ULTRASOUND AS A SOLE OR SYNERGISTIC DISINFECTANT IN DRINKING WATER

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ABSTRACT

Chlorine as a disinfectant reacts with natural organic matter to produce undesired and possibly carcinogenic halogenated disinfection by-products (DBPs), which are regulated by the U.S. Environmental Protection Agency under the Disinfectant/Disinfection By-products Rule (DBPR). In order to comply with the increasingly stringent regulations, alternative disinfectants such as ozone, UV irradiation, and chloramines have been investigated. Unfortunately, these alternatives have their own limitations and disadvantages as well. Sonication is another alternative that has not yet received adequate research. The hydroxyl radicals, tensile stresses, and fluid shear generated during sonication may inactivate microorganisms. The goals of this research were to evaluate the effectiveness of sonication alone and combined sonication and chlorination for inactivation of *E. coli*.

Four stages of disinfection experiments were conducted: chlorine alone, sonication alone, combined sonication and chlorination, and heating alone. Experiments were conducted in laboratory prepared phosphate buffered saline. The variables tested included the chlorine dose, chlorine contact time, sonication time, sonication system (probe or bath), sonication power-to-volume ratio, and sonication frequency. *E. coli* was enumerated by use of pour plates and/or membrane filtration before and after disinfection.

Substantial temperature and turbidity increases were recorded after sonication, especially at 900 W/L. After 10 minutes of sonication at 900 W/L, the temperature and turbidity of

the experimental solution rose up to 77°C and 23 NTU, respectively. At both 180 W/L and 900 W/L, sonication alone demonstrated little inactivation (less than 1 $\log_{10}$) of *E. coli* for temperatures below 60°C and greater than 7 $\log_{10}$ inactivation at temperatures over 60°C. The results from heating only experiments confirmed that temperature was responsible for the inactivation rather than other ultrasonic wave effects.

Sequential application of sonication and chlorination was ineffective at inactivating *E. coli*. Chlorination alone achieved higher levels of *E. coli* inactivation than the combination of both disinfectants. When sonication and chlorination were applied simultaneously, the inactivation was greater than the additive effect of two disinfectants, indicating that there were synergistic effects between sonication and chlorination. For example, at 900 W/L, chlorination alone at 0.6 mg/L for 2 minutes provided 1.2 $\log_{10}$ inactivation and sonication for 2 minutes alone provided less than 1 $\log_{10}$ inactivation of *E. coli*. When the two disinfectants were applied simultaneously, 4.5 $\log_{10}$ was achieved. Sonication may have weakened the cell membranes, causing them to be more susceptible to chlorine disinfection.

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INTRODUCTION

1.0 STATEMENT OF THE PROBLEM

Both surface waters and groundwaters are used as municipal drinking water supplies in the United States. However, these waters can be contaminated by waste inputs from point sources such as wastewater treatment plant discharges and non-point sources such as agricultural discharges. As a result, pathogenic microorganisms that can cause adverse health effects on human beings may be found in drinking waters. Therefore, disinfection of drinking waters is important in order to ensure public health.

Chlorine has been widely used as a disinfectant in drinking water over the past century because it is a strong disinfectant and is also cost effective. It is effective at inactivating most types of microorganisms found in raw water sources when the appropriate chlorine dosage, contact time, and pH are used. Chlorine also leaves a disinfectant residual which helps to minimize microbial regrowth in the water distribution system. However, the disinfection effectiveness of chlorine decreases when microorganisms are protected from chlorine contact by attachment to other organisms or particulate matter. Also, some organisms are more resistant to chlorination than other organisms.

Another disadvantage of chlorination is the formation of undesired halogenated disinfection by-products (DBPs) when free chlorine reacts with natural organic matter. Trihalomethanes (THMs) and haloacetic acids (HAAs) are common disinfection by-products from chlorine disinfection, and these DBPs are believed to be carcinogenic to

human beings. These by-products are regulated by the U.S. Environmental Protection Agency (U.S. EPA) under the Disinfectants and Disinfection By-products Rule (DBPR).

The Stage 1 DBPR was established on February 16, 1998 and became effective as of February 16, 1999. The maximum contaminant level (MCL) for total trihalomethanes was set at 0.080 mg/L and the MCL for haloacetic acids (five) at 0.060 mg/L. The future Stage 2 DBPR will be more stringent than the existing regulations. For many treatment plants, disinfection methods other than chlorine will be needed in order to meet the Stage 2 rule.

Some alternative disinfectants of interests include ozone disinfection, UV irradiation, chloramination, or the application of multiple disinfectants. Ozone is a strong oxidant; however, it also produces disinfection by-products. For those microorganisms that are resistant to other disinfectants, UV irradiation may be effective. Chloramine is a weaker disinfectant than free chlorine, therefore it is ineffective if used as a primary disinfectant. Sonication, applied alone or as a synergistic disinfectant, is an alternative that has not received sufficient analysis.

Sonication is the application of ultrasonic waves (high frequency sound waves). Ultrasound has been widely used in cleaning jewelry and in medical fields, but it has not yet been applied as a disinfectant in drinking water treatment plants. Ultrasonic waves may inactivate microorganisms directly by fluid shear, tensile stresses, and the formation of hydroxyl radicals. When used with other disinfectants, sonication may enhance

inactivation by breaking up floc material, disrupting cell membranes, and increasing the diffusion rate of gases into cells. This research investigated the inactivation of *E. coli* by sonication alone and by combined sonication and chlorination.

1.1 RESEARCH OBJECTIVES

There were two main goals of this research. The first goal was to investigate the ability of sonication to inactivate *Escherichia coli*. The second goal was to determine the combined effect of two disinfectants, sonication and chlorination, on *E. coli*. Synergy between the two disinfectants might allow a treatment plant to use less chlorine to achieve the same amount of inactivation, which would reduce the formation of undesired disinfection by-products. In order to achieve the goals, the following objectives were completed:

1) determine the inactivation of *E. coli* by chlorination alone,
2) determine the inactivation of *E. coli* by sonication alone,
3) determine the combined effects of sonication and chlorination by applying the disinfectants sequentially or simultaneously, and
4) determine the effect of heating on the inactivation of *E. coli*.

1.2 SCOPE OF RESEARCH

The sonication experiments were conducted in the laboratory using a sonication probe and a sonic bath. All experiments were well controlled in the laboratory and were

conducted at a starting temperature of approximately 22°C. Phosphate buffered saline was used as the water matrix throughout the entire experimental plan. Temperature, pH, turbidity, and output power (sonication probe only) were recorded before and after disinfection. *E. coli* concentrations were determined before and after disinfection using pour plate and/or membrane filtration techniques to determine the inactivation achieved by sonication.

To study the effects of sonication alone, *E. coli* suspensions were subjected to disinfection with either an ultrasonic probe or bath system. Power-to-volume ratios of 180 W/L or 900 W/L were used, as were sonication times from 10 seconds to 60 minutes. The effect of heating versus heating plus ultrasonic waves was also studied as substantial temperature increases was recorded during sonication, especially at 900 W/L.

The inactivation of *E. coli* by chlorine was also studied. This allowed comparison of inactivation with a single disinfectant to inactivation in the combined disinfection experiments. Various chlorine dosages were tested, ranging from 0.2 mg/L to 1.0 mg/L. The chlorine contact time varied from 10 seconds to 5 minutes. Chlorination at elevated temperatures (32°C and 39°C) was also investigated. In addition to the parameters measured in the sonication only experiments, free and total chlorine concentrations were also measured.

Combined application of sonication and chlorination experiments were performed to study the synergistic effects of sonication. The disinfectants were applied either

sequentially or simultaneously. The variables tested included chlorine dose, sonication time (sequential), disinfection time (simultaneous), and power-to-volume ratio (180 W/L or 900 W/L). Pre and post-disinfection measurements included temperature, pH, turbidity, and *E. coli* concentration. Free and total chlorine concentrations were also measured.

1.3 OVERVIEW OF THESIS

The following chapter is the literature review, which includes a discussion of current drinking water regulations, various drinking water disinfectants, sonication, and indicator organisms. The methods chapter provides details on how the research experiments were carried out. Then, the results of the experiments are presented and analyzed according to the disinfection scheme (chlorination only, sonication only, combined sonication and chlorination, and temperature effects). This report ends with conclusions and recommendations. Lastly Appendix A contains spreadsheets of all experimental results.

CHAPTER 2
LITERATURE REVIEW

2.0 INTRODUCTION

Chlorine is the most common chemical used in the disinfection process. It is effective in inactivating most types of microorganisms found in raw water, given suitable pH conditions, dosages, and contact times (Sobsey, 1989). Unfortunately, chlorine reacts with natural organic matter to form undesirable disinfection by-products (DBPs), most of which are considered carcinogenic. These DBPs are federally regulated by the U.S. Environmental Protection Agency under the Stage 1 Disinfectants/Disinfection By-products Rule. Disinfection alternatives, such as ozone, chloramines, ultraviolet irradiation, or a combination of disinfectants, have therefore become an area of interest.

This chapter begins with an introduction of various federal regulations on drinking water, followed by a discussion of the most commonly used disinfectant, chlorine, as well as other disinfection alternatives. In addition, the theory of ultrasonic disinfection and prior sonication research is presented in detail. Finally, information about pathogen indicators is provided.

2.1 DRINKING WATER REGULATIONS

2.1.1 Surface Water Treatment Rule

The Surface Water Treatment Rule (SWTR) was established in 1989. It became effective on December 31, 1990 (U.S. EPA, 1989). This rule applies to all public water systems

(PWSs) that use surface water or groundwater under the direct influence of surface water as water sources. The U.S. EPA set the maximum contaminant level goal (MCLG) to be zero for *Giardia lamblia*, viruses, and *Legionella* (U.S. EPA, 1989). Under the SWTR, all PWSs that use surface water sources are required to provide filtration and disinfection. A treatment plant may avoid filtration by complying with several criteria set up by the U.S. EPA (U.S. EPA, 1989). These criteria include a high quality source water, protection of the watershed, and the ability to meet all disinfection requirements. For PWSs that must filter, filtration technologies such as conventional/direct filtration, slow sand filtration, diatomaceous earth (DE) filtration, or other effective filtration methods are available. The Surface Water Treatment Rule also requires 99.9% (3 log) inactivation or removal of *Giardia lamblia* cysts and 99.99% (4 log) inactivation or removal of viruses. In addition, disinfection residuals have to be maintained and monitored above a certain concentration in the water distribution system. All PWSs that use surface water sources, whether providing filtration or not, must monitor the turbidity every four hours. For systems that practice conventional or direct filtration, turbidity measurements must be less than 0.5 Nephelometric Turbidity Units (NTU) in at least 95% of samples taken monthly and must not exceed 5 NTU as a maximum (U.S. EPA, 1989).

2.1.2 Interim Enhanced Surface Water Treatment Rule

The U.S. Environmental Protection Agency promulgated the Interim Enhanced Surface Water Treatment Rule (IESWTR) on December 16, 1998 (U.S. EPA, 1998a). In conjunction with the IESWTR, the Stage 1 Disinfectants and Disinfection Byproducts Rule (Stage 1 DBPR) was also promulgated on this date (U.S. EPA, 1998b). The

IESWTR strengthens the requirements of the Surface Water Treatment Rule that was established in 1989. The IESWTR applies to public water systems (PWSs) using surface water or groundwater under the direct influence of surface water that serve 10,000 people or more (U.S. EPA, 1998a). The goal of IESWTR was to improve public health by eliminating microbial contaminants, especially *Cryptosporidium*, by setting its Maximum Contaminant Level Goal (MCLG) to be zero and requiring 99% (2 log) physical removal of *Cryptosporidium* for any PWS that provides filtration. Systems that do not filter must implement a watershed control program. Apart from regulating *Cryptosporidium*, turbidity regulations were also enhanced. The maximum effluent turbidity from conventional and direct filtration was set at 0.3 Nephelometric Turbidity Units (NTU) in a minimum of 95% of samples taken each month and the turbidity must not exceed 1 NTU (U.S. EPA, 2001a). In addition, this rule requires all states in which public water systems use surface water or groundwater under the direct influence of surface water to conduct sanitary surveys, even for systems serving less than 10,000 people. The deadline for all systems to comply with the all IESWTR provisions was January 1, 2002.

2.1.3 Long Term 1 Enhanced Surface Water Treatment Rule

The U.S. EPA promulgated the Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) on January 14, 2002 (U.S. EPA, 2002b). This rule was built upon the SWTR and IESWTR to provide additional protection from *Cryptosporidium*. The major provisions are the same as the ones listed in the IESWTR, however the LT1ESWTR affects public water systems (PWSs) that use surface water or groundwater under the direct influence of surface water and serve fewer than 10,000 people. Additional

guidelines and standards on turbidity for systems using different types of filters, such as slow sand, diatomaceous earth, and other alternative filters, are also provided. All PWSs that are affected by the Long Term 1 Enhanced Surface Water Treatment Rule must fully comply with the applicable provisions by January 14, 2005. Moreover, according to the SWTR, the U.S. EPA has to promulgate Stage 2 Disinfectant and Disinfection Byproducts Rule within 18 months after the establishment of LT1ESWTR (U.S. EPA, 2001b).

2.1.4 Long Term 2 Enhanced Surface Water Treatment Rule

The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) will be based on the water treatment requirements set by the IESWTR and the LT1ESWTR (U.S. EPA, 2001b). The LT2ESWTR is expected to provide more control over microbial contaminants, particularly *Cryptosporidium*, as well as control the production of disinfection by-products formed by disinfection processes that use chemicals. Specific requirements may include source water monitoring for *Cryptosporidium* and additional treatment (inactivation or removal) based on source water concentrations. With the advancement in ultraviolet light disinfection, treatment plants will be allowed to utilize UV as a disinfectant. Research has shown that *Cryptosporidium* is sensitive to low doses of UV. The LT2ESWTR will apply to all community and non-community water systems that utilize surface water or groundwater under the direct influence of surface water. This rule was scheduled to be finalized in May 2002; however, it is now anticipated that the proposed rule will be published in mid-2003 and the final rule in mid-2004 (Roberson, 2002).

2.1.5 Stage 1 Disinfectants and Disinfection By-Products Rule

In order to protect the public from disinfection by-products, the U.S. EPA established the Stage 1 Disinfectants and Disinfection By-Products Rule (Stage 1 DBPR) on February 16, 1998 and it was effective as of February 16, 1999 (U.S. EPA, 1998b). This rule applies to community water systems (CWSs) and non-transient non-community water systems (NTNCWs) where a chemical disinfectant is used in primary or secondary (residual) disinfection. By January 1, 2002, all PWSs that use surface water or groundwater under the direct influence of surface water and serve 10,000 or more people were required to comply with the requirements set by the Stage 1 DBPR (U.S. EPA, 2001c). Systems serving fewer than 10,000 people and other groundwater systems that are bound to this rule must comply with the Stage 1 DBPR requirements by January 1, 2004. Table 1 shows the maximum residual disinfectant level goals (MRDLGs) and the maximum residual disinfectant levels (MRDLs) for chlorine, chloramine, and chlorine dioxide set by the U.S. EPA.

Table 1. Stage 1 DBPR maximum disinfectant residual concentrations (U.S. EPA, 1998b)

Disinfectant residual	**MRDLS (mg/L)**	**MRDL (mg/L)**
Chlorine	4 (as Cl_2)	4.0 (as Cl_2)
Chloramine	4 (as Cl_2)	4.0 (as Cl_2)
Chlorine Dioxide	0.8 (as ClO_2)	0.8 (as ClO_2)

Under the Stage 1 DBPR, the U.S. EPA also finalized a set of maximum contaminant level goals (MCLGs) and maximum contaminant levels (MCLs) to limit the production of disinfection by-products, such as total trihalomethanes (TTHMs), five haloacetic acid

compounds (HAA5), chlorite, and bromate (U.S. EPA, 1998b). TTHMs include chloroform, bromodichloromethane, dibromochloromethane, and bromoform. An MCL was set for HAA5 (monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid), while MCLGs were provided for dichloroacetic acid and trichloroacetic acid. The MCLGs and MCLs are listed in Table 2.

Table 2. Stage 1 DBPR MCLGs and MCLs (U.S. EPA, 1998b)

Disinfection by-products	**MCLG (mg/L)**	**MCL (mg/L)**
Total trihalomethanes (TTHM)	Not applicable	0.080
Chloroform	0	
Bromodichloromethane	0	
Dibromochloromethane	0.06	
Bromoform	0	
Haloacetic acids (five) (HAA5)	Not applicable	0.060
Dichloroacetic acid	0	
Trichloroacetic acid	0.3	
Chlorite	0.8	1.0
Bromate	0	0.010

In addition to disinfection residuals and disinfection by-products, organic substances (as total organic carbon) are also regulated under the Stage 1 DBPR because they react with chemical disinfectants to form disinfection by-products. This rule requires public water systems that use surface water or groundwater under the direct influence of surface water and practice conventional filtration methods to remove a certain percentage of total organic carbon (TOC, as mg/L), depending on the source water concentration of TOC and alkalinity (as $CaCO_3$). The removal of organic materials can be achieved by practicing enhanced coagulation or enhanced softening.

By promulgating the Stage 1 DBPR, the U.S. EPA believed that about 140 million people would be protected from disinfection by-products (U.S. EPA, 1998c). Also, the TTHM levels would be reduced by 24 percent on average nationwide.

2.1.6 Stage 2 Disinfectants and Disinfection By-Products Rule

Compared to the Stage 1 DBPR, the Stage 2 DBPR is expected to be more stringent on the levels of disinfection by-products (U.S. EPA, 2001d). The main purpose of promulgating the Stage 2 DBPR is to reduce peak disinfection by-product concentrations. This will be done by changing the monitoring requirements set forth by the Stage 1 DBPR. The Stage 1 DBPR allows the public water system to average the concentrations of DBPs from all monitoring locations in order to meet the MCLs, but the Stage 2 DBPR will require the system to meet the compliance limits at every individual sampling location. The Stage 2 DBPR is expected to be proposed in mid-2002, with the final rule published in mid-2004 (Roberson, 2002).

2.2 INACTIVATION METHODS

2.2.1 CT Concept

The "CT" factor is one of the most widely used methods to determine the germicidal efficiency of a particular disinfectant. The definition of CT is the product of disinfectant residual concentration C (in mg/L) and the contact time T (in minutes) (U.S. EPA, 1999b). The desired degree of microbial inactivation can be achieved by adjusting the disinfectant residual concentrations and contact times accordingly. The U.S. EPA has

developed tables of CT factors for different disinfectants (such as free chlorine, ozone, and chloramine), pathogens (such as viruses and *Giardia* cysts), temperatures and pH values. The CT factor is based on Chick's Law, which was developed by H. Chick in 1908. Chick's Law is represented as:

$$r = -kN$$

where r is the inactivation rate, k is the inactivation rate constant and N is the concentration of viable organisms (AWWA, 1999). In 1908, H. Watson also found the relationship between the inactivation rate constant k, and the concentration of disinfectant, C,

$$k = k'C^n$$

where k' is presumed independant of the disinfectant and microorganism concentrations and n is the coefficient of dilution.

2.2.2 DBP Formation

Disinfection by-products are generated as a result of primary or secondary disinfection (U.S. EPA, 1999c). Different types of DBPs are formed when different disinfectants react with natural organic matter (NOM) that is present in water (U.S. EPA, 1999b). The species and concentration of DBPs formed are associated with the type and concentration of disinfectant used, the duration of disinfection, temperature, pH, and where in water treatment process the disinfectant is applied (Bellar *et al.*, 1974; Rook, 1974; McGuire *et al.*, 1990). Natural organic matter reacts with free chlorine and free bromine to form predominantly halogenated by-products. The free bromine comes from the oxidation reaction of chlorine with bromide ions present in the source water. Trihalomethanes

(THM) and haloacetic acids (HAA) are the common disinfection by-products due to chlorine disinfection. THMs include chloroform, bromodichloromethane, dibromochloromethane, and bromoform. The HAA species of particular health concern include dichloroacetic acid and trichloroacetic acid. Selected data on cancer classifications associated with disinfection by-products are presented in Table 3.

Table 3. Status of health information for disinfectants and DBPs (U.S. EPA, 1999c)

Contaminant	**Cancer Classification***
Chloroform	B2
Bromodichloromethane	B2
Dibromochloromethane	C
Bromoform	B2
Dichloroacetic acid	B2
Trichloroacetic acid	C

*Note: Group B2 - Probable Human Carcinogen with sufficient evidence from animal studies.
Group C - Possible Human Carcinogen with limited evidence from animal Studies and inadequate or no data on humans.

Disinfectants other than chlorine also produce DBPs when they react with NOM. The DBPs from ozonation are mainly aldehydes, ketones, and inorganic by-products, such as bromate, which is produced if bromide ions are found in the water (Bellar *et al.*, 1974; Rook, 1974; McGuire *et al.*, 1990). Chlorine dioxide also forms chlorate and chlorite as by-products when it reacts with NOM. Both bromate and chlorite are regulated under the Stage 1 DBPR (U.S. EPA, 1998b).

2.2.3 Chlorine

Chlorine was first used as a disinfectant in water treatment in the United States in 1908, at Bubbly Creek (Chicago) and the Jersey City Water Company (AWWA, 1999). Shortly after that, chlorine was applied in several large cities in North America, such as New York City, Montreal, Milwaukee, Cleveland, Nashville, Baltimore, and Cincinnati, and also some smaller water treatment facilities within two years time. With chlorine disinfection, the number of typhoid cases dropped substantially.

Chlorine is primarily used as a disinfectant, but it also serves as an oxidant (U.S. EPA, 1999c). As chlorine has been used for nearly 100 years, characteristics and application techniques are well understood. Recently, studies have focused on the combination of chlorine and other disinfectants. By combining disinfectants, better microbial inactivation may be achieved while also controlling disinfection by-product production.

Chlorine gas, sodium hypochlorite, or calcium hypochlorite are the three forms of chlorine commonly used in the disinfection process (U.S. EPA, 1999c). For chlorine gas, hypochlorous acid (HOCl), hydrogen ions, and chloride ions are formed when chlorine gas is hydrolyzed in water. The H^+ ion produced as a result of hydrolysis reduces the pH of water. The following reaction demonstrates this phenomenon.

$$Cl_{2(g)} + H_2O \rightarrow HOCl + H^+Cl^-$$

Since hypochlorus acid (HOCl) is a weak acid having a pK_a of approximately 7.5, it dissociates into hydrogen ions and hypochlorite ions, as shown in the reaction below.

$$HOCl \Leftrightarrow H^+ + OCl^-$$

Incomplete dissociation of HOCl to OCl^- occurs between pH 6.5 and 8.5, but no dissociation take place at a pH lower than 6.5 (U.S. EPA, 1999c). At 20°C and pH below 7.5, the HOCl species dominates (AWWA, 1999). At pH 7.5 or above, the OCl^- species dominates. HOCl is a much stronger germicide than OCl^-, therefore a lower pH is preferred for disinfection.

Sodium hypochlorite (NaOCl) is produced by dissolving chlorine gas in a sodium hydroxide solution (U.S. EPA, 1999c). A typical sodium hypochlorite solution contains 12.5% chlorine, meaning that one pound of chlorine is found in one gallon of 12.5% sodium hypochlorite solution. Similar to the hydrolysis of chlorine gas, when sodium hypochlorite combines with water, hypochlorous acid, sodium ion, and hydroxyl ions are generated. The hydroxyl ions produced are responsible for raising the overall pH of water. This is illustrated in the following reaction.

$$NaOCl + H_2O \rightarrow HOCl + Na^+ + OH^-$$

Calcium hypochlorite is produced by dissolving chlorine gas in a solution of calcium oxide (lime) and sodium hydroxide (U.S. EPA, 1999c). Calcium hypochlorite is available in granular form, which usually contains 65% of chlorine. One pound of chlorine is contained in one and a half pounds of calcium hypochlorite. When calcium hypochlorite is added to water, hypochlorous acid, calcium ions, and hydroxyl ions are formed (U.S. EPA, 1999c). As with sodium hypochlorite situation, the hydroxyl ions raise the pH of water. Following is the reaction for calcium hypochlorite in water.

$$Ca(OCl)_2 + 2H_2O \rightarrow 2HOCl + Ca^{2+} + 2OH^-$$

The function of chlorine differs when it is applied in different locations during water treatment. Table 4 illustrates the possible points of chlorine addition and the uses of chlorine at each of these application points.

Table 4. Typical chlorine points of application and uses (U.S. EPA, 1999c).

Point of Application	**Typical Uses**
Raw Water Intake	Zebra mussel and Asiatic clam control, control biological growth
Flash Mixer (prior to sedimentation)	Disinfection, iron and manganese oxidation, taste and odor control, oxidation of hydrogen sulfide
Filter Influent	Disinfection, control biological growth in filter, iron and manganese oxidation, taste and odor control, algae control, color removal
Filter Clearwell	Disinfection
Distribution System	Maintain disinfectant residual

Table 5 shows the typical dosages of the three chlorine compounds used as disinfectants at water treatment plants. All of the three forms of chlorine compounds can serve as disinfectants or oxidizing agents; however, it is not economical for small water treatment facilities to use sodium and calcium hypochlorite because of the higher costs associated with the chemicals.

Table 5. Typical chlorine dosages at water treatment plants (U.S. EPA, 1999c).

Chlorine Compound	**Range of Doses**
Calcium hypochlorite	0.5 – 5 mg/L
Sodium hypochlorite	0.2 – 2 mg/L
Chlorine gas	1 – 16 mg/L

The actual concentration of the chlorine residual when treated water enters the distribution system varies from plant to plant. However, the U.S. EPA requires a minimum of 0.2 mg/L chlorine residual and the maximum residual disinfectant level (MRDLs) for chlorine is set to 4.0 mg/L (U.S. EPA, 1999b).

There are a number of advantages to using chlorine as a disinfectant. Chlorine is an effective disinfectant for many pathogens commonly found in drinking water (U.S. EPA, 1999c). Bacteria are extremely sensitive to chlorine, followed by viruses. Chlorine is not as effective at inactivating protozoa. According to a study performed in 1984, *Giardia* cysts are two orders higher in magnitude in resistance to chlorine than viruses, and more than three orders higher than bacteria (Hoff *et al.*, 1984). Chlorine is also a strong oxidizing agent that can be also used to correct taste and odor problems, prevent algal growth, maintain clear filter media, remove iron and manganese, destroy hydrogen sulfide, and suppress slime growth and hence preserve the quality of water in the distribution system. Chlorine also leaves a disinfectant residual such that microbial regrowth in the distribution system is minimized. The capital and operating costs of chlorine disinfection are relatively inexpensive compared to other disinfectants such as ozone and UV.

Chlorine also has several disadvantages. Chlorine reacts with organic and inorganic substances to produce disinfection by-products, some of which are believed to be carcinogenic (U.S. EPA, 1999c). Secondly, excessive doses of chlorine give rise to taste

and odor problems. Lastly, the handling of chlorine gas is dangerous, therefore a hazard exists when chlorine is used in the water treatment process.

2.2.4 Chloramines

Chloramines are formed by the combination of ammonia and aqueous chlorine (HOCl). There are three chloramine compounds: monochloramine, dichloramine, and nitrogen trichloride (U.S. EPA, 1999d). The reactions below show the formation of chloramines.

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

$$HOCl \Leftrightarrow OCl^- + H^+$$

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O \text{ (monochloramine)}$$

$$NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O \text{ (dichloramine)}$$

$$NHCl_2 + HOCl \rightarrow NCl_3 + H_2O \text{ (nitrogen trichloride)}$$

In the early 1900s, it was discovered that chloramines could act as disinfectants (U.S. EPA, 1999d). The first use of chloramines was for controlling tastes and odors. Later on, it was found that chloramines were more stable than chlorine and more effective in prohibiting bacterial regrowth in the distribution system.

Chloramines are usually used as a secondary disinfectant because they are a weaker disinfectant compared to chlorine. For example, at pH of 7 or below, monochloramine is 200, 200, 50, and 2.5 times less effective than chlorine in the inactivation of bacteria, viruses, spores, and cysts, respectively (U.S. EPA, 1999d). The CT values for

chloramines are much higher than that of chlorine or ozone, which means that much longer contact times or chloramine doses are needed.

Since dichloramine and nitrogen trichloride create tastes and odors, monochloramine is the preferred chloramine compound for drinking water disinfection (U.S. EPA, 1999d). The chlorine to ammonia ratio is typically set to 4:1, but ranges from 3:1 to 5:1. This ratio minimizes nitrification, the development of biofilm problems due to excess ammonia, and the production of tastes and odors. The formation of monochloramine from chlorine is pH dependent. The time required for 99% conversion of chlorine to monochloamine is shortest at pH 7 and pH 8.3, taking only 0.2 seconds and 0.069 seconds, respectively. At pH 2 the conversion takes 421 seconds and at pH 12 it takes 33.2 seconds. The dosage of monochloramine usually ranges from 1.0 – 4.0 mg/L, with a minimum of 0.5 mg/L of residual maintained in the water distribution system. It is suggested that a minimum dosage of 2.0 mg/L monochloramine should be used to prevent nitrification from occurring in the water distribution system (U.S. EPA, 1999d).

Comparing chloramines to free chlorine or chlorine dioxide, chloramines are less likely to react with organic compounds. Therefore, fewer disinfection by-products such as trihalomethanes are formed and fewer taste and odor problems arise (U.S. EPA, 1999a). Monochloramine residuals are more stable and better at controlling biofilms. However, biofilms will be formed and nitrification will occur when excess ammonia is present in the water distribution system. Chlormaines are cost effective and can be generated without difficulty.

One of the disadvantages of using chloramines as a disinfectant is that chloramines are not as powerful as other disinfectants, such as chlorine, ozone, and chlorine dioxide (U.S. EPA, 1999a). Chloramines are not a strong enough oxidant to oxidize iron, manganese, and sulfides. Monochloramine is preferred, and dichloramine causes treatment problems such as taste and odor issues. Despite the ease of chloramine generation, it must be produced on-site.

2.2.5 Ozone

The Netherlands was the first country in Europe to use ozone in drinking water treatment in 1893 (U.S. EPA, 1999a). Soon after that, ozone was widely applied for drinking water disinfection as well as oxidation in Europe. Ozone oxidation/disinfection technology reached the United States almost a century after the Netherlands first used it, but the number of water treatment facilities that utilize ozone has increased substantially. As of 1998, there were 264 water treatment plants in U.S. using ozone and 149 of them were small systems treating less than 1 mgd. During the early days, the United States used ozone mainly for oxidation purposes, such as removing color and controlling tastes and odors from drinking water. More recently ozone has been applied as the primary disinfectant, because of the Surface Water Treatment Rule and the Disinfection By-products Rule that are more stringent on microbial inactivation and DBP formation, respectively.

Ozone, with the chemical symbol O_3, is a colorless gas with a pungent smell at room temperature (U.S. EPA, 1999a). It exists in the air at low concentrations of 0.02 to 0.05

ppm, which does not cause health hazards. Ozone is slightly soluble in water, but 12 times less soluble than chlorine. Ozone is a strong oxidant but highly corrosive and toxic.

The powerful oxidizing power of ozone makes it effective for inactivating viruses, protozoa, and bacteria (U.S. EPA, 1999a). In drinking water treatment, ozone can be applied as primary disinfectant before filtration but after sedimentation. When used as an oxidant, ozone may be applied before coagulation, before sedimentation, or before filtration. One of the advantages of applying ozone in drinking water treatment is that it requires shorter contact time and doses, because ozone is a stronger and more powerful oxidant than other disinfectants such as chlorine and chloramine. Ozone is also effective in controlling tastes and odors and removing iron, manganese, and sulfides. The microbial inactivation mechanisms are not affected by pH.

The disadvantage of ozone application is that it cannot be used as a secondary disinfectant because it does not leave a residual. Therefore ozone is limited to being a primary disinfectant (U.S. EPA, 1999a). In addition, organic disinfection by-products are produced as a result of ozone oxidation with natural organic matter (NOM). Typically the by-products are aldehydes, organic acids, and aldo- and ketoacids. If bromide ion is involved, by-products such as hypobromous acid, hypobromite ion, bromate ion, brominated organics, and broamines will be generated. Installation and operating costs are high, because biologically activated filters have to be installed to remove

biodegradable by-products and assimilable organic carbon. Also, ozonation requires high energy inputs and has to be generated on-site as ozone is unstable.

2.2.6 Ultraviolet Irradiation

Ultraviolet (UV) irradiation is an electromagnetic wave having a wavelength of 100 – 400 nanometers (nm), which is between the X-ray and visible ray spectrums (U.S. EPA, 1999e). There are four types of UV, classified according to the wavelength: Vacuum UV (100 – 200 nm), UVC (200 – 280 nm), UVB (280 – 315 nm), and UVA (315 – 400 nm). When UV is used as a disinfectant, the UV range of 245 – 285 nm is applied, as it is the optimum range for inactivation of microorganisms. The device used for UV disinfection is called a UV lamp, which can be low-pressure (253.7 nm) or medium-pressure (180 – 1,370 nm). Medium-pressure lamps produce much greater UV intensity than low-pressure ones.

In order to quantify the microbial inactivation by UV, the dosage applied has to be calculated. The following formula illustrates the determination of UV dose:

$$D = I \times t$$

where D = UV dose ($mW \bullet s/cm^2$), I = intensity (mW/cm^2), and t = contact time (seconds) (U.S. EPA, 1999e).

UV radiation is not a chemical microbial inactivation method. Rather, when UV light penetrates through the cell wall of the microorganism, a photochemical reaction

irreversibly destroys or injures the nucleic acids and vital cell components, such as DNA and RNA.

UV has several advantages over other chemical disinfectants. First, it is very effective in inactivating bacteria and viruses. Low dosages of 5 – 25 mW•s/cm^2 are required for 2 log inactivation of bacteria and viruses, while 90 – 140 mW•s/cm^2 provides 4 log inactivation (U.S. EPA, 1999e). A recent study showed that an UV dose as low as 19 mJ/cm^2 caused significant oocyst inactivation (3.9 log) (Bukhari *et al.*, 1999). Greater than 4.5 log of oocyst inactivation was achieved when 66 mJ/cm^2 of UV dose was applied. Second, minimal concentrations of disinfection by-products are generated as the result of UV disinfection. Only low levels of formaldehydes are formed when UV is applied to most surface waters. It is believed that humic substances are responsible for the production of low levels of formaldehydes.

Although UV sounds superior, there are a few shortcomings when it is used in drinking water disinfection. First, UV radiation does not have a disinfectant residual, so it can only be used as a primary disinfectant and a different secondary disinfectant that provides a residual is required. Second, high concentrations of calcium, iron, turbidity, and phenols in source waters may impede UV disinfection as they absorb UV light, reducing the dose for microorganisms.

2.2.7 Sonication

Sonication is the application of ultrasonic sound waves, having a frequency of 20,000 Hz or above. The normal human ear cannot hear ultrasonic waves, because the frequency is above the upper limit of human hearing (Diaz, 1996). Ultrasound has been widely used in the medical field as well as the cleaning of jewelry, however, limited studies have been carried out to investigate the germicidal effects of ultrasound. Sonication as a disinfectant in water and wastewater treatment is discussed in detail in Section 2.3.

2.3 GERMICIDAL EFFECTS OF ULTRASOUND

As ultrasonic waves are introduced to a liquid, acoustic cavitation occurs (Hua and Thompson, 2000). Due to the sinusoidal pressure differences induced by ultrasonic waves, the bubbles in the liquid expand and contract, and finally collapse. Cavitation happens on a microscopic level, with the lifetime of a single bubble on the order of microseconds and its radius on the order of micrometers. During the process of cavitation, the bubbles expand in the "rarefaction" half cycle of the sound wave and collapse in the compression half cycle. Stable cavitation and transient cavitation are the two types of cavitation. Stable cavitation is less vigorous than transient cavitation.

The bubbles that undergo transient cavitation collapse in such a quick and violent manner that high temperatures and pressures are experienced near the collapse site. When the bubbles in the liquid medium collapse, the velocity of liquid surrounding the collapse region is approximately the speed of sound (Riesz *et al*., 1985). Microscopically, the temperature of the liquid when bubbles collapse increases to 2000 – 4000 K and the

pressure increases to greater than 330 atm (Hua and Thompson, 2000). Inside the bubbles, sonoluminescence, the emission of light from the bubbles due to cavitation, occurs as a result of the extreme conditions exhibited by those bubbles. Such extreme temperature and pressure conditions may cause the formation of hydrogen atoms and hydroxyl radicals and the generation of shear and tensile stresses (Riesz and Kondo, 1992).

Microorganisms may be inactivated by several mechanisms. First, cell membranes can be disrupted as a result of the stresses produced by microstreaming, which occurs when bubbles vibrate and the medium next to them flows (Scherba *et al.*, 1991). Second, the combined effects of fluid shear, tensile stresses, and hydroxyl radicals may lead to the inactivation of microorganisms. Third, sonication effects can be combined with other disinfectants. During the free radical attack, the cell membranes of the microorganisms are ruptured physically as a result of bubble implosion, and then disinfectants or chemical oxidants can diffuse into the cell and destroy the essential structures (Hua and Thompson, 2000). Lastly, sonication enhances the break up of floc materials or microorganisms that clump together, thus they are more susceptible to disinfection.

Apart from hydroxyl radical attack, the hydroxyl radicals can combine to form hydrogen peroxide (H_2O_2), which has oxidizing capabilities as well (Hua and Thompson, 2000). However, this inactivation mechanism may not be very effective, because the upper limit of H_2O_2 production is still low in concentration compared to what is needed for the

inactivation of microorganisms. Also, the enzymes such as peroxidase and catalase produced by aerobic microorganisms destroy H_2O_2, lowering its concentration in general.

2.3.1 Prior Work on Sonication Alone

Previous research has shown that sonication alone can inactivate microorganisms. The inactivation efficiency using sonication depends on several factors, which include sonication time, intensity, and frequency. Each of these factors is discussed in this section.

Several researchers found that the percent of microorganism inactivation increases as sonication time increases. Nakanishi *et al*. (2001) studied the inactivation of *Cryptosporidium parvum* oocysts in drinking water and their ability to infect mice when subjected to high intensity ultrasonic waves at 28, 45, and 100 kHz. Oocysts were suspended in purified water. They discovered that some oocyst walls were broken and nuclei leaked after sonication treatment, which was believed to be the result of cavitation. After 2, 10, and 20 minutes of sonication at 28 kHz, approximately 40%, 97%, and 99% of the oocysts lost their nuclei, respectively. Also, after 2 and 10 minutes of sonication, the infectivity of oocysts dropped to approximately 30% and 0.1%, respectively. Phull *et al.* (1997) found similar results for the inactivation of *E. coli* suspended in sterile saline solution and bacteria suspended in stream water. Their experiments started at approximately 20^{o}C. Several sonication intensities and frequencies were tested with both probe and bath sonication systems. They found that inactivation increased with the duration of microorganism exposure to ultrasound. Using a 20 kHz probe at 15 W/cm^3,

maximum inactivation was achieved after 15 minutes (the longest time tested). They concluded that 15 minutes of sonication time in large water treatment plants would be uneconomical based on the power output employed in their experiments.

Sonication intensity is also a key factor in the inactivation of microorganisms. Hua and Thompson (2000) tested the inavtivation of *E. coli* with sound intensity ranging from 4.6 – 74 W/cm^2. The results showed that inactivation rates were highest at the highest intensity tested, which was 74 Wcm^{-2}. For example, after 30 minutes of sonication at 20 kHz, 1.6 $\log_{10}$ inactivation of *E. coli* was observed at the highest sonication intensity of 74.1 W/cm^2, while 1.3 $\log_{10}$ and 1 $\log_{10}$ inactivation was found at 4.6 W/cm^2 and 18.5 W/cm^2, respectively. Nakanishi *et al*. (2001) tested the inactivation of *Cryptosporidium parvum* oocysts and concluded that high sonication intensities had physical and chemical effects associated with cavitation, which caused local high temperatures and pressures in the liquid. Scherba *et al*. (1991) conducted a study on microorganism inactivation in common-use water facilities, for example, hot tubs and whirlpools. All of their experiments were conducted at a temperature of $39^oC \pm 0.3^oC$ and a sonication frequency of 26 kHz. The organisms of concern were bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, as well as some viruses and fungi. Their results showed that with 60 minutes of ultrasound exposure, a significant decrease in fungal growth was detected, with better results as intensity increased. In general, the percent inactivation of bacteria increased with intensity level, except for *E. coli*. Thacker (1973) studied the effects of ultrasonic power and frequency on the survival of yeast cells. Two types of yeast cells were tested: haploid (dividing and

non-dividing) and diploid cells. These cells were treated in either yeast extracted-peptone-dextrose or 0.85% saline. Ultrasonic intensities of 1, 2, and 4 W/cm^2 and frequencies of 20 kHz and 1 MHz were tested. Fewer yeast cells survived with increasing intensity. Thacker also found out that the dividing and the largest cells were the most susceptible to ultrasonic disinfection. Lastly, the inactivation of yeast cells was due to the mechanical stresses caused by cavitation.

The frequency of ultrasonic waves plays an important role in disinfection effectiveness. Hua and Thompson (2000) conducted a study on ultrasonic wave frequencies on *E. coli*. The frequencies they tested were 205, 358, 618, and 1017 kHz. Their results indicated that 205 kHz was the most effective frequency. This frequency had the highest inactivation rate coefficient of 0.078 min^{-1}, which was approximately twice as large as that at 1017 kHz (0.030 min^{-1}). Hua and Thompson (2000) also found out that hydrogen peroxide formation rates at frequencies of 205 kHz and 358 kHz were 3.7 $\mu M\ min^{-1}$ and 4.7 $\mu M\ min^{-1}$, respectively. These formation rates were higher than the formation rates of 2.2 $\mu M\ min^{-1}$ and 1.4 $\mu M\ min^{-1}$ at 618 kHz and 1071 kHz, respectively. Nakanishi *et al*. (2001) studied the inactivation of *Cryptosporidium* oocysts at frequencies of 28, 45, and 100 kHz. They discovered that the lowest frequency (28 kHz), was the most effective in inactivating oocysts. After 10 minutes sonication time at 28 kHz, 10% of the total oocysts disappeared. The oocyst cell wall ruptured and the nuclei burst from 97% of the remaining oocysts. The infectivity was less than 1% of the base line, compared to 40% and 100% infectivity at frequencies of 45 kHz and 100 kHz, respectively. Phull *et al*. (1997) also conducted research on frequency with respect to inactivation of

microorganisms. Since their studies involved the application of ultrasound as well as other chemical agents, the results are presented in Section 2.3.2. Thacker (1973) tested the effects of ultrasound on yeast cell inactivation at frequencies of 20 kHz and 1 MHz. As shown in other studies for other organisms, the rate of yeast cell inactivation was higher when they were exposed to 20 kHz of ultrasonic treatment compared to the higher frequency.

2.3.2 Prior Work on the Synergistic Effect of Sonication

Phull *et al*. (1997) found a synergistic effect between sonication and chlorination. *E. coli* suspended in saline solution and raw stream water were used in their experiments. After 5 minutes of treatment time, the application of 1 mg/L chlorine inactivated 43% of the bacteria in the sample stream water and sonication alone inactivated 19%. When sonication was applied followed by chlorination, 86% inactivation of bacteria was achieved. After 20 minutes, 100% inactivation of bacteria was achieved for combined sonication and chlorination. Their research also indicated that increasing the sonication power from 12 W/cm^2 to 21 W/cm^2 increased the bacterial kill by 40% for a 5 minute treatment time in the presence of chlorine. Frequency also affected the percent inactivation of bacteria. Using the same amount of power, they concluded that higher ultrasonic wave frequency (800 kHz) was more effective than low frequency (25 kHz). With 1 minute of sonication followed by 5 minutes chlorine contact time and under the same sonication power, 75% of the bacteria survived after treatment at 25 kHz while only 20% survived at 800 kHz. Phull *et al*. (1997) suggested sonication followed by

chlorination is a better choice than chlorination followed by sonication, because the latter one causes a degassing effect, leading to lower chlorine concentrations.

Burleson *et al*. (1975) found a synergistic effect between sonication and ozonation in the inactivation of three types of viruses and six types of bacteria. The microorganisms were suspended in phosphate buffered saline (PBS) and secondary effluent. The effluent had a biological oxygen demand (BOD) of 20 mg/L and chemical oxygen demand (COD) of 45 mg/L. Four types of disinfection techniques were employed: ozonation alone, simultaneous application of ozonation and sonication, sonication alone, and sonication during oxygenation. Sonication alone and sonication during oxygenation for 10 minutes did not lead to inactivation of bacteria suspended in PBS or secondary effluent. However, all six types of bacteria were completely inactivated after 15 minutes of ozone contact or simultaneous application of ozone and ultrasound when they were suspended in PBS. When bacteria were suspended in secondary effluent, it required longer ozone or combined ozone and sonication contact time to achieve complete inactivation. In secondary effluent, the combination of ozonation and sonication provided more effective bacterial inactivation than ozonation alone in all bacterial strains tested. Since sonication alone did not inactivate the bacteria, but combined ozonation and sonication showed promising inactivation, this may be explained as a synergistic effect. Burleson *et al*. (1975) also found that the application of ultrasound reduced oxidizable organic material, thus reducing the amount of ozone needed for inactivation. The total inactivation of microorganisms could be enhanced by sonication, as sonication broke up clumps of bacteria and particulate organic material, causing microorganisms to be more exposed to

the ozone. It was believed that cavitation due to sonication also enhanced inactivation. The authors also suggested that simultaneous ozone and ultrasound application was an effective treatment process for microbial inactivation because of the instability of ozone.

Dahi (1976) also found synergistic effects with sonication and ozonation. A study was conducted on the inactivation of *E. coli* when subjected to ozonation alone, simultaneous ozonation and sonication, and sonication followed by ozonation. The behavior of ozone with or without sonication was also observed. A sonic probe system was used, which had an ultrasonic wave frequency of 20 kHz and output power of 160 W. Three types of water were used in the experiments: redistilled water with intermediate treatment with $KMnO_4$ then made isotonic and buffered with phosphate, sterilized secondary effluent from a biological sewage treatment plant, and sterilized secondary effluent diluted five times. The results showed that with the same given disinfection time, ozonation alone provided the least inactivation of *E. coli* compared to the combination of ozonation and sonication. Sonication followed by ozonation was more efficient in inactivating *E. coli* than simultaneous application of the two disinfectants. Ultrasonic waves enhanced microbial inactivation with ozone and also chemical oxidation processes caused by the free radicals generated from the decomposition of ozone. According to the experimental results, the aeration constant (K_La) for the disinfection system was increased by 15 – 45% after sonication, indicating the ozone interphase transfer was intensified.

2.4 INDICATOR ORGANISMS

There are a number of different types of microorganisms that exist in drinking water sources, including pathogenic and non-pathogenic organisms. The pathogenic microorganisms in drinking water may cause adverse health effects to humans and have to be inactivated before distributing the water to consumers. Because of the high costs and technical expertise needed to identify some pathogens, such as *Cryptosporidium*, routine testing for pathogens is not feasible. However, many non-pathogenic microorganisms, such as total coliforms, fecal coliforms, *Escherichia coli*, bacteriophages, and *Bacillus subtilis*, can be identified easily and economically with current technologies. Therefore, it is desirable to use non-pathogenic microorganisms as indicator organisms to indicate the expected fate of pathogens through water treatment processes such as disinfection.

Bacteria, viruses, and protozoa have different resistances to disinfection. Usually bacteria (such as *E. coli* or coliform bacteria) serve as indicators for the pathogenic bacteria group, bacteriophages (such as MS2 coliphage) for the pathogenic virus group, and aerobic spore-forming bacteria (such as *B. subtilis*) for pathogenic protozoa group.

2.4.1 Bacteria Indicators

Several researches have shown that non-pathogenic bacteria respond to disinfection in a similar manner as pathogenic bacteria. Chang *et al.* (1985), Harris *et al.* (1987) and Hassen *et al.* (2000) studied the inactivation of *E. coli* and *Streptococcus faecalis* by UV disinfection. Chang *et al.* (1985) also tested the germicidal efficiency of UV on total

coliform and standard plate count microorganisms obtained from secondary effluent. The researchers suggested that the doses of UV needed to inactivate 99.9% of cultured vegetative bacteria, total coliforms, and standard plate count microorganisms were comparable, with the exception of *S. faecalis* which required a 1.4 times higher dose of UV to achieve the same amount of inactivation. They also suggested that, at least for this study, total coliforms are adequate to serve as an indicator of disinfection (Chang *et al.*, 1985). Harris *et al.* (1987) selected *E. coli* and *S. faecalis* for the research because they are common biological indicators of the disinfection efficiency in water treatment. Results showed similar inactivation levels for both bacteria. Giese and Darby (2000) studied the sensitivity of three species of coliform bacteria (*Citrobacter diversus*, *Citrobacter freundii* and *Klebsiella pneumoniae*) and the bacteriophage ϕX-174 to medium pressure UV disinfection at wavelengths of 254 nm, 280 nm, and 301 nm. Their results showed that at an UV wavelength of 280 nm, the inactivation efficiencies of the three coliform species and the bacteriophage tested were similar with no significant differences. Giese and Darby (2000) concluded that the germicidal efficiency of one bacteria or virus species may be used to represent the relative inactivation of all bacteria and viruses when subjected to medium pressure UV irradiation.

2.4.2 Virus Indicators

Battigelli *et al.* (1993), Giese and Darby (2000), and Wilson *et al.* (1992) studied the use of virus indicators as surrogates for the disinfection of viral pathogens. Battigelli *et al.* (1993) indicated that traditional bacteriological indicators were not sufficient to provide protection against non-bacterial contamination of drinking water. They tested the

inactivation efficiencies of bacteriophages MS2 and ϕX-174, Hepatitis A, coxsackievirus, and rotavirus with various doses of UV irradiation. Their results showed that MS2 was the most resistant virus to UV irradiation compared to the other viruses inactivated. Apart from coliform bacteria, Giese and Darby (2002) also studied the behavior of ϕX-174 when subjected to medium pressure UV irradiation. The results and conclusions were discussed in the previous section (Section 2.4.1). Wilson *et al.* (1992) studied the use of MS2 coliphage as a test surrogate for the inactivation of various pathogenic bacteria and viruses by UV irradiation. They also concluded that MS2 was the most resistant to UV disinfection among the various pathogens tested. Their results suggested that 99.5% inactivation of MS2 corresponds to 99.9999% inactivation of bacterial pathogens or 99.99% inactivation of viral pathogens.

2.4.3 Protozoa Indicators

Barbeau *et al.* (1999) and Facile *et al.* (2000) used aerobic spore forming bacteria (*Bacillus subtilis* and environmental strains) as indicators to evaluate the inactivation of protozoa by chlorination and ozonation. Barbeau *et al.* (1999) found that the bacterial spores were actually more resistant to chlorination than *Giardia* and that the spore resistance increased with temperature. The CT value for 3 $\log_{10}$ inactivation *B. subtilis* was approximately 3 times the CT value for the same level of inactivation of *Giardia*. Facile *et al.* (2000) compared the CT values for 2 $\log_{10}$ inactivation of the aerobic spores obtained from their ozonation experiments with the CT values for the same amount of inactivation of *Giardia* and *Cryptosporidium* obtained from other literature. At near neutral pH and temperatures between 20 – 25^{o}C, the CT for 2 $\log_{10}$ inactivation of

Cryptosporidium ranged from 2.0 – 5.0 mg-min/L. At pH 6.3 and temperatures between 20 – 22^{o}C, the CT for 2 $\log_{10}$ inactivation of *B. subtilis* was 3.18 mg-min/L. These values were comparable, indicating that *B. subtilis* may be a suitable surrogate for *Cryptosporidium*. Since the CT values for 2 $\log_{10}$ inactivation of *Giardia* were several times smaller than *B. subtilis* or aerobic spore formers in general, then spore formers provide a conservative indicator for *Giardia*. The estimated inactivation of pathogenic protozoa based on this indicator due to ozonation would therefore be an underestimate.

2.4.4 Indicator Organism Conclusion

Research has shown that non-pathogenic bacteria, such as *E. coli*, total coliforms, and *Streptococcus faecalis*, are good indicators for the response of pathogenic bacteria to disinfection. Therefore, *E. coli* was chosen as a surrogate in this research and the results can be extrapolated to include various pathogenic bacteria and some types of viruses as well.

CHAPTER 3
METHODOLOGY

This chapter begins with an overview of the experimental plan. This is followed by the experimental procedures and finally, the analytical methods.

3.0 EXPERIMENTAL DESIGN

The goals of this research were to determine the inactivation of *E. coli* by sonication alone and by the combination of sonication and chlorination. A series of experiments were conducted to achieve these goals. As shown in Table 6, the disinfection experiments consisted of three major categories: chlorination alone, sonication alone, and the combination of chlorination and sonication. The disinfectants for the combined chlorination and sonication experiments were applied either sequentially or simultaneously. In addition, experiments were performed to study the effect of heating on the inactivation of *E. coli*. Experiments were conducted using E-pure water with a known starting concentration of *E. coli*.

Chlorine only experiments provide a basis for determining the synergistic effects of chlorination and sonication on the inactivation of *E. coli*. Based on preliminary experiments, chlorine doses of 0.4 to 1 mg/L were chosen. Chlorine doses greater than 1 mg/L inactivated all of the *E. coli* and hence produced undetectable counts; lower chlorine dose did not provide sufficient inactivation and samples had to be diluted many times before plating. Based on these considerations, a chlorine dose of 0.6 mg/L was

Table 6. Experimental plan for experiments using E-pure water.

Experiment Parameters	**Range of Variable**
Chlorine Only	
Dose	0.4 – 1 mg/L
Contact time	10 seconds – 5 minutes
Temperature	Room temperature (22 - 23^{o}C), 32^{o}C, 39^{o}C
Sonication Only	
Sonic time	10 seconds – 60 minutes
System	Probe or bath
Power-to-volume ratio	180 W/L or 900 W/L
Frequency	20 kHz for probe; 42 ± 6% kHz for bath
Output power	Approximately 90 W for probe; 70 W for bath
Temperature	Started at room temperature (22 - 23^{o}C)
Sonication + Chlorination	
Application sequence	Sequential or simultaneous
System	Probe
Power-to-volume ratio	180 W/L or 900 W/L
Chlorine dose	0.4 – 1.0 mg/L (mainly 0.6 mg/L)
Contact time	10 seconds - minutes
Temperature	Started at room temperature (22 - 23^{o}C)
Heating Only	
Temperature	Started at room temperature and increased to mimic heating by sonication

determined to be the most suitable concentration among the tested doses. Contact times from 10 seconds to 5 minutes were tested. Most of the chlorine only experiments were conducted at room temperature. Experiments were also completed at 32^{o}C and 39^{o}C because sonication caused the temperature to rise and thus chlorination occurred at elevated temperature for the sequential sonication plus chlorination experiments.

Sonication only experiments were conducted using both the probe and bath systems operating at power-to-volume ratios of 180 W/L and 900 W/L. Since the maximum

sonication power outputs were fixed for both sonic systems, the power-to-volume ratios were adjusted by changing the volume of the test samples. The sonication times tested ranged from 10 seconds to 60 minutes. A wide range of sonication times were tested so as to fully characterize the inactivation of *E. coli* over time. All of the sonication only experiments were started at room temperature and allowed to heat up during sonication.

The combined sonication and chlorination experiments were performed to study the synergistic effects of sonication on the inactivation of *E. coli* using the probe system. Two application sequences were tested: sequential (sonication followed by chlorination), and simultaneous (both sonication and chlorination applied at the same time). The majority of combined sonication and chlorination experiments focused on simultaneous disinfection, at both 180 W/L and 900 W/L, since the results showed that sequential disinfection was not effective. The chlorine doses tested in the combined sonication and chlorination experiments ranged from 0.4 to 1 mg/L; however, 0.6 mg/L was mainly used because the concentrations of *E. coli* that survived disinfection at this dose were still within the countable range.

3.1 EXPERIMENTAL PROCEDURES

This section describes in detail the preparations and procedures for all experiments, which include chlorination alone, sonication alone, and combined sonication and chlorination experiments.

3.1.1 *E. coli* Preparation

For each experiment performed, *E. coli* was grown in nutrient broth, centrifuged to decant the broth, and resuspended in phosphate buffered saline (PBS) or chlorine demand free PBS. Then a certain volume of the resuspended *E. coli* was added to the experimental water to obtain the desired starting concentration of 3×10^7 cfu/mL.

Two days prior to experimentation, two sterile 125-mL Erlenmeyer culture flasks, each containing 50 mL of tryptic soy broth, were transferred from the refrigerator to the incubator and incubated at 35°C overnight. One day prior to the experiment, *E. coli* from the frozen stock culture was transferred to culture flasks using a wire loop. The inoculated flask was then put on a rotating platform in the incubator at 35°C, shaking at a constant rate of 100 revolutions per minute (rpm). The inoculated culture with *E. coli* was allowed to grow in the incubator overnight for 16 – 18 hours.

On the day of an experiment, the *E. coli* culture was centrifuged to remove the broth. First, the centrifuge (Marathon 21000R, Fisher Scientific, Pittsburgh, PA) was cooled down to 4°C. Then one of the culture flasks was taken out of the incubator. The 50 mL in the flask was split into two autoclaved centrifuge tubes (Oakridge 50 mL centrifuge tubes 3119-0050 PPCO, Nalge Comapny, Rochester, NY). The two tubes were then centrifuged at 3,650 rpm for 20 minutes at 4°C.

After twenty minutes, the broth in one of the tubes was decanted, leaving behind only the pellet of *E. coli* at the bottom of the tube. The pellet was resuspended in a milk bottle

containing 25 mL of 0.01 M PBS or chlorine demand free (CDF) PBS. A small volume of the PBS was poured into the centrifuge tube and shaken until the pellet was completely dissolved. The solution was then poured back into the milk bottle. The resuspended *E. coli* was either used immediately or stored in the refrigerator for up to three hours until use. According to the results from trial experiments, the resuspended *E. coli* solution had an approximate concentration of 4 x 10^9 cfu/mL.

To perform an experiment, a certain volume of resuspended *E. coli* was spiked into the test water to achieve the desired initial concentration of *E. coli* (3 x 10^7 cfu/mL). The volume of resuspended *E. coli* added to the test water was determined by the following equation:

$$4\times10^{9}\ \frac{\text{cfu}}{\text{mL}}\times \text{vol. of resuspended } E.coli\ (\text{mL}) = 3\times10^{7}\ \frac{\text{cfu}}{\text{mL}}\times \text{vol. of test water (mL)}$$

After spiking *E. coli* into the test water, 1 mL of test water was withdrawn for pre-disinfection *E. coli* counts and 50 mL was removed for temperature, turbidity, and pH measurements. The test water was then ready for disinfection experiments.

3.1.2 Chlorine Only Experiments

For each experiment performed, a sterile 1-L media bottle containing 350 mL 0.01M CDF PBS (test water) was brought to starting temperature (22°C, 32 °C or 39 °C) by use of the water bath (Isotemp220, Fisher Scientific, Pittsburgh, PA). The CDF PBS was

warmed for 30 minutes to ensure the proper temperature was reached. Then, 2.6 mL of the resuspended *E. coli* was spiked into the test water. Fifty mL was withdrawn for temperature, pH, and turbidity measurements and 1 mL was withdrawn and transferred to a sterile dilution test tube containing 9 mL 0.01M PBS. The test tube was immediately placed in the refrigerator and was used to determine the pre-disinfection *E. coli* concentration.

Chlorine was applied in a sterile CDF BOD bottle. The BOD bottle contained a sterile magnetic stir bar, was wrapped with aluminum foil, and placed on a magnetic stirrer. Approximately 200 mL of the test water was poured into the BOD bottle. Then, the appropriate amount of chlorine was injected into the BOD bottle using a syringe dedicated to chlorine transfers (Hamilton Series 600/700 Fixed Needle Microliter Syringe, Hamilton Company, Reno, Nevada). The BOD bottle was filled with the remaining test water and capped headspace free. The stopwatch was started at the time of chlorine introduction. The volume of chlorine used was directly related to the target chlorine disinfection concentration, volume of the BOD bottle, and the concentration of the chlorine stock. The calculation is shown in the following equation:

$$\text{Conc. of } Cl_2 \text{ stock}\left(\frac{\text{mg}}{\text{mL}}\right) \times \text{Vol. of } Cl_2 \text{ used}(\mu\text{L}) = \text{Target } Cl_2 \text{ conc}\left(\frac{\text{mg}}{\text{L}}\right) \times \text{Vol. of BOD bottle}(\text{mL})$$

After the reaction period, 20 mL of the test water was removed for free and total chlorine measurements before quenching. The rest of the test water was then immediately quenched by pouring the water into an autoclaved beaker containing 0.3 mL of a 3%

sodium thiosulfate ($Na_2S_2O_3$) solution. Upon quenching, 1 mL of the post-disinfection water was transferred to a dilution tube and put in the refrigerator for post-disinfection *E. coli* enumeration. Free and total chlorine concentrations before and after quenching were measured. Post-disinfection temperature, pH, and turbidity readings were also recorded.

3.1.3 Sonication Only Experiments

3.1.3.1 Probe System

A probe-type sonicator (Sonicator 3000, Misonix Inc., Farmingdale, NY) was used for some of the sonication only experiments and all combined disinfectants experiments (sonication plus chlorination). The sonicator was set to the highest output power intensity of 10.0, which resulted in a power output of approximately 90 W. This power output along with the volume of test water was used to determine the power-to-volume ratio. Two power-to-volume ratios were tested: 180 W/L (500 mL of test water) and 900 W/L (100 mL of test water). The ultrasonic frequency was 20 kHz. It was constant and could not be altered. Since sonication only experiments did not involve chlorine, the PBS and glassware used in these experiments were sterile but did not have to be chlorine demand free.

All sonication only experiments started at room temperature (22 – 23^{o}C). For the 180 W/L experiments, 4.1 mL of resuspended *E. coli* was spiked into a 1 L media bottle containing 550 mL of sterile 0.01 M PBS. For 900 W/L experiments, 1.1 mL of resuspended *E. coli* was spiked into a 250-mL media bottle containing 150 mL of sterile

0.01 M PBS. Prior to disinfection, 50 mL of test water was removed for pre-disinfection temperature, pH, and turbidity measurements, and 1 mL was removed to determine the pre-disinfection *E. coli* concentration. Then, the test water was transferred to a beaker for sonication: 600 mL autoclaved beakers (Kimax #14000) were used for experiments at 180 W/L and 150 mL autoclaved beakers (Pyrex #1000) for experiments at 900 W/L. The probe was placed at the center of the beaker and approximately 1 inch below the water surface. The sonicator was started and the output power shown on the screen of the generator was monitored. After sonication, temperature was immediately recorded, followed by the removal of 1 mL of solution for post-disinfection *E. coli* enumeration. Turbidity and pH were measured on the remaining post-disinfection test water.

3.1.3.2 Sonic Bath

A sonic bath system (Branson 1510R-MT, Branson Ultrasonics Corporation, Danbury, Connecticut) was used in the sonication only experiments to compare the germicidal effectiveness of the bath versus the sonic probe systems. The sonic bath provided 42 ± 6% kHz and 70 W power output and these parameters could not be adjusted. Similar to the experiments with the sonic probe, sterile beakers were used for disinfection. The initial volume of test water (0.01 M PBS) and volume of resuspended *E. coli* to add were calculated to produce the power-to-volume ratios (180 W/L or 900 W/L) used in the probe experiments so that a direct comparison of bacterial inactivation between the sonic probe and bath systems could be achieved. The low power-to-volume ratio was achieved with 390 mL test water (prepared 440 mL sample with 3.3 mL *E. coli*) and the high

power-to-volume ratio was achieved with 80 mL test water (prepared 130 mL sample with 0.95 mL *E. coli*)

Prior to sonication, 50 mL of the test water spiked with *E. coli* was removed for pre-disinfection temperature, pH, and turbidity measurements, and 1 mL was removed for pre-disinfection *E. coli* enumeration. The remaining 390 mL or 80 mL of experimental water was transferred to a 600-mL or 150 mL beaker, respectively. The beaker was then placed in the sonic bath and the water level in the bath was adjusted to match with the liquid level in the beaker. One mL samples were withdrawn for *E. coli* enumeration at various time intervals. Temperature measurements were taken at the end of each time interval, while pH and turbidity were measured at the last experimental time interval.

3.1.4 Sonication Plus Chlorination Experiments

In this section, methods for sonication and chlorination experiments are introduced. There are two ways that the experiments were carried out: sequential or simultaneous application of ultrasound and chlorine. Sequential application means that sonication was applied followed by chlorination, whereas simultaneous application means that sonication and chlorination occurred at the same time.

3.1.4.1 Sequential Application of Sonication and Chlorination

Sequential application of sonication and chlorination was performed in the same manner as a sonication only experiment (sonic probe system) followed by a chlorination only

experiment. First, a sonication experiment was performed as described in Section 3.1.3.1 (sonication only). Immediately after sonication, the test water was subjected to chlorination according to the methods described in Section 3.1.2. The only difference occurred for experiments using 180 W/L sonication. For this case, instead of a 300-mL BOD bottle, a smaller BOD bottle that holds about 60 mL was used for chlorination. For each experiment, pH, turbidity, temperature, and *E. coli* concentrations were measured before sonication, after sonication, and after chlorination. In addition, after chlorination free and total chlorine (before and after quenching) were measured.

3.1.4.2 Simultaneous Application of Sonication and Chlorination

For simultaneous disinfection by sonication and chlorination, both disinfectants are applied at the same time. Disinfection is started by simultaneously starting the sonicator and injecting chlorine. The simultaneous disinfection experiments at both 180 W/L and 900 W/L were conducted in the same manner as the sonication only experiments (probe system), with the following exceptions: chlorine was injected at the beginning of the experiment and quenched immediately at the end of the sonication time interval. In addition, free and total chlorine concentrations before and after quenching were measured.

3.1.5 Heating Only

Since temperature increased substantially as sonication time increased, especially for experiments with a high power-to-volume ratio, additional experiments were conducted

to determine whether temperature or the ultrasonic waves inactivated the *E. coli*. Therefore, heating experiments were carried out without the introduction of chlorine or sonication to mimic the heating phenomenon observed during sonication.

3.1.5.1 E-pure Water

Heating experiments on E-pure water without the introduction of *E. coli* were performed to compare the resulting temperatures with those observed during high power-to-volume ratio sonication experiments. A close match in the temperature profile over time was desired to give a more accurate prediction of whether temperature effects or sonic waves inactivated the *E. coli*. For this preliminary experiment, the water bath was set to 80^{o}C. Then, 100 mL of E-pure water in a 250-mL media bottle at room temperature was placed in the water bath. Temperature in the sample was recorded every minute from 1 to 15 minutes and every 5 minutes from 15 minutes onwards. It was found that the temperature increase by heating only matched well with the temperatures observed during sonication (probe system) at 900 W/L, so there was no need to perform more experiements with E-pure water only.

3.1.5.2 With *E. coli*

To study the effect of temperature on the inactivation of *E. coli*, an experiment was set up in the same environment as the one without *E. coli*. The water bath was preheated to 80^{o}C. Approximately 100 mL of experimental water was prepared by adding 1.1 mL of resuspended *E. coli* into 150 mL of 0.01M PBS and removing 50 mL for initial

temperature, pH, and turbidity measurements and removing 1 mL for pre-disinfection enumeration of *E. coli*. The media bottle containing about 100 mL of experimental water was placed in the 80^{o}C water bath. At various time intervals, the temperature was recorded and 1 mL samples were withdrawn for *E. coli* enumeration.

3.2 ANALYTICAL METHODS

3.2.1 Introduction

Aseptic techniques were applied throughout all experiments, which included the culturing, transfer, disinfection, and enumeration of *E. coli*. This was done to prevent contamination of samples by other microorganisms. During all transfers, aseptic conditions were maintained by working in a clean bench and flaming all open containers. All work spaces used in disinfection and enumeration processes were sterilized by spraying with 50% ethanol. In addition, the thermometer and the sonication probe were wiped with 50% ethanol. All glassware, plasticware, and metalware were also sterile. Glassware was sterilized by autoclaving (Sterilmatic Sterilizer, Market Forge Industries Inc., Everett, MA). Pre-sterilized plasticware, including petri dishes and serological pipettes of various sizes were purchased. Finally, all culture media, enumeration media, and chemical reagents were sterilized by use of an autoclave.

3.2.2 Enumeration of *E. coli*

In all of the experiments performed, pour plates and membrane filtration were the two enumeration techniques used to determine the *E. coli* concentration before and after

disinfection. The samples were diluted to appropriate concentrations before plating so as to give countable numbers of *E. coli* colonies on each plate. Whether pour plates or membrane filtration was used, at least 3 different dilutions were plated for each sample, with three replicates for each dilution. A negative control, which consisted with PBS only without *E. coli*, was plated for each sample.

3.2.2.1 *E. coli* Rehydration

The *E. coli* culture was purchased in dehydrated form from the American Type Culture Collection (ATCC #11775). The dehydrated pellet of *E. coli* was received in a vial. First, the cap of the vial was opened by using a flamed tweezer. Second, 1 mL of tryptic soy broth (TSB) from an autoclaved test tube that contained 5 – 6 mL TSB was pipetted into the vial to rehydrate the *E. coli* pellet. Then, the rehydrated contents were poured from the vial back into the test tube, and the test tube was incubated at 35^{o}C for 48 hours. During the incubation period, 10 mL of 40% glycerol by volume was prepared by combining 4 mL of glycerol and 6 mL of E-pure water. The glycerol was then autoclaved. After the 48-hour incubation, the *E. coli* culture was transferred into a series of sterilized microcentrifuge tubes. Each tube consisted of 0.5 mL of the *E. coli* culture and 0.5 mL of 40% glycerol. The 12 microcentrifuge tubes were labeled as EC1 - EC12. All of the vials tubes were frozen in –70^{o}C alcohol, and then in a –70^{o}C freezer. The vial labeled EC1 was used for this research.

3.2.2.2 Dilution Series

Dilution series were prepared in test tubes with closures. Each test tube contained 9 mL of 0.01 M PBS. They were autoclaved before use. When 1 mL of undiluted sample was introduced into the first tube, the concentration of the first tube became 10^{-1} (diluted by 10 times compared to the original concentration). When 1 mL of sample from the 10^{-1} tube was transferred to the another tube that contained 9 mL of 0.01 M PBS, the concentration of the second became 10^{-2}. The diluting process was continued until the desired dilution had been reached.

3.2.2.3 Pour Plates

The procedures for pour plating are described in Standard Methods 9215B (APHA *et al.*, 1998). Pour plates were used for *E. coli* enumerations when determining concentrations of *E. coli* in dilutions from undiluted down to 10^{-7}. After the pre- and post-disinfection dilution series were completed, 1 mL of sample from each appropriate dilution was pipetted into a 100-mL petri dish. Three replicate plates were prepared for each dilution plus one negative control for each sample. The most diluted plates were placed in the back of the laminar flow hood and the most concentrated in the front. Approximately 10 – 12 mL of liquid tryptic soy agar at 47^{o}C was pipetted directly onto the 1-mL sample such that the sample was evenly distributed. The petri dish was covered and mixed in a figure eight motion. Then the cover was opened slightly and the agar was allowed to solidify for 5 minutes. All pour plates were incubated upside down at 35^{o}C for 22 - 24 hours and were counted after the incubation period was over. The ideal range of counts per plate was between 30 and 300. The dilution with counts in the ideal range was used

to determine the *E. coli* concentration. If no dilution was in the ideal range, countable plates were used or the experiment was repeated.

3.2.2.4 Membrane Filtration

Standard Method 9222B describes in detail the procedure for enumerating coliforms by membrane filtration (APHA *et al.*, 1998). The membrane filtration technique was used to enumerate *E. coli* when concentrations were below 30 cfu/mL. Low *E. coli* concentrations occurred with effective disinfection processes, such as high chlorine doses, long sonication times, and high temperatures associated with sonication. For this method, 50 mm petri dishes were pre-filled with 5 - 6 mL of tryptic soy agar and cooled to allow the agar to solidify. Dilution series were prepared if necessary. A 0.45 μm Millipore membrane filter (Millipore Corporation, Bedford, Massachusetts) was placed on the sterile filter tower, then 20 mL of sterile 0.01 M PBS was introduced into the tower before adding the sample so as to ensure even distribution of *E. coli* colonies on the filter. The appropriate volume of sample from each desired dilution was pipetted into the filter tower. The vacuum pump was turned on to suction the liquid through the filter. The membrane filter was then transferred to the petri dish. Each dilution was filtered in triplicate, and a negative control was filtered for each sample. All membrane filtration plates were incubated upside down at 35^{o}C for 22 – 24 hours. The ideal range of MF plate counts was 20 – 80 colonies and no more than 200 colonies per plate.

3.2.3 Turbidity

Turbidity was measured according to Standard Method 2130B (APHA *et al.*, 1998). A turbidimeter (2100N Turbidimeter, Hach Company, Loveland, Colorado) was used to measure the turbidity of water samples before and after disinfection. The turbidimeter was calibrated according to the procedures provided by the manufacturer every three months. Each day, the glass turbidity vials were coated with silicone to ensure a smooth vial surface. The sample was poured into a turbidity vial and the outer surface of the vial was cleaned and dried with Kimwipes to remove dirt or fingerprints from the glass. The vial was inverted gently two times and placed into the turbidimeter. The turbidity reading was taken when the reading stabilized.

3.2.4 pH

The procedure for measuring pH is described in Standard Methods 4500-H^+ B (APHA *et al.*, 1998). An Orion 420A pH meter was used to measure the pH of pre- and post-disinfection water samples (Orion Research Inc., Beverly, MA). The meter was calibrated before each use with standard buffers of pH 4.01, 7.00, and 10.01. The pH probe was rinsed with E-pure water before and after use. To take a pH measurement, the probe was immersed into the solution and the value recorded when the reading stabilized.

3.2.5 Chlorine

Chlorine used in this research was NaOCl purchased from the Fisher Scientific, with a concentration of approximately 6% by weight (60 mg/mL). The bottle of chlorine was

wrapped with aluminum foil so as to block out light that would cause it to decompose. To prevent contamination of the reagent bottle, approximately 35 mL of chlorine stock was poured into a 40-mL glass vial wrapped with aluminum foil for everyday use. Chlorine in the vial was used and refilled if needed. Since the concentration of chlorine stock applied in the disinfection experiments was so small, it was difficult to measure the small volume to be added to the test water. Therefore, the chlorine stock was diluted 10 times with CDF E-pure (called the 10% chlorine stock), and stored in a separate vial wrapped with aluminum foil. All of the chlorine vials and bottles were stored in the refrigerator at 4^{o}C.

3.2.6 Free and Total Chlorine

All glassware used to determine the concentration of both free and total chlorine, such as 125 mL Erlenmeyer flasks, 100 mL volumetric flasks, and test tubes, was chlorine demand free. This was done to ensure the chlorine applied would not be consumed by reactions with organic matter attached to the glass. CDF glassware was prepared by soaking glassware in a 100 mg/L chlorine bath. Just before use, the glassware was rinsed 3 times with E-pure water to remove any chlorine remaining on the glass.

3.2.6.1 Free Chlorine Calibration Curve

A free chlorine calibration curve was used to relate chlorine concentrations to absorbance values measured from a spectrophotometer. Standard Methods 4500-Cl G was used to measure both free and total chlorine residuals using the DPD colorimetric method

(APHA *et al.*, 1998). The preparation of a free chlorine calibration curve involved the use of spectrophotometer and titration. The spectrophotometer was set to a wavelength of 515 nm. Five Erlenmeyer flasks and five volumetric flasks were taken out of the 100 mg/L chlorine bath and rinsed with E-pure water. The volumetric flasks were filled up to the graduation line with CDF E-pure and labeled #1 through 5. A magnetic stir bar was put into each of the Erlenmeyer flasks, followed by adding 5 mL of DPD buffer solution and then 5 mL of DPD indicator solution. By use of a 10-μL syringe dedicated for chlorine transfers (Hamilton Series 600/700 Fixed Needle Microliter Syringe, Hamilton Company, Reno, Nevada), 2 μL of chlorine stock was transferred into the volumetric flask. The chlorine solution was immediately poured into the Erlenmeyer flask containing DPD buffer and indicator solutions and mixed. The solution turned pink. A 10-mm spectrophotometer cell was rinsed with the solution and filled with solution again, then it was placed in the spectrophotometer (Cary 50 Scan, Varian Australia Pty Ltd., Mulgrave, Victoria, Australia) for an absorbance reading.

Immediately after the absorbance value was obtained, the solution in the cell was poured back into the Erlenmeyer flask. The Erlenmeyer flask was placed on a magnetic stirrer and then titrated against the FAS solution until the pink color just disappeared as described in Method 4500-Cl F of Standard Methods (APHA *et al.*, 1998). The burette readings before and after titration were recorded and the volume of FAS used was determined.

The above processes were repeated for additions of 3, 4, and 5 μL of chlorine to the volumetric flasks. For the first volumetric flask, no chlorine was added and only the absorbance reading was taken for this blank solution. The volume of FAS used during each titration was used to determine the initial chlorine concentrations in the volumetric flasks. Using Microsoft Excel, a calibration curve was produced by plotting the chlorine concentration in the volumetric flasks (mg/L) on the y-axis and absorbance values (1/cm) on the x-axis. Both the equation and the R^2 value were obtained.

3.2.6.2 Total Chlorine Calibration Curve

The total chlorine calibration curve was produced in exactly the same way as the free chlorine calibration curve (see Section 3.2.6.1), except with the addition of 1.001 g potassium iodide (KI) to each of the Erlenmeyer flasks prior to adding DPD buffer solution and DPD indicator solution.

3.2.6.3 Residual Free Chlorine Measurement Using DPD Colorimetric Method

For any experiment that involved chlorine during disinfection, the free chlorine residual concentration was measured, before and after quenching. The DPD chlorimetric method #4500-Cl G in Standard Methods was used to perform this test (APHA *et al.*, 1998). Test tubes were taken out of the 100 mg/L chlorine bath and rinsed thoroughly with E-pure water. Then, 0.5 mL DPD buffer solution, 0.5 mL DPD indicator, and 10 mL of sample were added to a test tube in this order. The tube was then gently shaken. The solution in the tube was poured into a Varian 10-mm rectangular cell, rinsed with that solution, and

filled again. The cell was placed into the spectrophotometer and the absorbance value was taken. The concentration of free chlorine residual was calculated from the equation of the free chlorine calibration curve.

3.2.6.4 Residual Total Chlorine Measurement Using DPD Colorimetric Method

In addition to free chlorine residual concentration, the total chlorine residual concentration was also measured for any experiment that applied chlorine as a disinfectant, before and after quenching. The method of measuring the total chlorine residual was the same as the procedure for measuring free chlorine residuals (Section 3.2.6.3), with the addition of 0.1001 g KI to every CDF test tube prior to the introduction of DPD buffer and DPD indicator solutions.

3.2.6.5 Determination of Chlorine Stock Concentration

The total chlorine concentration of the 10% chlorine stock was checked prior to starting an experiment. This was to verify the concentration of the stock that would be applied to disinfection and to determine the exact amount of chlorine stock to add in order to provide the desired chlorine concentration in the test water. The process of checking the total chlorine concentration involved two testing methods: the DPD colorimetric (Section 3.2.6.2) and the DPD ferrous titrimetric methods (Section 3.2.6.2). However, for determining the concentration of the 10% chlorine stock, 50 µL of the 10% chlorine stock was added to a CDF volumetric flask using a pipette. As described previously, this solution was then poured into an Erlenmeyer flask with DPD buffer, DPD indicator, and

KI. The absorbance was measured and the solution titrated with FAS. The total chlorine concentration of the 10% stock was determined by plugging in the absorbance value into the equation from the total chlorine calibration curve and also by multiplying the volume of FAS used by two. The total chlorine concentrations determined from both methods should be the same.

3.2.7 Reagents and Glasswares

3.2.7.1 Tryptic Soy Broth

Tryptic soy broth (TSB) was a nutrient broth for culturing *E. coli*. It was prepared as indicated by the manufacturer by dissolving the dehydrated tryptic soy broth powder (DF0370-17-3, Becton, Dickinson and Company, Sparks, MD) in E-pure water, in the ratio of 30 g of powder to 1 L of water. Fifty mL of TSB was placed into each 125-mL culture flask with metal closures. The culture flasks containing TSB were autoclaved for 15 minutes at 121^{o}C and stored in the refrigerator at 4^{o}C for up to 2 weeks. The evening before *E. coli* inoculation, 2 flasks were transferred from the refrigerator to the 35^{o}C incubator.

3.2.7.2 Tryptic Soy Agar

Tryptic soy agar (TSA) was a medium for *E. coli* enumeration before and after disinfection. First, TSB was prepared according to the procedures described in Section 3.2.7.1. Second, 15 g of the dehydrated TSA powder (Bacto^{TM} Agar 214010, Dickinson and Company, Sparks, MD) was added to each liter of TSB. Third, the agar was brought

to a boil and then autoclaved for 20 – 30 minutes at 121°C, depending on the volume of agar being sterilized. The TSA was kept in media bottles with screw caps and stored in the refrigerator at 4°C for up to 3 months.

To prepare pour plates, the TSA was autoclaved again for 15 minutes and kept warm at 47°C in water bath. The agar was used within 3 hours for pour plating. Liquid TSA was also used to prepare 50 mm membrane filtration plates. About 5 – 6 mL of TSA at 47°C was pipetted carefully into each 50 mm petri dish to avoid air bubbles. The MF plates were stored in the refrigerator at 4°C upside down in sealed plastic bags for up to 2 weeks. The membrane filtration plates were transferred to the incubator at 35°C the evening before an experiment.

3.2.7.3 Phosphate Buffered Saline

The 0.1 M PBS stock was prepared by dissolving 80 g NaCl, 2.0 g KH_2PO_4, 2.0 g KCl, and 11.56 g anhydrous Na_2HPO_4 in E-pure water. The solution was brought up to 1 L in a volumetric flask and stirred using a magnetic stir bar until all solids were completely dissolved. The pH of the PBS was checked to verify that it was between pH 7.2 – 7.4. If not, the pH was adjusted to this range using 0.1 M HCl or 0.1 M NaOH. The 0.1 M PBS stock was autoclaved and stored in media bottles at room temperature.

The 0.1 M PBS stock was diluted 10 times for use as the test water and in dilution tubes by combining 1 part of 0.1 M PBS with 9 parts of E-pure water. The pH of the resulting 0.01 M PBS was checked to verify it was in the range of pH 7.2 and 7.4. The 0.01 M

PBS was then divided into various containers, such as media bottles, dilution tubes, and membrane filtration wash down bottles, and then autoclaved. If they were not used immediately, they were stored in the refrigerator at 4^{o}C for up to 3 months.

3.2.7.4 Chlorine Demand Free Phosphate Buffered Saline

Chlorine demand free (CDF) PBS was used in chlorination only and sonication plus chlorination experiments to ensure all of the chlorine applied was used for disinfection purposes and not on the reaction of chlorine with other constituents in the water. CDF PBS was made by chlorinating 0.01 M PBS with 5 mg/L chlorine for 24 hours in the dark with constant stirring and then dechlorinating by immersing a Pen-Ray UV Pen (34-0007-01 Lamp 8W germicidal 254 nm G8T5/S, UVP, Upland, CA) into the solution for 24 hours. Total chlorine residual was measured using the DPD colorimetric method (see Section 3.2.6.4). If the 0.01 M PBS was free from chlorine, it was then autoclaved and stored tightly capped in the refrigerator at 4^{o}C. If chlorine remained, the solution was irradiated with the UV pen for another 12 hours.

3.2.7.5 Chlorine Demand Free E-pure

Chlorine demand free E-pure water was used to generate results for free and total chlorine calibration curves. It was also used to check the concentration of 10% chlorine stock prior to performing an experiment. The methods of making CDF E-pure were exactly the same as the methods for CDF PBS, except that E-pure water was used instead

of 0.01 M PBS. The CDF E-pure water did not need to be autoclaved but was stored in the refrigerator at 4°C.

3.2.7.6 Chlorine Demand Free Glassware

Chlorine demand free glassware was prepared by soaking glassware, such as Erlenmeyer flasks, volumetric flasks, test tubes, milk bottles, and BOD bottles, in a 100 mg/L chlorine bath made from bleach. The glassware were taken out of the chlorine bath just before use and rinsed thoroughly at least 5 times with E-pure water.

3.2.7.7 Dilution Tubes

Dilution tubes were used in the pre- and post-disinfection dilution series. Each dilution tube consisted of 9 mL of 0.01 M PBS with a metal closure on the tube. The tubes were placed in a test tube rack and autoclaved for 15 minutes at 121°C. They were stored in the refrigerator at 4°C until use, with a maximum storage time of approximately 2 weeks.

3.2.7.8 Sodium Thiosulphate

Sodium thiosulfate ($Na_2S_2O_3$) was used for quenching chlorine so that exact chlorine disinfection time could be achieved. According to Method 9060A in Standard Methods (APHA *et al.*, 1998), 0.1 mL of a 3% $Na_2S_2O_3$ neutralizes up to 5 mg/L of residual chlorine in a 120 mL volume. A 3% solution can be prepared by dissolving 3 g of $Na_2S_2O_3$ in 100 mL of E-pure water. Since $Na_2S_2O_3 \bullet 5H_2O$ was used, 4.7069 g was needed to make a 3% solution. The solution was then autoclaved before use. 0.3 mL of

the 3% $Na_2S_2O_3$ solution was used to quench chlorine in the experiments where chlorine was involved.

3.2.7.9 DPD Indicator Solution

DPD indicator solution was used to measure free and total chlorine concentrations. It was purchased from a manufacturer (DPD Solution APHA, LabChem Inc., Pittsburgh, PA). The shelf life of the DPD indicator solution was 2 months.

3.2.7.10 DPD Buffer Solution

DPD buffer solution was used in conjunction with DPD indicator solution for free chlorine measurements and total chlorine measurements. With reference to Method 4500-Cl F in the Standard Methods (APHA *et al*, 1998), the solution was prepared by dissolving 24 g of anhydrous Na_2HPO_4 and 46 g of anhydrous KH_2PO_4 in E-pure water. Then it was combined with 100 mL E-pure water in which 800 mg of disodium ethylenediamine tetrascetate dihydrate (EDTA) was dissolved. The entire solution was diluted to a total volume of 1 L with E-pure water. The DPD buffer solution was stored in the refrigerator at 4^oC for up to 3 months.

3.2.7.11 Standard Ferrous Ammonium Sulfate (FAS) Titrant

The FAS solution served as a titrant to determine free and total chlorine concentrations using the titrimetric method. According to Method 4500-Cl F in Standard Methods (APHA *et al*, 1998), FAS titrant was made by dissolving 1.106 g of

$Fe(NH_4)_2(SO_2)_4 \bullet 6H_2O$ in E-pure water that already contained 1 mL of 1 + 3 H_2SO_4. The mixture was diluted up to 1 L with freshly boiled and cooled E-pure water. The FAS solution was stored in the refrigerator at 4^oC for up to 1 month.

CHAPTER 4
RESULTS

This chapter presents the results obtained from experiments conducted to determine the sole and synergistic effects of sonication on the inactivation of *E. coli*. It is divided into four sections according to the various disinfection methods applied in the experiments, which include disinfection by chlorine only, sonication only, the combination of sonication and chlorination, and heating only. Each experiment was repeated at least 3 times to ensure representative results were obtained. Three replicate plates were counted for each dilution so as to provide more reliable average counts. The graphs shown in this chapter present all experimental results, while the tables provide the average results from the replicate experiments. Detailed results are included in Appendix A.

4.0 DISINFECTION BY CHLORINE ONLY

Chlorine experiments were conducted mostly at room temperature ($22^oC – 23^oC$); however, elevated temperatures of 32^oC and 39^oC were also tested. The chlorine concentrations used for disinfection ranged from 0.2 to 1.0 mg/L and the contact time was 5 minutes. Since a chlorine concentration of 0.6 mg/L was considered the most suitable concentration for the combined sonication and chlorination experiments, additional experiments were performed using this chlorine dose and contact times of 10 seconds, 30 seconds, 1, 2, and 3 minutes.

The chlorine only experiments required the use of chlorine demand free phosphate buffered saline and glassware. The disinfection process was conducted in the dark, using

a CDF BOD bottle wrapped with aluminum foil, and with constant stirring using a magnetic stirrer. Temperature, pH, turbidity and *E. coli* concentration were measured before and after disinfection. Free and total chlorine residuals were measured after disinfection, both before and after quenching the experimental water with sodium thiosulfate.

4.0.1 Chlorination at Room Temperature

Figure 1 shows the $\log_{10}$ inactivation of *E. coli* by various chlorine doses with a 5 minute contact time. Less than 1 $\log_{10}$ inactivation was achieved at chlorine doses of 0.2 and 0.4 mg/L. When the chlorine dose was increased to 0.6 mg/L, on average 4.13 $\log_{10}$ inactivation of *E. coli* was achieved. If the chlorine dose was further increased to 1 mg/L, on average 5.25 $\log_{10}$ inactivation of *E. coli* inactivation was obtained. Based on these results, a chlorine dose of 0.6 mg/L was selected for further study. This chlorine dose was strong enough to achieve reasonable inactivation of *E. coli* while still providing countable results on the pour plates.

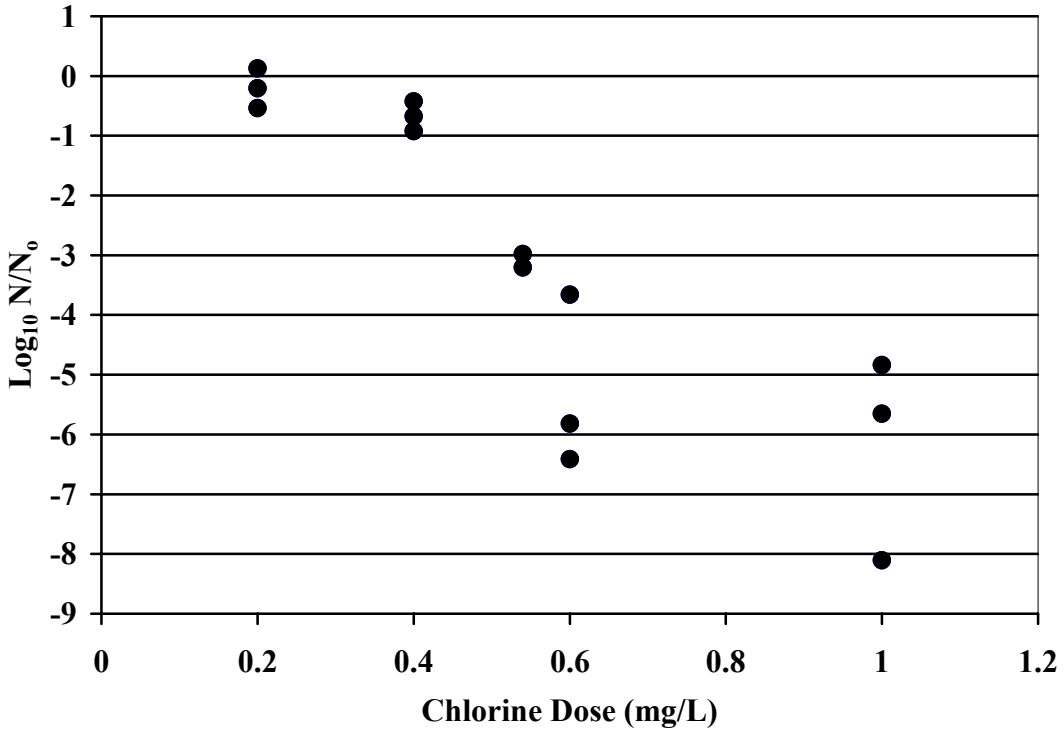

Figure 1. Log_{10} inactivation of *E. coli* at various chlorine doses (5 minute contact time, 22-23°C).

In addition to the 5 minute chlorine contact time, other disinfection times were also tested at a constant chlorine dose of 0.6 mg/L. The results are shown in Figure 2. Although the data shows some variability, up to 2 minutes resulted in approximately 1.3 log_{10} inactivation of *E. coli* on average. A noticeable increase in the inactivation of *E. coli* was observed after 3 and 5 minutes of chlorine disinfection, with 4.0 log_{10} and 4.1 log_{10} inactivation, respectively.

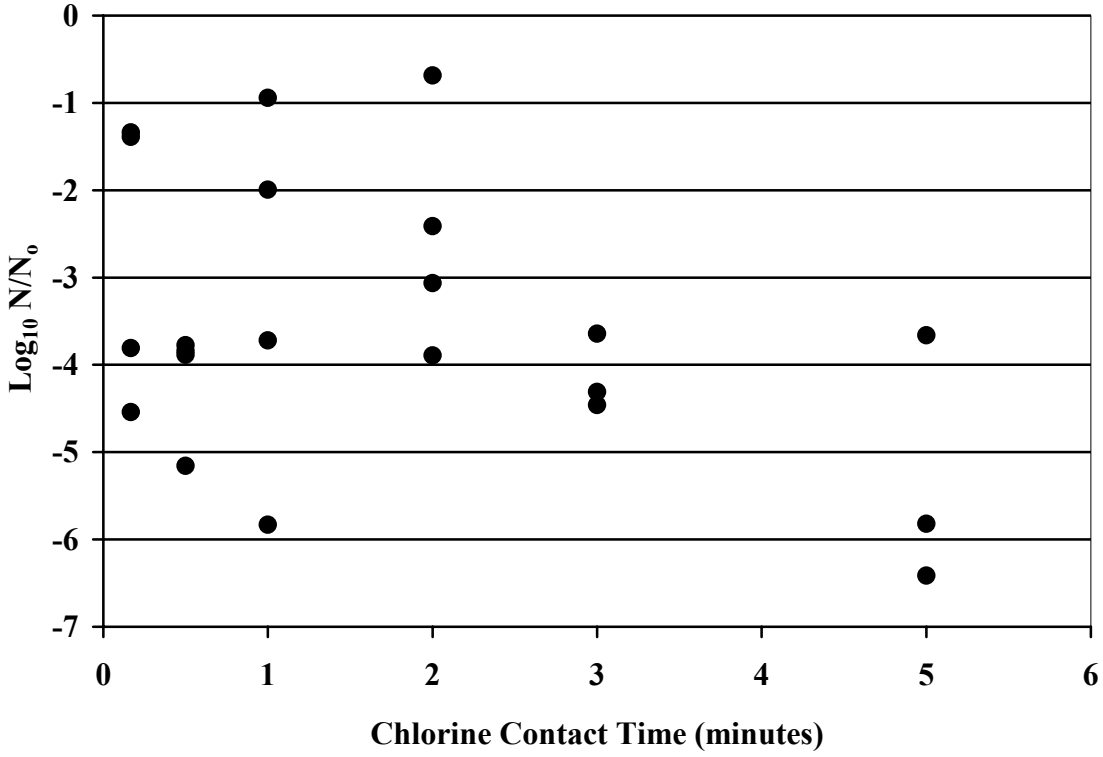

Figure 2. Log_{10} inactivation of *E. coli* at various chlorine contact times (chlorine dose of 0.6 mg/L, 22-23°C)

4.0.2 Chlorination at Elevated Temperatures

Sonication alone caused temperature increases (shown in later sections), therefore temperatures were higher at the start of the chlorination when sequential sonication plus chlorination experiments were conducted. As a result, chlorination at elevated temperatures was tested to have a suitable comparison to the combined sonication and chlorination experiments. Figure 3 shows the difference in the inactivation of *E. coli* by chlorination at room temperature and elevated temperatures. All experiments were

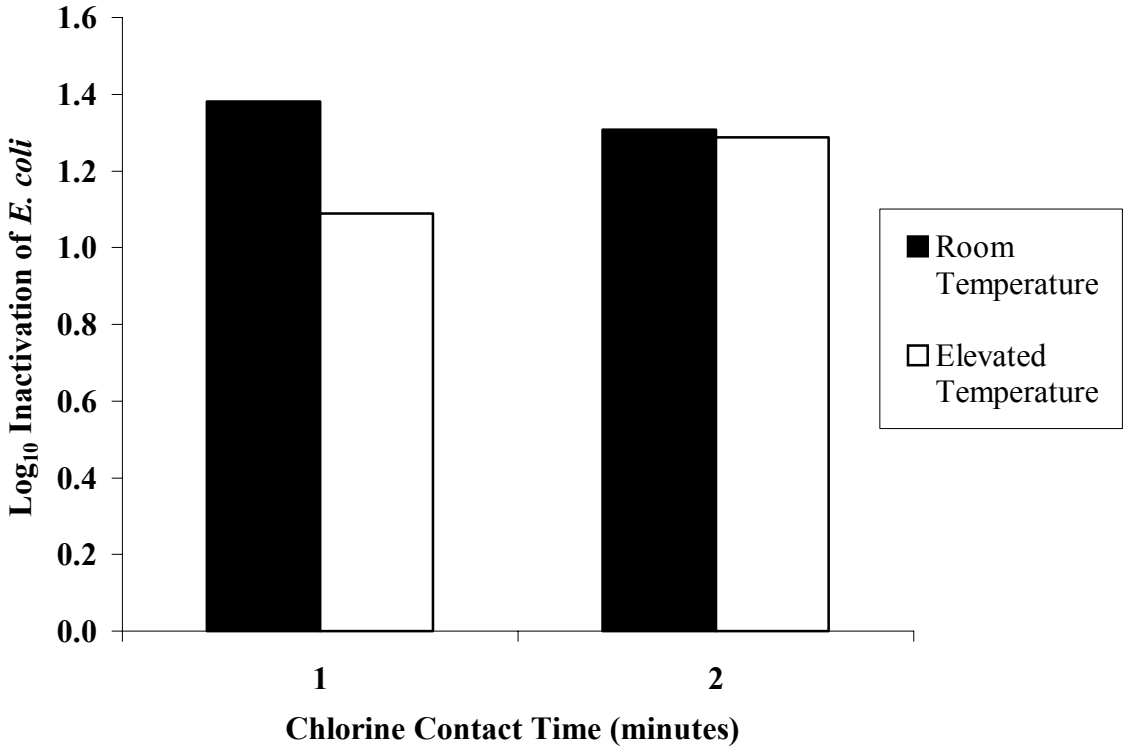

Figure 3. Log_{10} inactivation of *E. coli* at room and elevated temperatures with 0.6 mg/L chlorine.

conducted with a chlorine concentration of 0.6 mg/L. The elevated temperature tested for 1 minute was 32°C and for 2 minutes was 39°C. The results showed that temperature did not significantly affect the level of *E. coli* inactivation.

4.1 DISINFECTION BY SONICATION ONLY

Sonication experiments were carried out using either an ultrasonic probe or a sonic bath. All experiments started at room temperature and the samples were allowed to heat up

during sonication. Two power-to-volume ratios (180 W/L and 900 W/L) were tested for both the probe and the bath system. Measurements taken before and after disinfection included temperature, pH, turbidity, the output power from the probe-type sonicator, and *E. coli* concentration.

4.1.1 Probe System

The sonication times tested using the sonic probe ranged from 10 seconds to 60 minutes at 180 W/L and 10 seconds to 10 minutes at 900 W/L. The ultrasonic frequency of the sonication probe was 20 kHz.

4.1.1.1 Sonication at 180 W/L

Sonication alone at 180 W/L for 30 minutes or more inactivated *E. coli* down to detection limits. Figure 4 shows the inactivation of *E. coli* by sonication at 180 W/L, and the resulting temperature increase with sonication time. For sonication times of 10 minutes or less, 0.1 – 0.4 $\log_{10}$ inactivation was achieved, and the temperature rose up to about 40^{o}C after 10 minutes. The samples were heated up to about 45^{o}C and 50^{o}C with 15 and 20 minutes of sonication, respectively, and 0.6 – 0.8 $\log_{10}$ inactivation of *E. coli* was observed. After 30 minutes of sonication time, good inactivation of *E. coli* was obtained; the temperature rose to 60^{o}C and almost no growth was recorded after disinfection. For sonication times of 40 – 60 minutes, the temperature rose to 65 – 70 ^{o}C, and no growth was recorded. The $\log_{10}$ inactivation values shown in Figure 4 for these long sonication times were calculated based on the detection limits of the enumeration techniques.

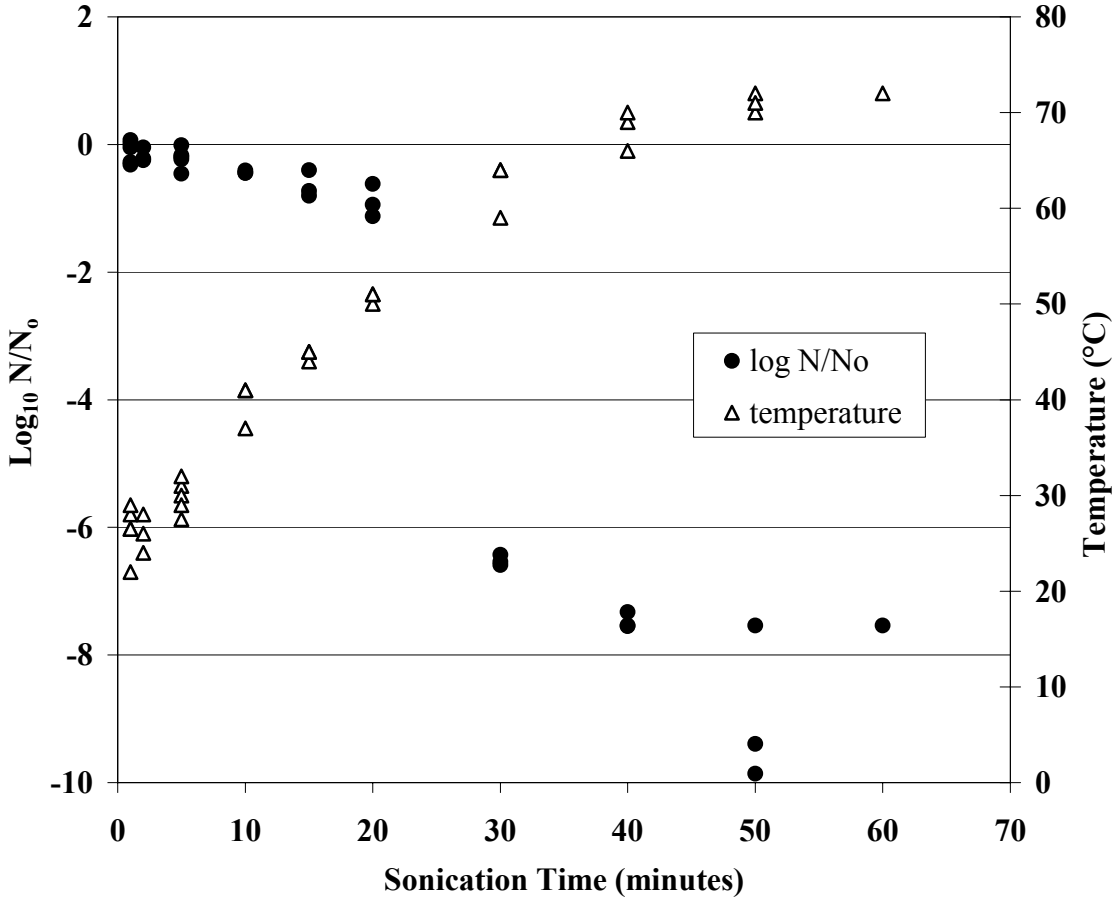

Figure 4. Log_{10} inactivation of *E. coli* with sonication at 20 kHz
(Power to volume ratio: 180 W/L; probe system)

In addition to temperature increase, the turbidity of the samples also increased over time. Table 7 shows this phenomenon during sonication. A slight and gradual increase from 2.1 NTU to 2.4 NTU was recorded for sonication times of 0.5 to 5 minutes, followed by a substantial increase to 7.2 NTU after 10 minutes of sonication, 12.3 NTU after 20 minutes, and 25.5 NTU after 60 minutes.

Table 7. Average $\log_{10}$ inactivation, temperature, and turbidity at various sonication times (180 W/L; probe system).

Sonication Time (min)	Average $\log_{10}$ Inactivation	Average Final Turbidity (NTU)	Average Final Temperature (^{o}C)
0.5	0.16	2.11	23.3
1	0.08	2.16	26.4
2	0.16	2.08	26.0
5	0.19	2.36	29.9
10	0.43	7.15	39.7
20	0.84	12.30	50.7
30	6.51	18.60	62.3
40	7.46	21.90	68.3
50	8.01	23.70	71.0
60	7.45	25.50	74.0

4.1.1.2 Sonication at 900 W/L

The results obtained for sonication only at 900 W/L and 20 kHz were similar to results at 180 W/L; however, dramatic increase in temperature, turbidity, and *E. coli* inactivation occurred with much shorter sonication times for the high power-to-volume ratio. As shown in Figure 5, less than one $\log_{10}$ of *E. coli* reduction was achieved with sonication times from 10 seconds up to 3 minutes. The temperature increased to approximately 50^{o}C and the turbidity to 10 NTU after 3 minutes of sonication time. Using high power sonication, only 5 minutes was needed for 6 $\log_{10}$ inactivation of *E. coli* (compared to 30 minutes at 180 W/L to achieve similar results). No *E. coli* growth was recorded after 10 minutes of sonication.

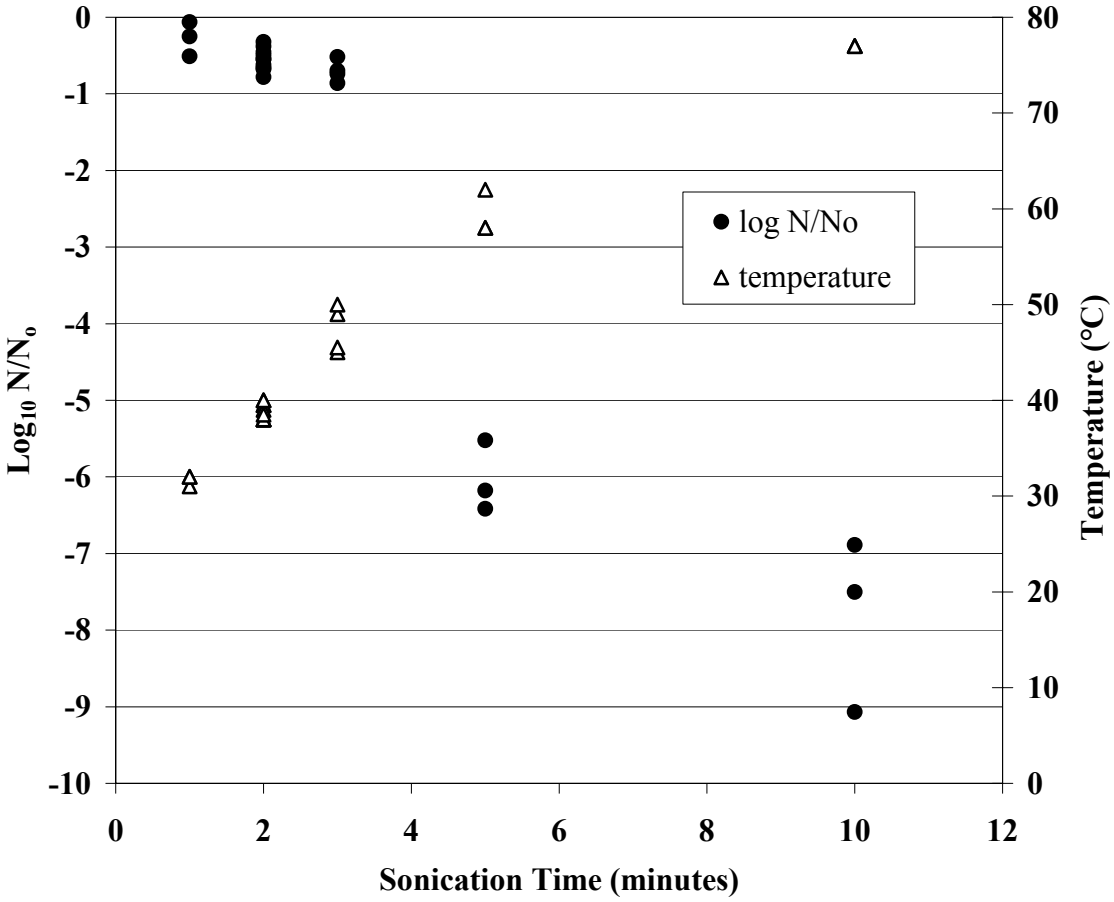

Figure 5. Log_{10} inactivation of *E. coli* with sonication at 20 kHz
(Power to volume ratio: 900 W/L; probe system)

Table 8 shows the average inactivation of *E. coli*, turbidity, and temperature after sonicating at 900 W/L for various time intervals. The final temperature and turbidity of samples subjected to 10 minutes of sonication was 77°C and 23.4 NTU, respectively. Using sonication at 180 W/L, less than 0.5 log_{10} reduction in *E. coli* was achieved at 10 minutes, and the temperature and turbidity were 40°C and 7.2 NTU, respectively.

Table 8. Average $\log_{10}$ inactivation, temperature, and turbidity at various sonication times (900 W/L; probe system).

Sonication Time (min)	Average $\log_{10}$ Inactivation	Average Final Turbidity (NTU)	Average Final Temperature (^{o}C)
0.1667	0.01	2.53	24.0
0.5	0.22	3.84	27.0
1	0.24	5.32	31.7
2	0.53	7.85	38.9
3	0.69	10.09	47.4
5	5.87	16.20	59.3
10	7.27	23.35	77.0

4.1.2 Bath System

In addition to the sonic probe system, *E. coli* inactivation was also studied with the use of a sonic bath with a 70 W power output and frequency of 42 kHz. The power-to-volume ratios tested using the bath were 180 W/L and 900 W/L, which were same as using the probe. The sonication times tested for 180 W/L sonication were 1 to 60 minutes and for 900 W/L were 1 to 50 minutes.

4.1.2.1 Sonication at 180 W/L

As can be seen in Figure 6, less than one $\log_{10}$ inactivation of *E. coli* was achieved for all sonication times tested from 1 to 60 minutes sonication. Therefore using a sonic bath to inactivate *E. coli* was not effective. A temperature increase was noted over time, but the extent was not as dramatic as was shown for the sonic probe system. The highest temperature achieved was 51^{o}C after 60 minutes. The average 0.26 $\log_{10}$ inactivation was achieved for this sonication time.

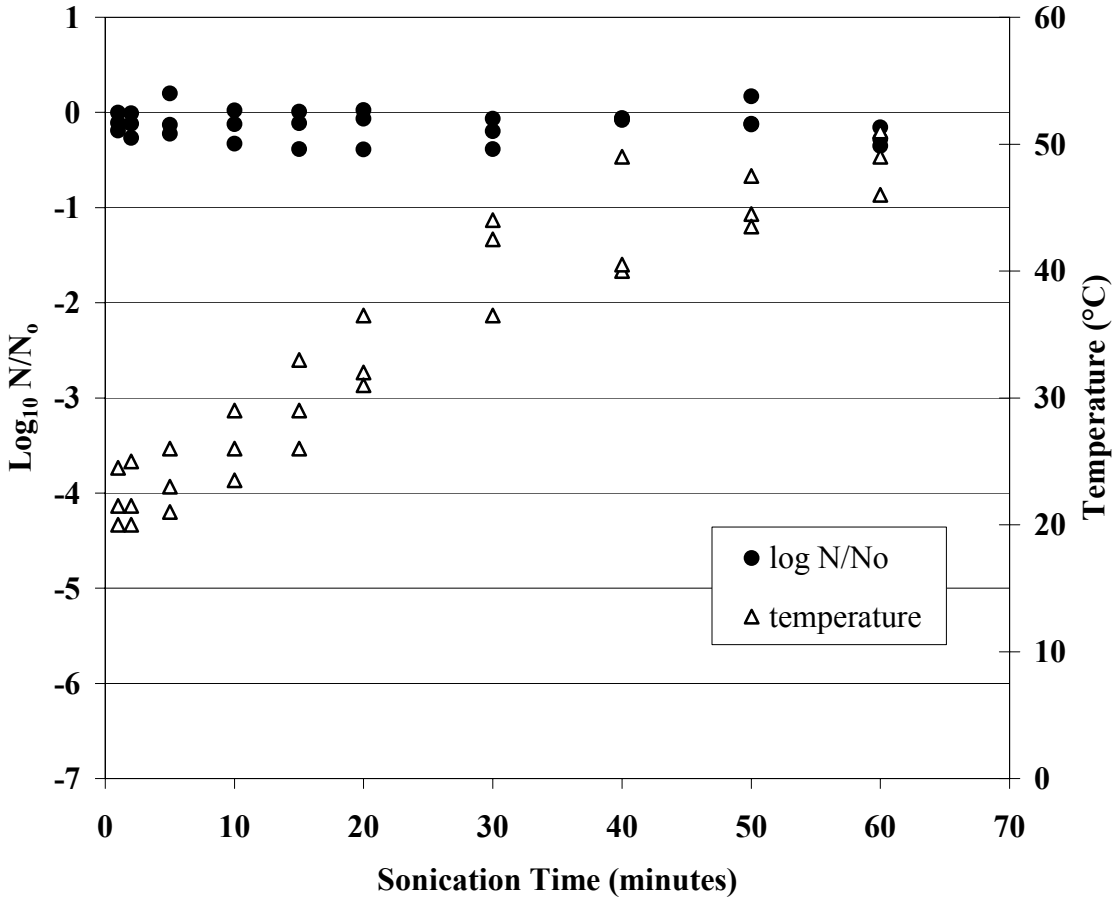

Figure 6. Log_{10} inactivation of *E. coli* with sonication at 42 kHz
(Power to volume ratio: 180 W/L; bath system)

4.1.2.2 Sonication at 900 W/L

The inactivation results using high power (900 W/L) sonication using a sonic bath were similar to results using 180 W/L. As shown in Figure 7, less than 1 log_{10} inactivation of *E. coli* was observed for all sonication times tested. However, the temperatures recorded were not consistent for the three replicate experiments, especially for 10 minutes of sonication time onwards. Inconsistent results in the log_{10} inactivation of *E. coli* at 40 and

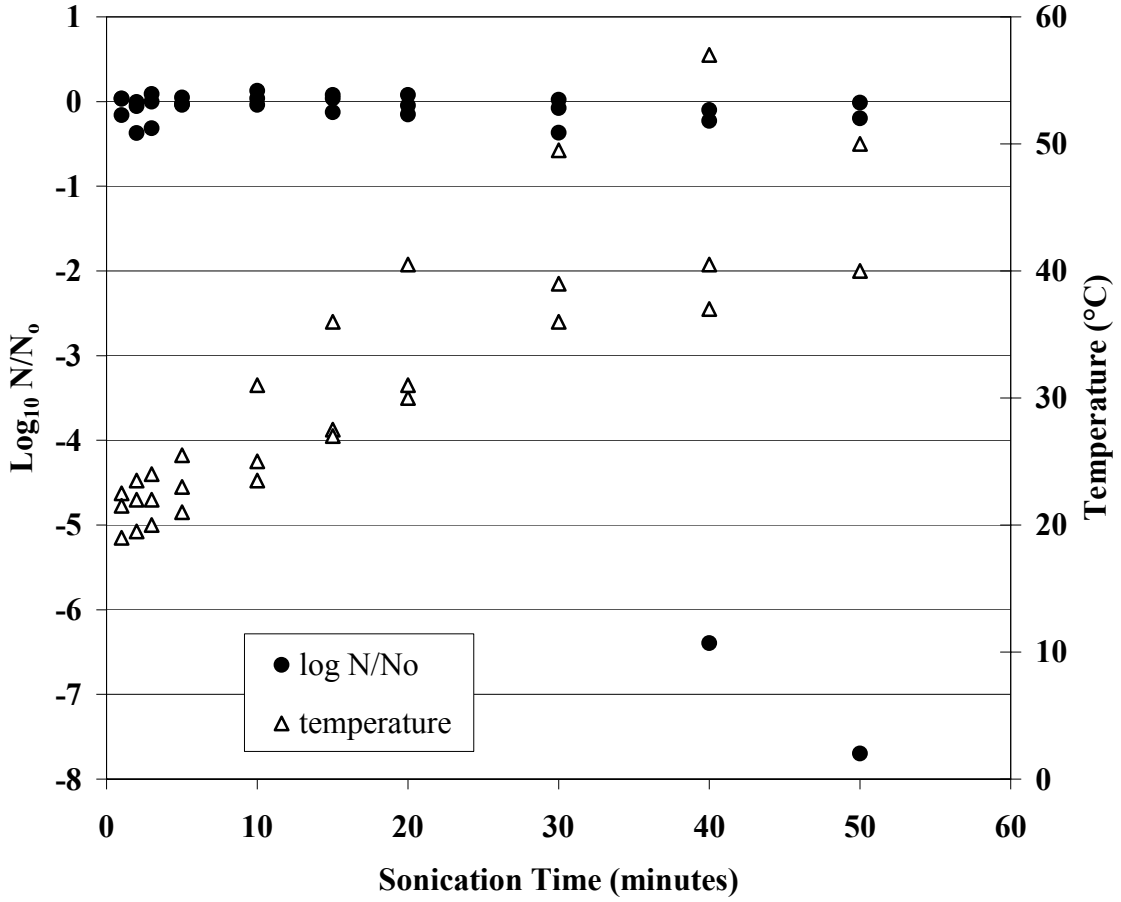

Figure 7. Log_{10} inactivation of *E. coli* with sonication at 42 kHz
(Power to volume ratio: 900 W/L; bath system)

50 minutes of sonication time were also observed. Because of the poor *E. coli* inactivation efficiency and the inconsistent temperature effects when using the sonic bath, all combined sonication and chlorination experiments were conducted using the sonic probe system.

4.2 DISINFECTION BY COMBINED SONICATION AND CHLORINATION

Combined sonication and chlorination experiments were carried out to determine the synergistic effects of the two disinfectants. These experiments were conducted in two ways. Sequential disinfection involved applying sonication followed by chlorination, and simultaneous disinfection was the application of sonication and chlorination at the same time. As with the sonication only experiments, both sequential and simultaneous disinfection experiments were performed at 180 W/L as well as 900 W/L, using the sonic probe system.

4.2.1 Sequential Application of Sonication and Chlorination

The sequential application of sonication and chlorination involved the application of sonication (180 W/L or 900 W/L) followed by chlorination. Temperature, pH, and turbidity measurements were taken before sonication, after sonication, and after chlorination. Free and total chlorine residuals were also measured before and after chlorine quenching. Since chlorine was added after sonicating for a certain amount of time, the temperature of the test water was elevated by the time chlorine was added for some experiments. The temperatures after sonication were higher for longer sonication times and the high power-to-volume ratio.

4.2.1.1 Sequential Application of Sonication and Chlorination at 180 W/L

Figure 8 shows the comparison of percent inactivation of *E. coli* by sonication only at 180 W/L, by chlorine only, and the sequential combination of sonication and

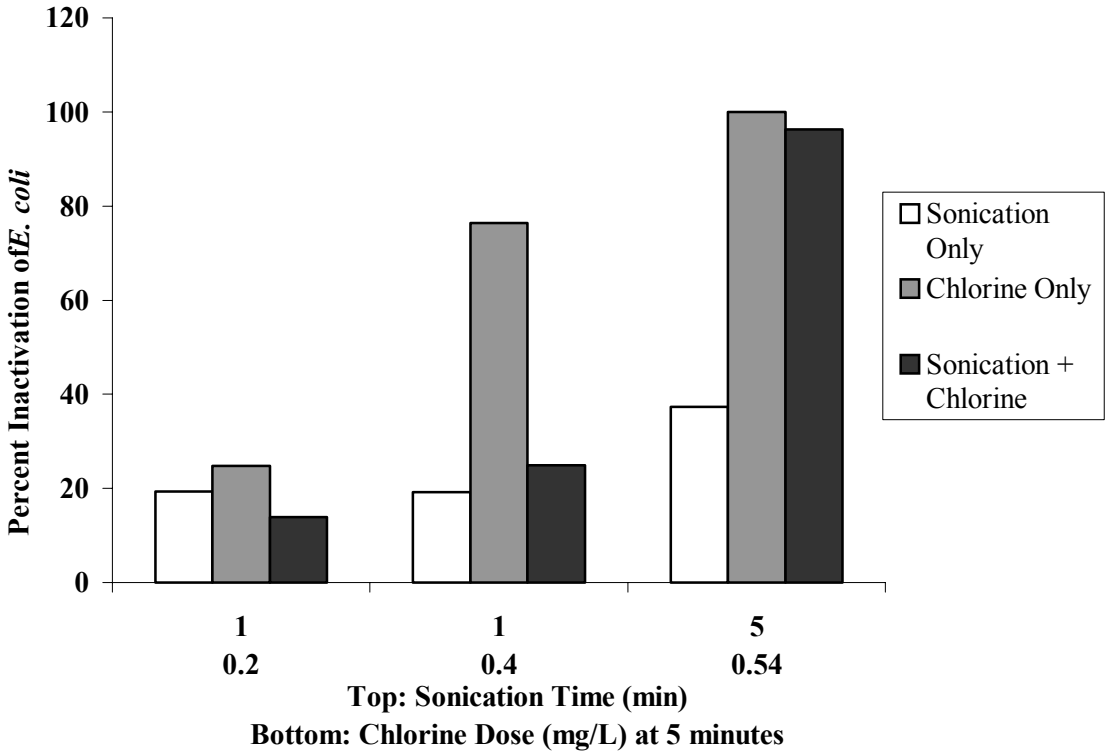

Figure 8. Percent inactivation of *E. coli* from sequential application of sonication and chlorination (Sonication power-to-volume ratio: 180 W/L; probe system)

chlorination. Chlorine alone provided better *E. coli* inactivation than the combination of sonication and chlorination. For example, with 1 minute of sonication at 180 W/L followed by 0.4 mg/L chlorination for 5 minutes, the overall inactivation of *E. coli* after both disinfection processes was only 25%, compared to 77% achieved by chlorine alone. Therefore, the sequential combination of the two disinfectants was shown to be ineffective, and no synergistic effects were observed. It is hypothesized that the *E. coli* were under stress during sonication and this may have caused the bacteria to have

enhanced repair mechanisms. Once sonication was over, the *E. coli* were more resistant to disinfection, hence they were not susceptible to chlorine disinfection after sonication.

In Figure 8, the chlorine only results are from experiments conducted at room temperature. Approximately a 3^{o}C increase in temperature was recorded after 1 minute sonication at 180 W/L and 4^{o}C after 5 minutes. Thus, chlorination was started at a slightly elevated temperature. However, this increase in temperature did not have significant impact on the inactivation of *E. coli* with regard to the chlorine only results at elevated temperatures (Section 4.0.2).

4.2.1.2 Sequential Application of Sonication and Chlorination at 900 W/L

Similar to the results obtained from the low power sequential application of sonication and chlorination, the results for high power (900 W/L) sequential disinfection were found to be ineffective in inactivating *E. coli*, as presented in Figure 9. Although the combination of 2 minutes sonication at 900 W/L followed by 5 minutes of chlorination at 0.4 mg/L provided more inactivation than sonication alone and chlorine alone, the overall reduction was still less than 1 $\log_{10}$ and thus was considered not effective. Again, the results from the sequential application of sonication at 900 W/L and chlorination did not show synergistic effects.

The temperature rose up to an average of 39^{o}C after 2 minutes sonication at 900 W/L and an average of 46^{o}C after 3 minutes. Although the chlorine only results in Figure 8 are shown for a temperature of 22 – 23^{o}C, the results are valid as it was previously shown

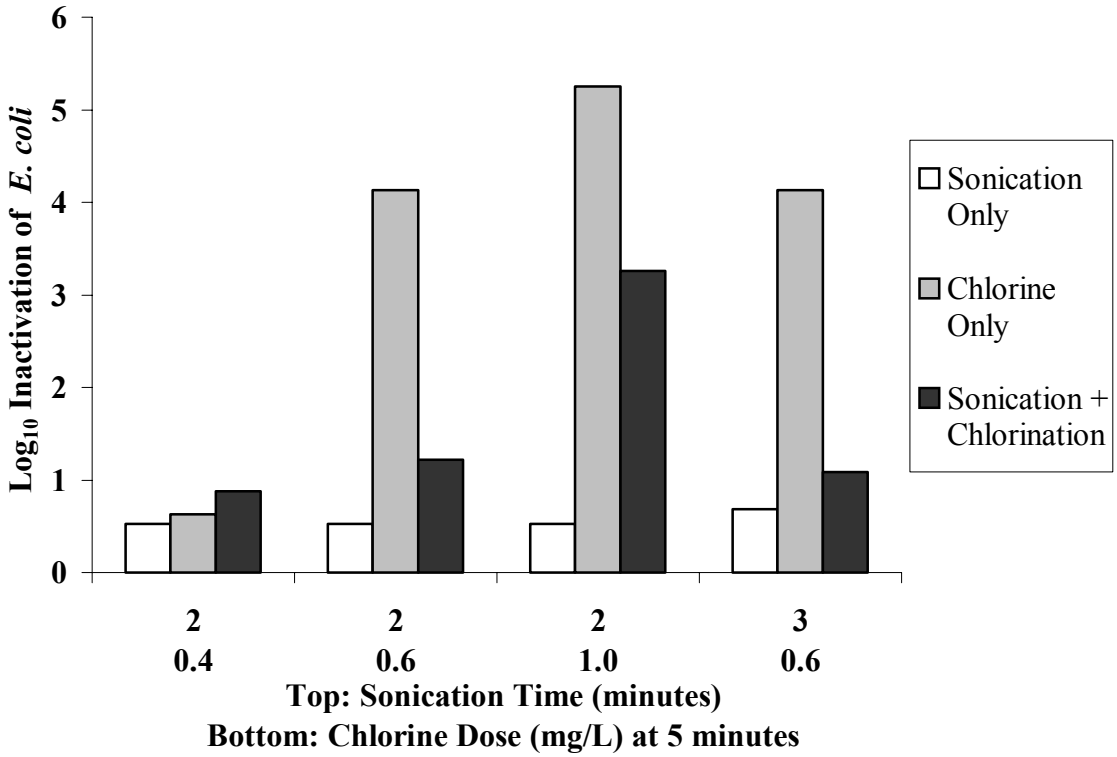

Figure 9. Log_{10} inactivation of *E. coli* from sequential application of sonication and chlorination (Sonication power-to-volume ratio: 900 W/L; probe system)

(Section 4.0.2) that this temperature increase did not significantly affect the results of chlorination.

4.2.2 Simultaneous Application of Sonication and Chlorination

Since the sequential application of sonication and chlorine failed to show improved inactivation of *E. coli* at both 180 W/L and 900 W/L, the effect of simultaneous application of the two disinfectants was tested. In these experiments, chlorine was

injected into the test water at the same moment the sonicator was started at room temperature (22 – 23°C). Since chlorine was involved, all simultaneous disinfection experiments were performed in the dark. The test water was allowed to heat up during sonication, so even though chlorine was applied at room temperature, the temperature of the test water that contained chlorine may have risen slightly by the end of the experiment.

4.2.2.1 Simultaneous Application of Sonication and Chlorination at 180 W/L

Selected results from the simultaneous application of sonication at 180 W/L and chlorination at 0.6 mg/L are shown in Figure 10. It should be noted that the chlorine only results presented are for room temperature (22 – 23°C) chlorination. The temperature effect was not significant in this case as sonication for 5 minutes at 180 W/L did not cause the temperature to rise substantially. Temperature rose up to approximately 39°C after 5 minutes of simultaneous disinfection at 180 W/L, this increase in temperature did not significantly impact the effect of chlorine on the inactivation of *E. coli*, with regard to Section 4.0.2. For all disinfection times tested, inactivation of *E. coli* by simultaneous sonication and chlorine was greater than what would be predicted based on the additive effect of sonication only and chlorination only. For example, 2 minutes of sonication alone achieved 0.16 $\log_{10}$ inactivation and 2 minutes of chlorination at 0.6 mg/L achieved 1.28 $\log_{10}$ inactivation of *E. coli*. When applied simultaneously, 5.10 $\log_{10}$ inactivation was achieved (compared to 1.44 $\log_{10}$ inactivation predicted if the results were additive). As a result, sonication provided a synergistic effect in the inactivation of *E. coli* when sonication and chlorination were applied simultaneously. It is possible that sonication

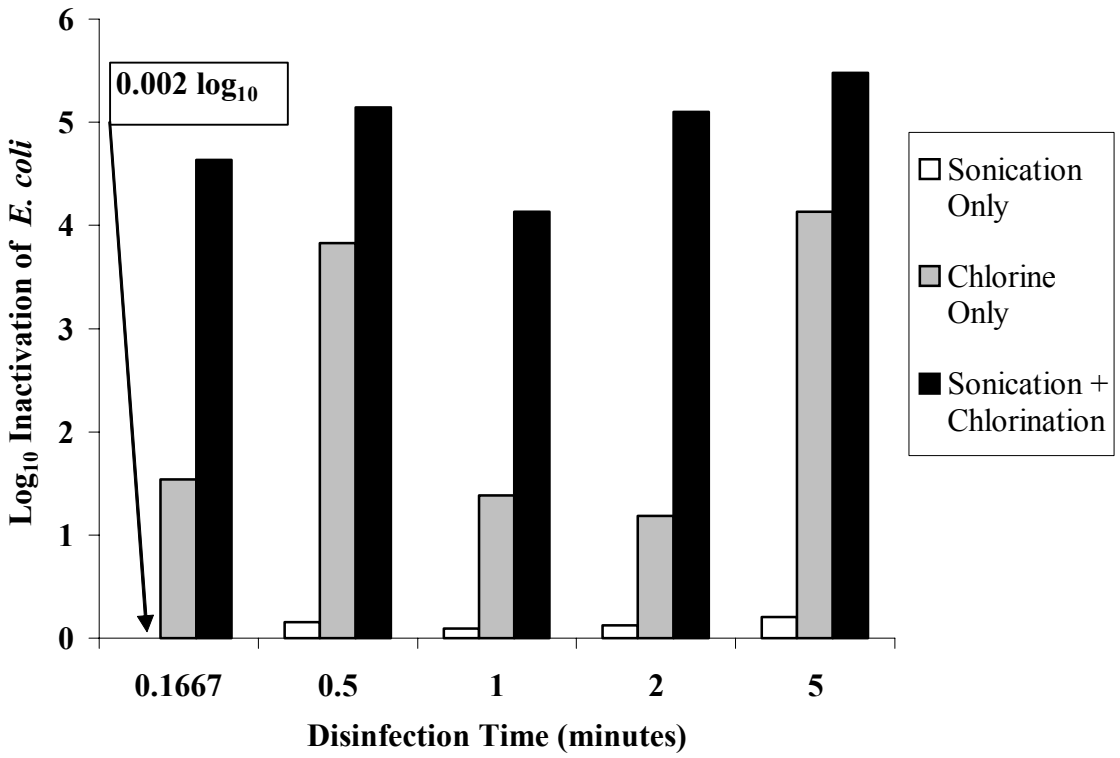

Figure 10. Log_{10} inactivation of *E. coli* from simultaneous application of sonication and chlorination (Sonication power-to-volume ratio: 180 W/L; probe system; chlorine dose 0.6 mg/L)

stressed the organisms, hence they were more susceptible to chlorine. For the 5 minute contact time, chlorine alone was already effective (4.13 log_{10} inactivation), therefore the synergistic effect of sonication and chlorination was not as pronounced as for the 1 and 2 minute contact times.

4.2.2.2 Simultaneous Application of Sonication and Chlorination at 900 W/L

Figure 11 shows the $\log_{10}$ inactivation of *E. coli* when it was subjected to sonication only at 900 W/L, chlorine only at 0.6 mg/L (at room temperature), and the simultaneous application of sonication at 900 W/L and chlorination at 0.6 mg/L. Results are similar to the results shown for simultaneous disinfection using 180 W/L sonication, as the synergistic effect of sonication can also be seen at this high power combination. For example at 2 minutes, 0.53 $\log_{10}$ inactivation of *E. coli* was achieved by sonication only at 900 W/L, 1.28 $\log_{10}$ inactivation of *E. coli* was obtained by chlorination only at 0.6 mg/L, but 4.5 $\log_{10}$ inactivation was recorded with simultaneous disinfection.

The shorter disinfection time periods chosen (10 seconds, 30 seconds, 1, 2, and 3 minutes) were used because the heating effect of sonication at 900 W/L was not yet significant. After 2 minutes of simultaneous disinfection at 900 W/L, the temperature rose to an average of 37.7^{o}C. At this temperature, the effects of ultrasonic waves predominate over the effects of heating alone, as shown in Section 4.3.3 and inactivation is minimal (approximately 0.1 $\log_{10}$). With reference to Figure 3 (chorination alone at room and elevated temperatures), a temperature of 39^{o}C did not have a significant effect on *E. coli* inactivation by chlorination alone for 2 minutes. It can be concluded that the temperature rise after 2 minutes of simultaneous disinfection at the high power-to-volume ratio did not significantly affect the germicidal efficiency of chlorine at the fixed dose of 0.6 mg/L. Therefore, ultrasonic waves, and not heating, resulted in synergistic effects with chlorine.

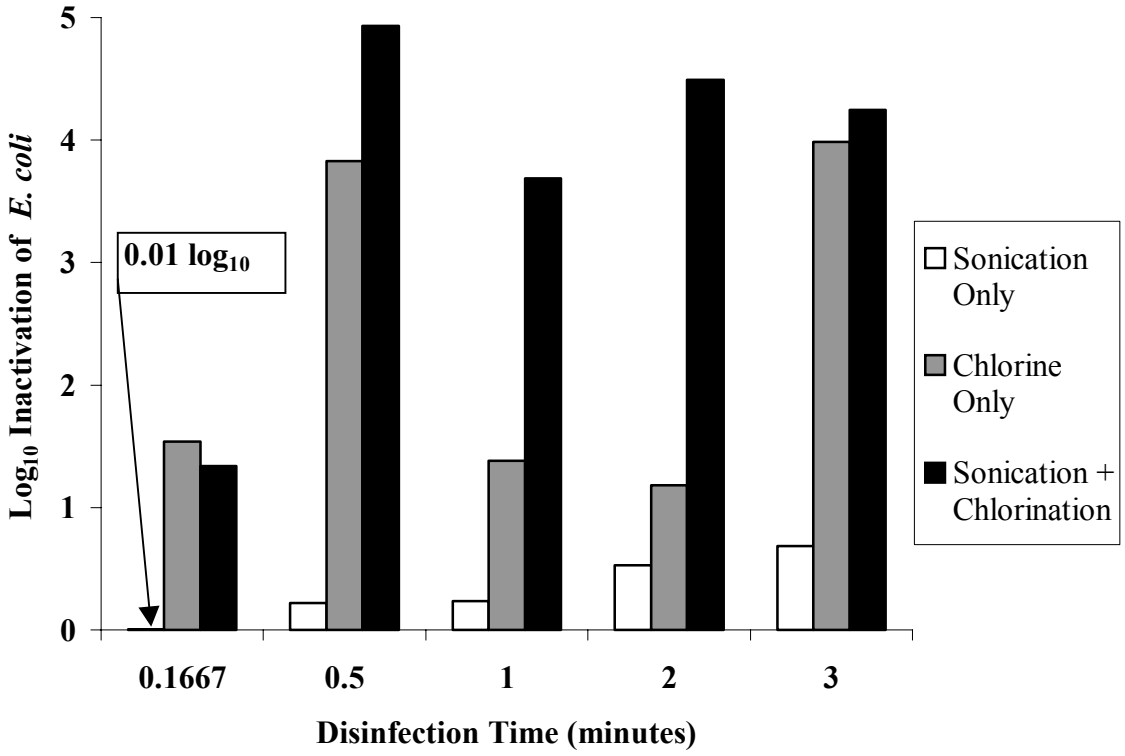

Figure 11. Log_{10} inactivation of *E. coli* from simultaneous application of sonication and chlorination (Sonication power-to-volume ratio: 900 W/L; probe system; chlorine dose 0.6 mg/L)

4.3 TEMPERATURE EFFECTS ON INACTIVATION

The purpose of performing heating alone experiments was to understand the effects of temperature on the inactivation of *E. coli*. Sonication using the probe system, particularly at 900 W/L, caused the temperature of the sample to rise up dramatically within a short period of time. It was uncertain whether the physical effect of the ultrasonic waves or the elevated temperature inactivated the *E. coli*. Results from the heating alone experiments provided means to separate the effects of sonication and temperature.

4.3.1 Temperature Profile Over Time in E-pure Water

Before investigating the effect of temperature on *E. coli*, an experiment was done using a water bath to attempt to mimic the temperature rise of E-pure water that was observed with the sonic probe. The water bath was warmed up to 80^{o}C, then a 250-mL media bottle containing 100 mL of E-pure water was placed in the center of the water bath. The temperature of E-pure water inside the media bottle was recorded over time.

Table 9 shows temperatures from the heating only experiment with E-pure water compared to the temperatures observed during high power sonication using the probe system. The temperatures due to heating only matched with the ones due to sonication only, in terms of the temperature rise pattern and the temperature values. It was also observed that E-pure water heated up slightly faster in the first 5 minutes than the *E. coli* solution, then rate of heating slowed down after that.

Table 9. Comparison of temperatures due to heating only and sonication only at 900 W/L. (preliminary experiment with E-pure water)

Time (minutes)	Temperature ^{o}C (Heating Only) with E-pure Water)	Temperature ^{o}C (Sonication Only, 900 W/L, probe)
1	35.0	31.7
2	44.5	38.9
3	52.0	47.4
5	63.0	59.3
10	71.5	77.0

4.3.2 Temperature Profile Over Time in *E. coli*

An experiment was performed to study the temperature effects on *E. coli* compared to sonication alone at 900 W/L. *E. coli* was spiked into PBS test water and the experiment was carried out in an 80^{o}C water bath. Three replicates were conducted for this experiment. The pattern and extent of temperature increase of the *E. coli* solution matched with that of the sonication only experiments, as illustrated below in Table 10.

Table 10. Comparison of temperatures due to heating only and sonication only at 900 W/L. (*E. coli* in PBS)

Time (minutes)	Temperature ^{o}C (Heating Only with *E. coli*)	Temperature ^{o}C (Sonication Only, 900 W/L, probe)
1	30.2	31.7
2	41.3	38.9
3	50.7	47.4
5	62.7	59.3
10	72.2	77.0

Figure 12 shows the $\log_{10}$ inactivation of *E. coli* due to heating alone versus time. In terms of *E. coli* inactivation, less than one $\log_{10}$ of reduction was achieved at temperature below 60^{o}C, which corresponded to the first 4 minutes of the experiment. At temperatures over 60^{o}C (5 - 10 minutes), the $\log_{10}$ inactivation of *E. coli* increased sharply and counts were at or below detection limits.

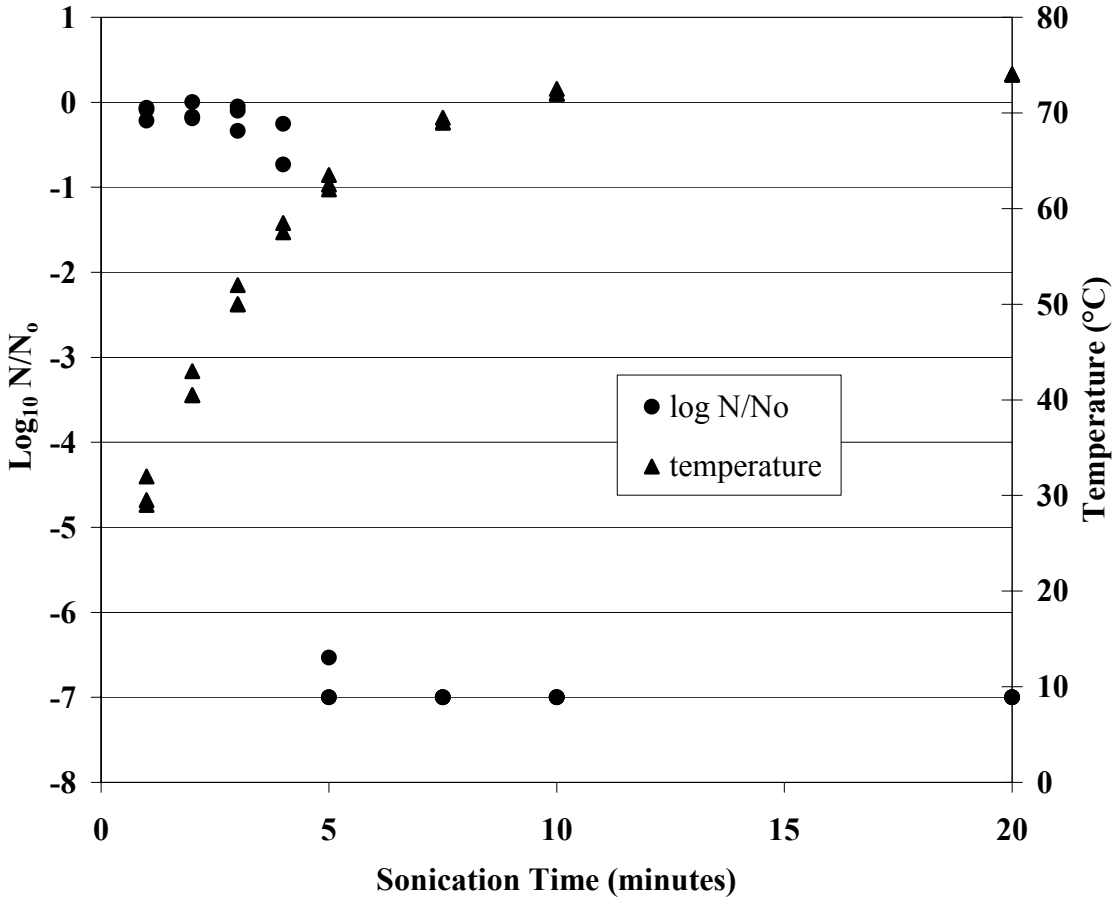

Figure 12. Effects of temperature on *E. coli* inactivation with respect to time.

4.3.3 Comparison of Inactivation by Sonication Versus Heating Only

Figure 13 compares the $\log_{10}$ inactivation of *E. coli* when it was subjected to heating alone versus sonication using the probe system at 900 W/L. The data shown in the Figure 13 are the average values of the results from the replicate experiments. The temperature profiles for heating only and sonication matched fairly closely. At temperatures lower than 60°C and when the disinfection time was 4 minutes or shorter,

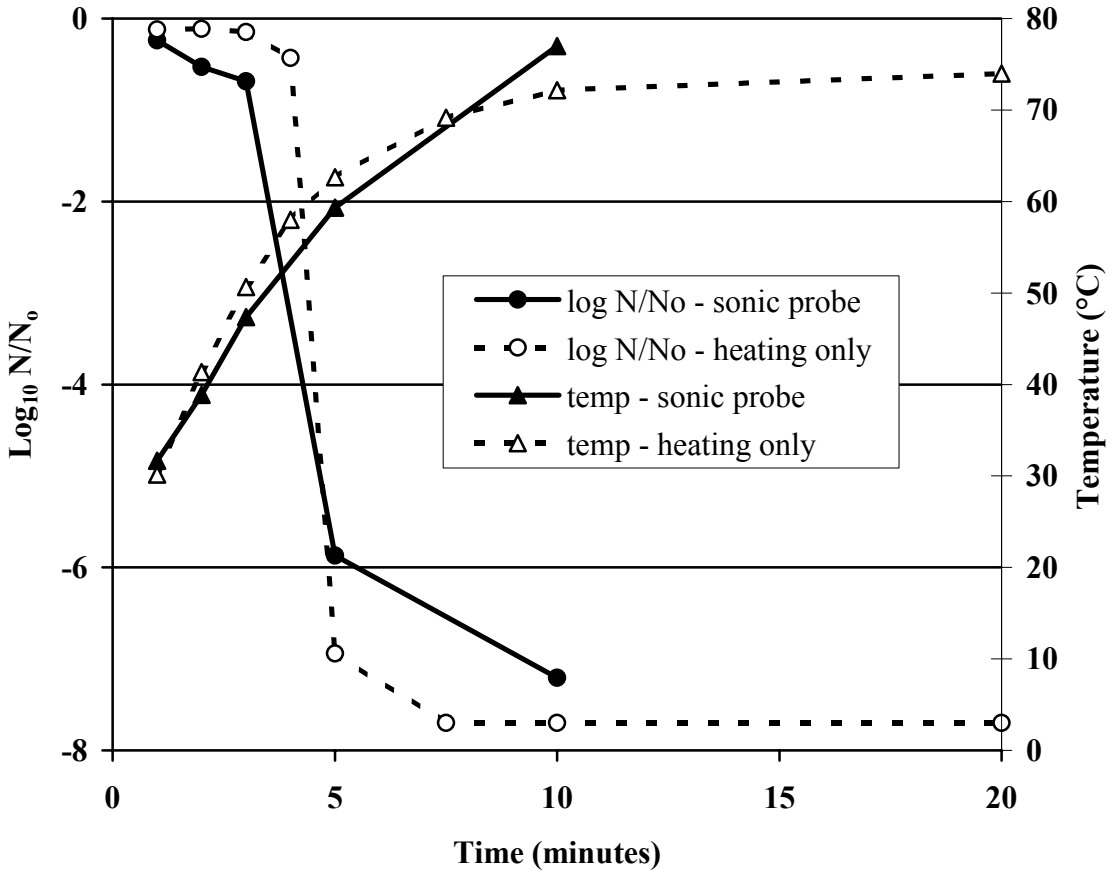

Figure 13. Comparison of temperature and $\log_{10}$ inactivation of *E. coli* by heating alone and sonication alone at 900 W/L.

sonication had a small effect on the inactivation of *E. coli*. Sonication provided about 0.3 $\log_{10}$ inactivation of *E. coli* while heating alone resulted in approximately 0.2 $\log_{10}$ inactivation. However, at temperatures higher than 60°C and disinfection time of 5 minutes or above, the temperature effect became significant, and *E. coli* was inactivated mainly due to heating.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.0 CONCLUSIONS

This research studied the inactivation of *E. coli* by chlorination, sonication, and combined disinfection (sonication and chlorination). Chlorine only experiments were conducted with various chlorine dosages, chlorine contact times, and starting temperatures. Sonication only experiments were performed using either a sonication probe or a sonic bath system. Two power-to-volume ratios were tested with a wide range of sonication times. For the combined sonication and chlorination experiments, the disinfectants were applied both sequentially and simultaneously using the probe system. Based on the experimental parameters tested in this study, the conclusions are as follows:

1. Under room temperature conditions and a 5-minute contact time, the inactivation of *E. coli* by chlorine increased with chlorine dose. Less than 1 $\log_{10}$ of inactivation was achieved with chlorine doses of 0.2 and 0.4 mg/L. When chlorine concentration was raised to 0.6 mg/L or greater, approximately 5 $\log_{10}$ inactivation of *E. coli* was achieved.

2. The data for fixed chlorine dose at 0.6 mg/L with varying contact times were inconsistent, therefore a definite conclusion could not be drawn.

3. Elevated temperatures (32^{o}C and 39^{o}C) did not significantly affect the $\log_{10}$ inactivation of *E. coli* by chlorine compared to the inactivation at room temperature.

4. The sonication probe system was more effective at inactivating *E. coli* than the sonic bath system. The sonic bath achieved less than 1 $\log_{10}$ inactivation of *E. coli* even with longest sonication time tested (60 minutes for 180 W/L; 50 minutes for 900 W/L). Therefore, the sonication probe system was used for in-depth sonication only studies as well as the combined sonication and chlorination experiments.

5. At both 180 W/L and 900 W/L, sonication alone with the probe system achieved inactivation over time. At 180 W/L, less than 1 $\log_{10}$ inactivation of *E. coli* was recorded within the first 20 minutes of sonication, but significant inactivation (greater than 7.5 $\log_{10}$) was achieved from 30 to 60 minutes. At 900 W/L, inactivation occurred more quickly, with greater than 7.5 $\log_{10}$ inactivation for 5 to 10 minutes of sonication time.

6. A substantial increase in sample temperature over time was recorded for both 180 W/L and 900 W/L sonication (probe system). Temperature increased at a faster rate at the high power-to-volume ratio. It took only 5 minutes for the *E. coli* samples to reach the pasteurization temperature (approximately 60^{o}C) after sonicating at the high power-to-volume ratio, compared to 30 minutes at the low power-to-volume ratio.

7. Turbidity increased substantially over time when sonication was applied. Similar to the temperature increase, the high power sonication caused the turbidity of the *E. coli*

sample solutions to rise much faster than low power sonication. After sonicating for 10 minutes at 180 W/L, the average final turbidity was approximately 7 NTU. Given the same duration of sonication at 900 W/L, the average final turbidity was 23 NTU.

8. Heating experiments showed that the inactivation of *E. coli* by sonication was primarily due to heating rather than the effect of ultrasonic waves.

9. Sequential application of sonication and chlorination was found to be ineffective, as chlorine alone provided more *E. coli* inactivation than the combination of two disinfectants.

10. The synergistic effect of sonication was observed when sonication and chlorination were applied simultaneously at both 180 W/L and 900 W/L using the sonication probe system. The resulting *E. coli* inactivation after simultaneous application of sonication and chlorination was greater than the additive inactivation achieved by chlorine alone and sonication alone.

11. Disinfection by sonication alone is not practical due to the long time or high power output required. However, simultaneous application of sonication and chlorine achieved significantly higher inactivation levels than chlorination alone. Thus, lower chlorine doses can be used to achieve the same inactivation. Ultrasound as a synergistic disinfectant has the potential to reduce the amount of chlorine used and the amount of halogenated disinfection by-products formed during chlorination.

5.1 RECOMMENDATIONS

Due to the limited time frame of this research, it is recommended that additional research on sonication is conducted. Specific recommendations are as follows.

1. Water obtained from raw water sources should be used to study the effects of natural organic matter on the disinfection methods carried out in this research.

2. More chlorination alone experiments are needed as the results were inconsistent.

3. Using a sonication probe system, other sonication frequencies should be tested to determine the most effective frequency for the simultaneous application of sonication and chlorination experiments to maximize the synergistic effects of sonication.

4. A cost analysis of sonication, whether ultrasound serves as a sole or synergistic disinfectant, is strongly recommended. This analysis should include capital costs and electricity costs based on the duration and intensity of sonication needed for a particular application.

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APPENDIX A

RESULTS FOR ALL EXPERIMENTS

Chlorination of *E. coli*
Performed in 300 mL BOD bottles

Exp #	Chlorine (mg/L)	Time (min)	Pre count (cfu/ml)	Post count (cfu/ml)	% inact.	$\log_{10}$ inact.	$-\log_{10} n/n_o$	Initial Temp (^oC)	Final Temp (^oC)	Initial Turb (NTU)	Final Turb (NTU)	AVERAGE % inact.	AVERAGE $\log_{10}$ inact.
16	0.2	5	2.53E+07	3.39E+07	-33.9921	-0.127	0.127	missed	missed	2.40	missed		
30	0.2	5	3.30E+07	2.06E+07	37.5758	0.205	-0.205	21.5	22.0	2.23	2.01		
31	0.2	5	2.23E+07	6.47E+06	70.9865	0.537	-0.537	21.5	21.5	2.08	1.95	24.857	0.124
17	0.4	5	3.47E+07	1.31E+07	62.2478	0.423	-0.423	missed	missed	2.44	missed		
33	0.4	5	3.77E+07	7.90E+06	79.0451	0.679	-0.679	22.0	22.0	2.59	1.99		
34	0.4	5	4.23E+07	5.03E+06	88.1087	0.925	-0.925	22.0	22.0	2.31	1.82	76.467	0.628
23	0.54	5	4.83E+07	3.03E+04	99.9373	3.203	-3.203	22.0	22.0	1.95	1.56		
24	0.54	5	6.17E+07	6.43E+04	99.8958	2.982	-2.982	22.0	22.0	2.05	1.68	99.917	3.078
10	0.6	5	7.77E+07	3.00E+01	100.0000	6.413	-6.413	missed	missed	2.30	missed		
11	0.6	5	2.56E+07	3.87E+01	99.9998	5.821	-5.821	missed	missed	2.19	missed		
35	0.6	5	4.23E+07	9.23E+03	99.9782	3.661	-3.661	22.0	22.0	2.17	1.86	99.993	4.134
8	1	5	7.23E+07	1.61E+02	99.9998	5.652	-5.652	missed	missed	2.28	missed		
9	1	5	5.97E+08	4.70E+00	100.0000	8.104	-8.104	missed	missed	2.17	missed		
36	1	5	3.43E+07	5.00E+02	99.9985	4.836	-4.836	22.0	22.0	2.12	2.55	99.999	5.252

Exp #	Chlorine (mg/L)	Time (min)	Pre count (cfu/ml)	Post count (cfu/ml)	% inact.	$\log_{10}$ inact.	$-\log_{10} n/n_o$	Initial Temp (^oC)	Final Temp (^oC)	Initial Turb (NTU)	Final Turb (NTU)	AVERAGE % inact.	AVERAGE $\log_{10}$ inact.
129	0.6	0.167	2.64E+07	1.21E+06	95.417	1.339	-1.339	22.0	22.0	2.47	1.56		
130	0.6	0.167	2.31E+07	3.60E+03	99.984	3.807	-3.807	22.0	22.0	2.44	1.85		
131	0.6	0.167	1.80E+07	7.33E+05	95.928	1.390	-1.390	22.5	22.5	2.44	1.75		
171	0.6	0.167	3.97E+07	1.14E+03	99.997	4.542	-4.542	22.0	22.0	1.99	1.43	97.831	1.664
126	0.6	0.5	2.05E+07	2.97E+03	99.986	3.839	-3.839	22.5	22.5	2.89	1.83		
127	0.6	0.5	1.79E+07	3.00E+03	99.983	3.776	-3.776	22.5	22.5	2.78	1.78		
128	0.6	0.5	2.38E+07	3.10E+03	99.987	3.885	-3.885	22.5	22.5	2.81	1.71		
172	0.6	0.5	5.17E+07	3.60E+02	99.999	5.157	-5.157	22.0	22.0	2.03	2.43	99.989	3.949
105	0.6	1	2.83E+07	3.23E+06	88.587	0.943	-0.943	22.0	22.0	2.17	1.80		
107	0.6	1	2.40E+07	2.43E+05	98.988	1.995	-1.995	21.0	21.0	2.45	1.68		
108	0.6	1	2.18E+07	4.17E+03	99.981	3.718	-3.718	21.0	21.0	2.43	1.62		
173	0.6	1	3.60E+07	5.33E+01	100.000	5.830	-5.830	22.0	22.0	1.86	1.47	96.889	1.507
109	0.6	2	2.35E+07	9.10E+04	99.613	2.412	-2.412	21.0	21.5	2.33	1.57		
110	0.6	2	2.83E+07	2.45E+04	99.913	3.063	-3.063	20.0	20.0	1.89	1.49		
112	0.6	2	2.93E+07	6.07E+06	79.283	0.684	-0.684	20.0	20.5	1.83	1.55		
174	0.6	2	3.40E+07	4.37E+03	99.987	3.891	-3.891	22.0	22.0	1.90	1.52	94.699	1.276
123	0.6	3	2.53E+07	5.77E+03	99.977	3.642	-3.642	22.0	22.0	2.10	1.66		
124	0.6	3	2.63E+07	1.29E+03	99.995	4.310	-4.310	22.0	22.0	1.93	1.57		
125	0.6	3	2.32E+07	8.07E+02	99.997	4.459	-4.459	22.0	22.0	1.85	1.58	99.990	3.983
10	0.6	5	7.77E+07	3.00E+01	100.000	6.413	-6.413	missed	missed	2.30	missed		
11	0.6	5	2.56E+07	3.87E+01	100.000	5.821	-5.821	missed	missed	2.19	missed		
35	0.6	5	4.23E+07	9.23E+03	99.978	3.661	-3.661	22.0	22.0	2.17	1.86	99.993	4.134
154	0.6	1	2.06E+07	1.63E+06	92.087	1.102	-1.102	32.0	30.0	1.76	1.66		
155	0.6	1	2.20E+07	4.00E+03	99.982	3.740	-3.740	32.0	30.0	1.76	1.56		
156	0.6	1	1.80E+07	4.43E+06	75.389	0.609	-0.609	32.0	30.0	1.77	1.65		
175	0.6	1	2.96E+07	1.50E+04	99.949	3.295	-3.295	32.0	31.0	1.91	1.78	91.852	1.089
157	0.6	2	1.72E+07	1.00E+04	99.942	3.236	-3.236	39.0	37.0	1.54	1.76		
158	0.6	2	2.25E+07	1.66E+06	92.622	1.132	-1.132	39.0	37.0	2.29	2.02		
159	0.6	2	1.69E+07	2.22E+06	86.864	0.882	-0.882	39.0	36.5	1.37	1.65		
176	0.6	2	6.33E+07	1.21E+04	99.981	3.719	-3.719	39.0	35.5	1.71	1.76	94.852	1.288

Sonication of *E. coli*
Low power to volume ratio (500 mL volume, highest power setting - 10)
Probe system
Bold numbers indicate no counts - detection limit value substituted

Exp #	Sonic Time (min)	Pre count (cfu/ml)	Post count (cfu/ml)	% inact.	$\log_{10}$ inact.	- $\log_{10}$ n/n$_o$	Initial Temp (^{o}C)	Final Temp (^{o}C)	Initial Turb (NTU)	Final Turb (NTU)	Output Power (W)	AVERAGE % inact.	AVERAGE $\log_{10}$ inact.	AVERAGE Final Turb.	AVERAGE Final Temp.
136	0.1667	3.67E+07	2.17E+07	40.872	0.228	-0.228	22.5	22.0	1.87	missed	93				
137	0.1667	2.21E+07	3.77E+07	-70.588	-0.232	0.232	22.5	22.0	1.61	missed	96				
138	0.1667	2.71E+07	1.86E+07	31.365	0.163	-0.163	22.5	22.0	1.80	missed	96-93	0.550	0.002	missed	22.0
136	0.5	3.67E+07	1.75E+07	52.316	0.322	-0.322	22.5	23.0	1.87	2.16	93				
137	0.5	2.21E+07	1.62E+07	26.697	0.135	-0.135	22.5	23.5	1.61	1.95	96-93				
138	0.5	2.71E+07	2.39E+07	11.808	0.055	-0.055	22.5	23.5	1.80	2.22	93	30.274	0.157	2.11	23.3
13	1	8.40E+07	4.03E+07	52.024	0.319	-0.319	24.0	26.5	2.18	2.19	96				
25	1	3.45E+07	1.83E+07	46.957	0.275	-0.275	20.0	22.0	2.00	missed	90				
18	1	2.09E+07	2.15E+07	-2.871	-0.012	0.012	25.5	28.0	2.05	2.01	90				
19	1	2.59E+07	3.04E+07	-17.375	-0.070	0.070	25.0	26.5	2.29	2.26	90				
20	1	3.31E+07	2.99E+07	9.668	0.044	-0.044	26.0	29.0	2.26	2.16	missed	17.681	0.084	2.16	26.4
14	2	3.53E+07	2.12E+07	39.943	0.221	-0.221	24.5	28.0	2.31	2.08	93				
25	2	3.45E+07	3.11E+07	9.855	0.045	-0.045	20.0	24.0	2.00	missed	90				
160	2	2.30E+07	1.31E+07	43.043	0.244	-0.244	23.0	26.0	2.34	3.38	93-90	30.947	0.161	2.73	26.0
15	5	2.98E+07	1.74E+07	41.611	0.234	-0.234	24.0	27.5	2.17	1.67	93				
21	5	2.51E+07	1.70E+07	32.271	0.169	-0.169	22.0	31.0	2.60	1.75	missed				
22	5	2.52E+07	2.45E+07	2.778	0.012	-0.012	22.0	32.0	1.96	1.54	missed				
37	5	3.83E+07	2.44E+07	36.292	0.196	-0.196	21.0	30.0	2.03	4.46	84				
25	5	3.45E+07	1.20E+07	65.217	0.459	-0.459	20.0	29.0	2.00	missed	93	35.634	0.191	2.36	29.9
25	10	3.45E+07	1.35E+07	60.870	0.407	-0.407	20.0	37.0	2.00	missed	90-81				
44	10	3.73E+07	1.33E+07	64.343	0.448	-0.448	23.0	41.0	2.16	7.22	84-81				
45	10	4.17E+07	1.51E+07	63.789	0.441	-0.441	23.0	41.0	2.01	7.07	84-76	63.001	0.432	7.15	39.7
25	15	3.45E+07	1.36E+07	60.580	0.404	-0.404	20.0	44.0	2.00	missed	81-78				
38	15	3.97E+07	6.23E+06	84.307	0.804	-0.804	21.0	45.0	2.23	missed	87-78				
39	15	2.60E+07	4.87E+06	81.269	0.727	-0.727	21.5	45.0	2.05	missed	84-75	75.385	0.609	missed	44.7
25	20	3.45E+07	8.33E+06	75.855	0.617	-0.617	20.0	50.0	2.00	missed	78-75				
38	20	3.97E+07	2.97E+06	92.519	1.126	-1.126	21.0	51.0	2.23	13.00	78-75				
39	20	2.60E+07	2.96E+06	88.615	0.944	-0.944	21.5	51.0	2.05	11.60	75	85.663	0.844	12.30	50.7
25	30	3.45E+07	**1.00E+01**	**100.000**	**6.538**	**-6.538**	20.0	59.0	2.00	missed	72-69				
161	30	2.51E+07	9.33E+00	100.000	6.430	-6.430	23.0	64.0	2.18	18.50	90-75				
162	30	1.94E+07	5.00E+00	100.000	6.589	-6.589	23.0	64.0	3.72	18.70	90-72	100.000	6.514	18.60	62.3
25	40	3.45E+07	**1.00E+00**	**100.000**	**7.538**	**-7.538**	20.0	66.0	2.00	missed	69-66				
167	40	2.13E+07	**1.00E+00**	**100.000**	**7.328**	**-7.328**	22.0	69.0	2.20	21.90	87-63				
168	40	3.53E+07	**1.00E+00**	**100.000**	**7.548**	**-7.548**	22.0	70.0	2.44	21.90	81-63	100.000	7.459	21.90	68.3
25	50	3.45E+07	**1.00E+00**	**100.000**	**7.538**	**-7.538**	20.0	70.0	2.00	missed	66-60				
163	50	4.83E+07	6.70E-03	100.000	9.858	-9.858	22.0	72.0	2.26	25.70	90-66				
166	50	3.23E+07	1.30E-02	100.000	9.395	-9.395	22.0	71.0	2.15	21.70	84-57	100.000	8.007	23.70	71.0
25	60	3.45E+07	**1.00E+00**	**100.00**	**7.538**	**-7.538**	20.0	72.0	2.00	missed	60-57				
164	60	2.35E+07	**1.00E+00**	**100.00**	**7.371**	**-7.371**	22.0	75.0	2.86	28.80	84-60				
165	60	2.80E+07	**1.00E+00**	**100.00**	**7.447**	**-7.447**	22.0	75.0	2.01	22.20	84-57	100.000	7.447	25.50	74.0

Sonication + Chlorination of *E. coli*
Low power to volume ratio (500 mL volume, highest power setting - 10)
Probe System

Sequential

	Sonic	Chlorine	Pre count	Initial	Initial	Post sonic	Post sonic	Post sonic	Post S+C	Post S+C	Post S+C	Sonic Only		Sonic + Chlorine		Average - S+C	
Exp #	Time (min)	(mg/L)	(cfu/mL)	Temp (^oC)	Turb (NTU)	count (cfu/mL)	Temp (^oC)	Turb (NTU)	count (cfu/mL)	Temp (^oC)	Turb (NTU)	% inact.	log_{10} inact.	% inact.	log_{10} inact.	% inact.	log_{10} inact.
18	1	0.4	2.09E+07	25.5	2.05	2.15E+07	28.0	2.01	1.57E+07	25.5	1.86	-2.871	-0.012	24.8804	0.124		
19	1	0.2	2.59E+07	25.0	2.29	3.04E+07	26.5	2.26	2.23E+07	24.0	2.29	-17.375	-0.070	13.8996	0.065		
20	1	0.6	3.31E+07	26.0	2.26	2.99E+07	29.0	2.16	TNTC	25.0	1.61	9.668	0.044	-	-	-	-
21	5	0.54	2.51E+07	22.0	2.60	1.70E+07	31.0	1.75	3.03E+04	missed	1.35	32.271	0.169	99.8793	2.918		
22	5	0.54	2.52E+07	22.0	1.96	2.45E+07	32.0	1.54	2.79E+06	28.0	1.45	2.778	0.012	88.9286	0.956		
37	5	0.54	3.83E+07	21.0	2.03	2.44E+07	30.0	4.46	7.77E+03	26.5	3.73	36.292	0.196	99.9797	3.693	96.2625	1.427

Simultaneous

	Sonic	Chlorine	Pre count	Initial	Initial	Post count	Post	Post	Output					Average		AVERAGE	
Exp #	Time (min)	(mg/L)	(cfu/mL)	Temp (^oC)	Turb (NTU)	count (cfu/mL)	Temp (^oC)	Turb (NTU)	Power (W)	% inact.	log_{10} inact.	$-log_{10} n/n_o$	% inact.	log_{10} inact.	Final Turb.	Final Temp.
133	0.1667	0.6	3.23E+07	22.5	2.35	1.23E+03	23.0	1.84	missed	99.996	4.418	-5.473				
134	0.1667	0.6	1.87E+07	22.5	2.38	4.53E+02	23.0	1.66	missed	99.998	4.615	-5.473				
141	0.1667	0.6	4.70E+07	22.0	1.92	3.07E+01	23.0	1.54	90	100.000	6.185	-5.473	99.998	4.677	1.68	23.0
135	0.5	0.6	2.26E+07	22.5	2.27	3.13E+02	23.5	1.63	missed	99.999	4.859	-5.473				
139	0.5	0.6	3.27E+07	22.0	2.16	1.62E+02	23.0	1.83	96-93	100.000	5.305	-5.473				
140	0.5	0.6	3.10E+07	22.0	1.94	8.97E+01	23.0	1.84	90	100.000	5.539	-5.473	99.999	5.141	1.77	23.2
89	1	0.6	2.74E+07	21.0	2.22	9.23E+01	23.0	2.12	90-87	100.000	5.473	-5.473				
90	1	0.6	3.47E+07	21.5	2.22	3.37E+01	23.5	2.12	90-87	100.000	6.013	-6.013				
91	1	0.6	2.72E+07	21.5	2.29	6.60E+03	23.5	2.24	87	99.976	3.615	-3.661	99.992	4.084	2.16	23.3
77	2	0.4	2.34E+07	20.0	2.10	1.50E+07	23.5	3.01	90-87	35.897	0.193	-0.193				
78	2	0.4	2.49E+07	20.0	2.10	1.56E+07	24.0	3.03	87-84	37.349	0.203	-0.203				
88	2	0.4	3.03E+07	20.0	2.15	4.97E+06	22.5	2.72	87-84	83.597	0.785	-0.785	52.281	0.321	2.92	23.3
79	2	0.6	2.29E+07	22.0	2.17	4.33E+02	26.5	2.65	90-87	99.998	4.723	-4.723				
83	2	0.6	2.42E+07	20.0	2.24	6.83E+01	24.5	2.67	87	100.000	5.549	-5.549				
84	2	0.6	2.38E+07	20.0	2.18	4.87E+01	24.0	2.47	87	100.000	5.689	-5.689	99.999	5.101	2.60	25.0
82	2	1.0	2.78E+07	20.0	2.15	1.77E+02	23.5	3.19	90	99.999	5.195	-5.195				
86	2	1.0	2.38E+07	20.0	2.26	4.63E+01	23.5	2.59	93-90	100.000	5.711	-5.711				
87	2	1.0	2.47E+07	20.0	2.14	1.87E+00	23.0	3.03	87-84	100.000	7.121	-7.121	100.000	5.553	2.94	23.3
92	3	0.6	2.27E+07	21.5	2.28	8.80E+01	27.5	3.06	87-84	100.000	5.412	-5.412				
93	3	0.6	3.00E+07	22.5	2.16	2.02E+02	28.5	3.01	90-87	99.999	5.172	-5.100				
94	3	0.6	2.67E+07	22.5	2.14	3.90E+02	28.5	3.07	87	99.999	4.835	-4.835	99.999	5.076	3.05	2802.0
95	5	0.6	2.46E+07	22.5	2.17	2.17E+02	32.0	4.27	90-84	99.999	5.054	-5.054				
97	5	0.6	2.40E+07	20.0	1.99	9.67E+00	28.0	3.89	87-84	100.000	6.395	-6.395				
98	5	0.6	2.23E+07	20.0	1.95	1.73E+01	28.5	3.80	87-84	100.000	6.110	-6.110	100.000	5.477	3.99	29.5
96	10	0.6	2.84E+07	22.5	2.14	2.43E+01	40.5	6.38	87-84	100.000	6.068	-6.068				
99	10	0.6	4.47E+07	22.0	2.03	2.03E+02	39.5	6.86	90-84	100.000	5.342	-5.342				
100	10	0.6	4.07E+07	22.0	2.04	3.83E+01	39.5	6.50	87-81	100.000	6.026	-6.026	100.000	5.675	6.58	39.8

Sonication of *E. coli*
High power to volume ratio (100 mL volume, highest power setting - 10)
Probe System
Bold numbers indicate no counts - detection limit value substituted

Exp #	Sonic Time (min)	Pre count (cfu/mL)	Post count (cfu/mL)	% inact.	$\log_{10}$ inact.	$-\log_{10} n/n_o$	Initial Temp (°C)	Final Temp (°C)	Initial Turb (NTU)	Final Turb (NTU)	Output Power (W)	AVERAGE % inact.	AVERAGE $\log_{10}$ inact.	AVERAGE Temp (°C)	AVERAGE Turb (NTU)
142	0.1667	3.40E+07	2.58E+07	24.118	0.120	-0.120	22.5	24.0	2.04	2.57	93				
143	0.1667	2.02E+07	2.00E+07	0.990	0.004	-0.004	22.5	24.0	2.02	2.49	93				
144	0.1667	2.13E+07	2.57E+07	-20.657	-0.082	0.082	22.5	24.0	2.00	2.54	93	1.483	0.0065	24.0	2.53
145	0.5	3.07E+07	1.83E+07	40.391	0.225	-0.225	22.0	27.0	1.96	3.74	93-90				
146	0.5	2.23E+07	1.91E+07	14.350	0.067	-0.067	22.0	27.0	2.09	3.94	93-90				
147	0.5	4.07E+07	1.46E+07	64.128	0.445	-0.445	22.0	27.0	2.21	3.84	90	39.623	0.2191	27.0	3.84
26	1	1.02E+08	3.14E+07	69.216	0.512	-0.512	22.0	31.0	2.50	4.80	87				
42	1	2.25E+07	1.95E+07	13.333	0.062	-0.062	23.0	32.0	2.25	5.53	87-84				
43	1	2.66E+07	1.50E+07	43.609	0.249	-0.249	23.0	32.0	2.26	5.62	84	42.053	0.2370	31.7	5.32
26	2	1.02E+08	1.69E+07	83.431	0.781	-0.781	22.0	38.0	2.50	7.40	87				
28	2	6.23E+07	1.99E+07	68.058	0.496	-0.496	23.0	39.5	2.39	8.86	87-84				
29	2	5.77E+07	1.37E+07	76.256	0.624	-0.624	22.0	40.0	2.14	7.82	87-84				
51	2	2.68E+07	7.63E+06	71.530	0.546	-0.546	22.0	39.5	1.89	7.58	90-87				
52	2	5.43E+07	1.14E+07	79.006	0.678	-0.678	22.0	39.0	2.20	7.91	90-87				
53	2	2.40E+07	1.14E+07	52.500	0.323	-0.323	22.0	38.0	2.01	7.90	90				
54	2	2.27E+07	9.43E+06	58.458	0.382	-0.382	22.0	38.0	1.85	7.35	90				
55	2	2.86E+07	8.10E+06	71.678	0.548	-0.548	23.0	40.0	1.79	7.35	87-84				
56	2	3.50E+07	7.63E+06	78.200	0.662	-0.662	21.5	38.0	2.32	8.06	90-87				
57	2	2.77E+07	9.73E+06	64.874	0.454	-0.454	22.0	38.5	2.57	8.24	90-87	70.399	0.5287	38.9	7.85
26	3	1.02E+08	1.41E+07	86.176	0.859	-0.859	22.0	45.0	2.50	10.60	87				
40	3	1.75E+07	5.33E+06	69.543	0.516	-0.516	22.0	49.0	2.51	8.87	87-81				
41	3	2.45E+07	4.90E+06	80.000	0.699	-0.699	24.0	50.0	2.19	10.50	87-81				
58	3	3.40E+07	6.17E+06	81.853	0.741	-0.741	21.5	45.5	2.20	10.40	90-87	79.393	0.6860	47.4	10.09
46	5	5.20E+07	1.56E+02	100.000	5.523	-5.523	21.0	58.0	2.08	13.10	90-78				
48	5	2.88E+07	1.10E+01	100.000	6.418	-6.418	25.0	62.0	2.25	17.60	87-72				
101	5	4.50E+07	3.00E+01	100.000	6.176	-6.176	22.0	58.0	2.22	17.90	90-78	100.000	5.8698	59.3	16.20
47	10	3.06E+07	9.67E-01	100.000	7.500	-7.500	22.0	77.0	2.00	19.30	90-63				
49	10	4.67E+07	4.00E-02	100.000	9.067	-9.067	23.0	77.0	3.11	missed	87-63				
102	10	3.37E+07	4.35E+00	100.000	6.889	-6.889	22.0	77.0	2.24	27.40	90-60	100.000	7.2688	77.0	23.35

Sonication + Chlorination of *E. coli*
High power to volume ratio (100 mL volume, highest power setting - 10)
Probe system

Sequential

	Sonic	Chlorine	Pre count	Post sonic	Post chlorine	Sonic Only		Sonic + Chlorine		Initial	Post sonic	Initial	Post sonic	Post chlorine	Output	AVERAGE S+C	
Exp #	Time (min)	(mg/L)	(cfu/mL)	count (cfu/mL)	count (cfu/mL)	% inact.	$\log_{10}$ inact.	% inact.	$\log_{10}$ inact.	Temp (^{o}C)	Temp (^{o}C)	Turb (NTU)	Turb (NTU)	Turb (NTU)	Power (W)	% inact.	$\log_{10}$ inact.
28	2	0.4	6.23E+07	1.99E+07	8.33E+06	68.058	0.496	86.629	0.874	23.0	39.5	2.39	8.86	5.9	87-84		
29	2	0.4	5.77E+07	1.37E+07	9.03E+06	76.256	0.624	84.350	0.805	22.0	40.0	2.14	7.82	6.55	87-84		
51	2	0.4	2.68E+07	7.63E+06	2.80E+06	71.530	0.546	89.552	0.981	22.0	39.5	1.89	7.58	6.78	90-87	86.844	0.881
52	2	0.6	5.43E+07	1.14E+07	1.56E+06	79.006	0.678	97.127	1.542	22.0	39.0	2.20	7.91	6.21	90-87		
53	2	0.6	2.40E+07	1.14E+07	1.79E+06	52.500	0.323	92.542	1.127	22.0	38.0	2.01	7.90	6.62	90		
54	2	0.6	2.27E+07	9.43E+06	1.79E+06	58.458	0.382	92.115	1.103	22.0	38.0	1.85	7.35	6.72	90	93.928	1.217
55	2	1.0	2.86E+07	8.10E+06	1.52E+04	71.678	0.548	99.947	3.275	23.0	40.0	1.79	7.35	4.75	87-84		
56	2	1.0	3.50E+07	7.63E+06	6.40E+03	78.200	0.662	99.982	3.738	21.5	38.0	2.32	8.06	6.53	90-87		
57	2	1.0	2.77E+07	9.73E+06	2.57E+04	64.874	0.454	99.907	3.033	22.0	38.5	2.57	8.24	7.04	90-87	99.945	3.262
58	3	0.6	3.40E+07	6.17E+06	2.80E+06	81.853	0.741	91.765	1.084	21.5	45.5	2.20	10.40	8.89	90-87	91.765	1.084

Simultaneous

	Disinf.	Chlorine	Pre count	Post sonic				Initial	Final	Initial	Final	Output	AVERAGE		AVERAGE	
Exp #	Time (min)	(mg/L)	(cfu/mL)	count (cfu/mL)	% inact.	$\log_{10}$ inact.	$-\log_{10} n/n_o$	Temp (^{o}C)	Temp (^{o}C)	Turb (NTU)	Turb (NTU)	Power (W)	% inact.	$\log_{10}$ inact.	Temp (^{o}C)	Turb (NTU)
148	0.1667	0.6	1.35E+07	1.86E+06	86.222	0.861	-3.730	22.5	24.0	2.37	2.51	93				
149	0.1667	0.6	1.71E+07	4.63E+02	99.997	4.567	-3.730	22.5	24.0	1.68	1.99	93				
150	0.1667	0.6	1.86E+07	9.13E+01	100.000	5.309	-3.730	22.5	24.0	1.95	1.98	93	95.406	1.338	24.0	2.16
151	0.5	0.6	2.18E+07	4.33E+02	99.998	4.702	-3.730	22.5	26.5	1.50	2.91	90				
152	0.5	0.6	3.67E+07	4.37E+02	99.999	4.924	-3.730	22.5	27.0	1.49	2.99	90				
153	0.5	0.6	2.37E+07	7.40E+01	100.000	5.506	-3.730	22.5	27.0	1.94	3.02	93-90	99.999	4.934	26.8	2.97
70	1	0.6	2.70E+07	5.03E+03	99.981	3.730	-3.730	23.0	33.0	2.42	5.29	87				
71	1	0.6	2.49E+07	6.20E+03	99.975	3.604	-3.604	22.5	31.0	2.15	4.55	90-87				
72	1	0.6	2.42E+07	4.30E+03	99.982	3.750	-3.750	22.5	31.0	2.18	4.45	90-87	99.980	3.690	31.7	4.76
62	2	0.4	2.36E+07	5.17E+06	78.093	0.659	-0.659	24.0	39.0	2.11	7.88	90-87				
64	2	0.4	2.22E+07	4.50E+06	79.730	0.693	-0.693	21.5	38.0	2.16	8.02	90-87				
76	2	0.4	2.14E+07	9.83E+04	99.541	2.338	-2.338	22.0	36.0	1.96	7.67	90-84	85.788	0.847	37.7	7.86
59	2	0.6	2.00E+07	6.63E+02	99.997	4.480	-4.480	21.5	36.0	2.00	7.90	90-87				
63	2	0.6	2.72E+07	9.40E+02	99.997	4.461	-4.461	24.0	39.0	2.08	7.27	90-87				
65	2	0.6	2.41E+07	6.90E+02	99.997	4.543	-4.543	21.5	38.0	2.11	7.86	90-87	99.997	4.493	37.7	7.68
66	2	1.0	2.51E+07	2.29E+02	99.999	5.040	-5.040	22.0	38.0	2.18	6.33	87-84				
67	2	1.0	2.53E+07	1.79E+03	99.993	4.151	-4.151	22.5	38.0	2.33	8.47	87				
68	2	1.0	3.37E+07	3.13E+03	99.991	4.032	-4.032	22.5	39.0	2.59	9.17	87-84	99.994	4.240	38.3	7.99
73	3	0.6	2.10E+07	2.22E+03	99.989	3.976	-3.976	25.0	46.5	2.26	11.00	90-84				
74	3	0.6	2.12E+07	1.26E+03	99.994	4.225	-4.225	25.0	46.5	2.31	11.20	90-84				
75	3	0.6	2.54E+07	1.33E+02	99.999	5.281	-5.281	21.5	47.5	1.93	10.90	90-84	99.994	4.246	46.8	11.03

Sonication of *E. coli*
Low power to volume ratio (390 mL volume)
Sonic Bath

Exp #	Sonic Time (min)	Pre count (cfu/mL)	Post count (cfu/mL)	% inact.	$\log_{10}$ inact.	- $\log_{10}$ n/n$_o$	Initial Temp (^{o}C)	Final Temp (^{o}C)	Initial Turb (NTU)	Final Turb (NTU)	AVERAGE % inact.	AVERAGE $\log_{10}$ inact.	AVERAGE Final Turb.	AVERAGE Final Temp.
103	1	4.13E+07	3.20E+07	22.518	0.111	-0.111	21.0	21.5	1.82	missed				
113	1	3.27E+07	2.11E+07	35.474	0.190	-0.190	22.0	24.5	2.29	missed				
116	1	2.42E+07	2.40E+07	0.826	0.004	-0.004	20.0	20.0	1.53	missed	19.606	0.09	missed	22.0
103	2	4.13E+07	2.23E+07	46.005	0.268	-0.268	21.0	21.5	1.82	missed				
113	2	3.27E+07	2.48E+07	24.159	0.120	-0.120	22.0	25.0	2.29	missed				
116	2	2.42E+07	2.38E+07	1.653	0.007	-0.007	20.0	20.0	1.53	missed	23.939	0.12	missed	22.2
103	5	4.13E+07	2.47E+07	40.194	0.223	-0.223	21.0	23.0	1.82	missed				
113	5	3.27E+07	2.43E+07	25.688	0.129	-0.129	22.0	26.0	2.29	missed				
116	5	2.42E+07	3.83E+07	-58.264	-0.199	0.199	20.0	21.0	1.53	missed	2.539	0.01	missed	23.3
103	10	4.13E+07	1.94E+07	53.027	0.328	-0.328	21.0	26.0	1.82	missed				
113	10	3.27E+07	2.47E+07	24.465	0.122	-0.122	22.0	29.0	2.29	missed				
116	10	2.42E+07	2.54E+07	-4.959	-0.021	0.021	20.0	23.5	1.53	missed	24.178	0.12	missed	26.2
103	15	4.13E+07	1.70E+07	58.838	0.386	-0.386	21.0	29.0	1.82	missed				
113	15	3.27E+07	2.52E+07	22.936	0.113	-0.113	22.0	33.0	2.29	missed				
116	15	2.42E+07	2.47E+07	-2.066	-0.009	0.009	20.0	26.0	1.53	missed	26.569	0.13	missed	29.3
103	20	4.13E+07	1.69E+07	59.080	0.388	-0.388	21.0	32.0	1.82	missed				
113	20	3.27E+07	2.80E+07	14.373	0.067	-0.067	22.0	36.5	2.29	missed				
116	20	2.42E+07	2.56E+07	-5.785	-0.024	0.024	20.0	31.0	1.53	missed	22.556	0.11	missed	33.2
103	30	4.13E+07	1.70E+07	58.838	0.386	-0.386	21.0	42.5	1.82	missed				
113	30	3.27E+07	2.07E+07	36.697	0.199	-0.199	22.0	44.0	2.29	missed				
116	30	2.42E+07	2.08E+07	14.050	0.066	-0.066	20.0	36.5	1.53	missed	36.528	0.20	missed	41.0
113	40	3.27E+07	2.86E+07	12.538	0.058	-0.058	21.0	49.0	1.82	missed				
114	40	2.97E+07	2.48E+07	16.498	0.078	-0.078	22.0	40.0	2.29	missed				
116	40	2.42E+07	2.07E+07	14.463	0.068	-0.068	20.0	40.5	1.53	missed	14.500	0.07	missed	43.2
113	50	3.27E+07	2.44E+07	25.382	0.127	-0.127	21.0	47.5	1.82	missed				
114	50	2.97E+07	4.40E+07	-48.148	-0.171	0.171	22.0	44.5	2.29	1.56				
116	50	2.42E+07	1.83E+07	24.380	0.121	-0.121	20.0	43.5	1.53	missed	0.538	0.00	missed	45.2
103	60	4.13E+07	1.84E+07	55.448	0.351	-0.351	21.0	49.0	1.82	1.66				
113	60	3.27E+07	1.71E+07	47.706	0.282	-0.282	22.0	51.0	2.29	1.68				
116	60	2.42E+07	1.69E+07	30.165	0.156	-0.156	20.0	46.0	1.53	1.46	44.440	0.26	1.60	48.7

Sonication of *E. coli*
High power to volume ratio (80 mL volume)
Sonic Bath
Bold numbers indicate no counts - detection limit value substituted

Exp #	Sonic Time (min)	Pre count (cfu/mL)	Post count (cfu/mL)	% inact.	$\log_{10}$ inact.	- $\log_{10}$ n/n_o	Initial Temp (^oC)	Final Temp (^oC)	Initial Turb (NTU)	Final Turb (NTU)	AVERAGE % inact.	AVERAGE $\log_{10}$ inact.	AVERAGE Final Turb.	AVERAGE Final Temp.
104	1	4.97E+07	3.43E+07	30.986	0.161	-0.161	22.0	22.5	2.16	missed				
115	1	2.22E+07	2.41E+07	-8.559	-0.036	0.036	21.5	21.5	2.08	missed				
117	1	2.58E+07	2.79E+07	-8.140	-0.034	0.034	20.0	19.0	1.76	missed	4.763	0.02	missed	21.0
104	2	4.97E+07	2.11E+07	57.545	0.372	-0.372	22.0	23.5	2.16	missed				
115	2	2.22E+07	2.20E+07	0.901	0.004	-0.004	21.5	22.0	2.08	missed				
117	2	2.58E+07	2.28E+07	11.628	0.054	-0.054	20.0	19.5	1.76	missed	23.358	0.12	missed	21.7
104	3	4.97E+07	2.41E+07	51.509	0.314	-0.314	22.0	24.0	2.16	missed				
115	3	2.22E+07	2.73E+07	-22.973	-0.090	0.090	21.5	22.0	2.08	missed				
117	3	2.58E+07	2.58E+07	0.000	0.000	0.000	20.0	20.0	1.76	missed	9.512	0.04	missed	22.0
104	5	4.97E+07	4.53E+07	8.853	0.040	-0.040	22.0	25.5	2.16	missed				
115	5	2.22E+07	2.47E+07	-11.261	-0.046	0.046	21.5	23.0	2.08	missed				
117	5	2.58E+07	2.42E+07	6.202	0.028	-0.028	20.0	21.0	1.76	missed	1.264	0.01	missed	23.2
104	10	4.97E+07	4.53E+07	8.853	0.040	-0.040	22.0	31.0	2.16	missed				
115	10	2.22E+07	2.97E+07	-33.784	-0.126	0.126	21.5	25.0	2.08	missed				
117	10	2.58E+07	2.80E+07	-8.527	-0.036	0.036	20.0	23.5	1.76	missed	-11.153	-0.05	missed	26.5
104	15	4.97E+07	3.73E+07	24.950	0.125	-0.125	22.0	36.0	2.16	missed				
115	15	2.22E+07	2.66E+07	-19.820	-0.079	0.079	21.5	27.5	2.08	missed				
117	15	2.58E+07	2.81E+07	-8.915	-0.037	0.037	20.0	27.0	1.76	missed	-1.262	-0.01	missed	30.2
104	20	4.97E+07	3.50E+07	29.577	0.152	-0.152	22.0	40.5	2.16	missed				
115	20	2.22E+07	2.66E+07	-19.820	-0.079	0.079	21.5	30.0	2.08	missed				
117	20	2.58E+07	2.31E+07	10.465	0.048	-0.048	20.0	31.0	1.76	missed	6.741	0.03	missed	33.8
104	30	4.97E+07	2.13E+07	57.143	0.368	-0.368	22.0	49.5	2.16	missed				
115	30	2.22E+07	2.33E+07	-4.955	-0.021	0.021	21.5	36.0	2.08	missed				
117	30	2.58E+07	2.17E+07	15.891	0.075	-0.075	20.0	39.0	1.76	missed	22.693	0.11	missed	41.5
104	40	4.97E+07	2.00E+01	100.000	6.395	-6.395	22.0	57.0	2.16	missed				
115	40	2.22E+07	1.76E+07	20.721	0.101	-0.101	21.5	40.5	2.08	missed				
118	40	2.86E+07	1.69E+07	40.909	0.228	-0.228	22.5	37.0	2.34	missed	53.877	0.34	missed	44.8
104	50	4.97E+07	**1.00E+00**	**100.000**	**7.696**	**-7.696**	22.0	60.5	2.16	2.60				
115	50	2.22E+07	2.16E+07	2.703	0.012	-0.012	21.5	50.0	2.08	missed				
118	50	2.86E+07	1.81E+07	36.713	0.199	-0.199	22.5	40.0	2.34	2.35	46.472	0.27	2.48	50.2
115	60	2.22E+07	1.67E+01	100.000	6.124	-6.124	21.5	56.5	2.08	2.05	100.000	6.12	2.05	48.8

Heating of *E. coli*

Temperature Effect Only (100 mL volume placed into 80°C water bath)

Exp #	Disinf. Time (min)	Pre count (cfu/mL)	Post count (cfu/mL)	% inact.	$\log_{10}$ inact.	- $\log_{10}$ n/n_o	Initial Temp (°C)	Final Temp (°C)	Initial Turb (NTU)	Final Turb (NTU)	AVERAGE % inact.	AVERAGE $\log_{10}$ inact.	AVERAGE Final Turb.	AVERAGE Final Temp.
120	1	5.00E+07	4.07E+07	18.60	0.089	-0.089	21.0	29.0	3.02	missed				
121	1	9.50E+07	5.80E+07	38.95	0.214	-0.214	19.5	29.5	3.15	missed				
122	1	3.40E+07	2.93E+07	13.82	0.065	-0.065	21.0	32.0	2.35	missed	23.79	0.12	missed	30.2
120	2	5.00E+07	5.03E+07	-0.60	-0.003	0.003	21.0	40.5	3.02	missed				
121	2	9.50E+07	6.40E+07	32.63	0.172	-0.172	19.5	40.5	3.15	missed				
122	2	3.40E+07	2.19E+07	35.59	0.191	-0.191	21.0	43.0	2.35	missed	22.54	0.11	missed	41.3
120	3	5.00E+07	3.97E+07	20.60	0.100	-0.100	21.0	50.0	3.02	missed				
121	3	9.50E+07	4.37E+07	54.00	0.337	-0.337	19.5	50.0	3.15	missed				
122	3	3.40E+07	3.02E+07	11.18	0.051	-0.051	21.0	52.0	2.35	missed	28.59	0.15	missed	50.7
121	4	9.50E+07	1.76E+07	81.47	0.732	-0.732	19.5	57.5	3.15	missed				
122	4	3.40E+07	1.90E+07	44.12	0.253	-0.253	21.0	58.5	2.35	missed	62.80	0.43	missed	58.0
120	5	5.00E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	62.5	3.02	missed				
121	5	9.50E+07	28	100.00	6.531	-6.531	19.5	62.0	3.15	missed				
122	5	3.40E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	63.5	2.35	missed	100.00	6.94	missed	62.7
120	7.5	5.00E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	69.5	3.02	missed				
121	7.5	9.50E+07	**1**	**100.00**	**7.000**	**-7.000**	19.5	69.0	3.15	missed				
122	7.5	3.40E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	69.0	2.35	missed	100.00	7.70	missed	69.2
120	10	5.00E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	72.5	3.02	missed				
121	10	9.50E+07	**1**	**100.00**	**7.000**	**-7.000**	19.5	72.0	3.15	missed				
122	10	3.40E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	72.0	2.35	missed	100.00	7.70	missed	72.2
120	20	5.00E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	74.0	3.02	8.11				
121	20	9.50E+07	**1**	**100.00**	**7.000**	**-7.000**	19.5	74.0	3.15	7.45				
122	20	3.40E+07	**1**	**100.00**	**7.000**	**-7.000**	21.0	74.0	2.35	7.45	100.00	7.70	7.67	74.0