PHILIPPINE ENGINEERING JOURNAL
PEJ 2009; Vol. 30, No. 1: 29-38

# EFFECT OF LOW FREQUENCY ULTRASONICATION AS A PRE-CHLORINATION TREATMENT IN THE ACTIVATION OF STREPTOCOCCUS FAECALIS IN DRINKING WATER

**Grace Ruth L. Salamat and Genandrialine L. Peralta**
*Environmental Engineering Graduate Program*
*University of the Philippines, Diliman*

## ABSTRACT

*Water disinfection has two goals: the inactivation of pathogens and the protection from subsequent intrusion of microorganisms downstream of the distribution system. Although excess disinfectant is necessary to prevent biological regrowth and inactivation of pathogenic organisms, the concern for the carcinogenic effects of disinfection by-products should also be addressed due to its environmental and health effects. Presently, ultrasonic disinfection is considered as an alternative method. However, ultrasonication alone cannot ensure non-intrusion of pathogens because it lacks a residual effect. Considering this, this study used ultrasonication as a pre-treatment method to reduce chlorine dose while maintaining effectiveness in the inactivation of Streptococcus faecalis. S. faecalis is a pathogenic organism that serves as an alternative indicator of fecal contamination in water. The experiments were divided into three phases: ultrasonication, chlorination, and sequential disinfection, where ultrasonication is used as pre-chlorination treatment. In ultrasonication, amplitude setting and exposure time were varied from 30-100 percent and 1-30 minutes, respectively. The US processor was operated at a fixed frequency of 20 kHz. At 100 percent amplitude, the recorded power was approximately 33 W. This phase resulted in an inactivation rate constant, $k_{strep}$ = 0.0293, and 0.399 log reduction units at 100 percent amplitude and 30 minutes of exposure time. Chlorination made use of different chlorine concentration, 0.5-10 ppm. 1ppm of chlorine yielded a 0.24705 log reduction units after 1 minute contact time. In sequential disinfection, 5 minutes of ultrasonication was followed by chlorination using different concentrations. A substantial increase in inactivation was observed from 1 min chlorination and sequential disinfection, from 0.24705 log reduction units to 0.46535 log reduction units. The use of sequential disinfection lowered chlorine concentration from 5 ppm to 1 ppm and shortened the contact time from 5 minutes to 1 minute while maintaining effectiveness of disinfection.*

## 1. INTRODUCTION

Water contributes to and affects health. It is in this regard that water quality becomes important. One way of ensuring quality of water is disinfection. Disinfection has two main goals: first is to inactivate pathogenic organisms and second is to provide disinfectant residual to prevent re-intrusion of pathogens downstream of the distribution system.

Among the conventional disinfection methods are chlorination, ozonation and UV irradiation. New methods are being looked at because of some problems encountered in conventional ones. For ozonation and UV irradiation, one problem is the lack of residual effect, leading to microbial regrowth. For chlorination, the main problem is the formation of harmful disinfection by-products.

Correspondence to: *Environmental Engineering Graduate Program, University of the Philippines, Diliman*



*Received: January 9, 2007*
*Accepted: December 15, 2011*

Among the alternative disinfection methods are gamma irradiation, activated carbon and ultrasonic disinfection. In the topic of disinfection, ultrasonication alone cannot prevent re-intrusion of pathogens because it lacks residual effect. In this regard, ultrasonication can be used as pretreatment to enhance effectiveness of other disinfection methods.

The general objective of this study is to look into the effects of ultrasonication as pre-chlorination treatment in the inactivation of *Streptococcus faecalis*. Specifically, the objectives are:

- To evaluate the effectiveness of ultrasonication and chlorination in the inactivation of *S. faecalis*, which is a more resistant type of bacteria
- To determine the relationship of ultrasonic exposure time in the inactivation of *S. faecalis*
- To ascertain whether ultrasonication can be used to lower chlorine dosage and if it can enhance chlorination efficiency

## 2. REVIEW OF RELATED LITERATURE

Among the disinfection methods, chlorination is proven to meet the two goals of disinfection mentioned earlier [Bryant et al., 1992]. Chlorination can be done using different chlorine disinfectants such as hypochlorite solutions, chloramines and chlorine dioxide. For this study, NaOCl was used because it's readily available. Between pH 6.5 and 8, which is the normal pH range of water, NaOCl dissociates to form HOCl. At pH>8, HOCl further disintegrates to form hypochlorite. Between the two, HOCl has a greater disinfecting power.

As any method there is, chlorination has its advantages and disadvantages. The advantages of chlorination include its effectiveness over a wide range of organisms, providing residual that inhibits microbial regrowth. Also, it has been proven cost-effective when compared to other disinfection methods. Some of the disadvantages of chlorination include its reduced effectiveness on chemotolerant organisms. Chlorine can also be blocked by bacterial flocs and other inert particles. And as much as the residual concentration is wanted, it can form harmful DBPs such as trihalomethanes [Ince et al., 2001; US EPA, 1999; Jolley et al., 1986].

Possible answer to these problems is to find a potent disinfectant that would leave a residual concentration without the harmful DBPs or to use a pretreatment method to enhance efficiency and or lower the required disinfectant dosage.

In this study, ultrasonication was used. Audible frequency range of sound is between 20-20kHz. Above this range is ultrasound [Young et al., 1996]. Ultrasound transmits a series of alternating compression and rarefaction, where bubbles form and expand at each rarefaction and contracts at compressions [Berg and Stork, 1995].

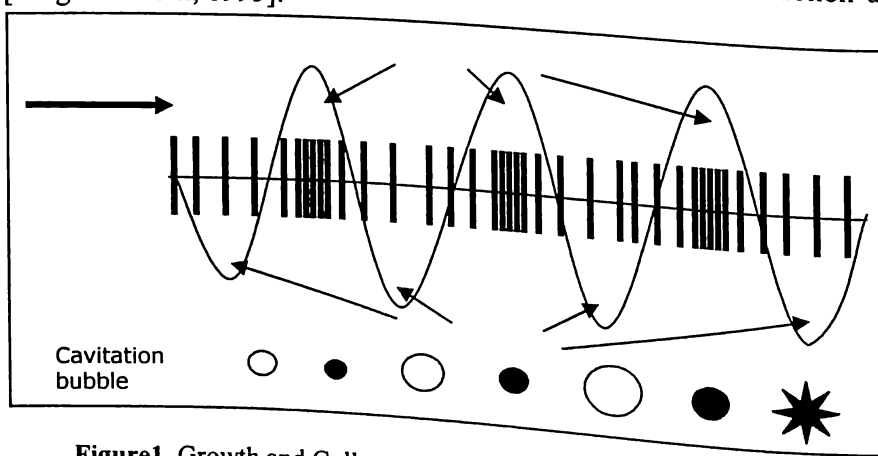


**Figure1.** Growth and Collapse of Cavitation Bubble [Battele Duxbury Operations Final Report 1998]

The amount of energy brought by sonication is usually in terms of power, which is the amount of energy per unit time.

$$P = k\omega^2 A^2 \qquad (1)$$

From this equation, it can be seen that power is directly proportional to both frequency and amplitude. Amplitude is described as the maximum displacement a particle can reach in a wave from equilibrium. Its implication lies in the degree of disturbance it causes in its medium [Young et al., 1996]. The greater the disturbance, the higher the amplitude, the higher the energy imparted.

Ultrasound can be classified through its frequency ranges. The 2 distinct ranges are the diagnostic ultrasound and the power ultrasound.

Diagnostic ultrasound is characterized by high frequency in the range of 2-10MHz, and the predominant mechanism in this range is the formation of free radicals, which further recombines to form oxidants. The other range, power ultrasound, is characterized by low frequency range of 20-100kHz. In this range, the predominant mechanism is cavitation [Mason, 1990; Hoffman et al., 1997; Tiehm, 1999; Ince et al., 2001; Neis et al., 2002].

Since the equipment used is limited to low frequency ultrasound, concentration will be given to cavitation.

The term cavitation means the cycle of formation, growth and collapse of bubbles caused by alternating positive and negative pressures. When this phenomenon is brought by sound waves, the term is acoustic cavitation [Pandit et al., 2000].

There are factors affecting cavitation: physical properties of the medium, temperature, presence of dissolved gases and irradiation power.

If the medium's vapor pressure is high, it volatilizes easily, causing cavitation bubbles to be cushioned by the vapor, reducing effective pressure upon collapse. Same is the case with temperature. The higher the temperature is, the higher the vapor pressure. This also explains the presence of dissolved gases, as dissolved gases could stifle the collapse of the bubbles, reducing the shock the cavitation provides [Petrucci et al., 1997; Mason, 1990].

Ultrasound, as disinfection, works in three ways: oxidant formation, disagglomeration of cell clusters and cell wall damage.

As mentioned earlier, oxidant formation is predominant in high frequency ultrasound, where free radicals are produced and recombined to form hydrogen peroxide. As previously stated, cavitation produces a shock. This shock disperses bacterial clusters, exposing individual cells. Further cavitation would shear the cell walls [Tsukamoto et al., 2004; Kalumuck et al., 2003; Neis et al., 2002; Weavers, 2001; Thompson et al., 1999; Hoffman et al., 1997].

Another aspect of the study is the bacteria used. Typically, coliform group is used as indicator organism. However, it is not always the best indicator for fecal contamination because non-fecal coliform exists [Mara et al., 2003; Schroeder et al., 1987]. For this reason, other bacteria are being used as indicator organism, one of which is *Streptococcus faecalis* of the fecal streptococcus group.

*S. faecalis* are usually arranged in pairs or in chains. Their cell walls are thicker than that of the coliform group, making them more resistant to disinfection. *S. faecalis* belongs to the group D of fecal streptococcus, which satisfies most requirements of an ideal indicator such as being exclusively associated to the intestinal tract, occurs in high numbers and more resistant to disinfection [Vincent, 2005; Neis et al., 2003; Mara et al., 2003].

 

## 3. METHODOLOGY

### *3.1 Sample Preparation*

*S. faecalis* was sub-cultured two days before being used to allow for proper incubation period (48 hours). Sterilized 0.1% (w/v) peptone water was inoculated with *S. faecalis* on the day of the experimental run. Aliquots of this prepared sample were used in the different disinfection procedures described in the following sections. Note that sub-culturing and inoculation were always done two days before and on the day of the experiment, respectively. This step ensures uniform cellular activity of *S. faecalis* in all samples.

### *3.2 Phase I : Sonication*

Sonication was done using fixed frequency (20 kHz), fixed sample volume (100 mL) and variable exposure time. A horn sonotrode (130 W, Cole–Parmer) with a 13mm titanium probe was used in conducting Phase I. In order to prevent extreme temperature increase, an ice-water bath was incorporated in the set-up. This was done to minimize temperature rise as most continuous systems would not incur the same temperature increase experienced in batch systems. Also, as stated earlier, an increase in temperature lowers the cavitation effects. As cavitation is the predominant mechanism in low frequency ultrasound, it was deemed better to maximize its effect. In this phase, there were two sets of experiment.

The first set of this phase intended to show the effect of varying amplitude setting, and hence power, to the inactivation of *S. faecalis*. A fixed exposure time of 10 minutes was used while the amplitude setting was varied from 30 to 100 percent.

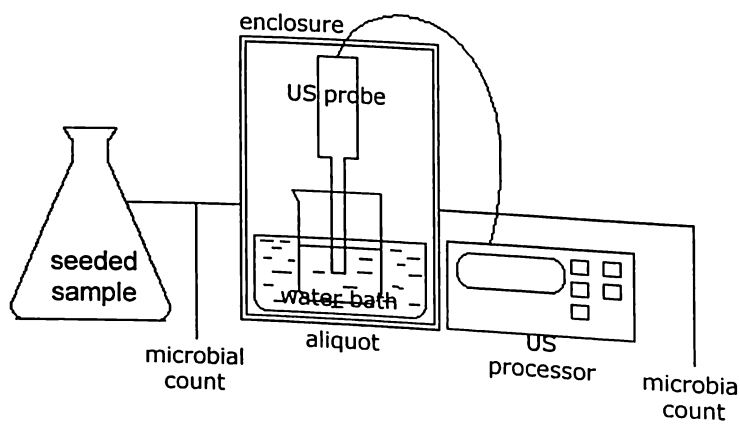


**Figure 2.** Phase I, Sonication Set-Up: Variable Exposure Time

After having established 100 % amplitude as most effective in the first set, the second set of experiment made use of this setting while varying exposure time from 1 to 30 minutes. This set aimed to establish the relationship of exposure time to the inactivation of *S. faecalis*.

### *1.3 Phase II : Chlorination*

pH of samples used in this phase has been adjusted to 6.5-7.5. 0.1 M calcium carbonate ($CaCO_3$) was used to adjust pH. This was done to simulate the pH of ordinary drinking water and to maximize effects of free chlorine in disinfection. Chlorination was done by stirring into the sample doses of chlorine. Variable concentrations of sodium hypochlorite solution (0.5, 1, 2, 5, 10 ppm) were used under fixed contact time (1 min / 5 min). Sodium thiosulfate ($Na_2S_2O_3$) was used to stop chlorine

reaction. Four sets using different sample: chlorine ratios were done (90:10, 75:25, 50:50 and 100:25). The result of this phase provided comparison for the third phase.

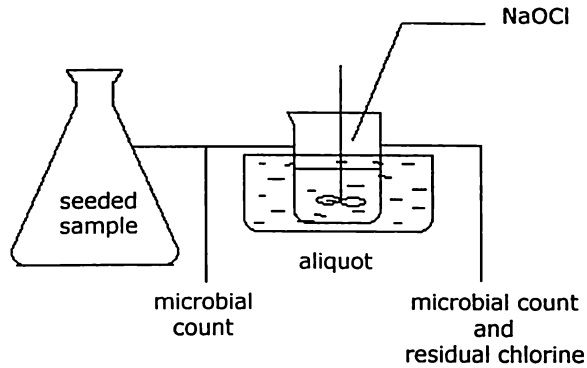


**Figure 3.** Phase II, Chlorination Set-Up

### *3.4 Phase III : Sequential Disinfection (US followed by Chlorine)*

For the third phase, all samples were subjected to ultrasonication for 5 minutes before undergoing chlorination for 1 and 5 minutes. Chlorination was done using different concentrations (0.5, 1, 2, 5 and 10 ppm), with a dilution factor of 0.2. Again, pH of samples was adjusted to 6.5-7.5.

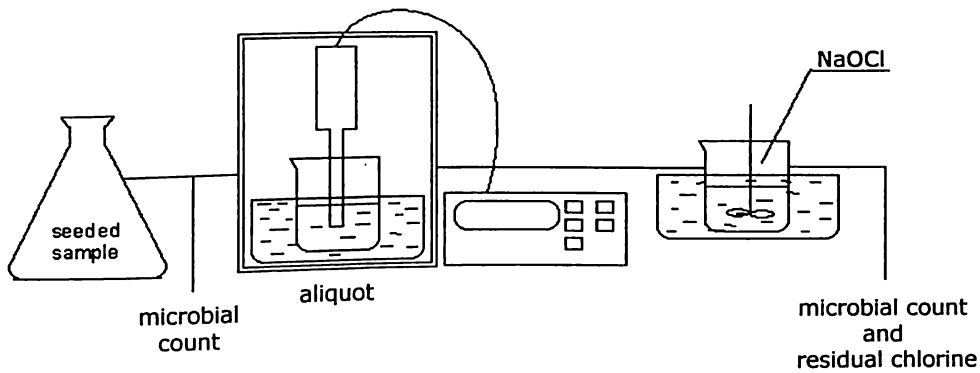


**Figure 4.** Phase III, Sequential Disinfection Set-Up

### *3.5 Microbial Analysis*

In all phases (I, II & III) of the study, microbial analysis was done after every process. Standard Method 9215 B: Pour Plate Technique, with some variations, was used in microbial analysis. This method was preferred due to its lower cost compared to Membrane Filtration Technique. And when compared to MPN methods, colony counting on solid medium exhibits better precision [Mara et al., 2003].

### *3.6 Residual Chlorine Analysis*

After every chlorination stage, all samples were subjected to a residual chlorine analysis. This was done using DPD Colorimetric Method through HACH colorimeter (DR/890 Portable Data Logging Colorimeter).

## 4. RESULTS AND DISCUSSION

Percent inactivation and log inactivation were calculated for all phases using equation 2 and 3, where No and N are the initial and final microbial count, respectively [Reyes, 2005].

$$\text{Percent Inactivation} = \left[1 - \frac{N}{N_o}\right] * 100 \qquad (2)$$

$$\text{Log inactivation} = \log \frac{N}{N_0} \qquad (3)$$

### *4.1 Sonication*

#### *4.1.a. Effects of Percentage Amplitude*

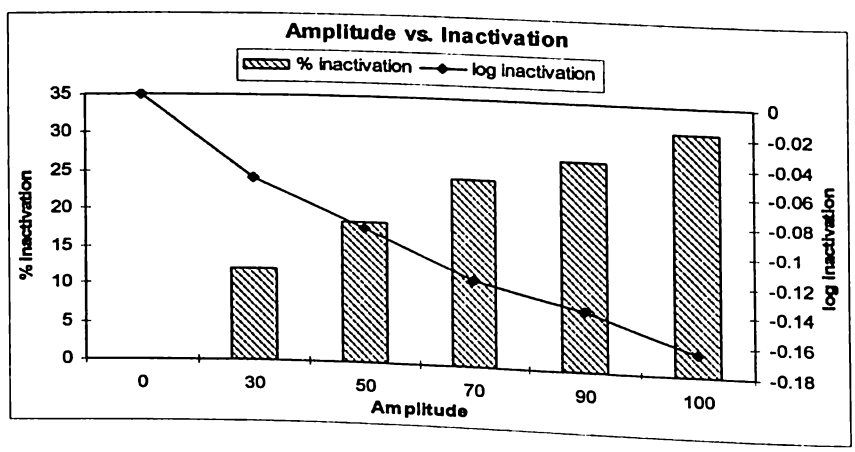


**Figure 5.** Percent Inactivation and Log Inactivation using Variable Amplitude

The effect of amplitude change in the inactivation of *S. faecalis* is evident in Figure 5. As amplitude increases, power increases, inactivation rate also increases [Reyes et al., 2005]. Inactivation rates increase with increasing power because higher energy is dissipated into and through the water to the bacteria.

#### *4.1.b. Effects of Exposure Time*

It can be seen that inactivation increases with exposure time. With increasing exposure time, more energy is being dissipated in the sample and into the bacteria. In Figure 6, a plot of ln(N/No) vs. exposure time illustrates a linear relationship. The inactivation rate constant, kstrep., calculated was 0.0293 min-1. This proportionality constant was calculated using the equation [Mara et al., 2003; Neis et al., 2002]:

$$-kt = \ln\left(\frac{N}{N_o}\right) \quad (4)$$

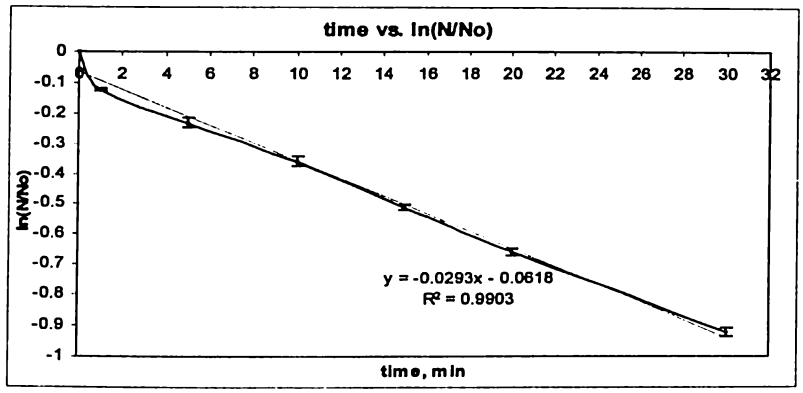


**Figure 6.** Correlation between Inactivation of *Streptococcus faecalis* and Exposure Time at 100 Percent Amplitude

## *4.2. Chlorination*

### *4.2.a. Effects of Concentration*

As expected, inactivation rate increases with increasing disinfectant concentration (Figure 7). Logically, when one increases the concentration, free chlorine in the solution also increases; thus, higher inactivation occurs.

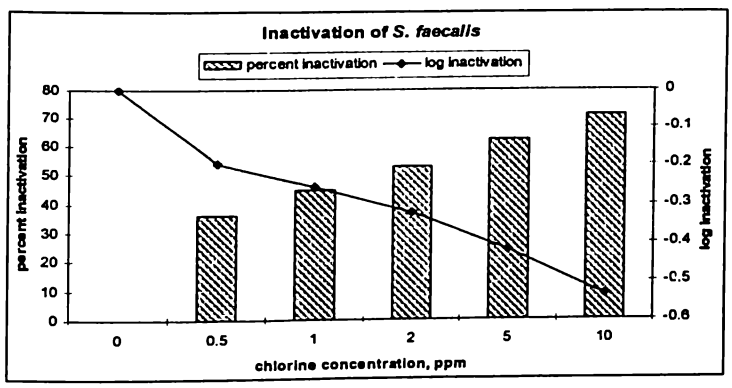


**Figure 7.** Percent and Log Inactivation of *Streptococcus faecalis* using Different Concentrations of Chlorine at n = 0.25

### *4.2.b. Effects of Chlorine:Sample Volume Ratio, n*

Volume ratio is explained by the volume ratio of the sample and the disinfectant, given by the equation:

$$n = \frac{V_{Cl\_sol'n}}{V_{total}} \quad (5)$$

As volume ratio increases, actual chlorine concentration in the solution is also higher. The free chlorine present in the solution also increases.

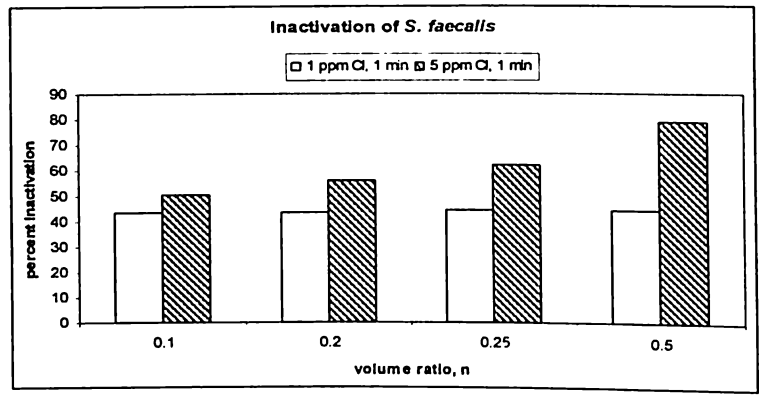


**Figure 8.** Percent Inactivation of *Streptococcus faecalis* using Volume Ratio, n

*4.2.c. Effects of Contact Time*

Normally, the effect of contact time would yield an inactivation rate constant, k. However, in this experiment, the change in concentration of disinfectant was not monitored as often as necessary. The effect of contact time, in this case, will just be dependent on the results shown in Figure 9.

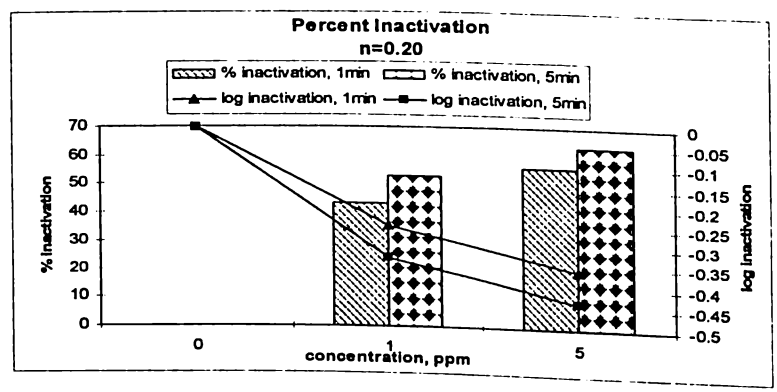


**Figure 9.** Percent and Log inactivation using Different Concentrations of Chlorine and Contact Time

Figure 9 shows that the difference between 1 min and 5 min chlorination is not large. It can be seen that the inactivation of *S. faecalis* was more notable for 1 minute than 5 minutes. This can be explained by the initial amount of *S. faecalis* in the sample. The higher number of bacteria in the solution, the higher demand for chlorine. This means, that during the initial stages of chlorination, the demand was significantly higher than in the succeeding minutes. Thus, chlorine concentration will drop considerably after the first minute, leaving lesser demand and lesser inactivation of *S. faecalis* in the subsequent minutes.

*4.3. Sequential Disinfection*

*4.3.a. Effects of Ultrasonication as Pretreatment to Chlorination*

Comparison between sonication, chlorination and sequential disinfection is seen in Figure 10. A substantial increase in inactivation was observed from 1 min unaided chlorination and the sequential disinfection, from 0.247 log reduction units to 0.465 log reduction units.

In chlorination alone, 5 ppm chlorine used for 5 minutes yielded 63.47% inactivation of *S. faecalis* (Figure 10). With 5 min US pretreatment, followed by chlorination using 1 ppm chlorine for 1 min, achieved 65.75% inactivation of *S. faecalis* (Figure 10). From this, it shows that US pretreatment can reduce both chlorine dosage and contact time, while maintaining effectiveness of disinfection.

This increase can be explained by the effects of sonication in the bacteria. First, if there are bacterial clusters present, they will be disagglomerated by the shearing forces of the cavitation bubbles [Tsukamoto et al., 2004]. Second, hydromechanical shearing damages cell walls. By being disagglomerated and with damaged cell walls, individual cells will be more susceptible to disinfectant penetration.

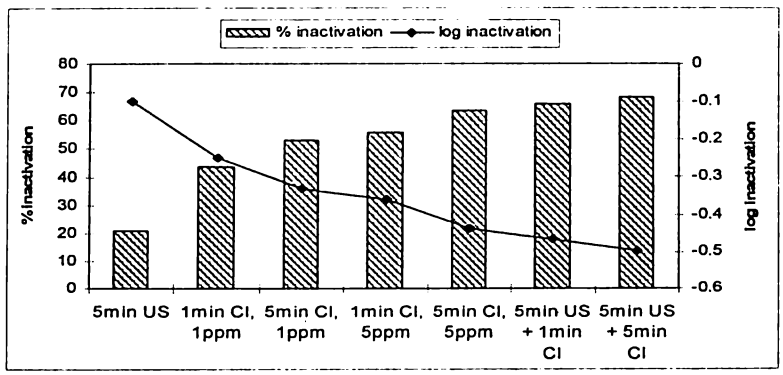


**Figure 10.** Comparison between the 3 Phases: Sonication (5min), Chlorination (1ppm) and Sequential Disinfection (5min US, 1ppm Cl)

## 5. CONCLUSIONS

Ultrasonication has a potential as a disinfection method on its own or in conjunction with chlorination, depending on the degree of desired inactivation.

In evaluating the effectiveness of ultrasonication and chlorination in the inactivation of *S. faecalis*, which is a more resistant type of bacteria, the following conclusions are made:

- For 100% amplitude, at 20 kHz, correlation of inactivation of *S. faecalis* to exposure time yields an inactivation rate constant, $k_{strep} = 0.0293$, having an $R^2 = 0.9903$.
- There is an increase in inactivation rate of *S. faecalis* between 1 min chlorination and the sequential disinfection, from 0.24705 log reduction units to 0.46535 log reduction units.

In determining the relationship of ultrasonic exposure time in the inactivation of *S. faecalis*, it was found that:

- as percent amplitude setting increases, dissipated power increases, the rate of inactivation also increases.
- inactivation rate increases with increasing exposure time.

In ascertaining whether ultrasonication can be used to lower chlorine dosage and if it can enhance chlorination efficiency, the following can be inferred:

- Ultrasonication can maintain an effective inactivation of ≈ 65% *S. faecalis* while reducing the chlorine concentration requirement from 5 ppm to 1 ppm.
- While ultrasonication can reduce chlorine dosage, chlorination is still necessary for the inactivation of a more resistant organism, *S. faecalis*.

## REFERENCES


1. Bryant E., Fulton G., Budd G. (1992), *Disinfection Alternatives for Safe Drinking Water*, Van Nostrand Reinhold, New York, p.518
2. Ince N., Belen R. (2001), *Aqueous Phase Disinfection with Power Ultrasound: Process Kinetics and Effects of Solid Catalysts*, Environmental Science and Technology, 35 (9), p.1885-1888
3. US Environmental Protection Agency (1999), *Wastewater Technology Fact Sheet : Chlorine Disinfection*
4. Jolley R., Bull R., Davis W. (1986), *Water Chlorination: Chemistry, Environmental Impact and Health Effects Vol. 5*, Lewis Publishers, Inc.
5. Young H., Freedman R. (1996), *University Physics 9th Edition*, Addison-Wesley Publishing Company, Inc., New York, p.1484
6. Berg R., Stork D. (1995), *The Physics of Sound, 2nd ed.* Prentice Hall
7. Battele Duxbury Operations (1998), *Ballast Water Secondary Treatment Technology Review: Final Report to Northeast Midwest Institute*, Duxbury (www.nemw.org)
8. Mason T. (1990), *Chemistry with Ultrasound*, Elsevier Science Publishers Ltd., London, p.195
9. Hoffman M., Hua I. (1997), *Optimization of Ultrasonic Irradiation as an Advanced Oxidation Technology*, Environmental Science and Technology, 31 (8), p.2237-2243
10. Neis U., Blume T. (2002), *The Effect of Ultrasound on Particulate Matter, Especially Micro-organisms in Complex Water and Waste Water Media (Lecture)*, Technical University Hamburg-Harburg, Hamburg
11. Neis U., Blume T., Martinez I. (2002), *Wastewater Disinfection Using Ultrasound and UV Light*, TU Hamburg-Harburg Reports on Sanitary Engineering, 35, p.117-128
12. Pandit A., Jyoti K. (2001), *Water Disinfection by Acoustic and Hydrodynamic Cavitation*, Biochemical Engineering Journal, 7, p. 201-212
13. Pandit A., Jyoti K. (2004), *Effect of Cavitation on Chemical Disinfection Efficiency*, Water Research, 38, p.2249-2258
14. Petrucci R., Harwood W. (1997), *General Chemistry: Principles and Modern Applications*, Prentice-Hall, Inc., New Jersey, p.989
15. Tsukamoto I., Constantinoiu E., Furuta M., Nishimura R., Maeda Y. (2004), *Inactivation Effect of Sonication and Chlorination on Saccharomyces cerevisiae. Calorimetric Analysis*, Ultrasonics Sonochemistry, 11, p.167-172
16. Kalumuck K., Hsiao C., Chahine G., Choi J. (2003), *Remediation and Disinfection of Water using Jet-Generated Cavitation*, Fifth international Symposium on Cavitation, Osaka, p.1-7
17. Weavers L. (2001), *Use of Power Ultrasound in Water Treatment (Lecture)*, Research Group Ohio State University, Ohio,(www.ceegs.ohio-state.edu/~lweavers/sonochem.htm)
18. Thompson J., Hua I., (2000), *Inactivation of Escherichia Coli by Sonication at Discrete Ultrasonic Frequency*, Water Research, 24 (15), p.3888-3893
19. Mara D., Horan N. (2003), *Handbook of Water and Wastewater Microbiology*, Academic Press, London, p.819
20. Schroeder E., Tchobanoglous G. (1987), Water Quality, Addison-Wesley Publishing Company, Inc., Massachusetts, p.768
21. Vincent W. (2005), *Overview of the Genus Streptococcus*, Quest Diagnostics, 12 (3), p.13-19
22. Neis U., Blume T. (2003), *Ultrasonic Disinfection of Wastewater Effluents for High-Quality Reuse*, Water Science and Technology: Water Supply, 3 (4), p.261-267
23. Peralta G., Reyes R., Escoto M. (2004), *Use of a Variable Intensity Tank Sonicator for Inactivation of Coliform in Drinking Water*, Asian Regional Conference on Environmental Pollution Control Technologies, Chia Nan University fo Pharmacy and Science, Taiwan, p.I1-I11
24. Reyes R. (2005), *Use of a Variable Intensity Tank Sonicator for Inactivation of Coliform in Drinking Water (Master's Thesis)*, University of the Philippines, Diliman, Quezon City