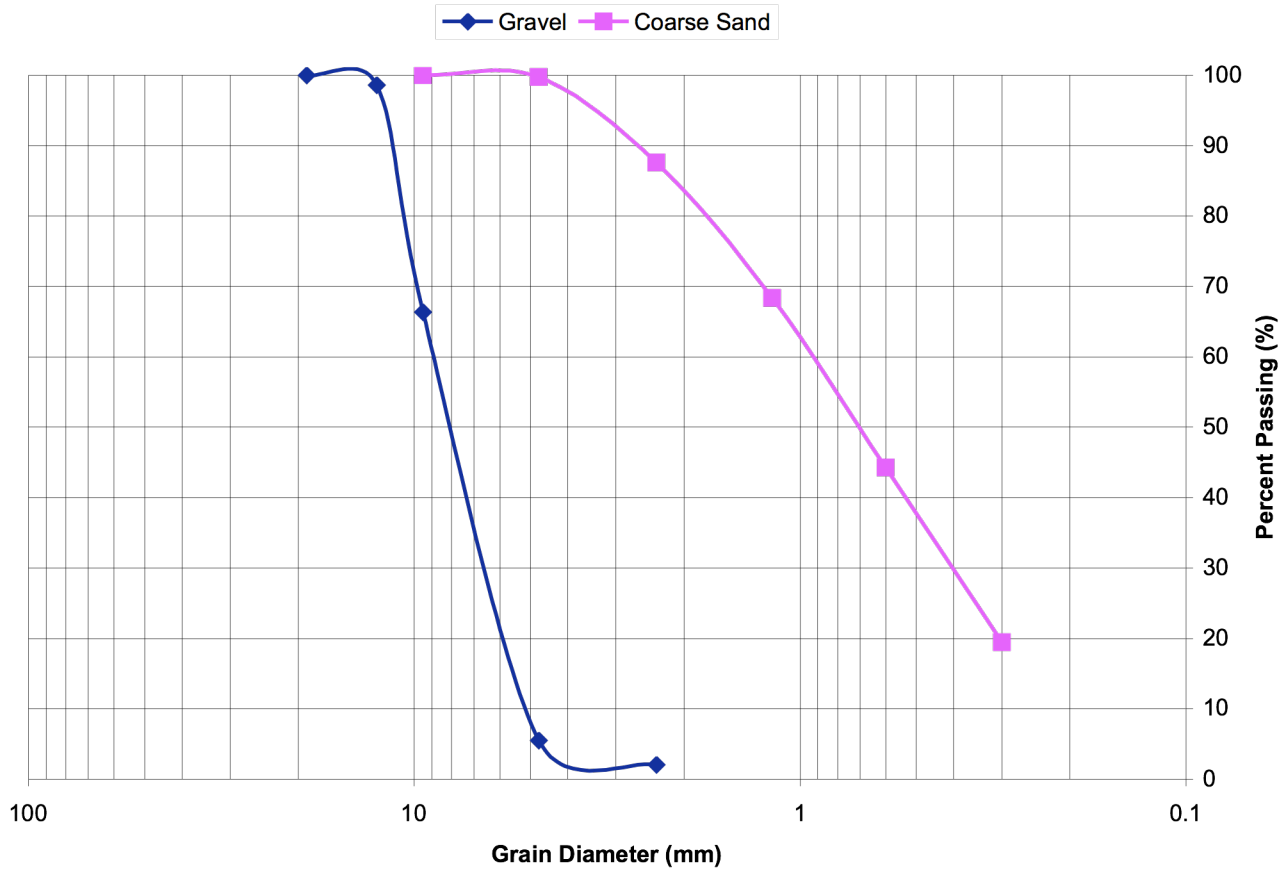
The average *E. coli* concentrations for the Light Bottles after 24 hours are shown in the next table. The difference between the average *E. coli* concentrations for the bottles cleaned with lime juice and the bottles rinsed with soap and the other treatment averages is larger than the LSD (0.82). Hence, the lime juice and the soap treatment are statistically different from each other and the other treatments.

Light Bottles	Average CFU/mL
Alcohol	1.07
Lime Juice	4.28
Soap	1.91
Positive Control	1.08

APPENDIX 6: SIEVE ANALYSIS

The following table and figure show the standard sieve analysis for the gravel and coarse sand used in the Roughing Filter Experiments and the Filter and Sunlight Experiments.

Sieve	Weight Retained		% Retained		Cumulative % Passing	
	Gravel	Sand	Gravel	Sand	Gravel	Sand
¾"	0		0.0		100.0	
½"	346.7		1.4		98.6	
⅜"	8142.6	0	32.2	0.0	66.4	100.0
No.4	15378.5	234.5	60.9	0.3	5.5	99.7
No.8	863.8	10548.6	3.4	12.1	2.1	87.6
No.16		16726.3		19.2		68.4
No.30		20986.1		24.1		44.2
No.50		21571.6		24.8		19.4
Pan	520.8	16916.2	2.1	19.5	0	0

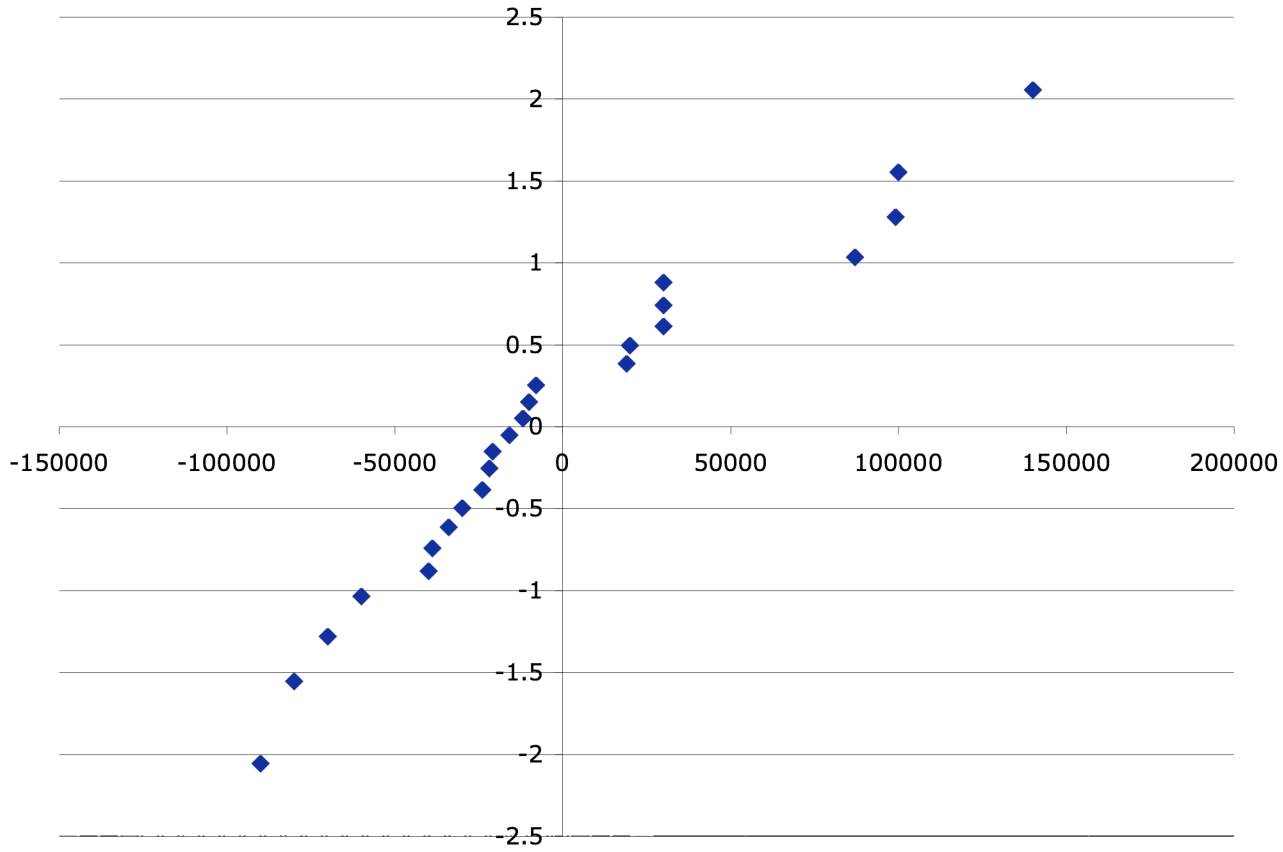

Gravel
Coarse Sand
100
90
80
70
60
50
40
30
20
10
0
Percent Passing (%)
100
10
1
0.1
Grain Diameter (mm)

APPENDIX 7: ANOVA FOR DARK CONTROL BOTTLES DURING ROUGHING FILTER AND SUNLIGHT EXPERIMENTS

The table below shows the analysis of variance (ANOVA) for time zero. From the ANOVA table it is clear that the null hypothesis can be rejected for the statement "all treatments are the same"; the F_{obs} value is 23.99, which is less than the F_{table} value, 4.32.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	8.80×10^{10}	8.80×10^{10}	23.99	4.32
Within Cleaning Agents	22	8.07×10^{10}	3.67×10^{09}		
Total	23	1.69×10^{11}			

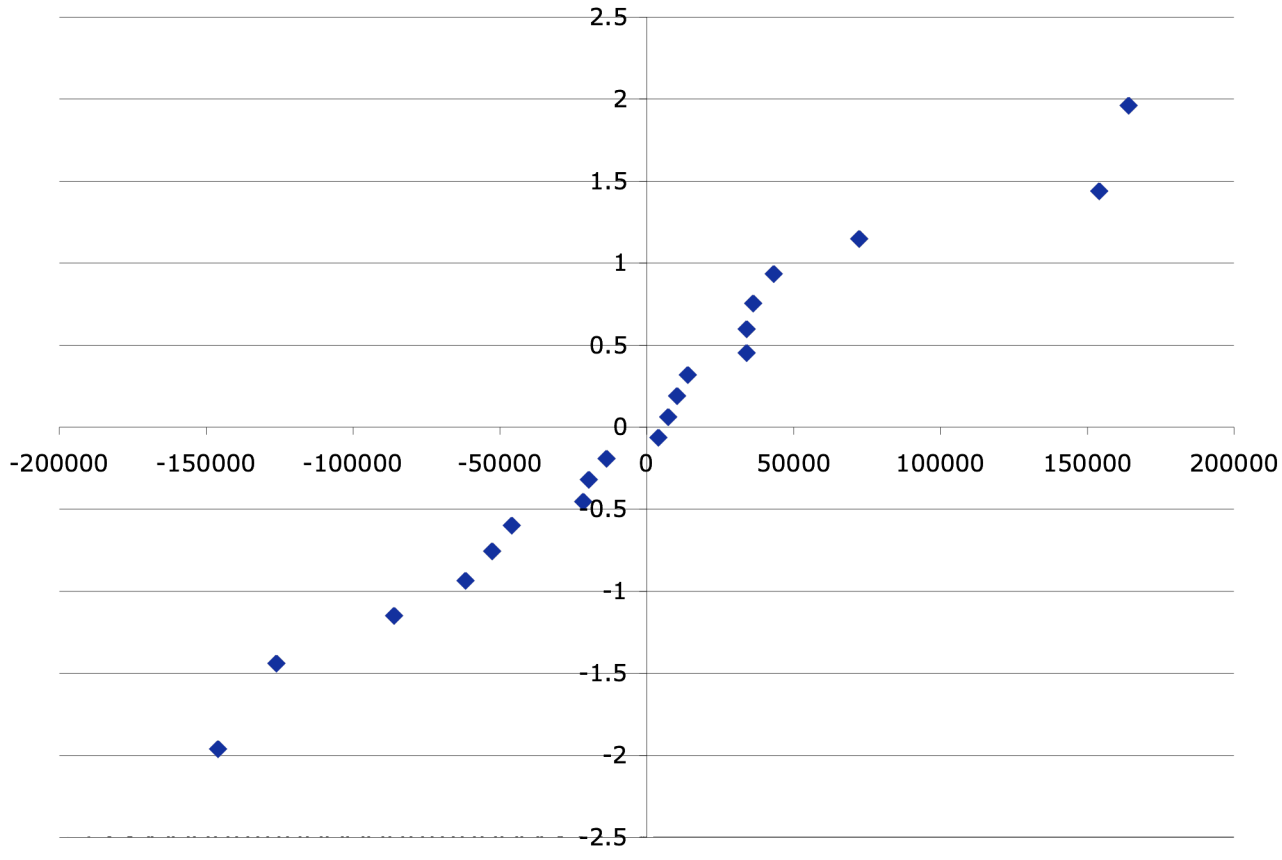
The next figure shows the normalized residual plot for time zero. Since the residuals lie on a straight line, they are normally distributed.



The next table shows the ANOVA for the 2.5-hour sample. From the ANOVA table it is clear that the null hypothesis must be rejected; the F_{obs} value is 12.37, which is slightly greater than the F_{table} value, 4.43.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	7.98×10^{10}	7.98×10^{10}	12.37	4.43
Within Cleaning Agents	18	1.16×10^{11}	6.45×10^{09}		
Total	19	1.96×10^{11}			

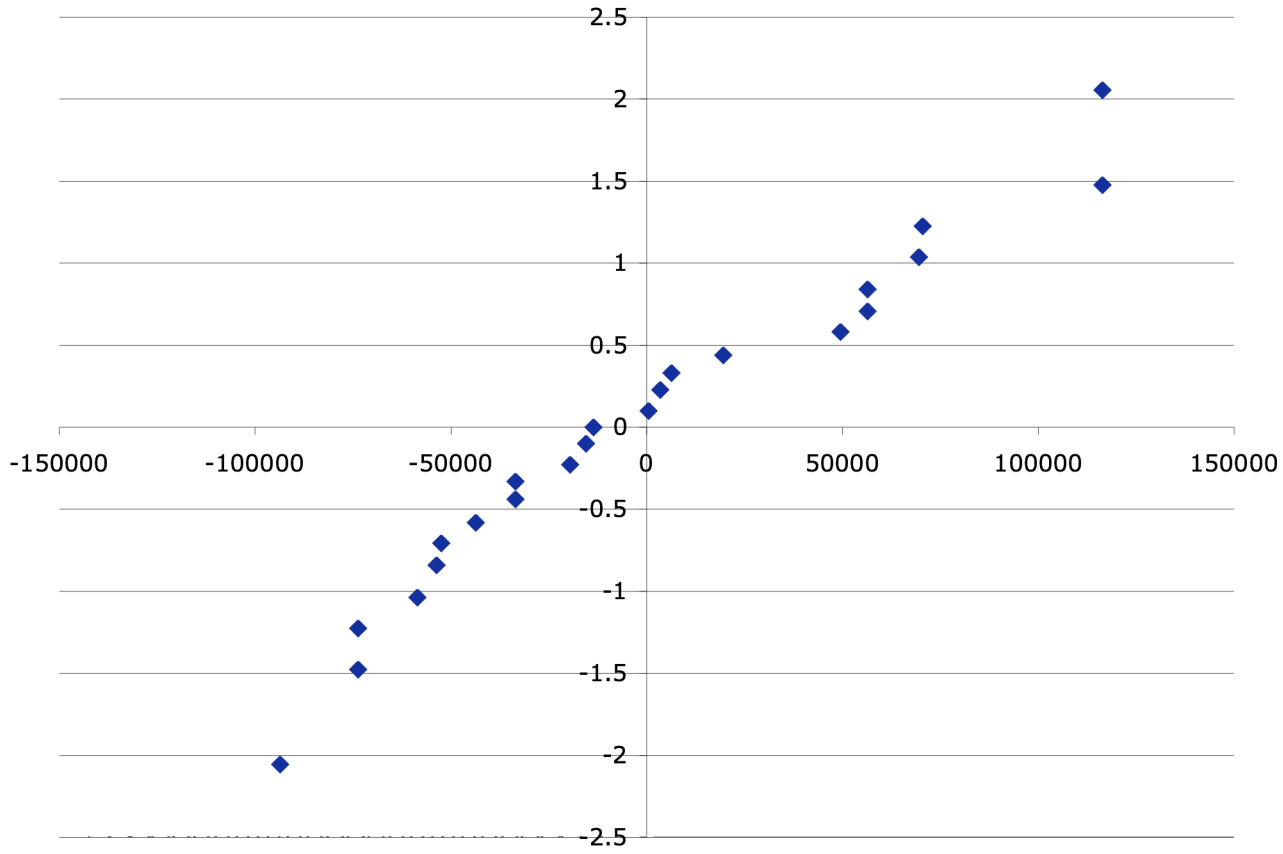
The figure below is a normalized residual plot for time 2.5 hours. The residuals lie on a straight line and are thus normally distributed.



The following table shows the ANOVA for the 5-hour sample time. From the ANOVA table it is clear that the null hypothesis must be rejected for the statement "all treatments are the same"; the F_{obs} value is 31.84, which is greater than the F_{table} value, 4.32.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	1.21×10^{11}	1.21×10^{11}	31.84	4.32
Within Cleaning Agents	21	7.97×10^{10}	3.80×10^{09}		
Total	22	2.01×10^{11}			

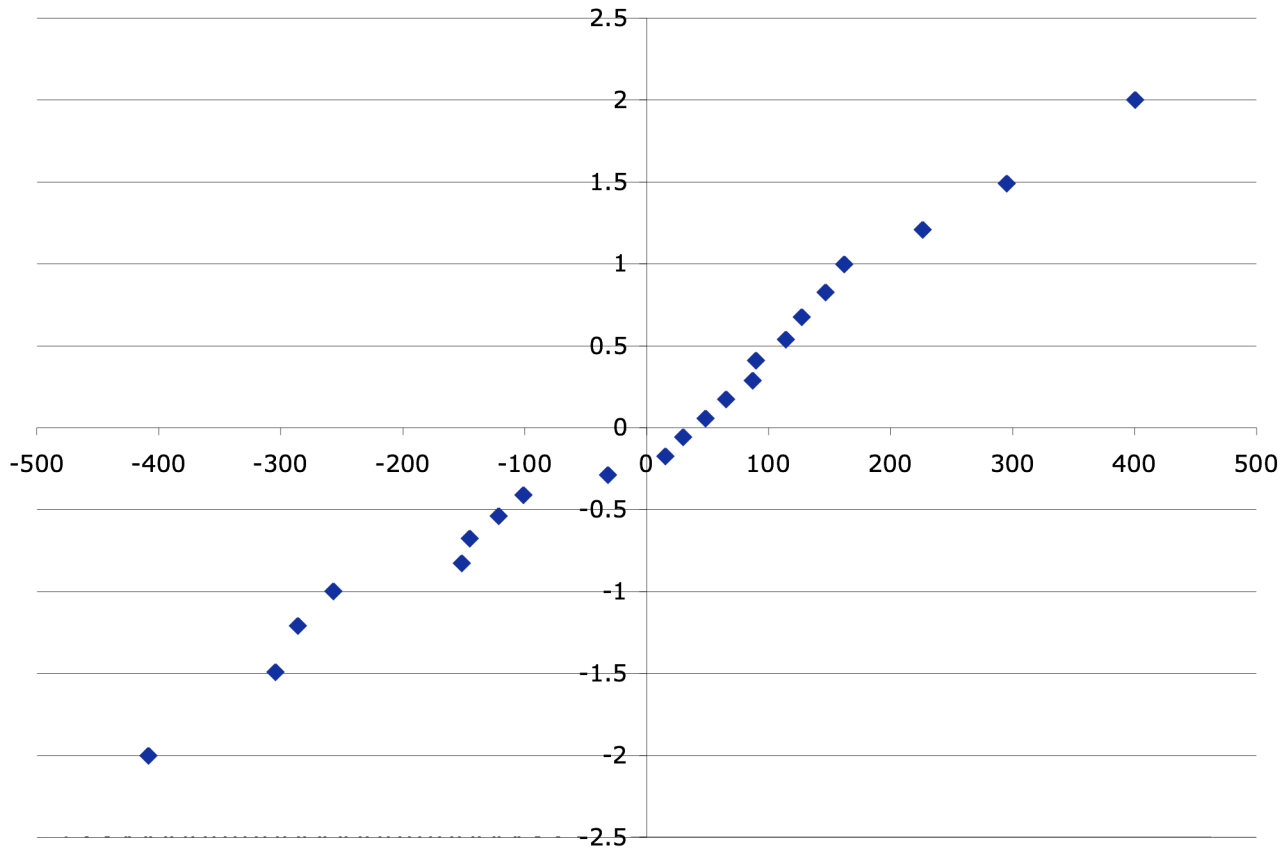
The next figure shows the normalized residual plot for time five hours. Since the residuals lie on a straight line, they are normally distributed.



The table below shows the ANOVA for the sample take at 24 hours. The data was transformed using y^{λ}, where λ was 0.5, before the analysis was carried out. This transformation was used since the data from the non-filtered water bottles was much greater than that from the filtered water bottles. The transformation allowed an appropriate comparison of the data. From the ANOVA table it is clear that the null hypothesis must be rejected; the F_{obs} value is 56.55, which is greater than the F_{table} value, 4.35.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	2.48×10^{06}	2.48×10^{06}	56.55	4.35
Within Cleaning Agents	20	8.76×10^{05}	4.38×10^{04}		
Total	21	3.35×10^{06}			

The figure below is a normalized residual plot for the 24 hour sample time. The residuals lie on a straight line; hence, they are normally distributed.

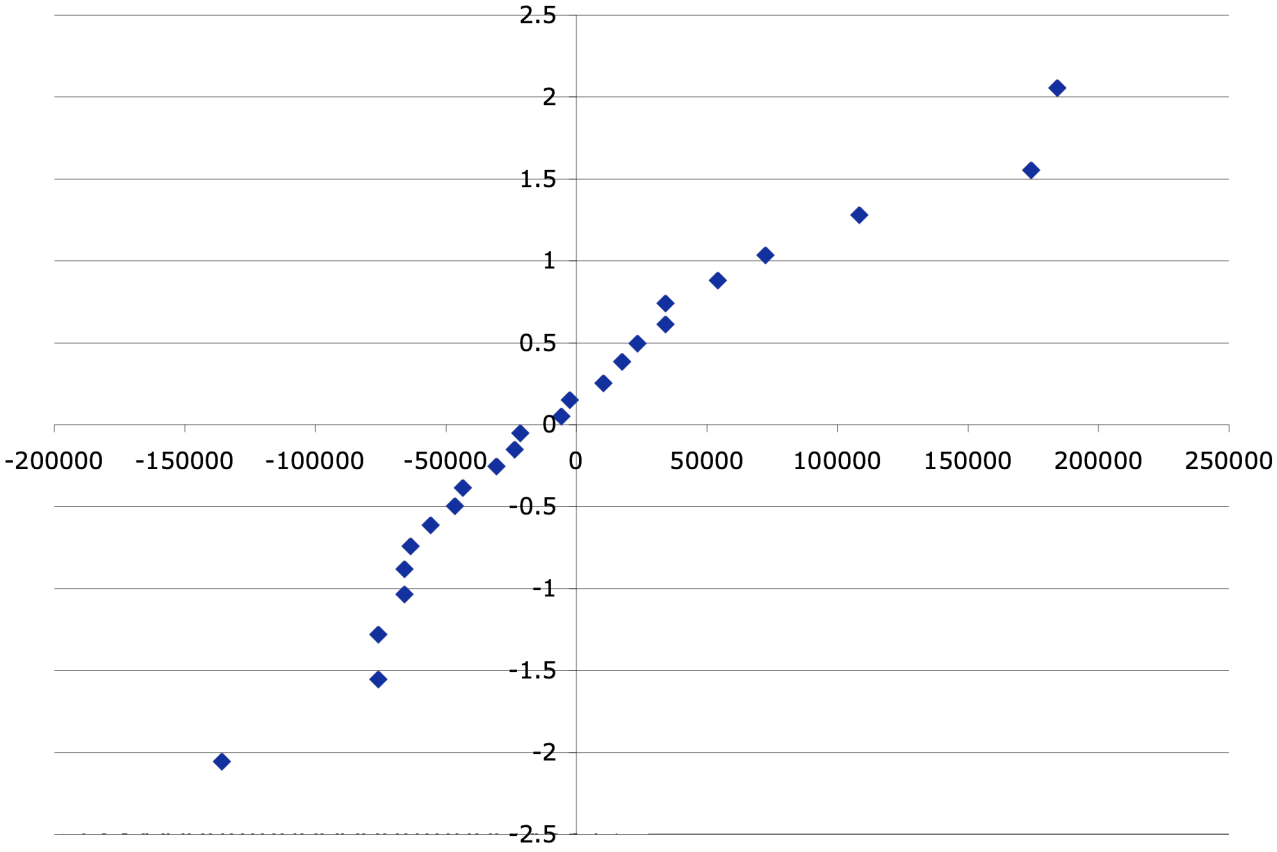


APPENDIX 8: ANOVA FOR LIGHT BOTTLES DURING ROUGHING FILTER AND SUNLIGHT EXPERIMENTS

The table below shows the analysis of variance (ANOVA) for time zero. From the ANOVA table it is clear that the null hypothesis must be rejected for the statement "all treatments are the same"; the F_{obs} value is 12.58, which is less than the F_{table} value, 4.31.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	7.97×10^{10}	7.97×10^{10}	12.58	4.31
Within Cleaning Agents	22	1.39×10^{11}	6.33×10^{09}		
Total	23	2.19×10^{11}			

The figure below shows the normalized residual plot. The points lie on a straight line indicating that the residuals are normally distributed.



It should be noted that for the following sampling times, transformations were used. These transformations were used since the *E. coli* concentrations from some of the bottles were much greater than those in the other bottles. The transformation allows for an appropriate comparison of the data.

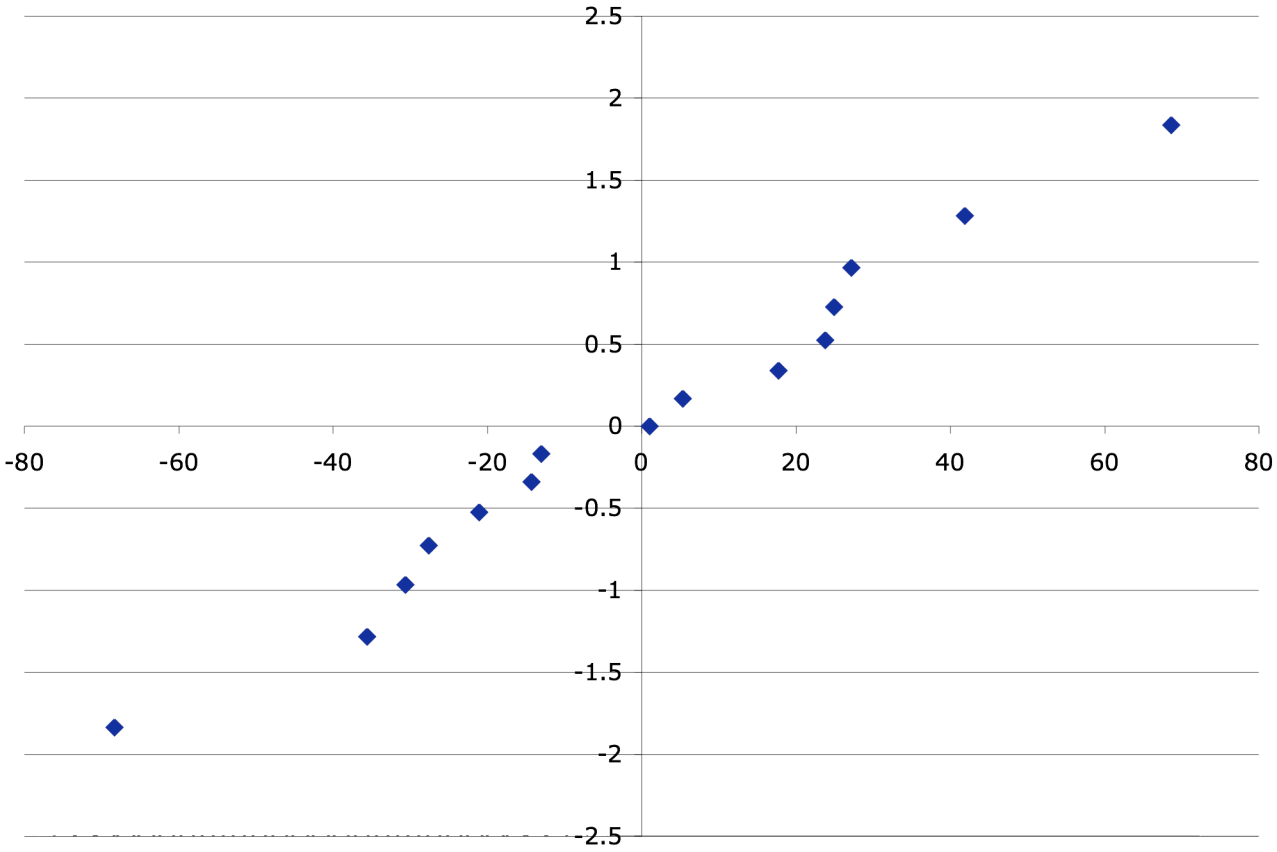
The next table shows the ANOVA for the 0.5-hour sample time. The transformation used for this sampling point was y^{λ}, λ equalled -0.5. The null hypothesis cannot be rejected since the F_{obs} value is 2.67, which is less than the F_{table} value of 4.54.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	7.65×10^{-06}	7.65×10^{-06}	2.67	4.54
Within Cleaning Agents	14	4.02×10^{-05}	2.87×10^{-06}		
Total	15	4.78×10^{-05}			

The following table shows the ANOVA for the samples taken after one hour. The transformation used for this sampling point was y^{λ}, λ equalled 0.5. The null hypothesis must be rejected since the F_{obs} value is 7.28, which is greater than the F_{table} value of 4.62.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	9.62×10^{03}	9.62×10^{03}	7.28	4.62
Within Cleaning Agents	13	1.72×10^{04}	1.32×10^{03}		
Total	14	2.68×10^{04}			

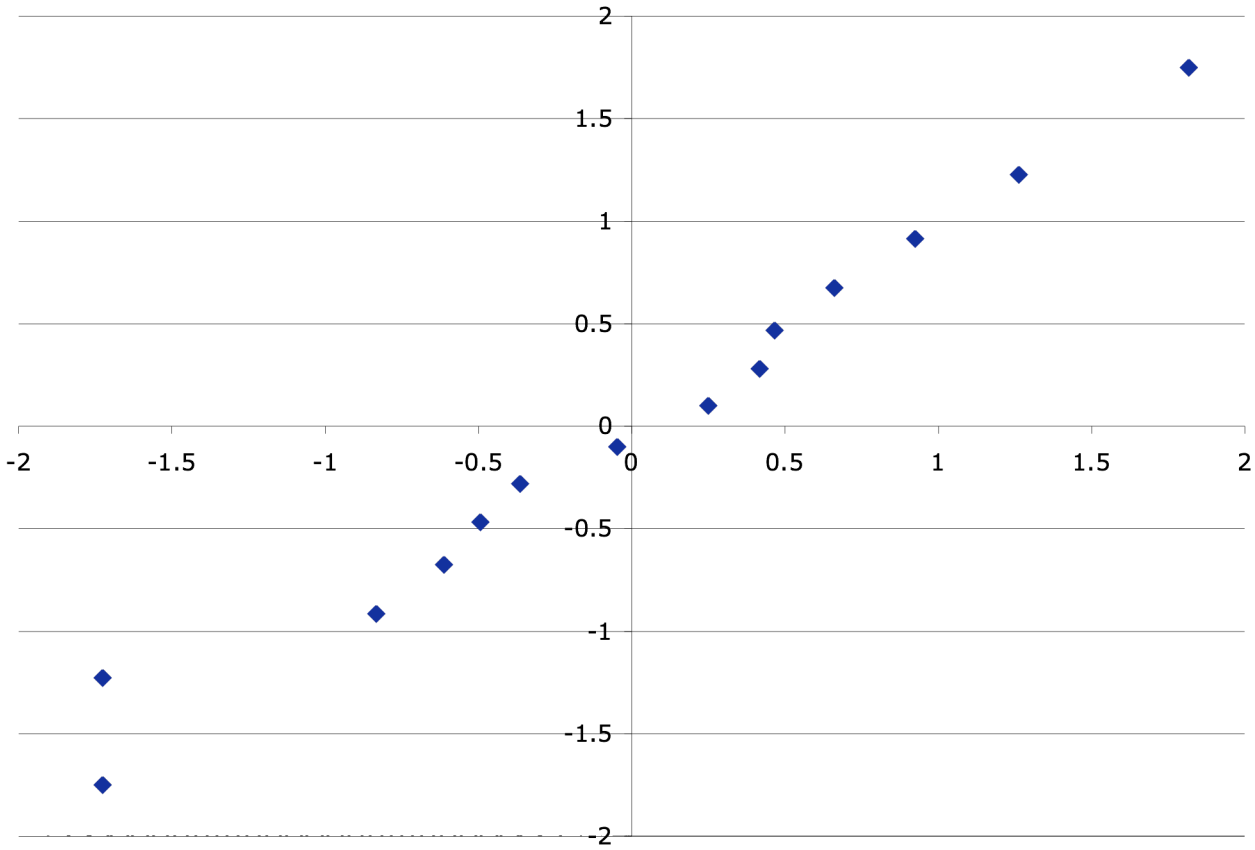
The figure below shows the normalized residuals for the one-hour sample time. The residuals lie on a straight line indicating they are normally distributed.



The ANOVA below is for the sample taken after 1.5 hours. The transformation used for this sampling point was y^{λ}, λ equalled 0.25. The null hypothesis must be rejected since the F_{obs} value is 16.70, which is greater than the F_{table} value of 4.70.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	19.54	19.54	16.70	4.70
Within Cleaning Agents	12	14.05	1.17		
Total	13	33.59			

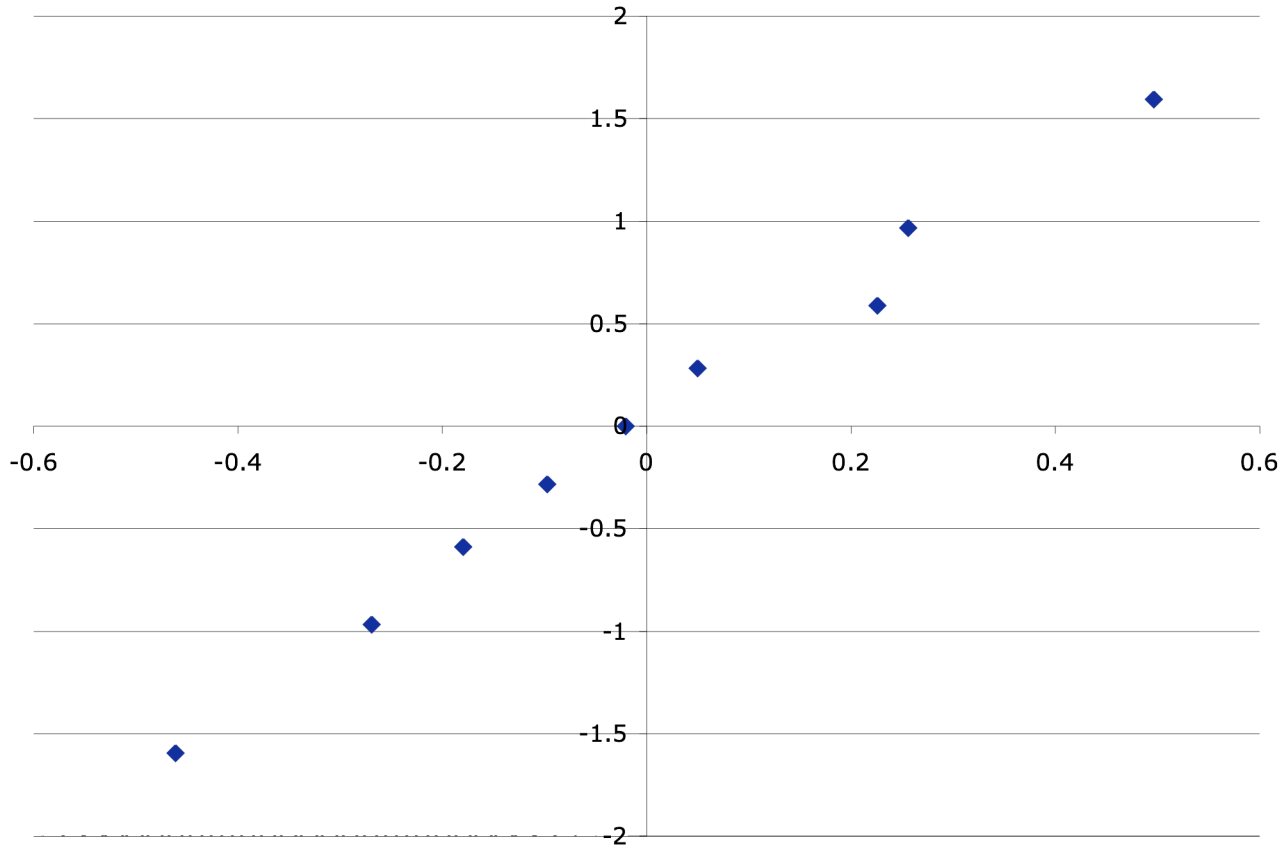
The next figure shows the normalized residuals. Since the points lie on a straight line, the residuals are normally distributed.



The next table is the ANOVA for the two-hour sample time. The transformation used for this sampling point was y^{λ}, λ equalled 0.25. The null hypothesis must be rejected since the F_{obs} value is 87.79, which is greater than the F_{table} value of 5.32.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	8.68	8.68	87.79	5.32
Within Cleaning Agents	7	0.69	0.10		
Total	8	9.37			

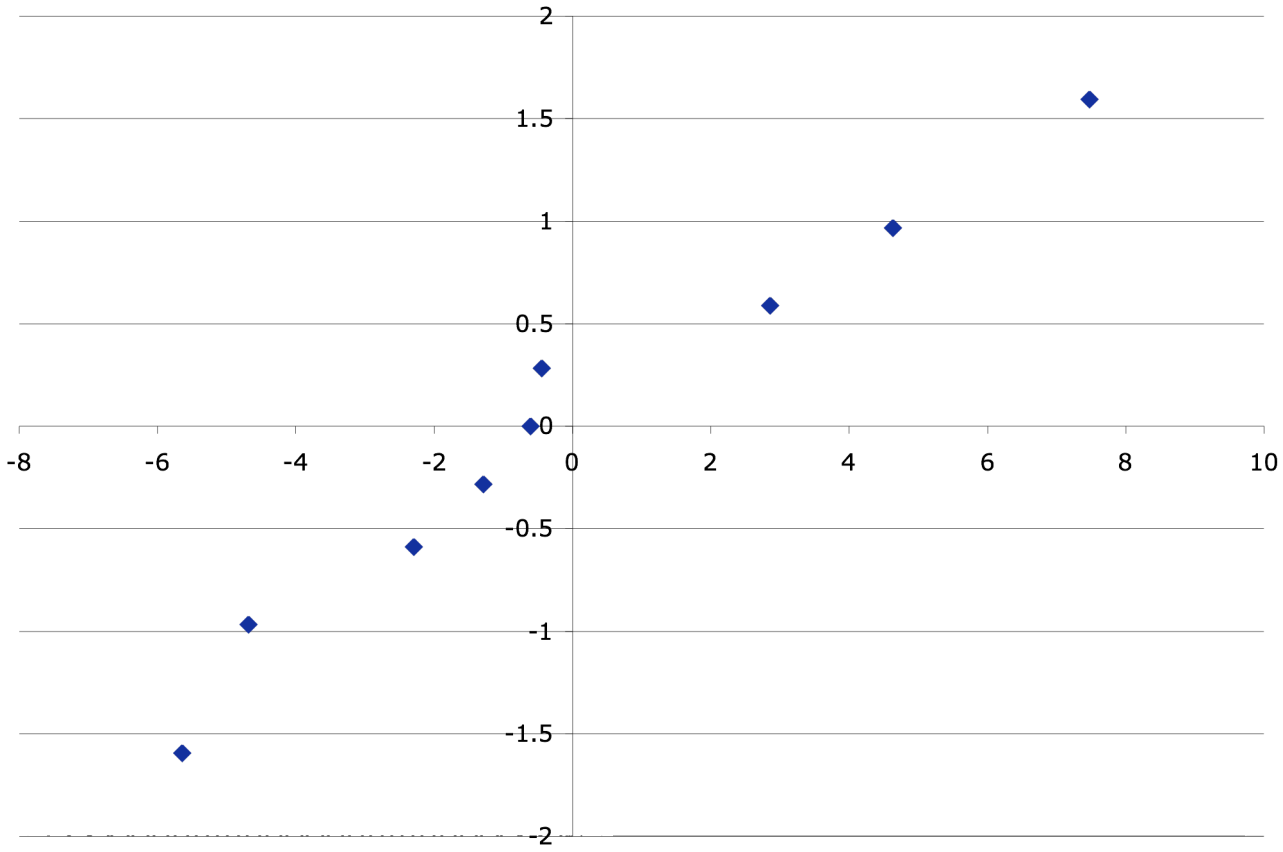
The following figure depicts the normalized residuals for the two-hour sample time. As can be seen, the points lie on a straight line; this feature suggests that the residuals are normally distributed.



The ANOVA for the 2.5-hour sample is shown next. The transformation used for this sampling point was y^{λ}, λ equalled 0.5. The null hypothesis must be rejected since the F_{obs} value is 20.89, which is greater than the F_{table} value of 5.32.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	438.15	438.15	20.89	5.32
Within Cleaning Agents	7	146.79	20.97		
Total	8	584.95			

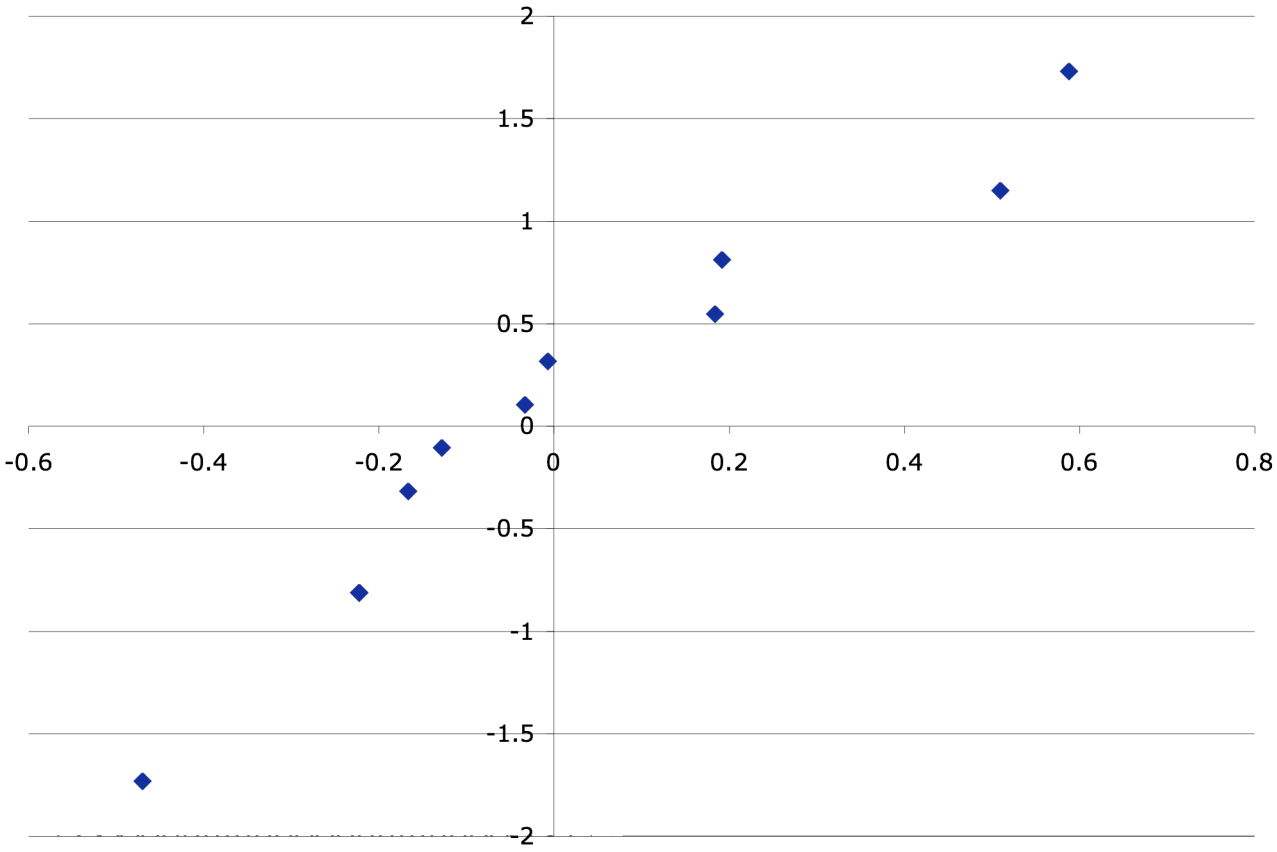
The figure below shows the normalized residuals. The points lie on a relatively straight line; this indicates that the residuals are normally distributed.



The ANOVA below is for the sample taken after three hours. The transformation used for this sampling point was y^{λ}, λ equalled 0.25. The null hypothesis must be rejected since the F_{obs} value is 281.70, which is greater than the F_{table} value of 4.88.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	30.71	30.71	281.70	4.88
Within Cleaning Agents	10	1.09	0.11		
Total	11	31.80			

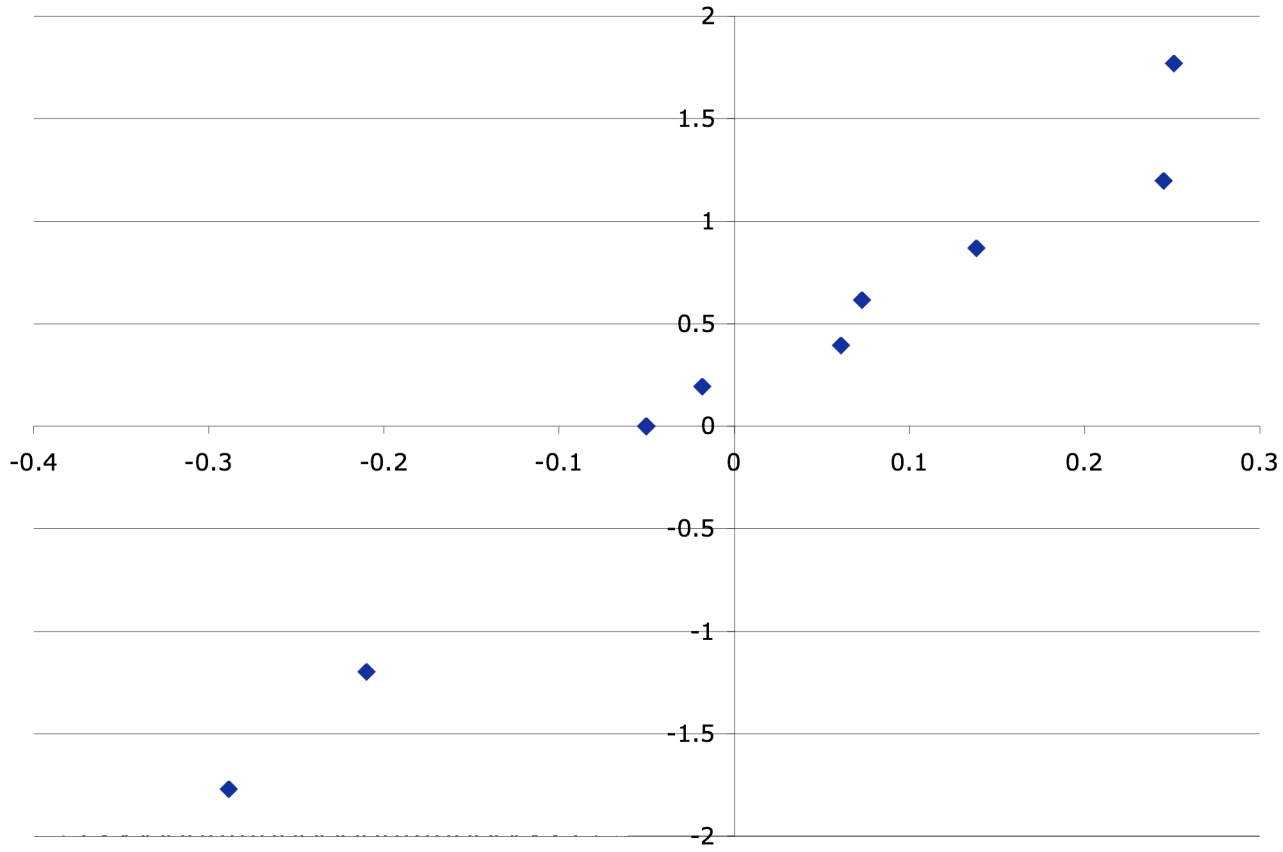
The next figure shows the normalized residuals. Since the points lie on a straight line, the residuals are normally distributed.



The next table is the ANOVA for the four-hour sample time. The transformation used for this sampling point was the log-transformation. The null hypothesis must be rejected since the F_{obs} value is 729.61, which is greater than the F_{table} value of 4.80.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	19.32	19.32	729.61	4.80
Within Cleaning Agents	11	0.29	0.03		
Total	12	19.61			

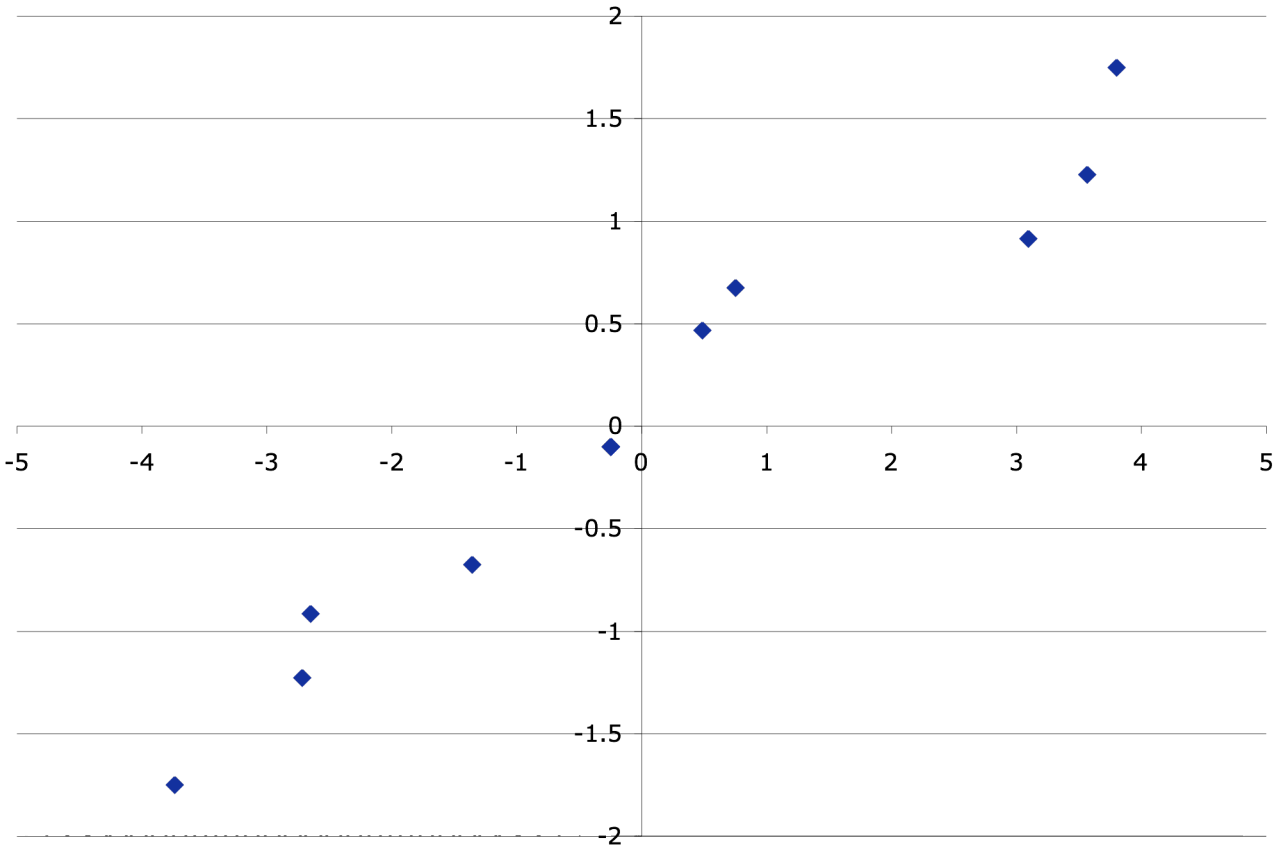
The following figure depicts the normalized residuals for the four-hour sample time. As can be seen, the points lie on a straight line; this feature suggests that the residuals are normally distributed.



The next table shows the ANOVA for samples taken after five hours. The transformation used for this sampling point was y^{λ}, λ equalled 0.5. The null hypothesis must be rejected since the F_{obs} value is 170.75, which is greater than the F_{table} value of 4.70.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	968.34	968.34	170.75	4.70
Within Cleaning Agents	12	68.05	5.67		
Total	13	1036.39			

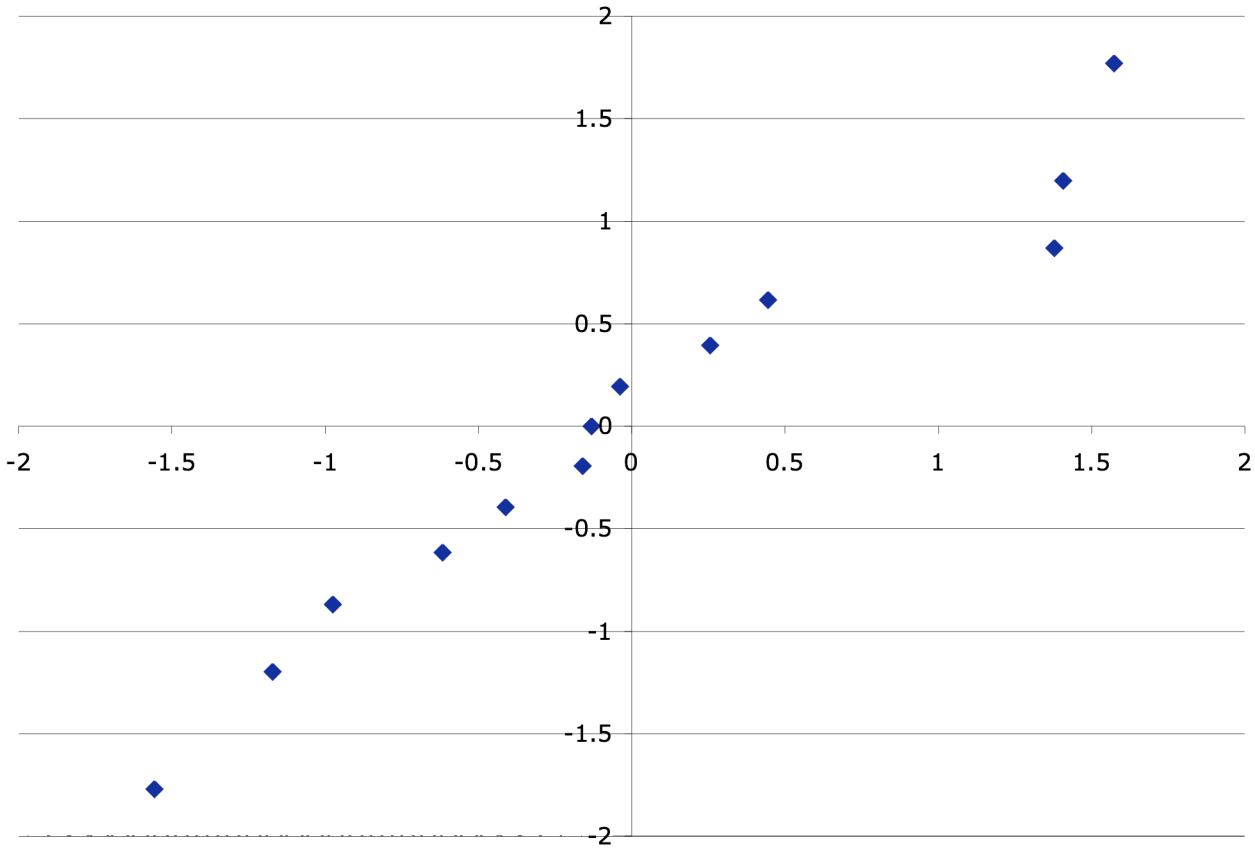
The figure below is a normalized residual plot. As shown, the points lie on a straight line. This indicates that the residuals are normally distributed.



Finally, the next table shows the ANOVA for the 24-hour sample time. The transformation used for this sampling point was y^{λ}, λ equalled 0.15. The null hypothesis must be rejected since the F_{obs} value is 65.18, which is greater than the F_{table} value of 4.80.

Source	df	Sum of Squares	Mean Square	F_{obs}	F_{table}
Cleaning Agents	1	70.85	70.85	65.18	4.80
Within Cleaning Agents	11	11.96	1.09		
Total	12	82.81			

The figure below shows the normalized residuals. The points lie on a straight line, showing that the residuals are normally distributed.


2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2