# APPLICATIONS OF HIGH FREQUENCY ULTRASOUND FOR WATER DISINFECTION[2]

Roberto S. Chamul and Juan L. Silva[1]
[1]Department of Food Science and Technology
Mississippi Agricultural and Forestry Experiment Station
Mississippi State University, Mississippi State, MS 39762

ABSTRACT

Recently there has been a trend in the search for new technologies that will inactivate microorganisms without the use of heat or the use of chemical additives. One technology with potential is high frequency ultrasound (HFU). It is used to kill microorganisms in liquid systems by the principle of cavitation. At high frequencies, cavitation causes smaller bubbles with higher energy. When these bubbles collapse, very high temperatures at these microenvironments result. This leads to ionization effects and formation of radicals.

The objectives of this project were to study the effect of different intensities of high frequency ultrasound to disinfect water in order to reduce the chances of waterborne pathogens being present. To do this, evaluation of the quality of groundwater in an aquifer in northeast Mississippi was studied, and the effect of HFU on microbial load of this water was conducted.

Groundwater used for this treatment from an aquifer in northeast Mississippi was sampled to evaluate its physical-chemical and microbial quality. It was found that this water varied in quality with season, probably due to rainfall and other environmental pressures. Three different types of HFU resistant bacteria were isolated from the water (Flavobacferium, Acinetobacfer baumannii, and Enferobacter cloacae) and used to evaluate the effect of HFU. Column of water and amplitude that produced maximum cavitation were chosen using an ultrasound generator K80 with a frequency of 850 kHz. Continuous ultrasound was used to calculate destructive values for *Flavobacterium* and Acinetobacter baumannii. Enterobacter cloacae was used for the pulsed experiment since it survived the continuous process.

Continuous high frequency ultrasound has a better chance to be used to disinfect water than pulsed ultrasound because the conditions to produce cavitation are not ideal in the water and ultrasound per se is not able to accomplish any disinfection. Another possibility is to use HFU in conjunction with low levels of disinfectants to increase efficiency and guarantee total kill.

Possible applications of this technology are in groundwater disinfection, food processing water and wastewater treatment, and recirculation of processing water.

INTRODUCTION

Surface water quality keeps changing due to nutrients and other chemicals from agricultural runoff, wastewater treatment plants, and industry leading municipalities to turn to groundwater (Pote and Daniel, 1997). Since 1978, the National Water Assessment described the major water problems in the United States as being 'inadequate-water supply' and 'overdraft of groundwater' (McArthur and Brammer, 1984). Northeast Mississippi and Western Alabama correspond to the Cretaceous clastic sediments. Geologic structure and topography exert a profound influence on the movement of water in the aquifer system (Mallory, 1993). The most extensive aquifers in this area have not been fully developed. The average pumpage from these aquifers is about 378 MLD. These aquifers are mainly recharged by precipitation. Temperature of this groundwater aquifers ranges from 17 to 37°C (Mallory, 1993).

The last Committee Report from The American Water Works Association Research Division Microbial Contaminant Committee reviewed the greatest concerns to the water industry. Included in this list are enterovirus, calicivirus and Norwalk virus, and hepatitis; Cyclospora cayetanensis, *Toxoplasma gondii*; and cyanobacteriai toxins. From these organisms, the ones with moderate priority for the water industry are Norwalk virus and calicivirus, Cyclospora, and algal toxins. These organisms become of interest because the

treatment to eliminate them from water is unknown or they are just not removed by conventional treatments (AWWA, 1999).

Sonochemical processes are localized in the region of cavitation. As the bubble collapses, the speed of the cavitation bubble reaches several kilometers per second; reaching temperatures in the order of 5000 K. Ultrasound requires only the presence of a liquid to transmit its power. Power ultrasound enhances chemical reactivity in a liquid through the generation of cavitation bubbles. Collapsing of bubbles generate very high local temperatures and pressures in excess of 98 MPa (Mason, 1993). High power ultrasound deals with frequencies in the range of 15kHz to 1 MHz (Hoffman et al., 1996). The dynamic temperature and pressure changes at the bubble interface and their effects on chemical reactions need more study. They reported that this region is likely to have temperatures and pressures in excess of 647 K and 2.21 MPa for periods of microseconds to milliseconds. They also observed that the collapsed bubble is an instantaneous source of heat, that conduction is the means of heat transfer, and that the heat capacity, thermal conductivity and density of the collapsed bubble are the same as the surrounding water at room temperature.

Mason (1998) divided the uses of power ultrasound into two categories. First, mechanical effects which include crystallization, degassing, destruction of foams, extraction of flavors, mixing, homogenization, and tenderization of meat. Second, chemical and biochemical effects that include bactericidal action, effluent treatment, modification of growth of living cells, oxidation, and sterilization of equipment. Chendke and Fogler (1975) have reported applications of ultrasound in the field of wastewater treatment. They reported that ultrasound breaks clumps of bacteria and viruses, and when used in combination with ozone, ultrasound waves keep bubbles from coalescing, thereby exposing the maximum surface area to oxidation attack. Reduction of 93% viruses and destruction of 100% bacteria using ultrasound and ozone were reported.

Mason (1993) reported the use of ultrasound to treat wastewater to remove chemical pollutants. Frequencies of 20,000 MHz have been reported to destroy *Cryptosporidium* and to disintegrate pesticides and organic compound residues. However the combination of ultrasound with ultraviolet light and/or peroxides was recommended (Anonymous, 1993).

The objectives of this project were to study the effect of different intensities of high frequency ultrasound to disinfect industrial (well) water in order to reduce the chances of waterborne pathogens being present. To do this, evaluation of the quality of groundwater in an aquifer in northeast Mississippi was studied, and the effect of HFU on microbial load of this water was conducted.

## MATERIALS AND METHODS

### Sampling

Water from an aquifer in northeast Mississippi was collected in previously sterilized 0.5 L glass bottles. To sample, the main valve of a 7.6 cm hose was completely opened and water was allowed to run for at least 10 min. Samples were placed in an ice chest with ice and immediately transported to the MSU lab for analysis. All reagents were obtained from Fisher Scientific (Houston, TX) unless otherwise noted.

### Ultrasound Generator

An ultrasonic K80 generator (Meinhardt, Leipzig, Germany) was connected to an ultrasonic chamber (Meinhardt). The ultrasonic chamber had a useful inner diameter of 70 mm, 346 mL useful volume, 75 mm oscillator diameter, and 33 cm ultrasound area. A TDS 430A oscilloscope (Tektronix, Wilsonville, OR) with a 10X 400 MHz passive probe (Tektronix) were used to monitor the frequency and amplitude of ultrasound.

### Physical-Chemical Analyses

Temperature (°C) was measured with a mercury-filled thermometer. Sample was collected and temperature measured on site. Dissolved oxygen was measured using the Azide modification.To measure pH, the electrometric principle was used. The meter was standardized with pH buffer 4 and 7 before measuring pH of samples. A 25 mL aliquot from the sampling bottle was titrated with 0.01 M EDTA to measure hardness. Hardness was expressed as mg of $CaCO_3$ / L. To measure turbidity the nephelometric method was employed using a ratio/XR turbidimeter (HACH, Loveland, CO). Turbidity was expressed in Nephelometric turbidity units (NTU). For total solids, an aliquot of 25 mL was poured into a preweighed porcelain

dish and evaporated to dryness at 103 to 105°C. Results were expressed in mg of total solids per liter. All tests were performed according to APHA (1998).

**Isolation and Identification of Ultrasound Resistant Bacteria**

A volume of sampled water (57.72 mL) corresponding to 1.5 cm column height was sonicated at 850KHz for 10 minutes using a K80 ultrasound generator (Meinhardt, Leipzig, Germany) using an amplitude of 580 mV (intensity 3). Microbial counts were performed using the membrane filter technique as described above for either heterotrophic plate count (HPC) or total coliform count (TCC). Colonies were transferred to either nutrient agar (HPC) or eosin methylene blue agar (TCC). Gram stains were done. After cultures were purified, they were transferred to tryptic soy agar slants. The API 20E or 20NE (BioMerieux Vitek Inc., Hazelwood, MO) were used for rapid identification of cultures of organisms isolated from TCC and HPC colonies which had formed from cells that had survived sonication.

**Treatment Conditions**

An aliquant of 57.72 g (57.75 mL) of water equivalent to 1.5 cm column height was treated with high-frequency ultrasound (HFU) using intensity 3, with contact times ranging from 12 to 25 min. Three replications per time per dilution were carried out. To validate these results, water was treated for 10.2 minutes using a frequency of 850 kHz and amplitude of 580 mV. HPC and TCC were enumerated using the Millipore filter technique. *Enterobacter cloacae*, found resistant to continuous ultrasound was used for the pulsed study using intensity 4. The pulses tested were 1(880 mV), 4 (900 mV) and 7 (790 mV) per second with contact times of 15 and 20 min

**Microbial Analyses**

To quantify the inactivation of bacteria the Millipore technique was used (APHA, 1998). One mL of the treated water was mixed with 9 mL of 0.1% peptone water and the respective medium to enumerate HPC or TCC. Decimal dilutions were performed when necessary. All analyses were performed in duplicates. Results were expressed as log CFU/mL. Decimal reduction (D) values were calculated according to Singh (1996) for *Flavobacterium spp* and *Acinetobacter baumanni*.

**Statistical Design**

A completely randomized design was considered for this section of the study. The Statistical Analysis System ver 6.12 (SAS, 1997) was used to calculate means and differences among time for the physical-chemical and microbiological data. The data were analyzed using the ANOVA procedure and when significant, means were separated using Fisher's protected LSD (Steele and Torrie, 1980).

**RESULTS AND DISCUSSION**

Temperature of groundwater varied ($p \leq 0.05$) from June 1997 to August 1999, ranging from 15 (spring) to 26°C (summer). Turbidity was the only parameter that did not show any differences ($p > 0.05$). Dissolved oxygen ranged from 6.8 to 8.2 mg/L, pH from 6.57 to 7.97, hardness from 8 to 16 mg of $CaCO_3$/L, and total solids from 110 to 327 mg/L. Total coliform counts varied from 0.0 to 3.0 log CFU/mL and HPC from 0.7 to 3.2 log CFU/mL (Table 1).

Microbial results agreed with Bitton and Gerba (1984) who stated that wells up to 138 m deep might show up to 5 log CFU/mL. Falcao et al. (1993) added that HPC can vary from 1 to 5 log CFU/mL in artesian or non-artesian wells. Therefore, the HPC from this particular aquifer falls into this range. However, it has to be noticed how the temperature of the water has a profound influence on the microbial load. These changes in temperature and microbial density may be related to infiltration of storm or runoff water from the surface. As Keswick (1984) mentioned, possible sources of microbes in groundwater is stormwater, agricultural practices, land disposal sewage, and septic tanks.

Heterotrophic (HPC) population of this aquifer was shown to be 79% Gram-positive and 21% Gram-negative. After water was treated for 10 min using intensity 3 (580 mV) the HPC surviving bacteria were: *Flavobacterium spp.*, *Pseudomonas spp.*, and *Chrysomonas spp.*; whereas TCC surviving bacteria were *Acinetobacter bauxmanii* and *Enterobacter spp.* (Table 2). Watchalotone (1997) reported that *Acinetobacter spp.*, *Flavobacterium spp.*, *Aeromonas spp.*, and *Pseudomonas spp.* are normally found in processed catfish fillets. Schaule and Flemming (1997) reported that *Klebsiella spp.*, *E. coli*, *Pseudomonas spp.*, and *Mycobacterium spp.* develop biofilms under oligotrophic conditions. Moreover, Kim (1999)

reported that *Acinetobacter spp.*in channel catfish fillets was resistant to ozone and hydrogen peroxide treatments and that *Flavobacterium spp.* was resistant to hydrogen peroxide. Cotton and Marshall (1998) found *Aeromonas* on deheaders, conveyors, and cutting boards, and *Pseudomonas* from automated filleting machines.

The inactivation curves showed D values of 4.1 to 4.2 min for *Flavobacterium* (Figure 1) and 5.1 min for *Acinetobacter baumanii* (Figure 2). Validation runs showed that there were no significant differences in the reduction of HPC and TCC. Reductions ranged from 0.95 to 1.49 log CFU/mL for HPC and 1.46 to 1.92 log CFU/mL for TCC (Table 3). However, the major portion of survivors (Table 4) during the first week was *Enterobacter cloacae* (33.3%) followed by *Chryseomonas luteola* (11.1%) and *Pseudomonas spp* (16.8 %). During the third week, the most resistant survivor bacteria were *Aeromonas spp* (31.4%) followed by *Acinetobacter* (25.0%). This shows that this particular aquifer has a very dynamic microbial ecology that could be explained by infiltrations from the surface.

Failing to reduce the initial density of microorganisms meant that the surviving bacteria needed a higher contact time than the one calculated for *Flavobacterium* and *A. baumannii* or the input of a higher amount of energy. For this, pulsed ultrasound was used in order to reduce the changes in temperature of the volume of water sonicated. Figure 3 shows that increasing the contact time increased the reduction of bacteria. It also shows that at the minimum contact times used and using one pulse per second there is no reduction, just the opposite, there is an increase in the log CFU/mL. This can be explained since one of the effects of ultrasound is the rupture of microscopic clumps of bacteria or any other type of solid material. However, it can be appreciated that after 20 min of contact time, the maximum log reduction was 0.3 log CFU/mL. Therefore, in order to disinfect water using pulsed ultrasound, longer contact times should be used and this would render this technique uneconomical from a practical standpoint. Otherwise a combination of HFU with heat, ozone, chlorine, $H_2O_2$ or others is needed.

## CONCLUSIONS

Quality of this aquifer changes continuously over time. This might be because this particular aquifer is under the influence of surface water like most of the aquifers in northeast Mississippi since they are recharged by rain. Even when aquifers are recharged from rain, quality of groundwater has always been considered optimum, especially from the microbial point of view.
However, the low concentration of calcium and magnesium in this aquifer and the constant temperature fluctuations are good indicators of the velocity at which this water source is recharging.

High frequency ultrasound at 580 mV inactivates bacteria. However, in order to achieve at least 4 log reductions, longer contact times are needed and that will render the process not feasible from the economic point of view. Pulsed ultrasound does not increase water temperature but fails to provide the conditions to produce cavitation that eventually will inactivate bacteria. Therefore, it is necessary to reduce the volume of water above the transducer to a minimum and look the engineering design of a more suitable piece of equipment that will provide a better disinfection for water (thin film continuous unit). Also, the combination of HFU with other technologies should be evaluated.

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TABLE 1

TABLE OF MEANS FOR THE PHYSICAL-CHEMICAL AND MICROBIAL VARIABLES EVALUATED FROM AN AQUIFER IN NORTHEAST MISSISSIPPI

| Date | Temperature[1] | Dissolved Oxygen[2] | pH | Turbidity[3] | Hardness[4] | Total Solids[5] | Total Coliforms[6] | Heterotrophic Plate Count[7] |
|---|---|---|---|---|---|---|---|---|
| 6/17/97 | 26.0 a | NP | 6.74 de | 0.03ns | 16.0 a | 110 b | 2.0 c | 1.5 h |
| 6/18/97 | 26.0 b | NP | 6.57 e | 0.04 | 16.0 a | 115 b | 2.0 c | 1.9 f |
| 3/12/98 | 15.0 g | NP | 7.42 b | 0.03 | 12.0 bc | 316 a | 0.0 f | 1.7 g |
| 3/26/99 | 15.0 g | 8.2 a | 7.02 cd | 0.02 | 8.0 d | 127 b | 0.0 f | 0.7 i |
| 4/9/99 | 25.0 c | 6.8 b | 7.32 bc | 0.13 | 12.0 bc | 271 a | 2.4 b | 3.0 b |
| 4/16/99 | 21.0 f | 6.9 b | 6.62 e | 0.09 | 10.0 cd | 121 b | 1.9 c | 2.5 c |
| 5/12/99 | 22.0 e | 7.4 b | 7.79 a | 0.08 | 14.0 ab | 175 b | 3.0 a | 3.2 a |
| 5/28/99 | 22.0 e | 7.4 b | 7.97 a | 0.55 | 8.0 d | 276 a | 1.5 e | 2.1 d |
| 8/9/99 | 24.0 d | 7.4 b | 7.30 bc | 0.07 | 14.0 ab | 327 a | 2.4 b | 2.0 e |
| LSD(0.05) | 0.0 | 0.53 | 0.33 | 0.53 | 3.91 | 84 | 0.07 | 0.05 |

abcdefg : means in a column not followed by the same letter differ ($P \leq 0.05$)
ns : not significant ($P \geq 0.05$)
[1] expressed in °C
[2] expressed in mg/L
[3] expressed in Nephelometric turbidity units (NTU)
[4] expressed as mg $CaCO_3$/L
[5] expressed as mg/L
[6,7] expressed as log CFU/mL
NP : not performed

TABLE 2

BACTERIA SURVIVING 10 MIN TREATMENT WITH 850 kHz HIGH FREQUENCY ULTRASOUND AT 580 mV

| Group | Bacterium | Gram Rx | No. Isolates |
|---|---|---|---|
| Heterotrophs | *Flavobacterium spp.* | - | 5 |
| | *Pseudomonas spp.* | - | 2 |
| | *Chrysomonas spp.* | - | 2 |
| | Unknown | - | 1 |
| Coliforms | *Acinetobacter bauxmanii* | - | 3 |
| | *Enterobacter spp.* | - | 1 |
| | Unknown | - | 1 |

TABLE 3

TEMPERATURE, pH, TURBIDITY, HPC, AND TCC FOR WATER TREATED FOR 10.2 MIN AT 850 kHz AND 580 mV (VALIDATION RUNS)

| Week | Temperature (°C) | pH | Turbidity (NTU) | HPC (Log CFU/mL) | TCC |
|---|---|---|---|---|---|
| 1 | 24.0 ns | 7.19 ns | 0.07 a | 0.95 ns | 1.46 ns |
| 2 | 22.5 | 6.81 | 0.03 b | 1.10 | 1.70 |
| 3 | 24.2 | 7.19 | 0.04 b | 1.49 | 1.92 |
| LSD(0.05) | 1.93 | 0.38 | 0.02 | 1.02 | 0.98 |

TABLE 4

SURVIVING BACTERIA FOUND IN GROUNDWATER IN NORTHEAST MISSISSIPPI DURING THE VALIDATION PERIOD OF THE EXPERIMENT

| Week | Culture | Number of Isolates | Percentage |
|---|---|---|---|
| 1 | *Enterobacter cloacae* | 6 | 33.3 |
| | *Chryseomonas luteola* | 2 | 11.1 |
| | *Pseudomonas aeruginosa* | 1 | 5.6 |
| | *Pseudomonas aurefaciens* | 1 | 5.6 |
| | *Pseudomonas fluorescens* | 1 | 5.6 |
| | Unknown | 7 | 38.8 |
| 3 | *Aeromonas spp.* | 11 | 34.4 |
| | *Acinetobacter spp.* | 8 | 25.0 |
| | *Moraxella spp.* | 2 | 6.3 |
| | *Pasteurella spp.* | 1 | 3.1 |
| | *Xanthomonas spp.* | 1 | 3.1 |
| | *Weeksella spp.* | 1 | 3.1 |
| | *Pseudomonas spp.* | 1 | 3.1 |
| | Unknown | 7 | 21.9 |

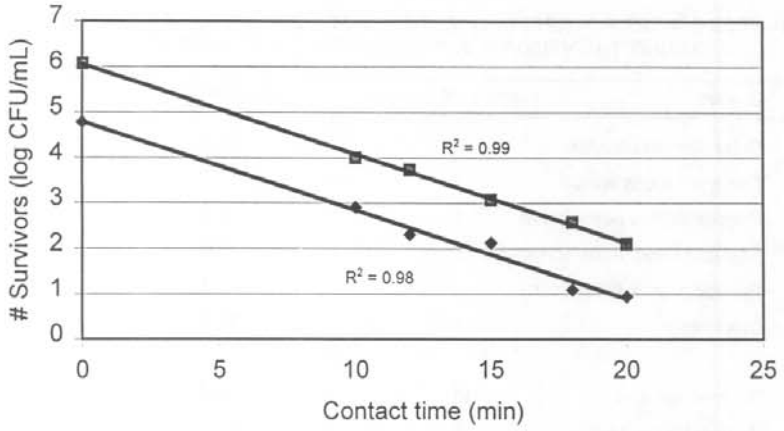


Figure 1. Inactivation curves for *Flavobacterium* spp. using high frequency ultrasound at a frequency of 850 kHz, 580 mV of amplitude and water column height of 1.5 cm (■=$10^4$ CFU/mL, ♦=$10^6$ CFU/mL)

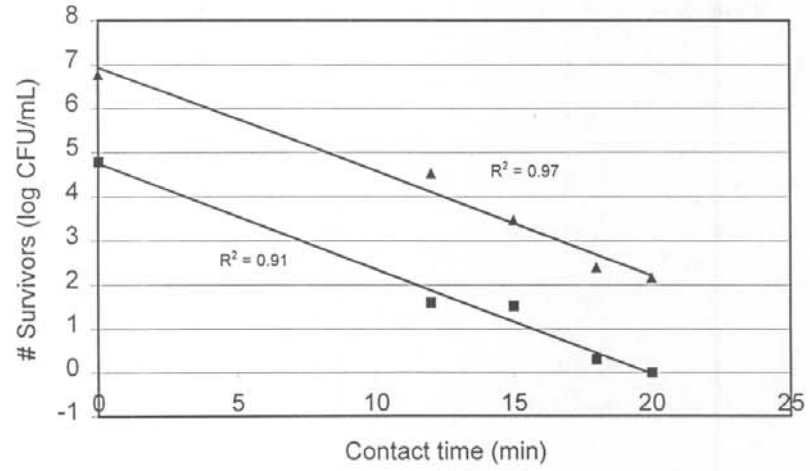


Figure 2. Inactivation curves for *Acinetobacter baumanii* using high frequency ultrasound at a frequency of 850 kHz, 580 mV of amplitude and water column height of 1.5 cm (■=$10^4$ CFU/mL, ♦=$10^6$ CFU/mL)

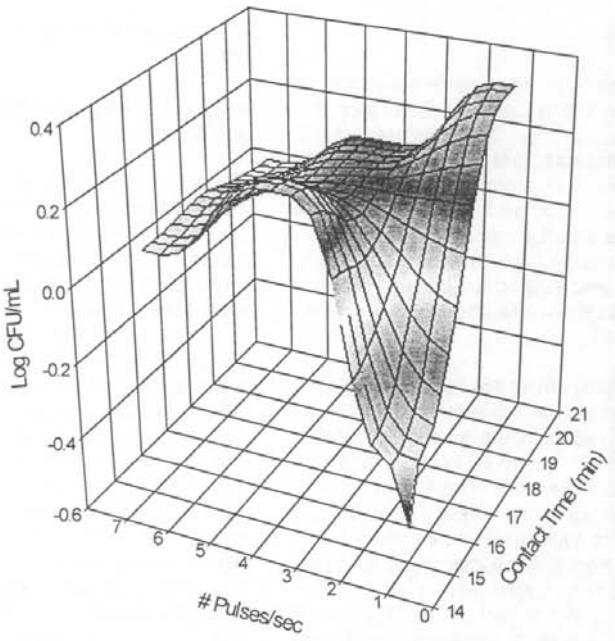


Figure 3. Effect of pulsed high-frequency ultrasound on reduction (+) or increase (-) of *Enterobacter cloacae* as affected by contact time and number of pulses