INACTIVATION OF *E. COLI* IN A FLOW THROUGH SONICATION SYSTEM

By

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Abstract

Drinking water sources are vulnerable to a broad range of contaminant threats. Recent U.S. legislation has focused on protecting public health from pathogens while also managing disinfection byproducts (DBPs) and organic contaminants. Chlorine is known to react with organic matter to form DBPs, thus alternative disinfection schemes are desirable. The goal of our research was to evaluate synergistic inactivation of *E. coli* with chlorine and sonication in a flow through system. Laboratory experiments were conducted to determine the impact of chlorine dose (0 to 1 mg/L), cavitation intensity (90 to 150 watts) and contact time (0 to 16 minutes) on inactivation. Tests were conducted with a probe system and a flow through cavitation device. Results showed that sonication alone was ineffective for the conditions tested. Sonication applied simultaneously with chlorine did not improve inactivation compared to each disinfectant alone.

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1 Introduction

1.1 Statement of Problem

Drinking water sources in the United States are vulnerable to contamination from point sources (e.g. wastewater treatment plants) and non-point sources (e.g. agricultural discharges). As a result of these waste inputs, pathogenic microorganisms that can cause adverse health effects on human beings may be found in drinking water sources. It is therefore important to disinfect waters intended for drinking to prevent negative public health effects.

Chlorine is the most widely used disinfectant for drinking in the United States because it is a strong disinfectant and is also cost effective and easy to apply. With proper dosing, chlorine is effective at inactivating most microorganisms present in water supplies. Chlorine also leaves a disinfectant residual which helps to minimize microbial growth or contamination effects 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, such as *Giardia lamblia* and *Cryptosporidium parvum*, are resistant to chlorine disinfection.

Another issue with chlorine disinfection is the formation of potentially carcinogenic disinfection by-products (DBPs) when free chlorine reacts with natural organic matter. Trihalomethanes (THMs) and haloacetic acids (HAAs) are two groups of DBPs formed

from chlorine disinfection. These by-products are regulated by the U.S. Environmental Protection Agency (U.S. EPA) under the several DBP Rules which have become more stringent in recent years.

Some alternative disinfectants of interests include ozone disinfection, UV irradiation, chloramination, or the application of multiple disinfectants. Ozone is a strong oxidant which produces fewer DBPs than chlorine, but must be generated on-site. UV irradiation is effective for inactivation of *Giardia lamblia* and *Cryptosporidium parvum*, but expensive to retrofit in large plants. Chloramines are a weaker disinfectant than free chlorine. They are commonly used as a secondary disinfectant because they decay slowly and provide adequate protection in distribution systems. Sonication, applied alone or as a synergistic disinfectant, is an alternative that has not received yet sufficient analysis to consider use in a full scale flow through system.

Sonication is the application of ultrasonic waves to water. Sonication may inactivate microorganisms by fluid shear caused by velocity gradients in cavitation, but it also causes breakup of flocs of bacteria that may make disinfection easier. This research aims at investigating the germicidal effects of sonication and combined sonication and chlorination on *E. coli* in a custom made bench-scale flow through cavitation system. The objective is to provide data on the feasibility of using a similar design at a larger scale in a treatment plant in the future.

1.2 Objectives

The main goal of this research was to demonstrate inactivation by sonication in a flow-through system compared to a batch reactor. Synergy between chlorine and sonication, which has been demonstrated in batch reactors, might allow a treatment plant to use less chlorine to achieve the same amount of inactivation if similar synergy could be achieved in full-scale, flow through systems. In order to achieve the goals, the following tasks were completed:

1) determine the effects of chlorine alone on the inactivation of *E. coli* in both static and flow through systems;
2) determine the inactivation of *E. coli* by sonication alone in both static and flow through systems; and
3) determine the combined effect of simultaneous sonication and chlorination in both static and flow through systems.

1.3 Scope of Research

The sonication experiments were conducted in two laboratories using a sonication probe and a custom designed flow through sonication chamber. All experiments were well controlled in the laboratory and were conducted at a starting temperature of approximately 22^{o}C. Phosphate buffered saline was used as the water matrix throughout the entire experimental plan. *E. coli* was enumerated before and after disinfection using pour plate and/or spread plate 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 flow through system. Power-to-volume ratios of 180 W/L to 300 W/L were used, as were sonication times up to 16 minutes. Temperature was monitored and controlled if necessary. The inactivation of *E. coli* was also studied by chlorine disinfection. This was to allow comparison of inactivation with a single disinfectant to inactivation in the combined disinfection experiments. Various chlorine dosages were tested, ranging from 0.4 mg/L to 1.0 mg/L with chlorine contact times up to 16 minutes. Combined application of sonication and chlorination experiments were performed to study the possible synergistic effects of sonication. The disinfectants were applied simultaneously. The experiments were conducted with the same variables and parameters as the single inactivation method experiments, and variables were the same to allow comparison.

1.4 Overview of Thesis

Due to the production of undesired halogenated DBPs as a result of chlorination, increasingly stringent Disinfectant and Disinfection By-products Rules, and limitations of some of the disinfection alternatives, there is a desire to discover another disinfection method to reduce DBPs while maintaining disinfection effectiveness. Limited research has been conducted on the effects of sonication on drinking water in a flow-through system. Therefore, the results of this research will provide an overview of ultrasound as a disinfectant, whether it is applied alone or combined with chlorine, and if less chlorine can be used to achieve the same amount of *E. coli* inactivation in a flow through system.

Followed by this introduction chapter is the literature review, which provides details on current drinking water regulations, various disinfectants, ultrasound, and prior sonication studies. The methods chapter immediately following describes the exact practices and procedures used in the study. Then, the results of the experiments are presented and analyzed. This report ends with recommendations for further study.

2 Literature Review

Drinking waters in the United States are regulated by the U.S. Environmental Protection Agency (U.S. EPA), which requires different levels of treatment based on water quality and source. Surface water sources must be filtered and disinfected, while groundwater sources generally require less treatment because of the protection afforded by the aquifer system the water has passed through. The majority of treatment plants use chlorine for disinfection; however, chlorine has several limitations as some pathogens are resistant to chlorine and chlorine reacts to form potentially carcinogenic disinfection by-products (DBPs). Therefore, alternative methods of disinfection are of interest to the drinking water community.

This chapter introduces relevant drinking water regulations, current disinfection methods, and a potential alternative disinfection option: sonication.

2.1 Drinking Water Regulations

The U.S. EPA was formed in December of 1970, after being established by President Nixon, and is responsible for regulating drinking water quality. The framework for these regulations is provided through the Safe Drinking Water Act (SDWA), which was first published in 1975. Specific regulations are provided for monitoring and treatment of surface waters and groundwaters as discussed below.

2.1.1 Safe Drinking Water Act

The Safe Drinking Water Act (SDWA) of 1975 was the first widespread drinking water regulation implemented in the United States. The SDWA required the U.S. EPA to establish the National Interim Primary Drinking Water Regulations (NIPDWRs). These regulations were to contain maximum contaminant levels (MCLs) for a specific set of contaminants known to exist at the time and commonly found in drinking waters. MCLs are set to protect public health based on the prevalence and effects of that contaminant while also considering the cost and availability of treatment for each contaminant. MCLs are enforceable standards that must be met by water providers. The SDWA also mandated that the U.S. EPA establish monitoring practices for treatment plants. Finally, the SDWA contained the mandate that the U.S. EPA evaluate the feasibility and success of the NIPDWRs and create the National Primary Drinking Water Regulations (NPDWRs). The NPDWRs are permanent standards that fully take into account cost-benefit analysis and public comment for determining contaminant limits.

2.1.1.1 1986 Amendments to SDWA

In 1986, the SDWA was amended in an effort to continue to update drinking water standards in the U.S. The amendments required the EPA to establish 83 MCLs within three years and to establish no less than 25 new MCLs every three years following. Also required was the establishment of maximum contaminant level goals (MCLGs). MCLGs take into account only human health effects, instead of being a balance of achievability, cost and human health effects. MCLGs are non-enforceable ideals that the U.S. EPA wishes to work toward.

2.1.1.2 1996 Amendments to SDWA

The 1996 amendments to SDWA established the current system that EPA uses to create MCLs. First, contaminant effects are fully considered in an attempt to establish the level at which contaminants are harmful to human health. Next, the proposed standards are published and public comments are accepted on the prospective regulations. Only after input from the public and professionals in the water industry are the MCLs established. New standards were mandated for arsenic and radon, and public water systems were required to annually distribute consumer confidence reports (CCRs) to all households receiving municipal water. CCRs provide details on the previous year's testing and water quality results and other relevant data about the water being delivered to consumers.

2.1.2 Surface Water Treatment Rule

The Surface Water Treatment Rule (SWTR) was established in 1989. It was effective as of December 31, 1990 (U.S. EPA 1989a). This rule applies to all municipal water providers that use surface water as a water source. The U.S. EPA set the maximum contaminant level goal (MCLG) to be zero for *Giardia lamblia*, viruses, and *Legionella* (U.S. EPA 1989a). Under the SWTR, all water systems 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, and these criteria include the quality of source water, protection of the watershed, and the ability to meet all disinfection requirements. 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. The SWTR also established requirements for turbidity, and required monitoring of this parameter. For systems that practice conventional or direct filtration, turbidity measurements are required to 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 1989a). Also established was a series of "credits" for practices other than inactivation that provide removal of regulated pathogens. These credits were a straightforward way of calculating the actual log-inactivation required by disinfection.

2.1.2.1 Interim Enhanced Surface Water Treatment Rule

In 1998, the Interim Enhanced Surface Water Treatment Rule (IESWTR) was promulgated to improve control of *Cryptosporidium* in surface waters. *Cryptosporidium* had emerged as pathogen especially resistant to chlorine disinfection. In 1993, an outbreak of *Cryptosporidium* in Milwaukee, Wisconsin, left over 100 people dead and over 1 million sick. While the source of the pathogen was never officially identified, an extensive rain event in the area created high turbidity levels in the raw water. This resulted in suboptimal coagulation although the plant was meeting current U. S. EPA requirements (MWH 2005). Based on concerns with C*ryptosporidium*, the IESWTR required a 2-log reduction in *Cryptosporidium*. The rule also required more specific monitoring of each filter in a treatment system to make sure all water was being treated adequately, rather than monitoring just the blended water after filtration. The maximum effluent turbidity from conventional and direct filtration was set at 0.3 NTU in a

minimum of 95% of samples taken each month, and the maximum allowable turbidity was set at 1 NTU (U.S. EPA 1998a). This regulation applied only to surface water systems serving more than 10,000 people (U.S. EPA 1998a).

2.1.2.2 Long Term 1 Enhanced Surface Water Treatment Rule

The Long Term 1 Enhanced Surface Water Treatment Rule (LT1) was published in 2002. The rule mandated that all surface water systems, including those serving less than 10,000 people, comply with the terms of IESWTR (U.S. EPA 2002).

2.1.2.3 Long Term 2 Enhanced Surface Water Treatment Rule

The Long Term 2 Enhanced Surface Water Treatment Rule (LT2), similarly to IESWTR and LT1, was established to further control *Cryptosporidium* in drinking water. In order to protect against possible future outbreaks, the LT2 requires monitoring of source waters for *Cryptosporidium.* Based on levels detected, systems may be required to meet higher levels of inactivation and removal than the LT1 previously required. Through the LT2, the U.S. EPA established a "toolbox" of options for controlling *Cryptosporidium*, including protecting the source water area, improving filtering, and adding UV or ozone disinfection. For smaller systems, however, many of these options are prohibited by monetary limitations (U.S. EPA 2006b).

2.1.3 Groundwater Regulations

The final Groundwater Rule was published in 2006. The rule requires sanitary surveys to identify systems and areas which are influenced by surface water or have other questionable sources, and can therefore contain more pollutants or microorganisms than typical groundwaters. Systems that were deemed to be at risk are required to provide 4-log inactivation of viruses (U.S. EPA 2006a).

2.1.4 Regulations for All Public Water Systems

In addition to the rules above that apply specifically to municipal water systems using either a surface or groundwater source, there are is a significant set of U.S. EPA regulations that apply to all drinking waters, regardless of source.

2.1.4.1 Total Coliform Rule

The Total Coliform Rule was published in final form in late 1989 and became effective in at the end of 1990. The rule requires that all water systems sample for coliforms based on the population they serve. For example, systems which serve fewer than 1000 people may test once a month, systems with 50,000 or more customers test 60 times per month and those with at least 2.5 million customers test at least 420 times per month (U.S. EPA 1989b). Coliforms are not pathogenic organisms, but their presence in water indicates that other pathogenic organisms may be present (MWH 2005). Because disease outbreaks have occurred with very low levels of coliforms detected (MWH 2005), the U.S. EPA set the MCLG for total coliforms at 0, and set a legally binding limit of positive coliforms in less than 5% of samples taken (U.S. EPA 1989b).

2.1.4.2 Stage 1 Disinfectants and Disinfection By-Products Rule

Disinfection by-products (DBPs) are potentially carcinogenic compounds created when disinfection chemicals react with natural organic matter present in source waters. In order to protect the public from disinfection by-products, the U.S. EPA established the Stage 1 Disinfectants and Disinfection By-Products (Stage 1 DBP Rule) in 1998. The rule only applied to surface water systems and groundwater systems found to be under the direct influence of surface water, because these sources typically contain enough organic matter to cause DBP production (MWH 2005). Larger systems (10,000 or more served) were required to become compliant by January 1, 2002. Systems serving fewer than 10,000 people and groundwater systems under the influence of surface waters must comply with the Stage 1 DBPR requirements by January 1, 2004. The rule established the maximum residual disinfectant level goals (MRDLGs) and the maximum residual disinfectant levels (MRDLs) for chlorine, chloramine, and chlorine dioxide (U.S. EPA 1998b).

Also included in the Stage 1 D/DBP Rule are 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 1989b). 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 are provided for

dichloroacetic acid and trichloroacetic acid. Both the MCLGs and the MCLs are listed in Table 2-1. It should be noted that the regulation allowed averaging of DBPs measured at different locations in the distribution system to occur. If DBP levels were high in one area of a distribution system, the system could remain in compliance if other areas were low enough that the average values were in compliance.

Table 2-1: Stage 1 DBPR MCLs and MCLGs (U.S. EPA 1998)

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

In an effort to further control DBPs, the U.S. EPA also regulated organics in water. This part of the rule requires some municipal systems to remove a higher percentage of total organic carbon depending on the source water concentration of TOC and alkalinity. The U.S. EPA did not specify how this removal needed to be accomplished.

2.1.4.3 Stage 2 Disinfectants and Disinfection By-Products Rule

The Stage 2 Disinfectants and Disinfection By-Products Rule built on the standards and goals outlined in the Stage 1 D/DBP Rule. While the MCLs did not change, the manner in which DBP concentrations were measured and calculated became stricter. First,

sample locations had to be established at points in the distribution system where DBP levels were highest. Second, running annual averages (RAAs) for the entire system were no longer adequate for compliance. Instead, RAAs for each sample location were required for compliance. The new regulations make compliance more difficult for public water systems, as small areas of high DBP concentrations can be caused by features in distribution systems such as low flow areas and dead ends (U.S. EPA 1998b).

2.1.5 Regulation Summary

Compliance with the above regulations can be difficult for water systems because the methods for solving problems under the regulations sometimes conflict. For instance, with systems using chlorine for disinfection, DBPs are lowered (to comply with the Stage 2 D/DBP Rule) most easily by reducing chlorine concentration. However, inactivation required by the LT2 is achieved most easily by adding more chlorine. Because of these conflicts that exist, there is interest in finding alternative disinfectants that would allow compliance with all regulations (MWH 2005).

2.2 Disinfection

Chlorine is the most common disinfectant used in the United States, with over 90 percent of current U.S. water supplies using it as all or part of their disinfection process (NDWC 1996), but other methods, such as ozone and UV light are also used. Chlorine disinfection can be achieved with gaseous chlorine, sodium hypochlorite, and calcium hypochlorite. Other disinfectants used in the United States include chlorine dioxide,

ozone and UV light. The following sections discuss the application and effectiveness of each of these disinfectants.

2.2.1 Chlorine

Chlorine is the most widely used disinfectant in the U.S. In 1908, the Jersey City Water Works began the first use of chlorine as a large scale disinfectant in the United States (NDWC 1996). Today, a large percentage of treatment plants use chlorine for primary or secondary disinfection (MWH 2005).

Chlorine is economical, and there is significant operator experience with chlorine chemicals, making it an attractive option for disinfection. Chlorine effectively inactivates a wide variety of waterborne pathogens (HDR Engineering 2001). It also removes some unpleasant tastes and odors from surface waters with high algal concentrations and it reduces coloration due to organic compounds in water (NDWC 1996). It is somewhat effective against viruses (including inactivations over 2 log), and *Giardia* (when inactivations of less than 2 log are necessary), but not as effective against C*ryptosporidium* or G*iardia* when more than 2 log inactivation is necessary (HDR Engineering 2001). Lastly, chlorine remains in the water in residual concentrations, for secondary disinfection in the distribution system. However, chlorine also has drawbacks. First, chlorine reacts with organic compounds in water to form carcinogenic disinfection byproducts, including THMs and HAAs as described in Section 2.1.4.2. Second, the safe transport and storage of certain types of chlorine, especially chlorine gas, prior to treatment can present problems. Lastly, some pathogens are resistant to chlorine

disinfection. These include *Cryptosporidium parvum* and *Giardia lamblia*, which require high doses (greater than 5.0 mg/L) of chlorine for inactivation.

Chlorine can be applied to drinking water in three ways: gaseous chlorine, sodium hypochlorite, and calcium hypochlorite. Chlorine gas reacts in water to form free Cl_2 and hypochlorus acid (HOCl), as shown in Reaction 1. Sodium hypochlorite forms hypochlorus acid when added to water, as shown in Reaction 2. Calcium hypochlorate dissolved in water also produces HOCl, as shown in Reaction 3. Additonally, HOCl undergoes acid-base reactions to form hypochlorite ion, as shown in Reaction 4. This reaction will not proceed at all at a pH of 6.5 or less, and it will proceed fully at 8.5 or more. Because hypochlorus acid is a far superior disinfectant, chlorination is substantially more effective at a lower pH (MWH 2005).

$$Cl_2(g) + H_2O \rightarrow HOCl + H^+ + Cl^- \qquad \textit{(Reaction 1)}$$

$$NaOCl + H_2O \rightarrow HOCl + Na^+ + OH^- \qquad \textit{(Reaction 2)}$$

$$Ca(OCl)_2 + 2H_2O \rightarrow 2HOCl + Ca^{2+} + 2OH^- \qquad \textit{(Reaction 3)}$$

$$HOCl \leftrightarrow H^+ + OCl^- \qquad \textit{(Reaction 4)}$$

2.2.2 Chloramines

Chloramines are compounds that are formed when ammonia reacts with dissolved chlorine. Chloromines can take three forms: mono (Reaction 7) and di (Reaction 8) chloramines and nitrogen trichloride (Reaction 9). Reactions 5 through 9 show how chloramines are formed.

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad \textit{(Reaction 5)}$$

$$HOCl \leftrightarrow OCl^- + H^+ \quad \textit{(Reaction 6)}$$

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O \quad \textit{(Reaction 7)}$$

$$NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O \quad \textit{(Reaction 8)}$$

$$NHCl_2 + HOCl \rightarrow NHCl_2 + H_2O \quad \textit{(Reaction 9)}$$

Chloramines were found to be effective as a secondary disinfectant because they are more stable than chlorine and remain in the distribution systems longer. However, chloromines are weaker than chlorine and would require large contact times or doses for primary disinfection (U.S. EPA 1999). Chloramines are as much as 100 times less effective than chlorine at low pHs, so they are rarely used as a primary disinfectant (MWH 2005).

When compared to free chlorine or chlorine dioxide, chloramines form fewer DBPs because they do not react as easily with organic compounds found in source water. Additionally, chloramines must be produced at the point of use, requiring more operator training and skill to use (U.S. EPA 1999). Another consideration is that significant safety procedures are necessary for facilities using chloramines because toxic gas is created when chlorine and ammonia are allowed to mix (HDR Engineering 2001).

2.2.3 Ozone

Ozone is one of the strongest oxidants available for use in water treatment. It is usually generated on-site, through an energy intensive process that converts pure oxygen gas to

ozone as shown in Reaction 10. The resulting gas is bubbled through the treated water in a contact tank that maximizes contact time (HDR Engineering 2001). After the gas passes through water in the treatment plant, it must go through an ozone destructor so that ozone is not released into the atmosphere (MWH 2005).

$$3O_2 \rightarrow 2O_3 \qquad \textit{(Reaction 10)}$$

One notable advantage of ozone is that because it is generated on-site, the need for transportation and storage of large amounts of a toxic substance is mitigated. Ozone produces fewer THMs and HAAs than traditional chlorine methods. However, it does produce some of the same DBPs produced by chlorine (aldehydes) and some unique to ozone treatment, such as bromate (BrO_3^-, which is regulated under the Stage 1 D/DBP rule), aldoketoacids and carboxylic acids (MWH 2005). Ozone is acceptable for inactivations of viruses, *Giardia,* and *Cryptosporidium* over a 2 log kill. It may be used in both small and large treatment systems, but does require very skilled operators because the gas must be generated, used and destroyed onsite(HDR Engineering 2001).

2.2.4 Ultraviolet Radiation

Ultraviolet radiation (UV) is a non-chemical method of disinfection. UV inactivates microorganisms though a unique process: organisms absorb the radiation and a photochemical reaction that occurs damages vital components of the cells, leaving them either dead or unable to reproduce. In addition to its effectiveness, one of the most attractive attributes of UV treatment is that it does not produce any known DBPs.

Earlier research in the 1990's indicated that UV was ineffective against certain microorganisms, especially *Cryptosporidium parvum* and *Giardia lamblia*. However, it was later determined that the methods used to determine the viability were inadequate. Subsequently, Bukhari *et al.* (2000) showed that UV is very effective against *Cryptosporidium parvum*, and Shin *et al.* (2000) showed that low pressure UV systems are very effective for inactivation of *Giardia lambia* at cost effective dosages. However, it can be expensive to retrofit large water treatment systems with UV because of the significant construction costs involved (MWH 2005). The operator skill required for UV systems medium (HDR Engineering 2001).

2.3 Sonication

Sonication is the process of applying sound waves at ultrasonic frequencies to water in an effort to inactivate microorganisms in that water. Sonication has been evaluated as a sole disinfectant, and also in conjunction with other disinfectants to enhance inactivation.

2.3.1 Sonication Alone

Several hypotheses exist for the inactivation mechanisms of sonication. Blume *et al* (2002) hypothesized that inactivation is a combination of shear forces caused by velocity gradients, breakdown of cell walls, and increasing vulnerability of pathogens by breaking up flocs or breaking microorganisms off particles to which they have attached. Additionally, free radicals and hydrogen peroxide produced when cells are exposed to sonication are potentially "cytotoxic and mutagenic" (Riesz and Misik 1999).

Clasen and Sobatta (1994) evaluated the inactivation of the microorganisms *Artemiasalina* and *Cyclops nauplii* by sonication applied at 22 kHz. *Artemiasalina* and *C. nauplii* concentrations were reduced by 40 to 60% in four seconds, and significantly more kill was observed as time was increased. The authors also showed that, because "from 20 kHz onwards the cavitation threshold increases with the transmitted frequency," application of ultrasound close to the 20 kHz frequency was most effective in causing cavitation.

Blume *et al*. (2002) evaluated ultrasound for inactivation of both *E. coli* and fecal streptococci organisms in wastewater. 0.9 and 2.9 log inactivation was achieved for *E. coli* and fecal streptococci, respectively. However, very long treatment times and high treatment intensities were required to accomplish this (see Figure 2-1). Therefore, this study demonstrated that sonication alone is not adequate as a solitary disinfectant from an economical standpoint (Blume, Martinez and Uwe 2002).

More recently, the inactivation of the protozoan pathogen *Cryptosporidium parvum* and *Giardia lablia* by sonication has been studied (Graczyk, et al. 2008). Ultrasound disintegration treatment was carried out on sewage sludge for 10 to 20 minutes. The concentration of *Cryptosporidium* oocysts were reduced from 13.5 oocysts/g to non-detectable levels for all treatment conditions tested, except for 10 minutes at 8 mm. *Giardia* cysts were reduced from 27.3 cysts/g to non-detectable levels for the higher amplitudes.

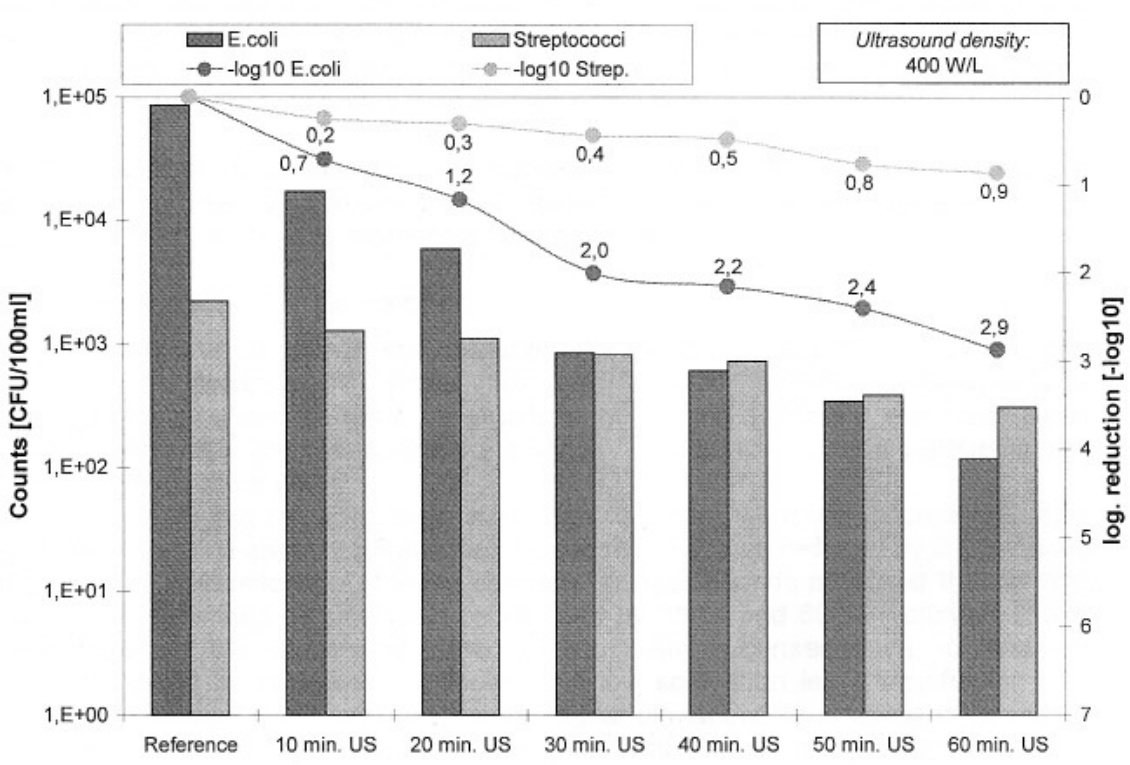

Figure 2-1: Inactivation on *E. coli* and fecal streptococci by sonication (Blume, Martinez and Uwe 2002)

2.3.2 Sonication with Ozone

The addition of sonication to ozonation creates a significant synergistic effect in the inactivation of microorganisms. Burleson *et al.* (1975) demonstrated a synergistic effect with combined sonication and ozonation for the inactivation of several microorganisms, including multiple viruses and bacteria. Four scenarios were evaluated: ozone treatment alone, sonication alone, simultaneous sonication and ozone treatment, and sonication with oxygenation (to demonstrate the synergistic effect was due to ozone, not just aeration). All tests were conducted with the microorganisms suspended in phosphate buffer solution (PBS) and also secondary effluent. Sonication and sonication with oxygenation showed no significant inactivation with treatment times up to 10 minutes.

When suspended in PBS, all types of bacteria tested were completely inactivated when exposed to ozone contact or simultaneous application of ozone and ultrasound for 15 minutes. Longer contacts times were necessary when bacteria were suspended in secondary effluent which the authors noted was more representative of real-world applications. A synergistic effect was noted for combined treatment. (Burleson, Murray and Pollard 1975)

Additional studies on *E. coli* inactivation have confirmed the synergistic effects of combined ozone and sonication. Dahi *et al.* (1976) quantified the inactivation of *E. coli* for three treatment scenarios: ozone alone, sonication followed by ozone treatment, and simultaneous ozone treatment with sonication. A sonic probe system was used with an output power of 160 W and an ultrasonic wave frequency of 20 kHz. Experiments were conducted in three different media: (1) redistilled water (treated with potassium permanganate, made isotonic and buffered with phosphate), (2) sterilized secondary effluent from a wastewater treatment plant, (3) diluted sterilized secondary effluent. The disinfection time was held constant. Results show that ozone treatment alone provided the least inactivation of *E. coli*. Sonication pretreatment with ozonation directly after was more effective at disinfecting *E. coli* than simultaneous application of ozone and ultrasound. Both combined treatment scenarios were more effective than sonication or ozonation alone. It was shown that ultrasonic waves enhanced microbial inactivation with ozone and also chemical oxidation processes caused by the free radicals generated from the decomposition of ozone. When the aeration constant ($K_L a$) was calculated from the experimental data, it showed an increase of 15 – 45% after sonication. While

inactivation still could be enhanced through the mechanical pathways discussed in Section 2.3.1, the implication of this data is that inactivation was also aided by the enhancement of the ozone transfer to liquid.

2.3.3 Sonication with Ultraviolet Light

Sonication is shown to have similar synergistic effects when applied in conjunction with ultraviolet light. Data in UV studies show that long contact times are necessary for inactivation through sonication only, and that these contact times make sonication ineffective alone (Blume, Martinez and Uwe 2002). However, because of the marked effect that particle size has on UV treatment, treatment of wastewater with sonication combined with UV treatment shows an 0.8 to 1.2 order of magnitude increase in inactivation of microorganisms when compared to treatment by UV light alone (Blume, Martinez and Uwe 2002). In addition, the use of combined UV and ultrasound increased the overall inactivation for a given amount of energy, thereby allowing for equal disinfection at a lower cost (Blume, Martinez and Uwe 2002).

2.3.4 Sonication with Chlorine

The most important and relevant effects to the research presented here is the advantages of using combined chlorine disinfection and sonication. As discussed above, recent regulations pose a challenge for drinking water treatment plants that rely on chlorine because of mandates to increase disinfection (when source water is poor) and simultaneously reduce DBP levels. Sonication may serve to alleviate these issues as

combined treatment with sonication and chlorine improves inactivation compared to the additive effects of the individual disinfectants.

Duckhouse *et al.* (2004), studied the inactivation of *E. coli* treated with simultaneous sonication and chlorination (using sodium hypochlorite) and pre-treatment with sonication before a period of chlorination. All experiments were conducted at 20℃. The article lacks information on exact chlorine dose and focuses on the comparisons shown between the different applications of sonication and chlorination. Sonication dose information is included in the article, and two scenarios were used: 1.22 watts of power applied at 850 kHz for a total intensity of 0.029 watts per square centimeter, and 22.34 watts applied at 20 kHz for a total intensity of 16.92 watts per square centimeter (Duckhouse, *et al.* 2004).

Figure 2-2 shows results of *E.* coli inactivation using a 20 kHz frequency. The control case (chlorine only) resulted in 2.5 log inactivation of *E. coli* with a 5 minute contact time. Pretreatment with sonication was ineffective in reducing inactivation levels to 1-log kill for 1 minute presonication and 1.7 log kill for 5 minute presonication. Simultaneous treatment showed enhanced inactivation. A five-minute simultaneous treatment resulted in 4.5 log reduction of *E. coli*, about 1.5 log better than the chlorine alone. A one-minute pretreatment showed nearly 5.0 log reduction overall, an increase of 4.5 log over chlorine alone (Duckhouse, *et al.* 2004).

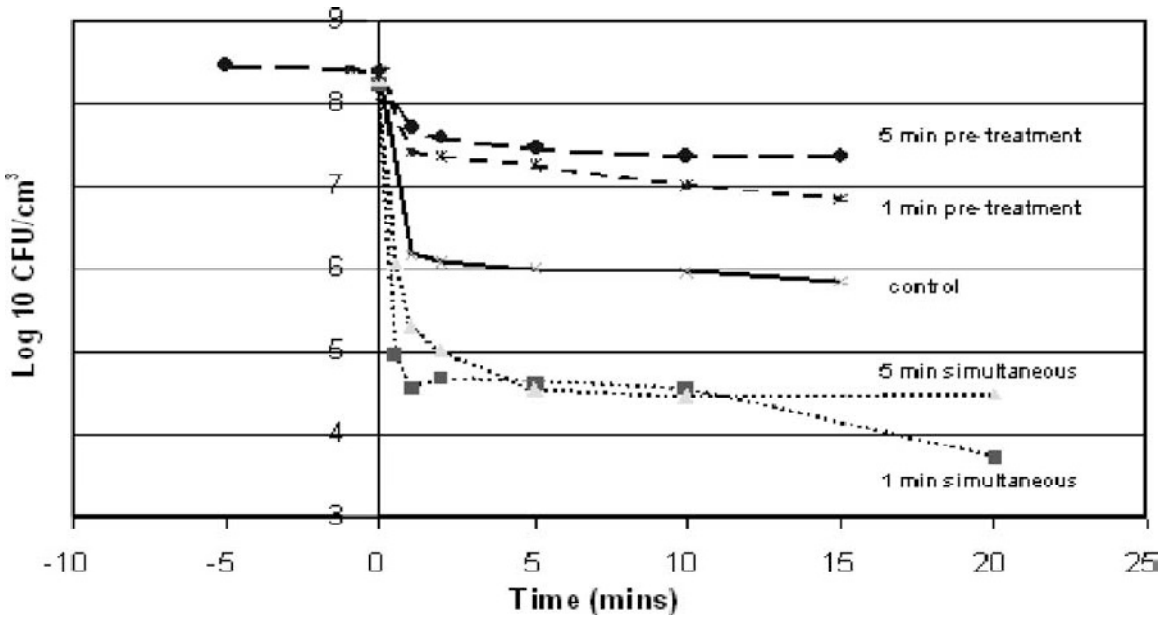

Figure 2-2: Chlorine inactivation of *E. coli* with pre- and simultaneous sonication at 20 kHz (Duckhouse, *et al.* 2004)

Combined sonication and chlorination was also studied at 850 kHz (see Figure 2-3). Results were dissimilar to the results at 20 kHz. At 850 kHz, simultaneous treatment resulted in poorer inactivation of *E. coli* compared to chlorine alone. In contrast, pretreatment with 850 kHz sonication showed similar inactivation levels to 20 kHz simultaneous sonication.

While inactivation results are quite similar for one minute of simultaneous treatment at 20 kHz and one minute of pre-treatment at 850 kHz, the viability of both of these options is quite different. The power applied in both situations was not the same: only 1.22 watts were used at 850 kHz and 22.43 watts were applied at 20 kHz. Given this information, Duckhouse *et al.* concluded that the best option for sonication was one-minute pretreatment at 850 kHz.

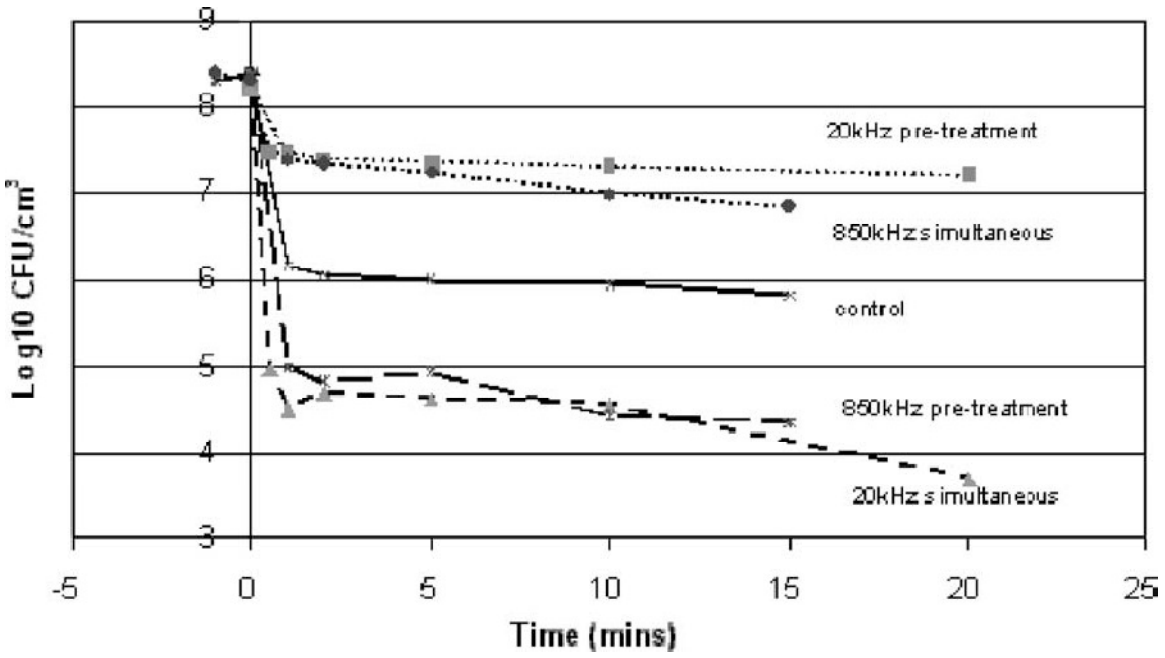

Figure 2-3: Chlorine inactivation of *E. coli* with pre- and simultaneous sonication for 1 minute *(Duckhouse, et al.* 2004)

Earlier work by Phull *et al.* (1997) had also demonstrated a synergistic effect between sonication and chlorination. *E. coli* suspended in both saline solution and raw stream water were used for comparison in their experiments. After 5 minutes of treatment time, 1 mg/L chlorine inactivated 43% of the bacteria in the stream water and sonication alone inactivated 19% of the same bacteria. When sonication was applied followed by chlorination, 86% inactivation of bacteria was achieved. After 20 minutes, 100% inactivation of bacteria was achieved with the application of combined sonication and chlorination. Their research also indicated that increasing the sonication power from 12 watts/cm^2 squared to 21 watts/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. These data suggested that sonication followed by chlorination is a better choice than chlorination followed by sonication, because the latter scenario causes a degassing effect, leading to lower chlorine concentrations (Phull, *et al.* 1997).

2.3.5 Sonication Summary

Preliminary research has demonstrated the possible synergistic effects of sonication and chlorination. For application to full-scale water treatment plants, a flow through sonication system is needed. The bench scale setup built for this project is the next step in developing sonication technology for use in drinking water treatment. The results of this research are of interest to the water treatment community because the need exists for alternative disinfection schemes that allow for adequate disinfection to comply the LT2 Rule while also lowering the levels of DBPs in finished waters. This research was undertaken to further evaluate sonication due to limited research on sonication as a potential alternative disinfectant.

3 Methods

The goals of this research were to determine the inactivation of *E. coli* by sonication alone and by the combination of sonication and chlorination in a bench-scale flow through cavitation system. A series of experiments were designed and conducted to achieve these goals. This chapter begins with an overview of the experimental plan for evaluating inactivation of *E. coli*. This is followed by the experimental procedures and finally, the analytical methods.

3.1 Experimental Design

Two sonication systems were used in the research: a probe system and a flow through system. The probe system was a Misonix system, which is shown in Figure 3-1. Prior research had demonstrated the effectiveness of the probe system in conjunction with low dose chlorination for the inactivation of *E. coli* and MS2 coliphage (Plummer and Long 2005). Thus, this system was used as a comparison to the newly designed flow through system.

The flow through cavitation system, shown in Figure 3-2, was designed and constructed by Harris Acoustics Products Corporation, East Walpole, Massachusetts. The sonication chamber consisted of a stainless-steel sonication chamber with 12 transducers mounted on the underside. A peristaltic pump circulated the test water through the system, which included sterile, chlorine demand free Tygon 3603 tubing and a custom copper heat-exchanging coil, which was placed in an ice bath to control temperature. Temperature

was monitored via a temperature probe mounted in the circulation system just after the sonication chamber.

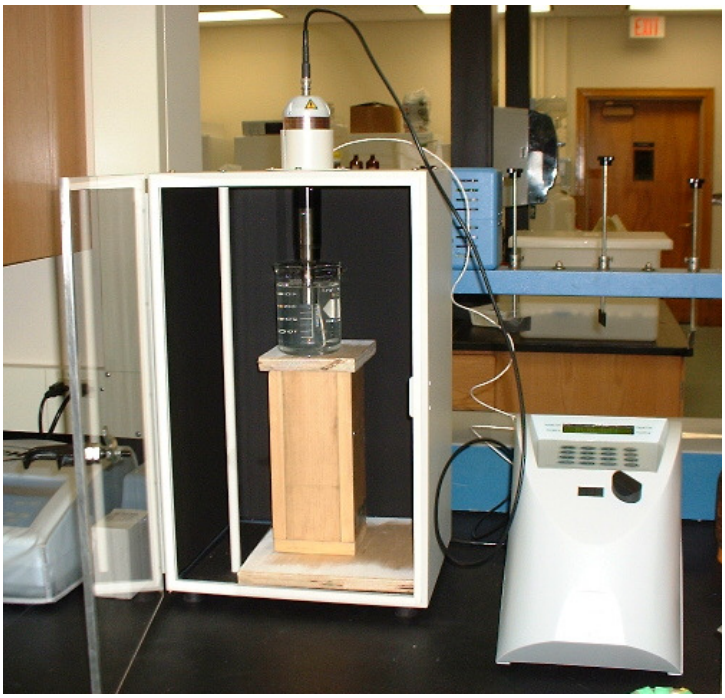

Figure 3-1: Misonix probe sonication system

The disinfection experiments conducted in this research consisted of four major categories: chlorination alone, sonication alone, the combination of chlorination and sonication, and control experiments. The disinfectants for the combined chlorination and sonication experiments were applied simultaneously. For experiments with extended sonication times, the temperature was controlled via the cooling coil to prevent the confounding impacts of heating on the inactivation of *E. coli*. All experiments were conducted in phosphate buffered saline (PBS) with a known starting concentration of *E. coli*. Table 3-1 summarizes all experiment parameters used in this research.

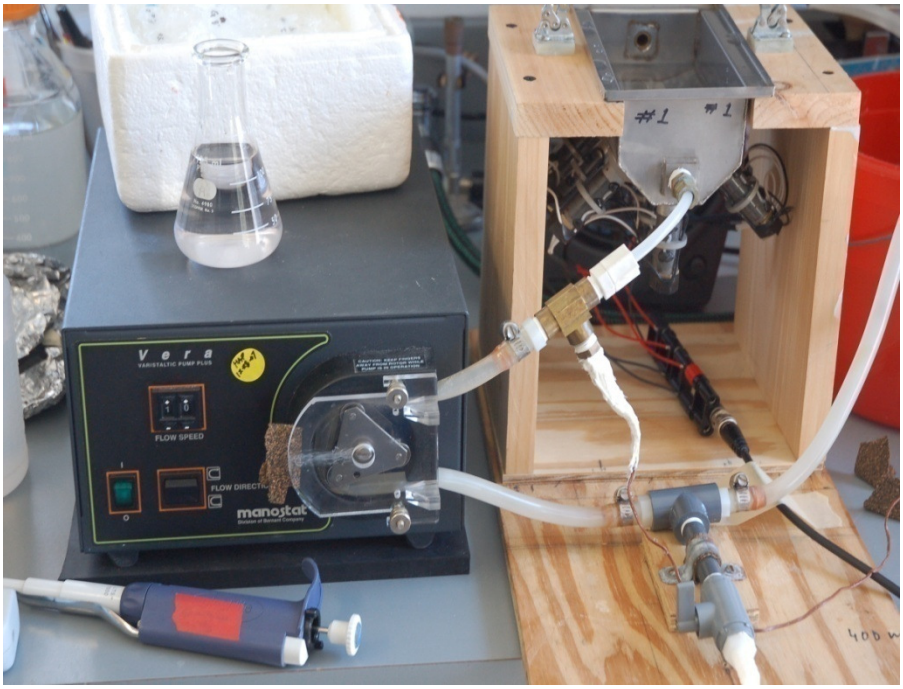

Figure 3-2: Harris Acoustic Products flow through sonication system

Table 3-1: Experimental variables

Parameter	*Range of Variable*		
	Chlorine Only	*Sonication Only*	*Chlorine and Sonication*
Chlorine Dose (mg/L)	0.4-1.0	NA	0.4-1.0
Sonication Time (min)	NA	0-16	0-16
Temp (°C)	22-23	Controlled below 25	Controlled below 25

NA – Not Applicable

Chlorine only and sonication only experiments were used to quantify inactivation of *E. coli* by the individual treatment techniques. These results were compared to results from

simultaneous chlorination and sonication experiments to determine if a synergistic effect occurred. Control experiments provided a baseline comparison to demonstrate any other pathways in the system that may cause removal or inactivation of microorganisms. Based on preliminary experiments, chlorine doses of 0.4 to 1 mg/L were chosen. Chlorine doses greater than 1 mg/L inactivated *E. coli* to undetectable counts; lower chlorine doses did not provide any significant inactivation. Based on these considerations, a chlorine dose of 0.6 mg/L was determined to be the most suitable concentration among the tested doses.

For sonication, contact times of up to 16 minutes were tested with samples taken at several intervals during exposure. For the probe system, treatment times were equal to actual times. For the flow through system, the volume of the cavitation chamber was 400 mL and approximately 100 mL of the sample was contained in the tubing and coil. Water in the tubing and coil is not exposed to sonication. Therefore, circulation times were longer than the treatment times to account for the time spent in the tubing and coil. Table 3-2 shows the actual circulation times used in the experiments, and the corresponding treatment times achieved. In the results section, times are presented as treatment times.

Control and chlorine only experiments were conducted at room temperature. For experiments with the probe system, experiments were started at room temperature ($22^{o}C$) and monitored through treatment. For experiments with the flow through system,

temperature was controlled to between 20°C and 25°C by immersing the copper coil in an ice bath.

Table 3-2: Treatment and circulation times in flow through system

Actual Circulation Time (min:sec)	Treatment Time in Chamber (min:sec)
1:16	1:00
2:32	2:00
5:04	4:00
10:08	8:00
20:16	16:00

A portion of the experiments were conducted at Worcester Polytechnic Institute (WPI) in Worcester, Massachusetts, and a portion at Tufts University Cummings School of Veterinary Medicine (Tufts) in North Grafton, Massachusetts. All experiments at WPI used *E. coli* as the test organism. Experiments at Tufts were conducted with *E. coli*, *Cryptosporidium parvum* and *Bacillus subtilis.* This thesis is focused on *E. coli* results, using data from both university laboratories.

3.2 Experimental Procedures

This section describes in detail the preparations and procedures for all experiments. First, preparation of *E. coli* is discussed. Then, methods for chlorination alone, sonication alone, and combined sonication and chlorination experiments with the probe system are provided. Control experiments are discussed, and lastly, the methods for the flow through system are detailed.

3.2.1 *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 (CDF) PBS. Then, a certain volume of the resuspended *E. coli* was added to the experimental water (CDF PBS) to obtain the desired starting concentration of 3 x 10^7cfu/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^{o}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^{o}C, shaking at a constant rate of 100 revolutions per minute (rpm). The inoculated flask 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 Company, 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 dilution bottle containing 25 mL of 0.01 M CDF PBS. A small volume of the CDF PBS was poured into the centrifuge tube and shaken until the pellet was completely dissolved. The solution was then poured back into the dilution 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 4×10^9cfu/mL.

To perform an experiment, a certain volume of resuspended *E. coli* was spiked into the test water (CDF PBS) to achieve the desired initial concentration of *E. coli* (3×10^7cfu/mL). The volume of resuspended *E. coli* added to the test water was determined by Equation 11:

$$C_{resuspension} * V_{resuspension} = C_{test\ water} * V_{test\ water} \quad \textit{(Equation 11)}$$

In this equation, $C_{resuspension}$ = 4 x 10^9 cfu/mL, $C_{test\ water}$ = 3 x 10^7 cfu/mL, and the volume of the test water varied for different experimental conditions. For experiments with the probe system, the test water was prepared in an autoclaved, CDF media bottle. 1 mL of test water was withdrawn from the media for a pre-disinfection *E. coli* count. For experiments with the flow through system, the test water was poured into the cavitation chamber. Then, the appropriate volume of resuspended *E. coli* was added to the cavitation chamber. The system was allowed to circulate for 90 seconds to ensure mixing and 1 mL of test water was withdrawn from the center of the cavitation chamber for pre-disinfection *E. coli* counts. The test water was then ready for disinfection experiments.

3.2.2 Experiments with Probe Sonication System

3.2.2.1 Chlorine Only Experiments

For each experiment performed, a CDF sterile 1-L media bottle containing 500 mL 0.01M CDF PBS (test water) was allowed to reach room temperature. Then, 3.75 mL of the resuspended *E. coli* was spiked into the test water. The bottle was gently inverted to mix, then 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 to the sterile CDF media bottle. The volume of chlorine stock solution added was calculated via Equation 12:

$$C_{Cl\ Stock} * V_{Cl\ Stock} = C_{test\ water} * V_{test\ water} \qquad (Equation\ 12)$$

In this equation, $C_{test\ water}$ ranged from 0.4 to 1.0 mg/L and $V_{test\ water}$ was 500 mL. After chlorine was added to achieve the desired dose, the bottle was gently inverted to mix. During the reaction period, 1 mL of test water was removed at specified intervals to determine the *E. coli* concentration. The 1 mL samples of test water were immediately quenched after sampling by adding a specified amount of sodium thiosulfate ($Na_2S_2O_3$) solution. Upon quenching, the post-disinfection water samples were transferred to dilution tubes and put in the refrigerator for post-disinfection *E. coli* enumeration.

3.2.2.2 Sonication Only and Combined Sonication/Chlorination

A probe-type sonicator (Sonicator 3000, Misonix Inc., Farmingdale, NY) was used for sonication only experiments and combined disinfectants experiments (sonication plus chlorination) as a comparison to the flow through system. 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. Using a 500 mL sample, the power-to-volume ratio tested was 180 W/L. The ultrasonic frequency was constant at 20 kHz 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. CDF materials were used for combined sonication and chlorination experiments.

All sonication only experiments started at room temperature (22 – 23^{o}C). For the experiments, 3.75 mL of resuspended *E. coli* was spiked into a 1 L media bottle containing 500 mL of sterile 0.01 M PBS. Prior to disinfection, 1 mL was removed to

determine the pre-disinfection *E. coli* concentration. Then, the test water was transferred to a 600 mL autoclaved beaker (Kimax #14000). The sonication 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. At each specified time interval, temperature was recorded, followed by the removal of 1 mL of solution for post-disinfection *E. coli* enumeration. Sodium thiosulfate was added to the post-disinfection samples. Although the sonication only samples did not have chlorine, this step was performed to ensure consistency in sample processing. For sonication and chlorination experiments, the same procedures were used as sonication only experiments with one exception: chlorine was dosed into the beaker at the same time that the sonication was started.

3.2.3 Experiments with Flow-through Sonication System

A sonication system as described in Section 3.1 was used to evaluate the germicidal effectiveness of a flow through cavitation device. The system operated at 25 ± 6% kHz and provided up to 150 W of power output, and these parameters were fully adjustable.

Prior to an experiment, the cavitation system was washed twice with sterile distilled water. To wash the system, approximately 500 mL of distilled water was poured into the chamber, circulated with the pump at high speed for 60 seconds, and then drained via the access port in between the pump and the coil. This step was repeated twice. The chamber with the transducers was then sprayed with a 50% ethanol solution and allowed to dry. Then, 500 mL of 0.01 M CDF PBS was added to the chamber and the pump

turned on to fill the tubing. The chlorine concentration of the test water was measured after 90 seconds of circulation to ensure no residual chlorine was present. The appropriate volume of resuspended *E. coli* was added to achieve the starting concentration and the water was circulated for an additional 90 seconds to ensure a uniform distribution of *E. coli*. A 1 mL sample was then taken from the center of the chamber for pre-disinfection counts.

Next, the appropriate treatment was started: no treatment for the control experiments, or the addition of chlorine and/or the start of sonication for the disinfection experiments. At the times listed in Table 3-2, 1 mL samples were taken from the center of the chamber for post-disinfection enumeration of *E. coli*. All samples were quenched with sodium thiosulfate. Temperature and power output were monitored for all experiments, and temperature was controlled as stated in Section 3.1.

3.3 Analytical Methods

3.3.1 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 the same autoclave.

3.3.2 *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 EC7 was used for this research.

3.3.3 Enumeration of *E. coli*

In the experiments performed at WPI, the pour plate method was 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. At least 3 different dilutions were plated for each sample, with two replicates for

each dilution. A negative control, which consisted of PBS only without *E. coli*, was plated for each sample, and agar only controls were plated for each bottle of agar. For the experiments performed at Tufts University, the spread plate method was used (details not provided).

3.3.3.1 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 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.

3.3.3.2 Pour Plates

The procedures for pour plating are described in Standard Methods 9215B (APHA, *et al.* 2005). After the pre- and post-disinfection dilution series were completed, 1 mL of sample from each appropriate dilution was pipetted into a 100-mm petri dish. Two replicate plates were prepared for each dilution plus one negative control for each experiment. 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°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. Log reductions of *E. coli* for all post disinfection scenarios were calculated.

3.3.4 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 small, it was difficult to measure the small volume to be added to the test water. Therefore, chlorine stock solutions of approximately 50 mg/L and 500 mg/L were prepared and used as needed to achieve desired chlorine concentrations in the test water. All of the chlorine vials and bottles were stored in the refrigerator at 4°C.

3.3.5 Free and Total Chlorine Concentrations

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 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.

3.3.5.1 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.* 2005). The preparation of a free chlorine calibration curve involved the use of spectrophotometer and titration. The spectrophotometer was set to wavelength of 515 nm. Five Erlenmeyer flasks and five volumetric flasks were taken out of the 100 mg/L chlorine bath and rinsed three times 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.* 2005). 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 consumed 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.

3.3.5.2 Residual Free Chlorine Measurement Using DPD Colorimetric Method

For selected experiments that involved chlorine during disinfection, the free chlorine residual concentration was measured, before and after quenching. The DPD colorimetric method #4500-Cl G in Standard Methods was used to perform this test (APHA, *et al.*

2005). Test tubes were taken out of the 100 mg/L chlorine bath and rinsed thoroughly with E-pure water three times. 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.

3.3.5.3 Residual Total Chlorine Measurement Using DPD Colorimetric Method

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

3.3.5.4 Determination of Chlorine Stock Concentration

The free chlorine concentration of the 500 mg/L and 50 mg/L chlorine stock solutions were 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. To determine the concentration of the 500 mg/L chlorine stock, 1 mL of the chlorine stock was added to a CDF volumetric flask using a pipette and brought up to 100 mL with CDF

E-pure water. As described previously, this solution was then poured into an Erlenmeyer flask with DPD buffer, DPD indicator, and a stir bar. The solution was titrated with FAS. The free chlorine concentration of the solution is equal (in mg/L) to the volume of FAS used (in mL) with this method. The results were then multiplied by 100 to account for dilution. The same procedure was followed for the 50 mg/L solution, but 10 mL of stock was added and brought up to 100 mL, and the results were multiplied by 10 instead of 100. These procedures of diluting the stock solution prior to titration were done to ensure the volume of FAS used was in the appropriate range (less than 5 mL) per Standard Methods.

3.3.6 Reagents and Glasswares

3.3.6.1 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.

3.3.6.2 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 3.3.6.1. Second, 15 g of the dehydrated TSA powder (Bacto™ Agar 214010, Dickinson and Company, Sparks, MD) was added to each liter of TSB. Third, the agar was brought to boil and then autoclaved for 20 – 30 minutes at 121°C, depending on the volume of agar being sterilized. The TSA was kept in the 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 a 47°C in water bath. The agar was used within 3 hours for pour plating.

3.3.6.3 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 7.2 - 7.4. The 0.01 M PBS was

then divided into various containers, such as media bottles and dilution tubes, and then autoclaved. If they were not used immediately, they were stored in the refrigerator at 4°C for up to 3 months.

3.3.6.4 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. If the 0.01 M PBS was free from chlorine, it was then autoclaved and stored tightly capped in the refrigerator at 4°C. If chlorine remained, the solution was irradiated with the UV pen for another 12 to 24 hours.

3.3.7 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 the 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.

3.3.7.1 Chlorine Demand Free Glassware and Tubing

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. If necessary, the glassware was then autoclaved. CDF tubing was prepared in the same manner. The tubing was used as part of the flow through sonication system.

3.3.7.2 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^{o}C. They were stored in the refrigerator at 4^{o}C until use, with a maximum storage time of approximately 2 weeks.

3.3.7.3 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 the Standard Methods (APHA, *et al.* 2005), 0.1 mL of a 3% $Na_2S_2O_3$ neutralizes up to 5 mg/L of residual chlorine. 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. Sufficient sodium thiosulfate was used to quench all chlorine as outlined by Standard Methods.

3.3.7.4 DPD Indicator Solution

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

3.3.7.5 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* 2005), 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 ethylenediaminetetrascetatedihydrate (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.

3.3.7.6 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 the Standard Methods (APHA, *et al* 2005), 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.

4 Results

Chapter four presents the results obtained from experiments conducted to determine the sole and synergistic effects of sonication on the inactivation of *E. coli*. The chapter is divided into three sections. First, experiments conducted with the WPI Misonix probe system are presented. Next, experiments conducted in the Harris Acoustics Products Corporation cavitation chamber without circulation are shown. Finally, experiments conducted in the cavitation chamber with circulation are presented. Full experimental results are provided in Appendix A of this thesis.

4.1 Probe System

Initial experiments were performed with the Misonix probe system with testing conducted at Tufts Univeristy. In order to determine if the vessel in which sonication was performed impacted results, experiments were conducted in a 500 mL glass beacker and in the Harris cavitation chamber (with no tubing or circulation and using the probe for cavitation rather than the transducers). Temperature was monitored but not controlled in all experiments. The starting temperature ranged from 21 to 23^{o}C, and the temperature after 4 minutes never exceeded 25^{o}C. Thus, temperature was not a factor in *E. coli* inactivation.

The results for the probe system experiments performed at Tufts University are shown in Figure 4-1. The sonication probe was operated at 90 W, providing 180 W/L of power to 500 mL samples of experimental water. Experiments were conducted with sonication only, sonication plus chlorine, and chlorine only, using 0.6 mg/L chlorine for all tests

with chlorine. Control experiments were also conducted with no treatment. The control experiment (no treatment) indicated no significant change in *E. coli* concentration over the 4 minute experiment time (log change less than 0.1 log). Chlorine at 0.6 mg/L resulted in a 1.04 to 1.22 log reduction in *E. coli* concentration with 1 to 4 minutes of contact time. Sonication alone was not effective in inactivating *E. coli*. Sonication performed in the beaker produced similar results to the control sample, while sonication in the cavitation chamber reduced *E. coli* only 0.21 to 0.37 log with a 1 to 4 minute contact time. Simultaneous sonication and chlorination was less effective than chlorine alone. For example, combined treatment in the chamber resulted in 0.28 log reduction of *E. coli* after 4 minutes, compared to 1.22 log reduction by chlorine alone with the same contact time.

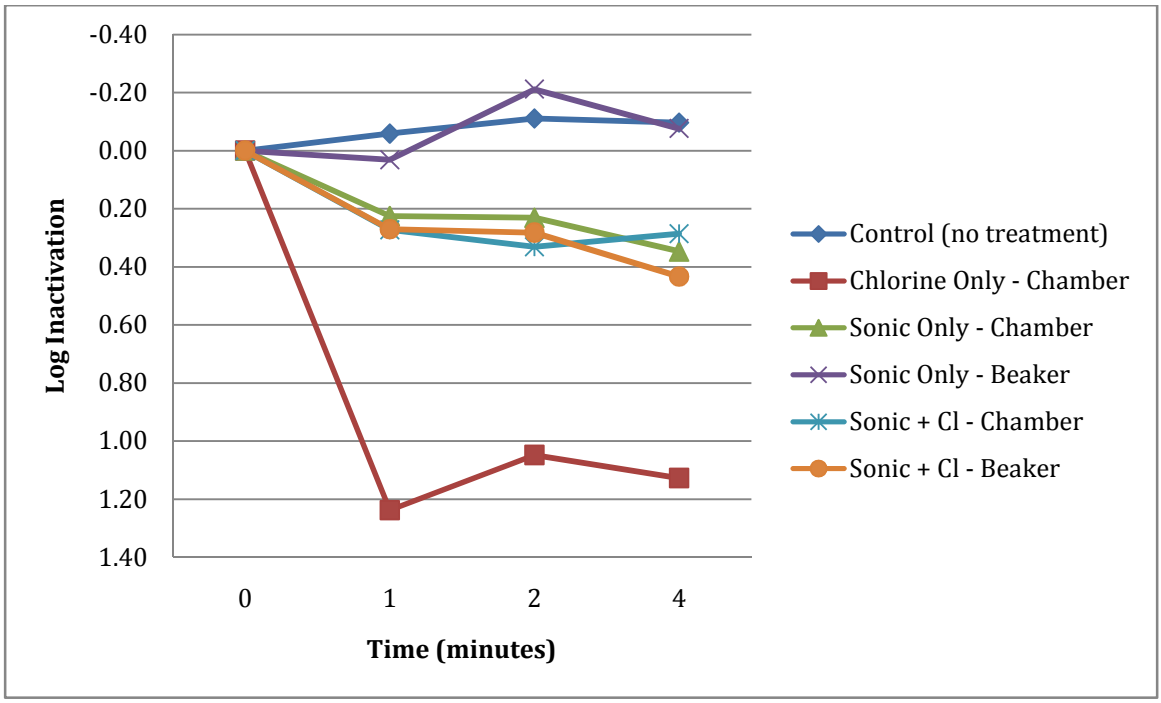

Figure 4-1: Log inactivation of *E. coli* with probe sonication system (performed at Tufts)

The data presented in Figure 4-1 contradict results from prior studies discussed in Section 2.3.4, none of which showed a reduction in the effectiveness of chlorine because of added sonication.

Because these results conflicted with established data, experiments were duplicated in the WPI environmental engineering laboratory and included chlorination in the CDF beaker as well. Again, temperature remained below levels which could impact inactivation for the *E. coli* (less than 25^{o}C in all experiments).

As shown in Figure 4-2, the control experiment showed no significant change in *E. coli* concentration with time. Sonication only in the beaker or Harris cavitation chamber also showed no significant impact on *E. coli*, with less than 0.1 log reduction at 4 minutes. In both the beaker and the cavitation chamber, chlorine at 0.6 mg/L reduced *E. coli* concentrations by 7-log with 2 minutes of contact time. When sonication and chlorination were applied simultaneously, 7-log reduction was observed after 2 minutes. Thus, results were similar for chlorine alone and combined treatment. While these data demonstrate that sonication does not interfere with chlorination, no synergistic impacts for combined treatment were observed.

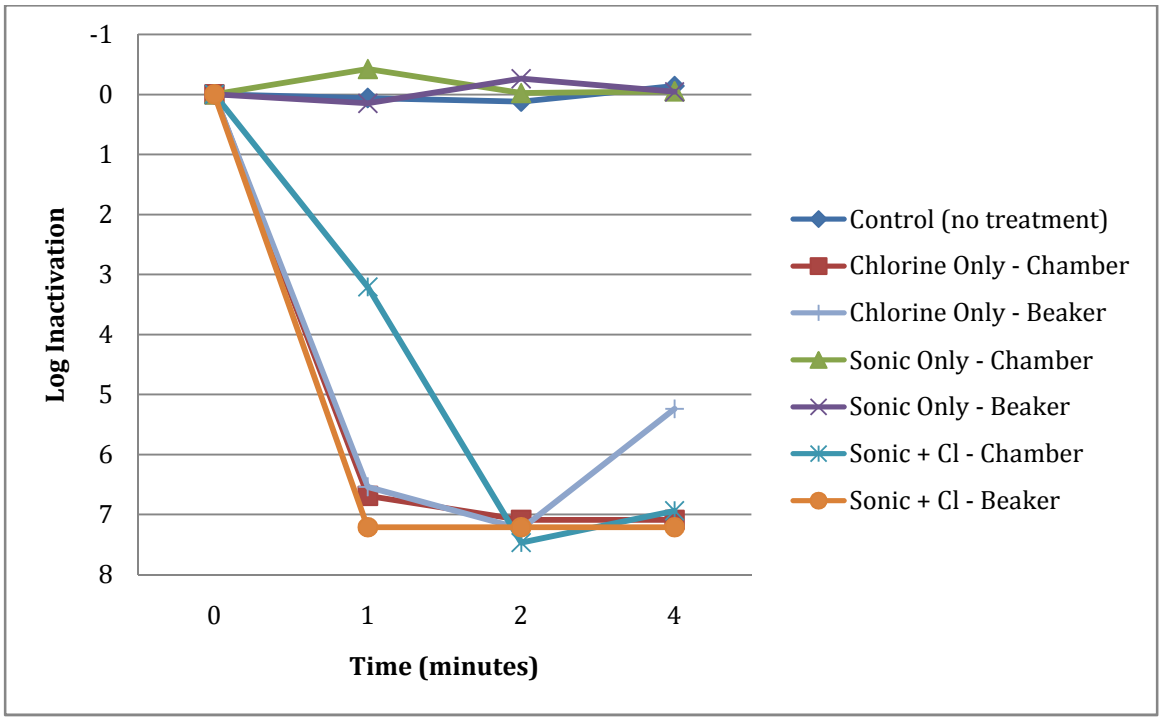

Figure 4-2: Log Inactivation of *E. coli* with Probe Sonication System (performed at WPI)

4.2 Cavitation System without Circulation

The next phase of experiments evaluated the effectiveness of the Harris Acoustic Products, Inc., cavitation system. This section presents results from experiments conducted in the cavitation chamber but without tubing or the cooling coil connected. The objective was to quantify inactivation by the transducers without potentially confounding influences introduced by circulation. Experiments were conducted both at WPI and at Tufts University. Temperature was below 25°C for all experiments. Cavitation was performed at 25 ± 6% kHz and up to 150 W of power output, with a power to volume ratio of 0.3 W/cm^2.

The first set of experiments with the cavitation chamber-based sonication system were performed at Tufts University. Results are shown in Figure 4-3. The control treatment and sonication alone both showed negligible inactivation effects on the *E. coli* (less than 0.2 log reduction at 4 minutes). Chlorine applied at 0.6 mg/L resulted in 0.81 to 1.22 log inactivation with 1 to 4 minute contact time. However, this inactivation is six orders of magnitude less than the same treatment tested in the first phase of the experiment using the WPI probe system. The chlorine only results should be comparable for the two phases of experimentation. As seen in earlier results, combined treatment provided similar inactivation to treatment with chlorine alone.

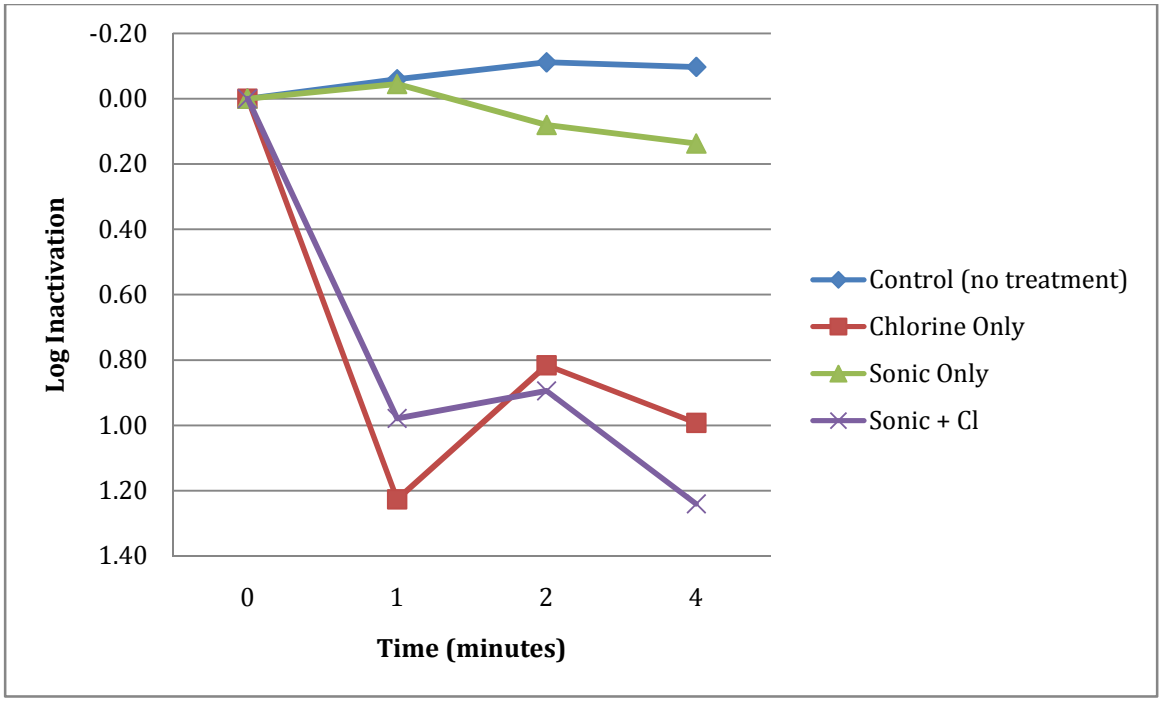

Figure 4-3: Log inactivation of *E. coli* with cavitation chamber system (performed at Tufts)

This set of experiments was repeated at WPI and the results are shown in Figure 4-4. Results are congruent with the WPI test results from the probe-based system given in

Section 4.1. Sonication only and no treatment both had negligible effects on the *E. coli.* Chlorination showed substantial log reduction, 5.2 to 7.2 log, during the tests. Sonication and chlorination together resulted in 4.6 to 5.6 log reduction of *E. coli*.

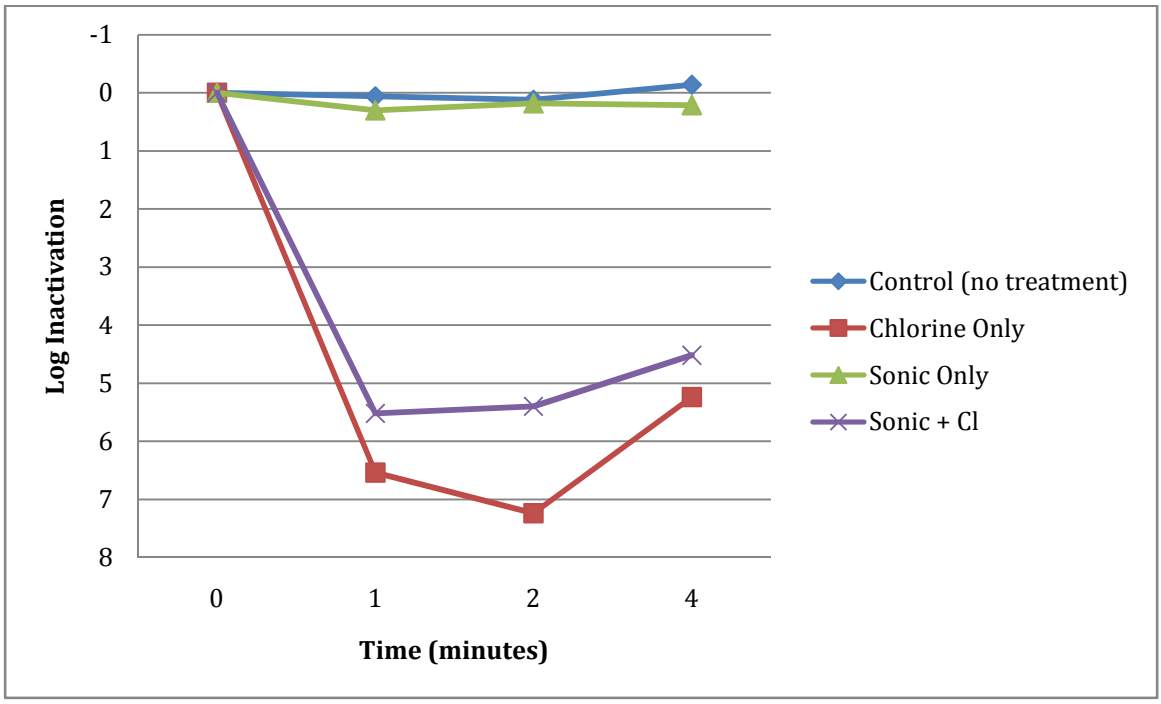

Figure 4-4: Log inactivation of *E. coli* with cavitation system (performed at WPI)

4.3 Cavitation Chamber System with Circulation

The Harris cavitation system was tested in flow through mode, as described in Chapter 3. Initial experiments (data not presented) indicated that the tubing exerted a chlorine demand. Thereafter, the tubing was autoclaved and chlorine demand free to reduce this impact as much as possible. As in the second phase of experiments, the cavitation system was operated at 25 ± 6% kHz and provided up to 150 W of power output, providing 0.3 W/cm^2. Experimental water was circulated through the tubing and cooling coil, and treatment times adjusted to account for the time spent out of the cavitation chamber.

Results are shown in Figure 4-5. As shown previously, the control experiment and sonication only had no notable effect on the inactivation of *E. coli*. Chlorination reduced *E. coli* by 4.5, 3.7 and 3.6 log at 1, 2 and 4 minutes, respectively. Similarly, combined sonication and chlorination resulted in 4.4, 3.5 and 3.1 log inactivation of *E. coli* for the three treatment times. These results are consistent with prior phases of research, showing similar effectiveness of chlorine only and sonication and chlorine combined. However, the actually log reductions in the flow through system were 1.2 to 2.5 log less than in non-circulating trials.

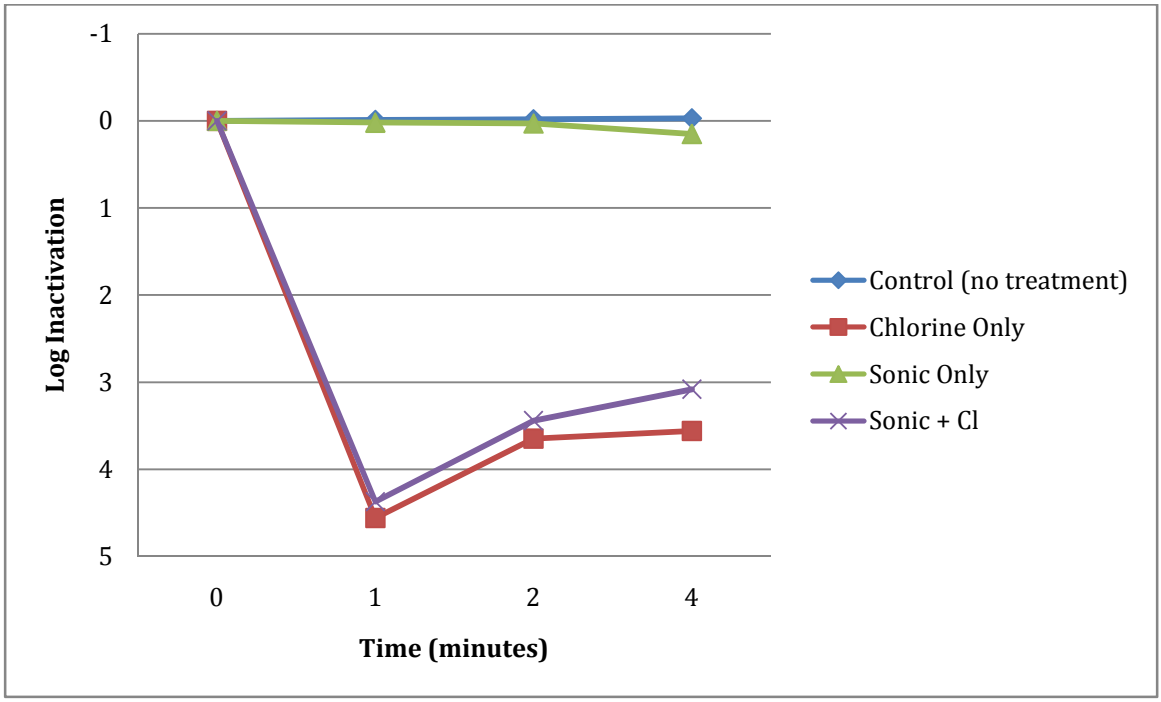

Figure 4-5: Log inactivation of *E. coli* with circulating cavitation system (preformed at WPI)

4.4 Comparison of Cavitation Systems

Tables 4-1 and 4-2 provide a comparison of chlorination and chlorination plus sonication effectiveness as cavitation systems were changed. Both tables demonstrate a significant

decline in inactivation in the cavitation system when circulation is occurring (as much as 2-log less with chlorine only, and nearly 3-log less with combined treatment). Additionally, both tables show that the effectiveness of treatment is not changed significantly (less than 0.5 log) when the reaction vessel is changed from the beaker to the cavitation chamber.

Table 4-1: Chlorine-only comparison

Chlorine (mg/L)	Sonicator	Sonic Vessel	% reduction			log reduction			
			1 min	2 min	4 min	0	1	2	4
0.6	-	Beaker	100	100	100	0	6.54	7.24	5.24
0.6	-	Chamber - Static	100	100	100	0	6.69	7.09	7.09
0.6	-	Chamber - Circulation	100	99.98	99.97	0	4.56	3.65	3.56

Table 4-2: Chlorination plus sonication comparison

Chlorine (mg/L)	Sonicator	Sonic Vessel	% reduction			log reduction			
			1 min	2 min	4 min	0	1	2	4
0.6	WPI	Beaker	100	100	100	0	7.21	7.21	7.21
0.6	WPI	Chamber - Static	99.94	100	100	0	3.21	7.47	6.94
0.6	Harris	Chamber - Circulation	100	99.96	99.92	0	4.37	3.44	3.08

5 Recommendations

More research is required if synergistic disinfection with sonication and chlorine is to be considered for application in a water treatment system. Because of the limited time and resources for this study, the following additional work is not feasible for completion as part of the data presented here, but has emerged as necessary because of the research results.

i. A sonication system should be designed that allows more flexibility and better control of experimental parameters. The system should include:
    a. Adjustable power output to at least 200W.
    b. Adjustable frequency of sonication (more than +/- 6%) so that multiple frequencies can be examined
    c. Removable components which contact experimental water. Specifically, any part of the apparatus which will contact experimental water should be chlorine demand free, and autoclaved.
    d. The ability to examine true flow through (point to point) treatment, rather than just circulation, to better emulate conditions in a treatment plant.

ii. The power required to run the sonication equipment should be monitored at all times in order to fully evaluate the economic feasibility of using a sonication system to achieve synergistic disinfection.

iii. The increase in temperature caused by sonication should be monitored at all times in order to fully evaluate the feasibility of using the sonication system in a

drinking water treatment system, where water cannot be provided at raised temperatures to consumers.

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Appendicies

Appendix 1: Results for Experiments Conducted at WPI

Exp.	Chlorine	Sonicator	Sonic Vessel	% reduction			log reduction			
	(mg/L)			1 min	2 min	4 min	0	1	2	4
A	-	WPI Probe	Beaker	28.43	-82.74	-10.15	0	0.15	-0.26	-0.04
							0	0.37	0	0.07
B	0.6	-	Beaker	100	100	100	0	6.54	7.24	5.24
							0	1.4	1.4	1.4
C	0.6	WPI Probe	Beaker	100	100	100	0	7.21	7.21	7.21
							0	6	6	6
D	-	-	Trough – no tubing	12.18	24.68	-38.78	0	0.06	0.12	-0.14
E	-	WPI Probe	Trough – no tubing	-161.43	-3.59	-11.66	0	-0.42	-0.02	-0.05
F	0.6	-	Trough – no tubing	100	100	100	0	6.69	7.09	7.09
G	0.6	WPI Probe	Trough – no tubing	99.94	100	100	0	3.21	7.47	6.94
H	-	Harris	Trough - no circ	21.67	16.39	37.22	0	0.11	0.08	0.2
							0	0.3	0.18	0.21
I	0.6	Harris	Trough - no circ	100	100	99.97	0	5.11	4.75	3.6
							0	5.52	5.4	4.52
J	-	Harris	Trough - circulation	4.98	5.98	28.57	0	0.02	0.03	0.15
							0	0.39	0.46	0.44
K	0.6	Harris	Trough - circulation	100	99.96	99.92	0	4.37	3.44	3.08
							0	5.18	4.08	3.85
L	0.6	-	Trough - circulation	100	99.98	99.97	0	4.56	3.65	3.56
							0	5.82	5.6	4.77
M	-	-	Trough - circulation	-3.21	-5	-7.5	0	-0.01	-0.02	-0.03
							0	-0.12	0.24	0.24

When two lines of data are presented, the first line represents data from WPI enumeration, the second line represents Tufts enumeration.

Appendix 2: Experiments Conducted at Tufts with Tufts *E. coli*

Exp #	Cl2 (mg/L)	Sonic (W)	Sonic (kHz)	Vessel	Time (min)	Raw Counts 1.00E-03	Raw Counts 1.00E-03	Time = 0 count	Ave Conc	% reduction	log reduction
II-53	na	na	na	Trough	0	36	30	3.30E+04	3.30E+04	0.00	0.00
					1	33	20	3.30E+04	2.65E+04	19.70	0.10
					2	28	39	3.30E+04	3.35E+04	-1.52	-0.01
					4	25	49	3.30E+04	3.70E+04	-12.12	-0.05
II-52	0.6	na	na	Trough	0	43	32	3.75E+04	3.75E+04	0.00	0.00
					1	55	52	3.75E+04	5.35E+04	-42.67	-0.15
					2	44	50	3.75E+04	4.70E+04	-25.33	-0.10
					4	72	81	3.75E+04	7.65E+04	-104.00	-0.31
II-58	0.6	na	na	Beaker	0	44	50	4.70E+04	4.70E+04	0.00	0.00
					1	24	52	4.70E+04	3.80E+04	19.15	0.09
					2	42	36	4.70E+04	3.90E+04	17.02	0.08
					4	20	67	4.70E+04	4.35E+04	7.45	0.03
II-54	na	90		Harris in trough	0	4	60	3.20E+04	3.20E+04	0.00	0.00
					1	31	48	3.20E+04	3.95E+04	-23.44	-0.09
					2	44	44	3.20E+04	4.40E+04	-37.50	-0.14
					4	25	56	3.20E+04	4.05E+04	-26.56	-0.10
II-51	na	90		WPI in trough	0	79	84	8.15E+04	8.15E+04	0.00	0.00
					1	37	68	8.15E+04	5.25E+04	35.58	0.19
					2	46	68	8.15E+04	5.70E+04	30.06	0.16
					4	25	22	8.15E+04	2.35E+04	71.17	0.54
II-57	na	90		WPI in beaker	0	72	50	6.10E+04	6.10E+04	0.00	0.00
					1	96	56	6.10E+04	7.60E+04	-24.59	-0.10
					2	60	52	6.10E+04	5.60E+04	8.20	0.04
					4	37	42	6.10E+04	3.95E+04	35.25	0.19
II-50	0.6	90		Harris in trough	0	59	98	7.85E+04	7.85E+04	0.00	0.00
					1	41	41	7.85E+04	4.10E+04	47.77	0.28
					2	24	42	7.85E+04	3.30E+04	57.96	0.38
					4	14	39	7.85E+04	2.65E+04	66.24	0.47
II-55	0.6	90		WPI in trough	0	96	94	9.50E+04	9.50E+04	0.00	0.00
					1	68	44	9.50E+04	5.60E+04	41.05	0.23
					2	25	21	9.50E+04	2.30E+04	75.79	0.62
					4	16	13	9.50E+04	1.45E+04	84.74	0.82
II-56	0.6	90		WPI in beaker	0	40	48	4.40E+04	4.40E+04	0.00	0.00
					1	62	60	4.40E+04	6.10E+04	-38.64	-0.14
					2	44	50	4.40E+04	4.70E+04	-6.82	-0.03
					4	36	22	4.40E+04	2.90E+04	34.09	0.18

Appendix 3: Experiments Conducted at Tufts with WPI *E. coli*

Exp #	Cl2 (mg/L)	Sonic (W)	Sonic (kHz)	Vessel	Time (min)	Raw Counts 1.00E-03	Raw Counts 1.00E-03	Time = 0 count	Ave Conc	% reduction	log reduction
II-67	na	na	na	Trough	0	56	40	4.80E+04	4.80E+04	0.00	0.00
					1	70	40	4.80E+04	5.50E+04	-14.58	-0.06
					2	60	64	4.80E+04	6.20E+04	-29.17	-0.11
					4	80	40	4.80E+04	6.00E+04	-25.00	-0.10
II-66	0.6	na	na	Trough	0	58	60	5.90E+04	5.90E+04	0.00	0.00
					1	6	1	5.90E+04	3.50E+03	94.07	1.23
					2	13	5	5.90E+04	9.00E+03	84.75	0.82
					4	4	8	5.90E+04	6.00E+03	89.83	0.99
II-59	0.6	na	na	Beaker	0	256	280	2.68E+05	2.68E+05	0.00	0.00
					1	26	5	2.68E+05	1.55E+04	94.22	1.24
					2	24	24	2.68E+05	2.40E+04	91.04	1.05
					4	22	18	2.68E+05	2.00E+04	92.54	1.13
II-65	na	90		Harris in trough	0	88	60	7.40E+04	7.40E+04	0.00	0.00
					1	100	64	7.40E+04	8.20E+04	-10.81	-0.04
					2	78	45	7.40E+04	6.15E+04	16.89	0.08
					4	52	56	7.40E+04	5.40E+04	27.03	0.14
II-64	na	90		WPI in trough	0	180	300	2.40E+05	2.40E+05	0.00	0.00
					1	36	250	2.40E+05	1.43E+05	40.42	0.22
					2	32	250	2.40E+05	1.41E+05	41.25	0.23
					4	26	190	2.40E+05	1.08E+05	55.00	0.35
II-60	na	90		WPI in beaker	0	200	144	1.72E+05	1.72E+05	0.00	0.00
					1	200	120	1.72E+05	1.60E+05	6.98	0.03
					2	300	260	1.72E+05	2.80E+05	-62.79	-0.21
					4	300	110	1.72E+05	2.05E+05	-19.19	-0.08
II-63	0.6	90		Harris in trough	0	200	200	2.00E+05	2.00E+05	0.00	0.00
					1	21	21	2.00E+05	2.10E+04	89.50	0.98
					2	24	27	2.00E+05	2.55E+04	87.25	0.89
					4	16	7	2.00E+05	1.15E+04	94.25	1.24
II-62	0.6	90		WPI in trough	0	360	390	3.75E+05	3.75E+05	0.00	0.00
					1	200	200	3.75E+05	2.00E+05	46.67	0.27
					2	150	200	3.75E+05	1.75E+05	53.33	0.33
					4	280	108	3.75E+05	1.94E+05	48.27	0.29
II-61	0.6	90		WPI in beaker	0	108	250	1.79E+05	1.79E+05	0.00	0.00
					1	96	96	1.79E+05	9.60E+04	46.37	0.27
					2	86	101	1.79E+05	9.35E+04	47.77	0.28
					4	48	84	1.79E+05	6.60E+04	63.13	0.43