Naval Surface Warfare Center
Carderock Division
West Bethesda, MD 20817-5700

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Survivability, Structures, and Materials Department

Technical Report

High-Power Ultrasound for Disinfection of Graywater and Ballast Water: A Beaker-Scale and Pilot-Scale Investigation

by

Robert A. Brizzolara, Eric R. Holm, and David M. Stamper,
(Naval Surface Warfare Center, Carderock Division)

and

Rachel Dudley, Julie Slaughter, and Jon Snodgrass
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14. ABSTRACT

We investigated the disinfection of several bacteria relevant to ship's graywater and sewage, and bacteria and zooplankton relevant to ship's ballast water, using high power ultrasound (HPU) driven by the magnetostrictive material, TERFENOL-D. TERFENOL-D is capable of generating high power ultrasound more efficiently than previously existing technologies. Initial experiments were carried out at the beaker scale. Results from these experiments were used to make recommendations for area of focus in pilot-scale experiments. Beaker-scale results were also used to specify the flow parameters of a scaled-up treatment system. Etrema Products,Inc. applied these flow parameters in designing and building a pilot-scale (50-gpm) treatment system based on a 3 kW TERFENOL-D transducer and cascade horn. Experiments at the pilot scale investigated the effects of contact time or flow rate, ultrasonic intensity or energy density, and pressure on the effectiveness of HPU for treating bacteria and zooplankton. Contact times and energy densities appear feasible for treatment of zooplankton such as *Artemia*, in the context of flow rate required for the ballast water application; however, contact times and energy densities were orders of magnitude higher for bacteria such as *E. coli*.

15. SUBJECT TERMS

ultrasound	bacteria	non-indigenous species	sewage
ship	environmental	TERFENOL	disinfection
ballast water	zooplankton	phytoplankton	graywater

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Administrative Information

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Executive Summary

We investigated the disinfection of several bacteria relevant to shipboard graywater and sewage wastestreams, as well as bacteria and zooplankton relevant to ballast water, using high power ultrasound (HPU) driven by the magnetostrictive material, TERFENOL-D. TERFENOL-D is capable of generating high power ultrasound more efficiently than previously existing technologies such as piezoelectric materials. Initial experiments were carried out at the beaker scale. The results from these experiments were used to define focus areas in pilot-scale experiments. Beaker-scale results were also used to specify design parameters of a scaled-up treatment system. Etrema Products Inc. applied these parameters in designing and building a pilot-scale (50 gpm) treatment system based on a 3 kW TERFENOL-D transducer and a cascade horn. Experiments at the pilot-scale investigated the effects of contact time (flow rate), ultrasonic intensity or energy density, and pressure on the effectiveness of HPU for treating bacteria and zooplankton. The conclusions of the first year's investigations (FY05) were three-fold:

1. *Zooplankton Disinfection in Ballast Water: Experimental results, which showed disinfection of ballast water organisms by HPU, are promising and further testing of the pilot-scale system using natural seawater or brackish water is recommended.*
2. *Bacterial Disinfection in Ballast Water: HPU disinfection of bacteria, while effective, is not likely to be practical based on the treatment cell size and power consumption expected for a full-scale system based on the experimental results. Two recommendations are made: (1) optimize treatment cell diameter to achieve more effective bacterial disinfection and (2) evaluate combined disinfection methods for their potential in achieving a more feasible bacterial disinfection approach.*
3. *Graywater: The use of HPU for disinfection of the effluent of membrane bioreactors, while effective, is not likely to be able to compete in terms of system size and power consumption with existing disinfection technology, such as ultraviolet (UV) light. HPU may be better-suited to disinfecting turbid water than UV; however, that capability is not typically required in current graywater treatment. It is recommended that the use of HPU to improve the biodegradability of graywater be evaluated.*

Introduction

There are multiple wastewater discharges from Navy ships that require safe, reliable, and cost-effective treatment. Graywater treatment must remove pathogens from the wastestream, reduce biological oxygen demand, and total suspended solids. Ballast water regulations may require removal of organisms to mitigate transport of nonindigineous species in the ships' ballast tanks. Acoustic devices, such as ultrasonics, represent a potentially useful technology for effective disinfection of water (National Research Council, 1996). At lower ultrasonic frequencies, ultrasound kills organisms via cavitation, resulting in high, localized mechanical forces on organisms in the water. Ultrasonic treatment systems typically employ piezoceramic transducers; however, magnetostrictive materials are attractive candidates for ballast water treatment using ultrasonic cavitation. Magnetostrictive materials change shape in response to an applied magnetic field. In particular, TERFENOL-D is unique among magnetostrictive materials in the extremely large strains it can generate, 20 times greater than traditional magnetostrictives such as nickel, and 2-5 times greater than traditional piezoceramics. It exhibits very high energy efficiency and density and is more robust than piezoelectric materials since magnetostrictive materials do not depolarize (Mason 2000; Bright 2000; and Moffett 1991). The U.S. Navy developed TERFENOL-D for high power sonar applications. These properties may also form the basis for more cost-effective ballast water treatment systems, utilizing either ultrasound alone, or in combination with other technologies. ETREMA Products, Inc. have recently developed systems utilizing TERFENOL-D for the treatment of hog waste, and have shown that the use of high power ultrasound (HPU) can enable compact, high volume systems capable of sanitizing a complex waste stream.

Ultrasound has long been known to provide biological disinfection of water. The effectiveness typically depends on both the frequency and intensity. Hua and Thompson (2000) demonstrated sonolytic inactivation of *Escherichia coli*, and characterized the effects of frequency and power. Experiments were performed at intensities ranging from 4.6 to 74 W/cm^2, total power ranging from 80 to 140 W, power per volume of 0.27-0.46 W/ml, and at frequencies of 20, 205, 358, 618, and 1071 kHz. It was found that the most effective frequency for inactivation was 205 kHz. This inactivation rate also increased with power intensity. Scherba et al. (1991) showed that 26 kHz ultrasonic energy had a germicidal effect against *E. coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*, fungus, and viruses. They characterized the germicidal effect as a function of time and power, showing that the mortality increased with both. Allison et al. (1996), using a 60 W transducer, found that 20 kHz ultrasound decreased cell viability of *E. coli* exponentially with time. Increasing the intensity also increased the rate of cell killing.

The objectives of this work were three-fold: (1) to understand and quantify the effects of high-power ultrasound on pure cultures of organisms of concern for ballast water and graywater treatment, (2) to determine if high-power ultrasound is a viable treatment candidate for graywater and ballast water, and (3) to determine basic parameters necessary for scale-up of the HPU treatment, including the contact time required to treat various organisms, the energy required per volume of water treated, and the power required for treatment of flowing water at various flowrates.

Materials and Methods

Bacteria

For the graywater beaker-scale experiments, a sewage sample and three bacteria, *Escherichia coli* ATCC 11775, *Enterococcus avium* ATCC 14025, and *P. aeruginosa* ATCC 10145, were treated with high power ultrasound. Cultures were grown to mid-log phase at room temperature (~ 22°C) in a rich medium (Luria broth or trypticase soy broth), harvested, washed, and resuspended to ~10^6cells/ml in the appropriate sonication medium. For graywater experiments, the chief sonication medium was a mineral salts base (50% strength phosphate buffered saline, PBS) with starch, gelatin, and laundry detergent. Variations on this composition were explored and other media included synthetic seawater and synthetic freshwater. Cultures, typically in 100 ml of the relevant medium, were exposed to HPU in a glass vessel with a tapwater-flushed jacket for temperature control at <30°C. Samples were removed over the course of exposure and enumerated by dilution plate counting. The results were expressed as the decimal reduction time (DRT) and decimal reduction energy density (DRED) for each organism tested. The DRT is defined as the time necessary to achieve one log kill of an organism. The DRED is defined as the energy density necessary to achieve one log kill of an organism. The DRED is equal to the product of the ultrasonic power and DRT, divided by the liquid volume. Cell survival was expressed in colony-forming units per ml ($CFU \cdot ml^{-1}$) and plotted versus time and energy density, with the slopes of the survival curves being proportional to the DRT or DRED, respectively. For the ballast water experiments, *Vibrio cholerae* was grown and treated as described for the graywater experiments, but resuspended in synthetic seawater. *B. subtilis* spores were supplied by NSWC Dahlgren Division and resuspended in synthetic seawater. *E.coli*, *E. avium,* and an unknown cyst were additional experimental subjects. These experiments were performed using 20 ml liquid volume.

Pilot-scale tests were conducted with *E. coli* in artificial seawater. *E. coli* was grown and treated as previously described. The treatment system was put into recirculating mode for the tests, and pressure varied from 10-20 psi. In all cases the power was (nominally) 3 kW or 1 kW calorimetric. The frequency of the transducer was fixed at 20.32 kHz. The treatment time was determined from the quotient of the treatment cell volume and flowrate. The DRT and DRED were determined in the same way as for the beaker-scale tests.

Plankton

Phytoplankton experiments were carried out by the Center for Applied Aquatic Ecology at North Carolina State University. Test species included the toxic dinoflagellate *Pfiesteria piscicida*, the toxic cyanobacterium (blue green alga) *Anabaena flos-aquae*, and the non-toxic diatom *Thalassiosira eccentrica*. Tests were carried out under static conditions in a 100 ml jacketed reaction vessel identical to that used in experiments with bacteria. Tests were run for 4 minutes, and were conducted at 3 levels of ultrasonic intensity, 14 W/cm^2, 16.8 W/cm^2, and 19.6 W/cm^2. *Pfiesteria* and *Thalassiosira* were tested in seawater (15 ppt and 30 ppt, respectively), while *Anabaena* was tested in BG-11 culture medium. At each time point a 1 ml sample was drawn from the reaction vessel. For the dinoflagellate and diatom, counts or estimates of the

number of live cells were made after staining, either visually or using a flow cytometer. In the case of the cyanobacterium, reculturing was used to determine the concentration of live cells. For all species, trials at a particular level of ultrasonic intensity were replicated 3 times.

Zooplankton species tested included the brine shrimp *Artemia* sp., cladoceran *Ceriodaphnia dubia*, and rotifers *Brachionus plicatilis*, *Brachionus calyciflorus*, and *Philodina* sp. Cladocerans and rotifers occur in ballast water; *Artemia* has been suggested as a surrogate test organism for ballast water treatment systems. Initial trials with *Artemia* suggested that testing under static conditions would be inappropriate due to the very short contact times required to generate significant levels of mortality. Consequently, zooplankton were treated using a flow-through reaction vessel in contrast to the bacteria and phytoplankton, which were tested in a static system.

Trials were carried out by adjusting the power input to the transducer to obtain the appropriate level of ultrasonic intensity, allowing the output to stabilize, then varying flow rate/contact time while holding power (and thus, ultrasonic intensity) constant. Power was then adjusted to the next desired level, and the flow rate tests repeated. The order in which power or intensity levels were tested, and the order of flow rate tests within each power level, was randomized. Ultrasonic intensities tested were 0 W/cm^2 (control), 13.5-16.7 W/cm^2 (Low), 19-21.2 W/cm^2 (Intermediate), and 23.9-25.5 W/cm^2 (High). Tests were conducted in either artificial seawater or artificial freshwater depending on the species.

Pilot-scale tests were conducted with *Artemia* sp. in artificial seawater. The treatment system was put into single-pass mode for the tests, (nominal) power varied from 0-3 kW, and pressure varied from 0-20 psi. The tests were run at 3 flow rates (12-48 gpm) corresponding to contact times ranging from approximately 0.4 to 1.8 seconds. The ultrasonic frequency was fixed at 20.32 kHz. The DRT and DRED were determined in the same way as described previously for bacteria.

Beaker-Scale HPU System

The HPU transducer (Model AU-12, Etrema Products, Inc., Ames, IA), based on TERFENOL-D magnetostrictive material, drove a 13.3 cm long titanium horn with a circular terminal face having an area 1.26 cm^2. Coolant was supplied to the transducer as low-pressure airflow (house air) during operation. Alternating current (AC) power was supplied by an HP3325B function generator (Hewlett Packard, Palo Alto, CA), amplified with an LVC2016 linear amplifier (AE Techron, Elkhart, IN). The AC voltage (V), current (I) and phase angle (θ) supplied to the HPU transducer were monitored by a 123 scopemeter (Fluke, Everett, WA) and logged to a personal computer *via* a serial port and Fluke software. The function generator output was adjusted manually to keep the phase angle as small as possible, which provided the maximum power efficiency to the transducer. The nominal driving frequency was 19 kHz, adjusted slightly as necessary. The electrical power provided to the transducer was determined from

$$P = V * I * \cos\theta \qquad (1)$$

where V is the voltage, I is the current, and θ is the phase angle between current and voltage.

The determination of the efficiency of ultrasonic power transmission into the water is necessary for determination of ultrasonic intensity. The efficiency of power transmission into the water was determined calorimetrically (Mason 1991). The temperature rise in an insulated beaker was monitored by a Fluke thermocouple probe meter. Transducer power consumption was monitored as described above over a time long enough to provide a 10-12°C rise in 200 ml of synthetic graywater. The efficiency was calculated using

$$\varepsilon = (\text{Power to medium})/(\text{Electrical power to transducer}) = (c*\Delta T*m)/(P*t) \quad (2)$$

where c is the specific heat of water (4.18×10^3 $J \cdot kg^{-1} \cdot °C^{-1}$), ΔT is the temperature change of the water (°C), m is the mass of the water (kg), and t is the time (in seconds) of ultrasonic exposure. It was empirically determined that the calorimetric efficiency (ε) depended on the electrical power input (P) to the device according to

$$\varepsilon = -0.149 * P + 50.7 \quad (3)$$

with $r^2 = 0.774$. It was assumed for these calculations that the specific heat of synthetic graywater is the same as that for water, and that the thermal energy involved in heating the titanium horn was negligible.

The ultrasonic intensity, i ($W \cdot cm^{-2}$), is properly determined from the ultrasonic power that the transducer delivers to the water, not the electrical power provided to the transducer (as is occasionally done in the literature). The ultrasonic power delivered to the water is the product of the electrical power delivered to the transducer and the efficiency:

$$i = (P * \varepsilon)/A \quad (4)$$

where ε is the calorimetric efficiency and A is the horn face area (1.26 cm^2).

For the bacterial and phytoplankton disinfection experiments, HPU was typically applied to 100 ml of medium (formulations described below) in a jacketed sonochemical reaction vessel (Figure 1, ACE Glass Part #9850, Vineland, NJ). This vessel is a conical shape tapering to a cylinder at the bottom, with a water jacket for temperature control. Additional experiments investigating the effects of changing volume and vessel shape and size on HPU disinfection utilized straight wall jacketed beakers (100 and 250 ml) and a custom 250 ml conical vessel with dimensions proportionate to the #9850 vessel. The HPU horn was consistently inserted to a depth of 0.5-1.0 cm below the surface of the medium, just sufficient to avoid entraining air into the medium during HPU operation. Cold tap water was run through the cooling jacket and was confirmed to maintain the temperature of the liquid below 25°C. For the zooplankton disinfection experiments, a glass flow reaction vessel was used (Figure 2, ACE Glass Part #9841).

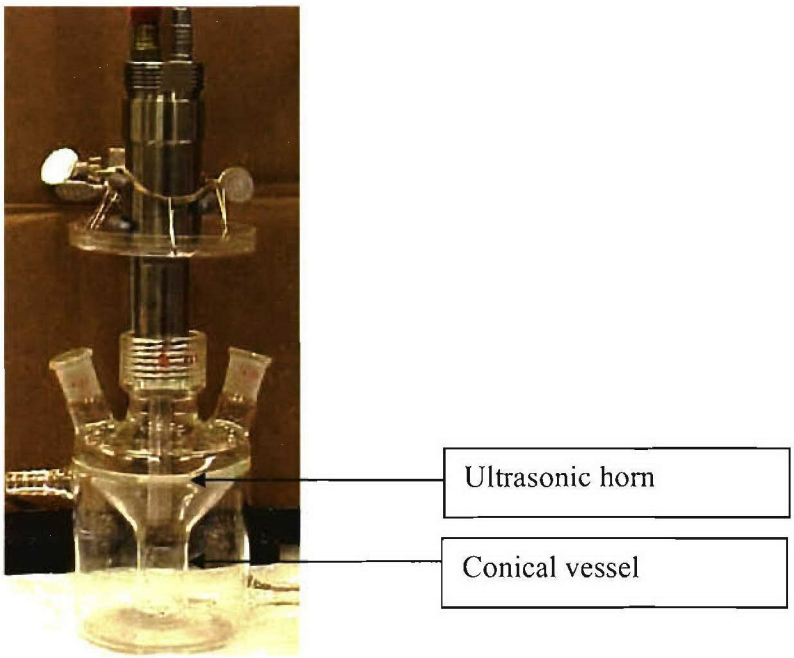


Figure 1. Conical vessel and HPU transducer used for beaker–scale experiments on bacteria and zooplankton.

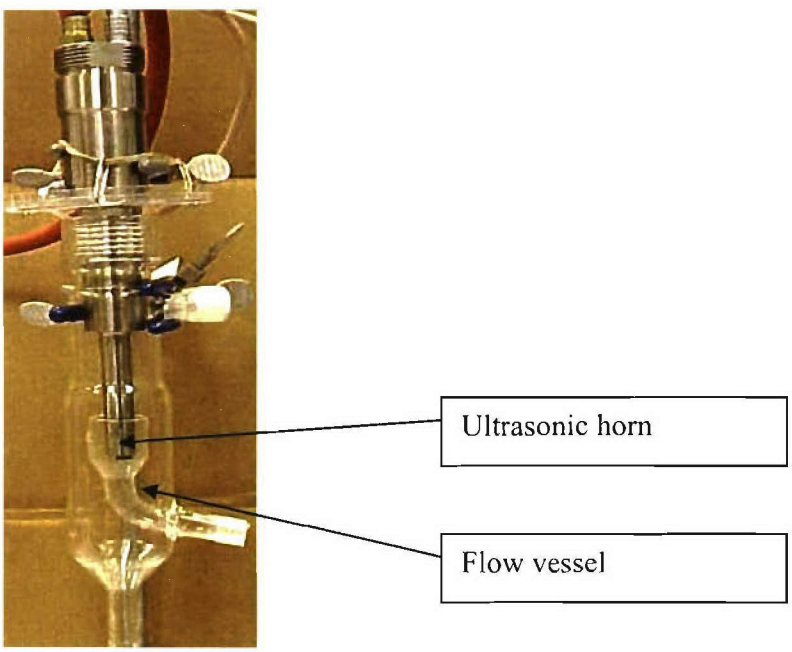


Figure 2. Flow vessel and HPU transducer used for bench-scale experiments on zooplankton.

Pilot-Scale HPU System

The pilot-scale high power ultrasonic system is a medium scale test bed for investigating ultrasonic treatment of various waste streams aboard Navy ships. Data from experiments conducted at the beaker-scale were used to develop design parameters for the pilot-scale treatment system. The flow rate capability of the system is up to 200 gallons per minute and therefore lends itself to transitional testing between the lab-scale environment and the full scale capacities that would be encountered on a ship. The system was built so that contact times or treatment durations suitable for both bacteria and organisms found in ballast water could be attained. This required a system that could recirculate the test stream to reach very long contact times, while also accommodating single-pass treatment for zooplankton disinfection. In addition, the device incorporated valves which enabled adjustment of the pressure from 0 psi to 20 psi, across the full range of flow rates. Initial experiments with the pilot-scale treatment system have examined the effects of contact time, pressure, and power on mortality of test organisms. Overall, there are two main components to the system: the MaXonics high-power ultrasonic system and the flow loop skid. The MaXonics system consists of a control cabinet (Figure 3), ultrasonic transducer (Figure 4), and cascade horn (Figure 5). The flow loop skid is shown in Figure 6.

The flow loop skid consists of the following components:

- two sample storage tanks
- variable frequency drive pump
- ultrasonic treatment flow cell
- diaphragm valve for system pressurization
- flow meter
- two thermometers
- piping and valves for various flow routing configurations
- electrical transformers and disconnect boxes for the MaXonics system and all skid components

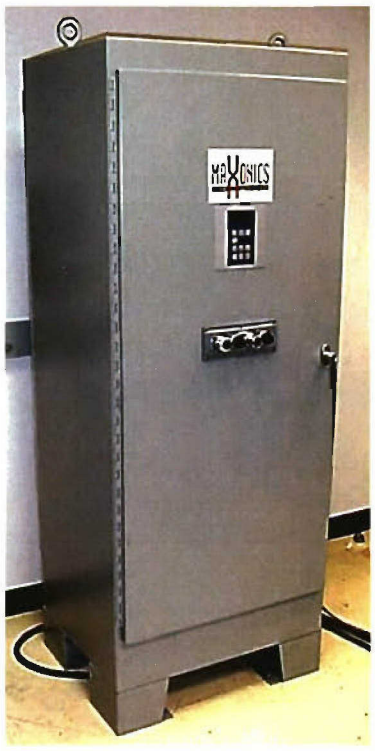


Figure 3. Maxonics High-Power Ultrasonic System Control Cabinet.

Figure 4. Ultrasonic Transducer of The Maxonics System.

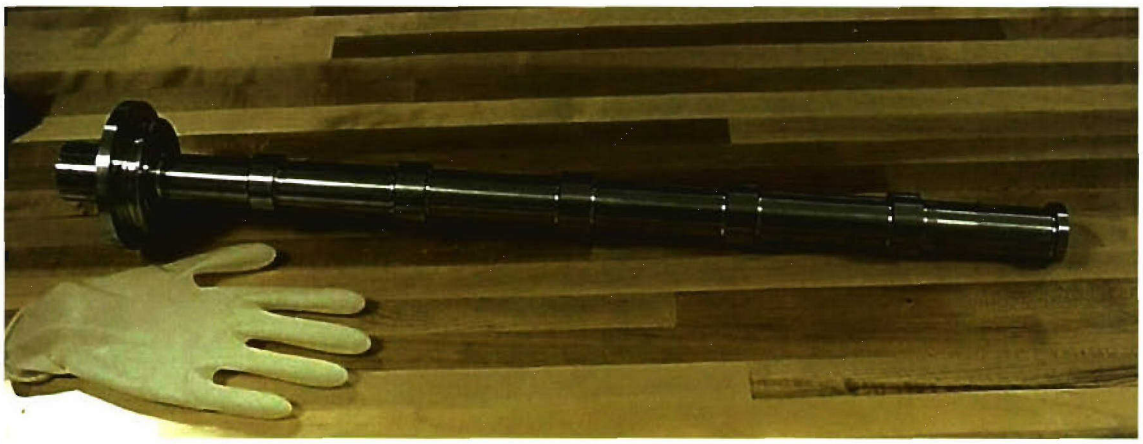
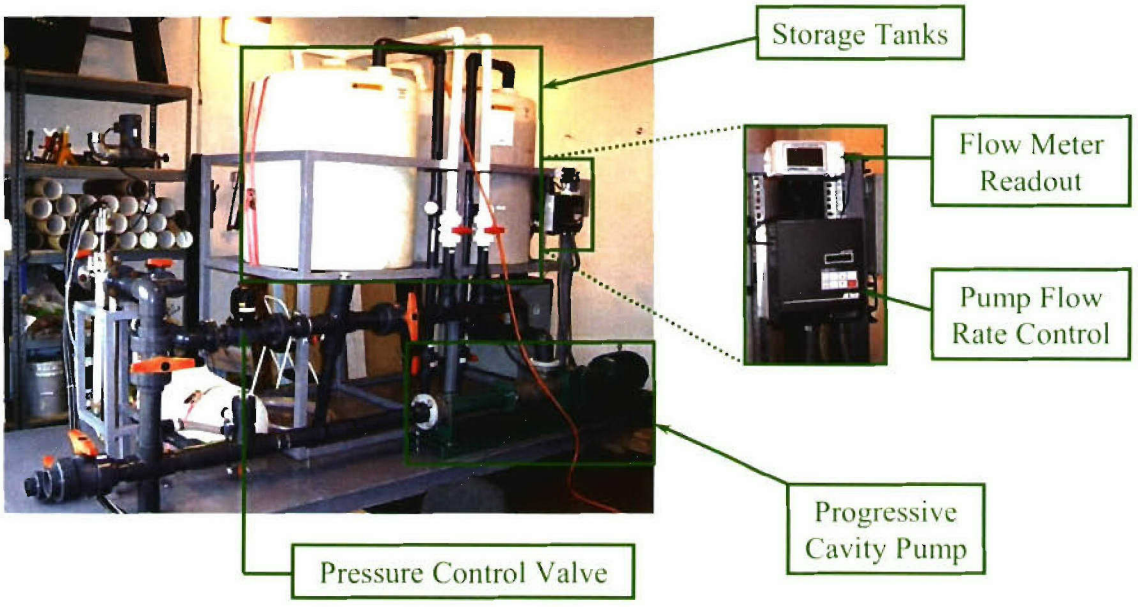


Figure 5. Ultrasonic Treatment Cascade Horn.



Notes: Storage tanks are used to hold either the waste stream to be treated or the treated material. Tanks have already been used to culture large volumes of test organisms including *E. coli* and *Artemia* sp.

Figure 6. Pilot-scale (50 gpm) HPU treatment system.

Results: Beaker-Scale Experiments

A summary of the experimental results, for both beaker-scale and pilot-scale experiments, is provided in Appendix A for ballast water and Appendix B for graywater.

Bacteria - Graywater

Bacterial disinfection experiments were performed at NSWC, Carderock. The ultrasonic intensity ranged from 23-26 W/cm^2. Decimal reduction time and decimal reduction energy density for the bacteria and the sewage sample were significantly different from one another ($P < 0.05$, Table 1). Of the tested pure cultures, *E. avium* had the highest DRT and DRED and *E. coli* had the lowest DRT and DRED. The DRT and DRED of *P. aeruginosa* were somewhat higher than *E. coli* (Table 1). Higher DRT and DRED values for *E. avium* may be due to the thicker, more mechanically robust cell wall present in Gram-positive bacteria, compared with the Gram-negative bacteria *E. coli* and *P. aeruginosa*. The significant difference between *P. aeruginosa* and *E. coli* is not explainable on the basis of cell wall structure.

Table 1. Summary of HPU beaker-scale results for bacteria in 100 ml of synthetic graywater, in the conical vessel.

Culture	Intensity (W·cm^{-2})	DRT (min) [(b)] ± 95% CI	r^2 [(a)]	DRED (J/ml) [(b)] ± 95% CI	r^2 [(a)]
E. coli stationary	25-26	5.2 ± 0.4	0.99	100 ± 5	0.99
E. coli log	23-25	5.0 ± 0.7	0.99	98 ± 10	0.98
E. avium stationary	25-26	16 ± 3	0.99	310 ± 40	0.97
E. avium log	26	14 ± 4	0.98	270 ± 40	0.97
P. aeruginosa stationary	25-26	7.5 ± 0.5	1.0	140 ± 10	0.99
Sewage	25-26	56 ± 18	0.94	1100 ± 250	0.90

Notes: Decimal reduction time (DRT) and decimal reduction energy density (DRED) for several pure cultures and a sewage mixed liquor sample.

[(a)] r^2 is the coefficient of determination for the kill curve regression analyses

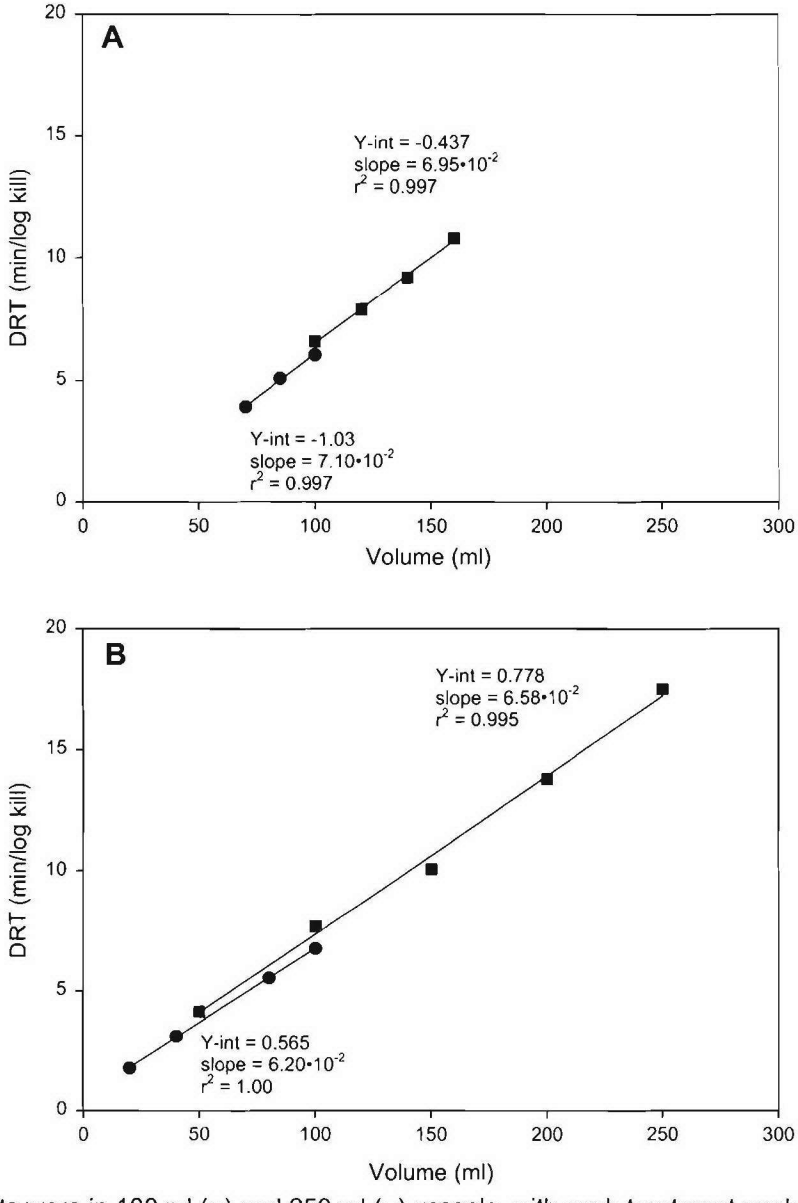
[(b)] per log kill

Growth phase of *E. coli* and *E. avium* had no effect on killing by HPU. No significant differences in DRT or DRED were found between stationary phase and log phase cultures for *E. coli* or *E. avium* (Table 1).

An initial attempt to determine the DRT and DRED for the sewage sample resulted in kill curves with poor correlation between the $CFU \cdot ml^{-1}$ and exposure time or energy density (data not shown). $CFU \cdot ml^{-1}$ increased over a 10 minute period, indicating fragmentation of filamentous and floc-forming bacteria that were evident in microscopic examination of the sewage sample. After taking into account this initial 'fragmentation' effect, the sewage sample took 3.5-11 times longer to kill than any of the pure cultures (Table 1).

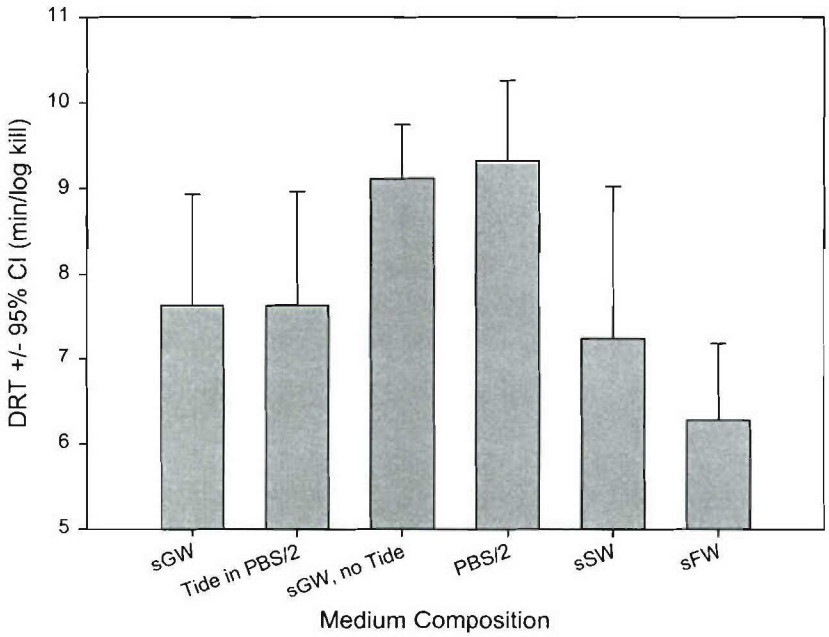
We also determined the effect of vessel geometry and volume on disinfection. The DRT for *E. coli* increased linearly with the volume of medium sonicated (Figure 7). The slope of this relationship did not change with the volume of the test container, whether the testing was conducted in synthetic graywater in cylindrical vessels (Figure 7A), or in synthetic seawater in cone-shaped vessels (Figure 7B).

Although the DRTs of *E. coli* in synthetic seawater and synthetic graywater were similar, disinfection rates by HPU were significantly affected by certain changes in medium composition (Figure 8). *E. coli* suspended in PBS/2 or synthetic graywater without detergent had the largest DRT/DRED. *E. coli* suspended in synthetic freshwater was killed by HPU more quickly. In the case of the significant variations in synthetic graywater, detergents, by affecting cell membrane stability, could make bacteria more susceptible to killing by ultrasound. The results show that the composition of the sonication medium is an important consideration for any sonication experimentation.



Note: Experiments were in 100 ml (•) and 250 ml (■) vessels, with each treatment analyzed separately. HPU exposure was 25-28 W/cm^2. Regression parameters in the figure are proximal to the relevant data set. Analyses of the regression parameters revealed no statistical differences between the slopes in each experiment (TEST statement, PROC REG, SAS/STAT).

Figure 7. DRT as a function of liquid volume for *E. coli* in synthetic graywater and cylinder shaped vessels (**A**) and in synthetic seawater and cone-shaped vessels (**B**).



Note: Intensity for each was 21-22 W/cm^2. Variations of synthetic graywater, as well as synthetic seawater and synthetic freshwater are shown. The DRTs for media marked by "*" or "**" are significantly different from that of sGW ($P \leq 0.05$ or 0.01, respectively; TEST statement, PROC REG, SAS/STAT). sGW=synthetic graywater, PBS=phosphate buffered saline, sSW=synthetic seawater, sFW=synthetic freshwater.

Figure 8. Comparison of DRT for *E. coli* in various sonication media.

Bacteria – Ballast Water

Bacterial disinfection experiments were performed at NSWC, Carderock. The survival of *Vibrio cholerae* and spores of *Bacillus subtilis* in artificial seawater was quantified. DRT for *V. cholerae* ranged from 0.75-2.2 minutes, depending on ultrasonic intensity which ranged from 19-31 W/cm^2. These DRT values are similar to what was found for *E. coli* in synthetic graywater (at 100 ml). In contrast, spores of *Bacillus subtilis* were extremely resistant to sonication; 213 minutes of treatment was required to reduce the number of viable spores by 90%. Exposure to ultrasound also had no effect on the heat resistance of the spores.

Table 2. Summary of HPU beaker-scale results for bacteria in 20 ml of synthetic seawater, in the conical vessel.

Culture	Intensity ($W \cdot cm^{-2}$)	DRT (min) ± 95% CI	$r^{2\,(a)}$	DRED (J/ml)# ± 95% CI	$r^{2\,(a)}$
V. cholerae	31	0.9 ± 0.1	0.99	97 ± 20	0.96
E. coli	31	1.4 ± 0.2	0.99	180 ± 20	0.99
E. avium	30	8.8 ± 2.5	0.97	930 ± 290	0.96
Bacillus subtilis	23	213 ± 105	0.80	21,000 ± 12,000	0.80

Notes: Decimal reduction time (DRT) and decimal reduction energy density (DRED) for several pure cultures

(a) r^2 is the coefficient of determination for the kill curve regression analyses

Phytoplankton – Ballast Water

Experiments that examined the efficacy of HPU in treating phytoplankton that may be transported in ballast water were performed at North Carolina State University, due to their experience in handling phytoplankton. Tests were carried out under static conditions in a 100 ml jacketed reaction vessel identical to that used in experiments with bacteria. Tests were run for 4 minutes, and were conducted at 3 levels of ultrasonic intensity, 14 W/cm^2, 16.8 W/cm^2, and 19.6 W/cm^2. *Pfiesteria* and *Thalassiosira* were tested in seawater (15 ppt and 30 ppt, respectively), while *Anabaena* was tested in BG-11 culture media. At each time point a 1 ml sample was drawn from the reaction vessel. For the *Pfiesteria* and *Thalassiosira*, counts or estimates of the number of live cells were made after staining, either visually or using a flow cytometer. In the case of the *Anabaena*, reculturing was used to determine the concentration of live cells. For all species, trials at a particular level of ultrasonic intensity were replicated three times. All species appeared to be as resistant to treatment by HPU as the graywater bacteria. DRT in 100 ml batch assays are reported in Table 3. For the *Pfiesteria* and *Anabaena* there was no variation in DRT due to ultrasonic intensity, but destruction of the *Thalassiosira* was dependent on the intensity of treatment.

Table 3. Summary of HPU beaker-scale results for phytoplankton in 100 ml of synthetic seawater in the conical vessel.

Ultrasonic Intensity (W/cm^2)	***Pfiesteria*** DRT (sec)	***Anabaena*** DRT (sec)	***Thalassiosira*** DRT (sec)
14-20	568	375	144

NOTE: Decimal reduction times (DRT) in seconds, for three species of phytoplankton. There was no significant difference in DRT for the three intensity values investigated for *Pfiesteria* and *Anabaena*; therefore, the DRTs are averaged over the intensities. For *Thalassiosira*, the DRT is for 16.8 W/cm^2

Zooplankton – Ballast Water

Zooplankton disinfection experiments were performed at NSWC, Carderock. The ultrasonic intensity was 19-21 W/cm^2, somewhat lower than that used for bacteria. Treatment with ultrasound was very effective in killing zooplankton relative to bacteria and phytoplankton. Estimated contact times of 1 - 4 seconds produced substantial mortality. Treatment yielded few 'moribund' individuals; depending on contact time, zooplankton either passed through the reactor (apparently) unharmed, were killed with varying levels of visible physical damage, or were reduced to debris (Figure 9). For all zooplankton species, for the range of ultrasonic intensities investigated, the percentage of organisms surviving was independent of ultrasonic intensity. Contact time, however, significantly affected survival in all cases except for 'delayed' counts for the freshwater rotifers. In this case sonication caused considerable mortality, but the level of mortality was highly variable. We used the regression coefficient for the contact time term to estimate DRT for each of the test species (Table 3). The decimal reduction times ranged from 3 to 9 seconds.

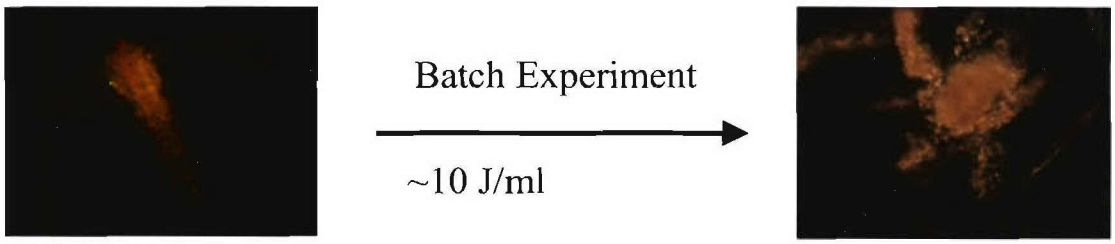


Figure 9. Micrographs of *Artemia*, before (left) and after (right) exposure to HPU in a batch treatment system.

Table 4. Summary of HPU beaker-scale results for zooplankton in flowing synthetic seawater.

Organism	DRT (sec) Immediate counts	DRT (sec) Delayed counts
Artemia sp.	4	4
B. plicatilis	9	5
B. calyciflorus/*Philodina*	9	ns
C. dubia	3	--

NOTE: The treatment vessel volume was 12.4 ml. Decimal reduction times for treatment of zooplankton by ultrasound, as determined by multiple regression of percentage survival (see text for details). Times are estimated for survival immediately after treatment and 5-9 h after treatment. NS = contact time not significant. There were no delayed counts for *C. dubia*.

Figure 10 is a plot of DRED measured for the various organisms examined in this work versus organism size. For phytoplankton and zooplankton, the DRED exhibits a monotonic decrease as a function of organism size, with the exception of the cyanobacterium. The three bacteria (*E. avium*, *E. coli,* and *V. cholerae*) have markedly different DRED despite being approximately the same size. There are several bacterial characteristics that may influence susceptibility to ultrasound. There have been reports that rod-shaped cells are more easily disrupted than spherical cells (Harrison 1991, Tiehm 2001). There have also been reports that gram-negative cells (which possess thinner cell walls) are more easily disrupted than gram-positive cells (Harrison 1991, Tiehm 2001). These facts are consistent with the spherical, gram-positive *E. avium* having a longer DRED than the rod-shaped, gram-negative *E. coli* and *V. cholerae.*

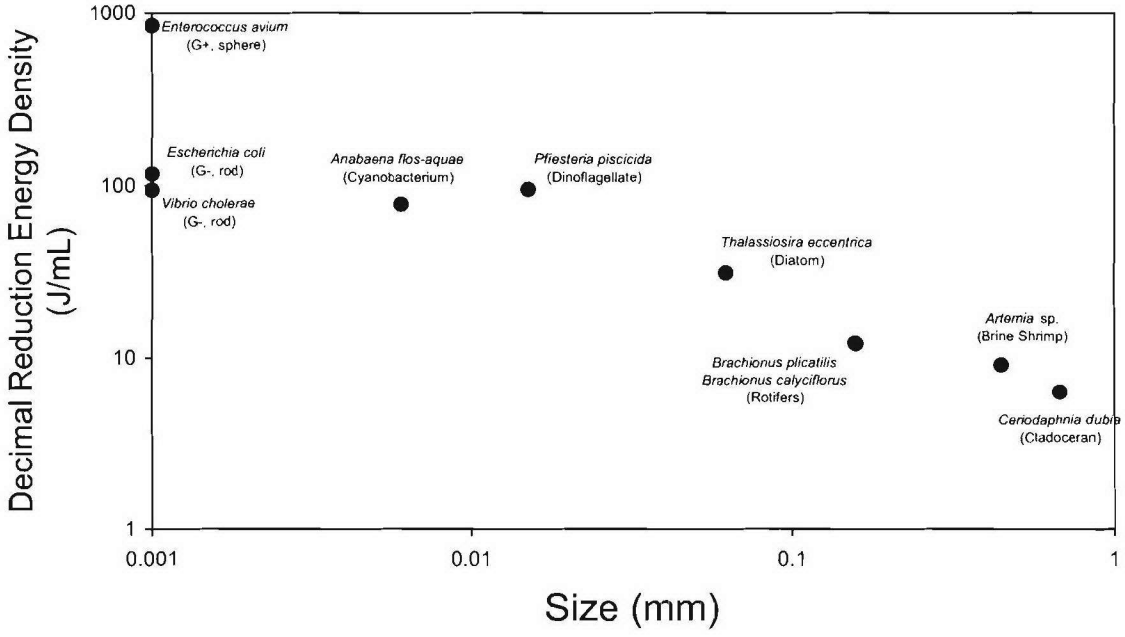


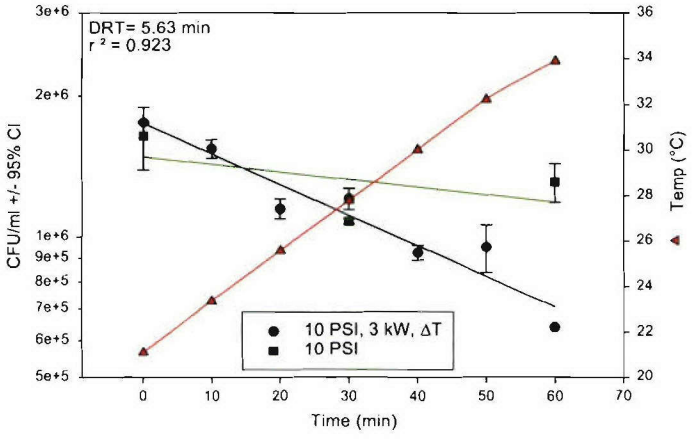
Figure 10. Plot of decimal reduction energy density versus organism size for the various organisms tested.

Results: Pilot-Scale Experiments

A summary of the experimental results, for both beaker-scale and pilot-scale experiments, is provided in Appendix A for ballast water and Appendix B for graywater.

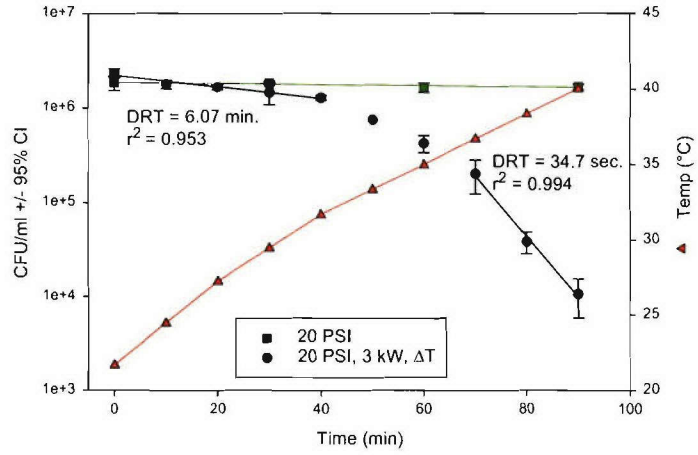
Bacteria

Decimal reduction times for *E. coli* in the pilot-scale system (with a 1.3L treatment volume) were significantly longer than those obtained in the beaker-scale tests – 5.63 minutes for the 10 psi treatment and 6.07 minutes for the 20 psi treatment whereas the DRT in the beaker-scale experiments was approximately 1.4 minutes in a 20 ml volume (Table 2). Decimal reduction energy density (DRED) for the pilot-scale system was higher than for the beaker-scale tests, 255-273 J/ml @ 10 W/cm^2 intensity, pilot-scale vs.164 J/ml @ 31 W/cm^2 intensity, beaker-scale with 20 ml liquid volume (Table 2). HPU caused both mortality of *E. coli* over time and significant increases in the temperature of the recirculating water (Figure 11). Longer exposures carried out during the tests at 20 psi indicate that temperatures from 35-40°C, well below the lethal temperature for *E. coli* (> 44.5°C), increased the kill rate (10 fold) from the HPU (Figure 12). This apparent synergistic effect is being investigated further.



NOTE: Figure shows change in number of colony forming units with application of ultrasound (●), control counts with no ultrasound but application of pressure (■), and change in temperature concomitant with application of ultrasound (▲).

Figure 11. Mortality of *E. coli* in pilot-scale HPU treatment system, when treated at 10 psi.



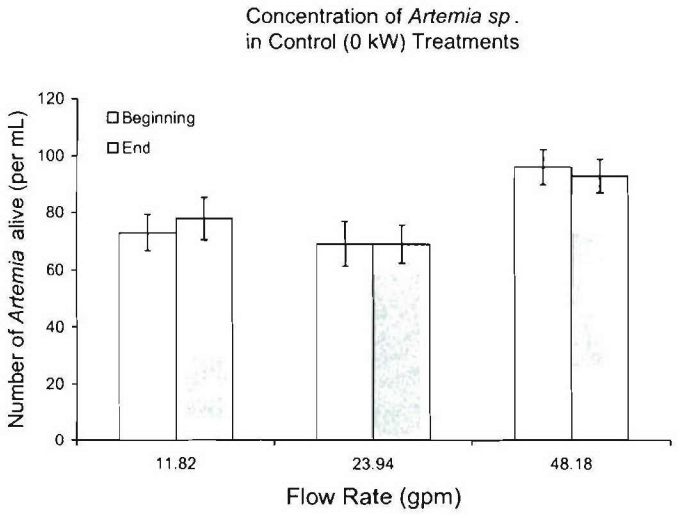
NOTE: Figure shows change in number of colony forming units with application of ultrasound (●), control counts with no ultrasound but application of pressure (■), and change in temperature concomitant with application of ultrasound (▲). Note the decrease in DRT as exposure time and test stream temperature increases above 60 minutes.

Figure 12. Mortality of *E. coli* in pilot-scale HPU treatment system, when treated at 20 psi.

Zooplankton

Control treatments (no ultrasound applied) indicated that the concentration of live *Artemia* in the treated test stream varied depending on the flow rate, with higher concentrations occurring at the highest flow rate (Figure 13). Concentration of *Artemia* remained constant at different flow rates and was constant over the course of an experiment consuming 150 gal of test feed (Figure 13). Counts of *Artemia* sp., for individual runs of the pilot-scale treatment system, Sept. 12-13, 2005 are provided in Appendix C.

In contrast to experiments carried out on the beaker scale, mortality of *Artemia* was affected by the level of power or ultrasonic intensity applied (Figure 14). Mortality increased as power increased. As well, the effect of flow rate or contact time changed depending on the ultrasonic intensity. Finally, system pressure altered the relationship between flow rate and mortality (Figure 14). Tests indicated that, under certain conditions of ultrasonic intensity and pressure, the numbers of *Artemia* could be reduced by approximately 99% at contact times < 1 second.



NOTE: Bars correspond to beginning and end of experiments consuming 150 gal of test feed.

Figure 13. Concentration of *Artemia* sp. in control treatments (no ultrasound applied) at various flow rates.

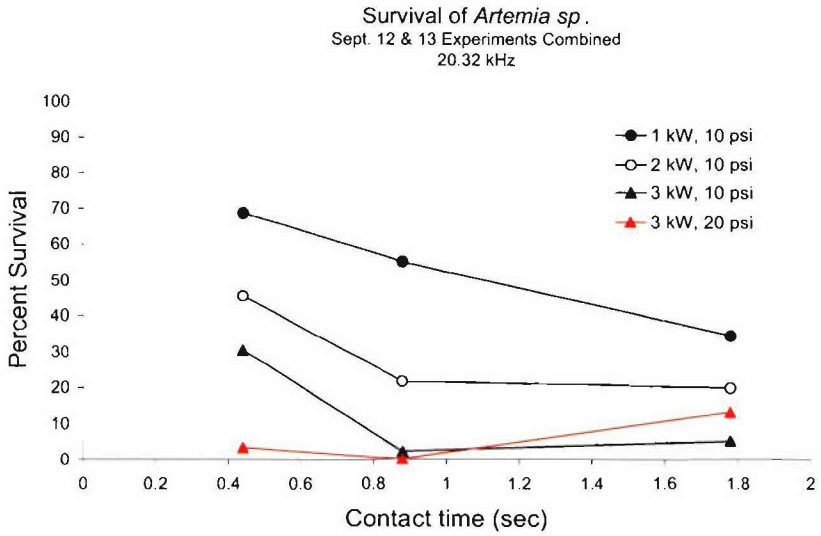


Figure 14. Mortality of *Artemia* sp. exposed to ultrasound at various flow rates and pressures.

Results: Cavitation Erosion Experiments

Experiments were performed to determine the extent of cavitation erosion that the treatment cell pipe wall and the cascade horn would be expected to incur, and to determine optimum materials of construction for these components. The ASTM G-32 test method was used, with 1-in. x 1-in. coupons in ASTM seawater. Ultrasonic intensity and horn-coupon separation distance were chosen to be representative of what would be used in a disinfection treatment cell. The ultrasonic intensity was 75 W/cm^2 (determined from electrical power consumption of the transducer). This is estimated to be equal to 30 W/cm^2 calorimetric power, roughly equal to the highest intensity used in the beaker-scale experiments and a factor of three greater than the highest intensity used in the pilot-scale experiments. Separation distances ranged from 0.1-in. x 1-in.

Three sets of experiments were performed. First, three materials were evaluated for resistance to cavitation erosion: alloy 686, commercially pure (CP) titanium, and 90:10 Cu:Ni. These runs were performed at a horn tip-sample separation of 0.03 in. The results (Figure 15) are expressed as the mass loss plotted versus the time of exposure to cavitation. Alloy 686 and titanium experienced no measurable mass loss under these conditions, but the CuNi experienced substantial mass loss. Figure 16 shows photographs of the coupons following 12 hours of exposure to cavitation. There is clear damage to the CuNi sample. There is evidence of slight damage to the alloy 686 coupon and slightly more damage to the titanium.

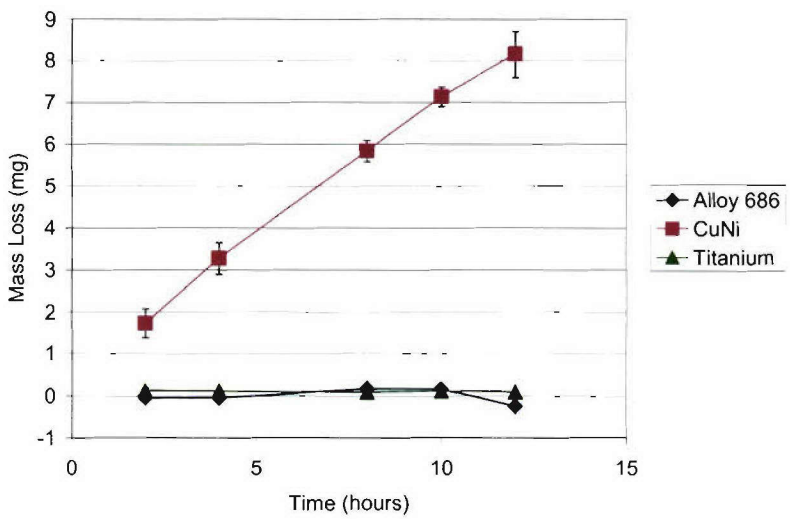
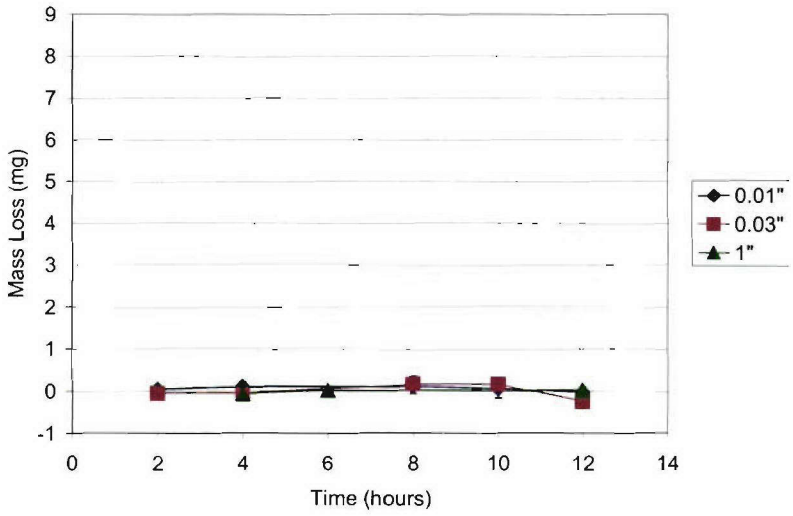


Figure 15. Cavitation erosion results for alloy 686, CP titanium, and CuNi at a various flow rates and pressures (separation distance 0.03-in.) The error bars represent the standard deviation of the mean of three trials and in most cases are smaller than the size of the datapoint.

Figure 16. Photographs of test coupons following 12 hours exposure to cavitation: alloy 686 (left), CuNi (center) and CP titanium (right).

The second set of tests examined the effects of separation distance on cavitation erosion in alloy 686. Even at a separation distance as small as 0.01 in., there was no measurable mass loss (Figure 17). Figure 18 shows photographs of the test coupons following 12 hours exposure to cavitation. Slight damage is seen on the coupon using 0.01-in. separation, less damage is seen on the 0.03" separation distance coupon. No damage can be seen on the 1-in. coupon.



Note: The error bars, which represent the standard deviation of the mean of three trials, are smaller than the size of the datapoints.

Figure 17. Cavitation erosion results for alloy 686 at separation distances of 0.01 inches, 0.03 inches, and 1 inch.

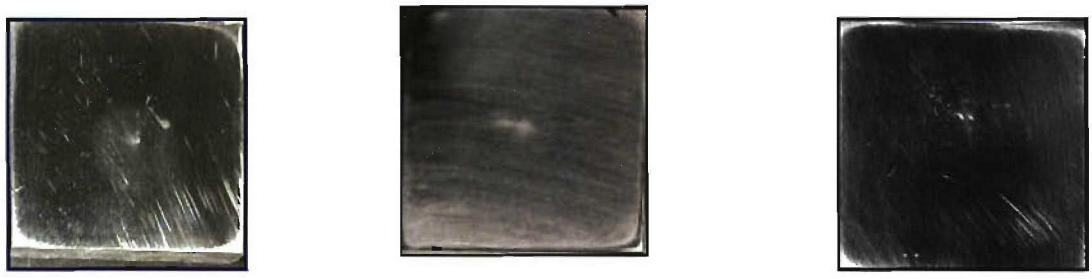
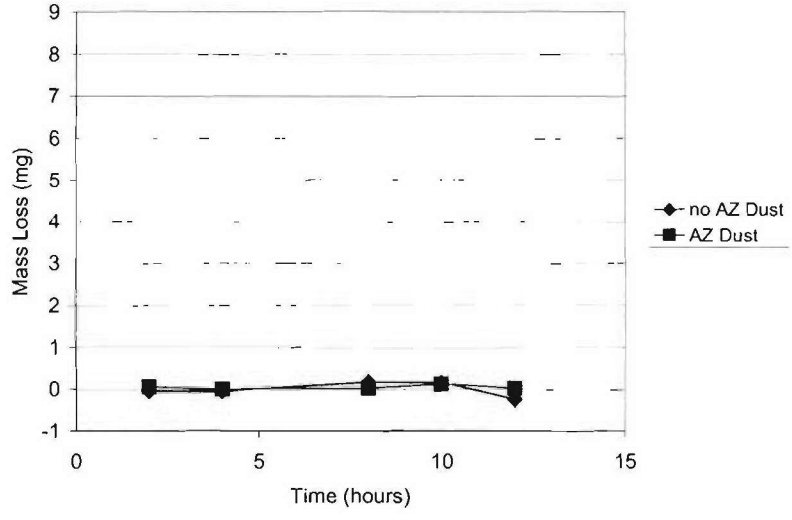


Figure 18. Photographs of test coupons following 12 hours exposure to cavitation: alloy 686 at separation distances of 0.01 inches (left), 0.03 inches (center), and 1 inch titanium (right).

The third set of tests examined the effects of Arizona Test Dust on the cavitation erosion of alloy 686 at a separation distance of 0.03 in. Fifty μg of Arizona test dust per ml of water were used to simulate the presence of silt or other erosive material in the water. The Arizona test dust did not have a measurable effect on the mass loss of the alloy 686 after 12 hours exposure (Figure 19). Photographs of the coupons are shown in Figure 20; the extent of damage appears the same on both.



Note: The error bars, which represent the standard deviation of the mean of three trials, are smaller than the size of the datapoints.

Figure 19. Cavitation erosion results for alloy 686 at a separation distance of 0.03 inches, with and without Arizona Test Dust.

Figure 20. Photographs of test coupons following 12 hours exposure to cavitation: alloy 686 at a separation distance of 0.03 inches with no Arizona Test Dust (left), and alloy 686 at a separation distance of 0.03 inches with Arizona Test Dust (right).

The results of the cavitation erosion experiments show titanium and alloy 686 are promising materials for fabrication of HPU treatment cells and cascade horns. There is some evidence, from the visual appearance of the coupons, that alloy 686 may perform somewhat better than titanium in this application. These tests show that material loss from the treatment cell wall and cascade horn, while not a short-term concern, may be an issue over longer-term operation of the HPU device under these conditions. A longer duration exposure in the ASTM G-32 test would result in a more precise estimate of the rate of mass loss experienced by alloy 686 and titanium due to cavitation erosion.

Conclusions: Outlook for the Use of HPU for Wastewater Treatment

Several conclusions can be drawn regarding the potential of high-power ultrasound for wastewater treatment. In drawing these conclusions, three quantities were examined. All three were derived from the measured DRTs and DREDs, at beaker and pilot-scale, and are referred to as "scale-up quantities." Calculations based on the pilot-scale system are considered to be more representative of a full-scale system, due to the larger system size and the use of the cascade horn. Calculations based on DRTs and DREDs measured at the beaker scale are included for comparison. The first quantity examined was the estimated full-scale treatment cell size necessary for a given wastestream flow rate. This was determined from the decimal reduction time, and an assumed flow rate, which was 1000 gpm for ballast water treatment and 5 gpm for graywater treatment, assuming the same ultrasonic intensity as in the beaker- and pilot-scale experiments. Both of these represent values that fall within the range of what would be found on a U.S. Navy ship. The second quantity was power consumption, which was determined from the decimal reduction energy density and the assumed flow rate of 1000 gpm for ballast water and 5 gpm for graywater. The third factor was energy consumption, which was determined from the decimal reduction energy, and an assumed water volume to be treated of one million gallons for ballast water and 10,000 gallons for graywater (50 gallon/day-Sailor * 200 Sailors). Again, both represent values that fall within the range of what would be found on a U.S. Navy ship. In looking at the potential of HPU for full-scale treatment system, the ballast water and graywater wastestreams were considered separately.

Ballast Water: The results of the scale-up calculations for HPU treatment of ballast water are shown in Table 5.

Table 5. Scale-up quantities for HPU disinfection of ballast water.

Organism	Treatment Cell Volume per 1000 gpm for 1 log kill (gallons)	Ultrasonic Power Required per 1000 gpm for 1 log kill (kilowatts)	Energy Required per million gallons of water for 1 log kill (kWh)
Benchtop System			
Vibrio cholerae	750	5572	92863
Escherichia coli	1400	10401	173343
Enterococcus avium	8900	63920	1065330
Bacillus globigii	213000	1173630	19560500
yeast	18000	64638	1077300
Artemia	72	578	9629
Marine Rotifers	99	768	12805
FW Rotifers	104	773	12881
FW Cladoceran	52	400	6659
Pfiesteria	10400	9760	162664
Thalassiosira	5600	5255	87588
Anabaena	2100	1971	32846
Pilot Scale System			
Artemia @3 kW and 0.88 sec	8	24	398
Artemia@2 kW and 1.8 sec	42	80	1328
Escherichia coli @3 kW & 2.1 min	5300	15200	253333

1. Zooplankton Disinfection: The treatment cell size, power consumption and energy consumption results based on the DRT and DRED measured using the pilot-scale system are considerably better than from the beaker-scale system. This is presumably due to the cascade horn design in the pilot-scale system treatment cell, which provides an extended treatment volume compared to the horn used in the beaker-scale experiments, which is close to a point-source geometry. The improved zooplankton disinfection performance in the pilot-scale system occurred despite the fact that the ultrasonic intensity (~10 W/cm^2) was approximately half that in the beaker-scale experiments. This indicates that 10 W/cm^2 is sufficient for zooplankton disinfection. As discussed below, this is likely not the case for bacterial disinfection. In the pilot-scale experiments, it was determined that the HPU operating parameters can make a large difference in the DRT and DRED. The table in Appendix C shows that *Artemia* disinfection at 3 kW power results in a DRT of 0.5 sec. and a DRED of 0.4 J/ml whereas *Artemia* disinfection at 2 kW results in a DRT of 2.5 sec. and a DRED of 1.3 J/ml.

For ballast water, the treatment cell volume necessary to treat 1000 gpm of ballast water varies over a wide range depending on which organism is being treated. For the calculations based on the pilot-scale system *Artemia* disinfection results, the treatment cell volumes are quite promising. An 8-gallon treatment cell, while very large, is feasible either as a single treatment cell, or multiple cells operating in parallel. The power and energy consumptions appear to be feasible as well. The next step in this line of investigation should be a determination of the disinfection performance of the pilot-scale system on natural seawater or brackish water.

2. Bacterial Disinfection: Treatment cell volume, power and energy consumption for bacterial disinfection are much higher than for zooplankton. The improvement in zooplankton disinfection performance for the pilot-scale system relative to the beaker-scale system did not extend to bacteria; the disinfection performance of the pilot-scale system against *E. coli* was somewhat worse than the beaker-scale system. In addition, in the beaker-scale experiments, it was found that the measured DRT depended linearly on the treated liquid volume. This indicates that the treatment container size in these experiments was larger than the disinfection zone. Since the size of the treatment container is important at the beaker scale, we expect that it will be important at the pilot-scale as well. If this hypothesis is correct, then the bacteria that pass through the treatment cell at a large radial distance from the cascade horn will not experience high enough ultrasonic intensity to be killed. Only the bacteria that travel close to the cascade horn are killed. Therefore, the ultrasonic intensity needs to be higher than 10 W/cm^2 and the treatment cell needs to have a smaller diameter to confine the bacteria to a region close to the horn. These hypotheses can be tested by performing experiments with disinfection cells of different diameters.

   Reducing the diameter of the treatment cell, as described in the previous paragraph, might improve the DRTs for bacteria. However, the treatment cell energy consumption likely would not improve, since for a given flow velocity (contact time) a treatment cell of smaller diameter will have larger energy per volume of water treated. For bacteria, combined treatment methods employing HPU and a second method may offer a route to disinfection of large water volumes and flow rates. Combined treatment approaches will be investigated at the beaker scale in FY06.

3. Phytoplankton Disinfection: Treatment cell volume, power, and energy consumption for phytoplankton disinfection are much higher than for zooplankton, as measured at the beaker scale. As with bacteria, optimizing the size of the treatment cell and the use of combined treatment systems may offer routes to improvement in disinfection performance.

Graywater

The utility of HPU disinfection in a graywater treatment system would be as a post-treatment, for example in disinfecting the effluent from a membrane bioreactor. In evaluating HPU for this application, it is noted from Table 6 that the treatment cell size, power, and energy consumption are not particularly large, due to the much smaller flow rates and volumes to be treated in this application relative to ballast water. However, it is unlikely that HPU could compete with ultraviolet disinfection for disinfection of membrane bioreactor effluent. HPU may well be competitive in other water disinfection applications, including disinfection of turbid water, for which UV is not effective.

Table 6. Scale-up quantities for HPU disinfection of graywater.

Organism	Treatment Cell Volume per 5 gpm for 1 log kill (gallons)	Ultrasonic Power Required per 5 gpm for 1 log kill (watts)	Energy Required per 10,000 gal of water 1 log kill (kWh)
Benchtop System			
Escherichia coli-stationary	26.2	31989	1066
Escherichia coli-log	25.1	28785	960
Enterococcus avium-stationary	79.5	97065	3235
Enterococcus avium-log	68.0	84652	2822
P. aeruginosa	37.5	45785	1526
Sewage (mixed liquor)	281.0	343084	11436

Besides disinfection, HPU has potential applicability to graywater treatment in causing physical and chemical degradation of materials in graywater. For example, it is expected that HPU would enhance the biodegradability of graywater by reducing particle size and increasing surface to volume ratio. In FY06, we will quantify the effect of HPU on biodegradability and the contact times and energy densities necessary to achieve the effect will be determined.

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Appendix A

Summary of Results: Ballast Water

Results summary for bacteria, phytoplankton and zooplankton in synthetic seawater, treated using beaker-scale and pilot-scale systems.

Organism	Test Format	Horn Area (cm^2)	Calorimetric Power (Watts)	Intensity (W/cm^2)	Assumed Treatment Volume (ml)	Power Density Calorimetric (W/ml)	Decimal Reduction Time (sec/log kill)	Decimal Reduction Energy Density (Calorimetric) watts/(ml/sec)/log kill ((J/ml)/log kill)
Beaker System								
Vibrio cholerae	Batch	1.26	39.1	31.0	20	1.96	54	106
Escherichia coli	Batch	1.26	39.1	31.0	20	1.96	84	164
Enterococcus avium	Batch	1.26	37.8	30.0	20	1.89	528	998
Bacillus subtilis (spores)	Batch	1.26	29.0	23.0	20	1.45	12780	18531
Artemia	Flow	1.26	26.1	20.7	12.4	2.11	4.3	9.1
Marine Rotifers	Flow	1.26	25.2	20.0	12.4	2.04	6.0	12.1
FW Rotifers	Flow	1.26	24.2	19.2	12.4	1.95	6.3	12.2
FW Cladoceran	Flow	1.26	25.0	19.8	12.4	2.02	3.1	6.3
Pfiesteria	Batch	1.26	21.2	16.8	100	0.21	568	120
Thalassiosira	Batch	1.26	21.2	16.8	100	0.21	144	30
Anabaena	Batch	1.26	21.2	16.8	100	0.21	375	79
Pilot Scale System								
Artemia @3 kW and 0.88 sec	Flow	103	1000	9.7	1325	0.75	0.5	0.4
Artemia@2 kW and 1.8 sec	Flow	103	667	6.5	1325	0.50	2.5	1.3
Escherichia coli @3 kW & 2.1 min	Flow	103	1000	9.7	1325	0.75	318.0	240.0

Appendix B

Summary of Results: Graywater

Results summary for bacteria in water and synthetic graywater, treated using beaker-scale system.

Organism	Test Format	Horn Area (cm2)	Calorimetric Power (Watts)	Intensity (W/cm2)	Assumed Treatment Volume (ml)	Power Density (W/ml)	Decimal Reduction Time (sec/log kill)	Decimal Reduction Energy Density watts/(ml/sec)/log kill ((J/ml)/log kill)
Beaker-Scale System								
Escherichia coli-stationary	Batch	1.26	32.1	25.5	100	0.32	314	101
Escherichia coli-log	Batch	1.26	30.2	24.0	100	0.30	301	91
Escherichia coli	Batch	1.26	33.4	26.5	20	1.67	120	200
Enterococcus avium-stationary	Batch	1.26	32.1	25.5	100	0.32	954	307
Enterococcus avium-log	Batch	1.26	32.8	26.0	100	0.33	816	267
P. aeruginosa	Batch	1.26	32.1	25.5	100	0.32	450	145
Sewage (mixed liquor)	Batch	1.26	32.1	25.5	100	0.32	3372	1083
Pilot-Scale System								
Escherichia coli								
Enterococcus avium								

Appendix C

Artemia Counts

Counts of *Artemia* sp., for individual runs of the pilot-scale treatment system, Sept. 12-13, 2005. Flow rate is measured in gpm, power in kW, pressure in psi, and volume in ml. Counts are the total number of *Artemia* found in a sample of the volume designated.

Flow Rate	Power	Pressure	Count	Volume
Sept. 12, 2005				
11.82 (start)	0	10	73	1
23.94 (start)	0	10	69	1
48.18 (start)	0	10	96	1
11.82 (end)	0	10	78	1
23.94 (end)	0	10	69	1
48.48 (end)	0	10	93	1
11.82	1	10	26	1
23.94	1	10	38	1
48.18	1	10	65	1
11.82	2	10	15	1
23.94	2	10	15	1
48.18	2	10	43	1
Sept. 13, 2005				
23.94 (start)	0	10	91	2
48.18 (start)	0	10	142	2
11.82	3	10	7	3
23.94	3	10	3	3
48.18	3	10	65	3
11.82 (end)	0	20	56	2
23.94 (end)	0	20	43	2
48.18 (end)	0	20	63	2
11.82	3	20	11	3
23.94	3	20	0	3
48.18	3	20	3	3

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