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Quantification of the energy required for the destruction of *Balanus amphitrite* larva by ultrasonic treatment

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Ultrasonic treatment, a relatively less explored technology in water disinfection, was used to quantify the energy required for the destruction of larvae of barnacle Balanus amphitrite*, which is a major marine fouling and a potential invasive organism. Since the power used and treatment time for disinfection are economically, and practically, the most important parameters, the energy required to pulverize the larvae into pieces ≤30 μm was determined as a function of the acoustic power density. The present investigation suggests that an ultrasonic system operating at 20 kHz and 0.0975 W/cm³ can effectively pulverize barnacle larvae having length (∼440 μm) and breadth (∼350 μm) within 45 seconds using 0.1 mJ/larva of pulverization energy. It was also observed that following pulverization of the larvae, the bacterial abundance increased and the rate of release of bacteria was dependent on power level and treatment time, which in turn decided the pulverization rate and hence the rate of release of bacteria.*

Keywords: *Balanus amphitrite*, ultrasonic treatment, bacteria, pulverization energy, acoustic power density, water treatment

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INTRODUCTION

Acoustic cavitation is a relatively less explored technique in the treatment of water and investigations are needed to ascertain its practical applicability especially in brackish/saline environment or with specific marine microbes. Apart from making water effective for drinking purposes, it can find utility in a ship to treat ship's ballast water, treatment of industrial effluent, etc. There are various existing, as well as, newly emerging technologies available for the disinfection of water, ranging from physical methods such as heating and UV treatment, to chemical treatment with biocides; however they have many inherent problems. Firstly, they are energy wise intensive and hence expensive, they can cause contamination of the treated water (due to addition of chemicals), and therefore, of the environment into which the water is subsequently released. Their mechanism of action is often complicated and poorly understood, and their effect varies with different organisms. Also there is a possibility of generation of treatment byproducts.

Although there are a number of illustrations regarding use of cavitation for waste water treatment, the theoretical analysis to develop the fundamentals and experimental studies to implement the same is a necessity. We have addressed the implication of ultrasonic treatment in disrupting and/or pulverizing the organisms that could be transported via ballast water. Ballast water is the seawater taken up by the ships in order to weigh down and/or balance the latter while unloading their cargo. Conversely, when loading cargo, they discharge the ballast water. In this process, ships transfer millions of tons of ballast water from one place to another worldwide, inadvertently discharging non-indigenous aquatic organisms into receiving waters (Carlton & Geller, 1993). Translocation of organisms through ships (bio-invasion) is considered to be one of the central issues that have plagued the naturally evolved biodiversity, the consequences of which are being realized increasingly in recent years (Gollasch *et al.*, 2000; Anil *et al.*, 2002). Marine invertebrate larvae are of major concern in ballast water treatment/management programmes and in addition they also harbour many bacteria within them. The release of bacteria while these large planktonic organisms are killed or destroyed by rupturing is an additional concern in the ballast water treatment. It has been found that most of the pathogens introduced to Chesapeake Bay came from bacteria associated with plankton rather than that in water (Ruiz *et al.*, 2000). In this context it is important to get the maximum extent of microorganisms released out of the planktonic organisms during the latter's destruction so that additional treatment measures can be effectively applied to reduce or eliminate them. Very little is known about the strength of their exoskeleton and the mechanical or thermal energy required for breaking or pulverizing them into small pieces (≤30 μm) so that most of the bacteria are released after the treatment. In view of this we chose a representative marine invertebrate larva of a major fouling barnacle which is also a potential invasive organism, *Balanus amphitrite* (∼440 μm in length and ∼350 μm in breadth) with a

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chitinous exoskeleton as a candidate organism for this study. This paper addresses the effectiveness of the ultrasonic treatment on *B. amphitrite* larvae and quantifies the energy required for their destruction, and this has implications in ballast water treatment.

MATERIALS AND METHODS

Ultrasonic equipment

The equipment used for ultrasonic treatment in this study is an ultrasonic homogenizer LABSONIC U developed by the B. Braun Biotech International Gmbh with an operating frequency of 20 kHz and a rated maximum power of 150 watts. The LABSONIC U consists of a generator, a transducer and a metal probe. Electrical power supplied to the generator is given out as a user-defined electrical output which then serves as an input to the transducer. The transducer then converts it into acoustic power which is supplied to the sample via the probe. The transducer is fitted with the titanium needle probe tip of length 127 mm and diameter 4 mm. In this paper, the term 'Power level' refers to the frequency generator output and 'Acoustic power' refers to the transducer output. The actual delivered power was determined calorimetrically by using the formula below

$$= \frac{(\mathrm{m.Cp.}\Delta\mathrm{T})}{\text{Electrical power} \times \text{Time of operation}} \times 100$$

where m is the mass of water in g, Cp is the specific heat in joules and ΔT is the rise in temperature in °C. For power levels of 30W, 50W, 80W and 110W, the actual mechanical delivered power was estimated to be 0.4W, 0.8W, 1.49W and 2.25W respectively.

Organism used

The barnacle, *B. amphitrite* was used for this experiment. The larval development in this organism includes six naupliar instars which feed on phytoplankton and a non-feeding cyprid instar specialized to explore suitable surfaces for settlement (Desai & Anil, 2004). The barnacles were maintained in the laboratory using *Artemia* sp. The larvae released by the adults were collected and mass reared in 2-l glass beakers using filtered seawater (FSW) of 35‰ on a diet of *Chaetoceros calcitrans*, a unicellular diatom, at a cell concentration of 2×10^5 cells ml^{-1}. The food organism was replenished every day while changing the water. Fourth instar larvae having length (~440 μm) and breadth (~350 μm) were used in the present study.

Samples

The larval samples subjected to the treatment were held in flat-bottom borosilicate tubes of dimensions: height = 5.6 cm, radius = 1.2 cm. The sample tube consisted of 5 ml of filtered seawater (0.22 μm filter) making a level of 16 mm height. A single barnacle larva was transferred into each tube (Figure 1). Before transferring the larvae, they were rinsed several times with autoclaved filtered seawater. After subjecting it to sonication with the probe of length 127 mm and diameter 4 mm, it was then observed under the dissection microscope and photographed wherever necessary. The above procedure was used for all the treatments.

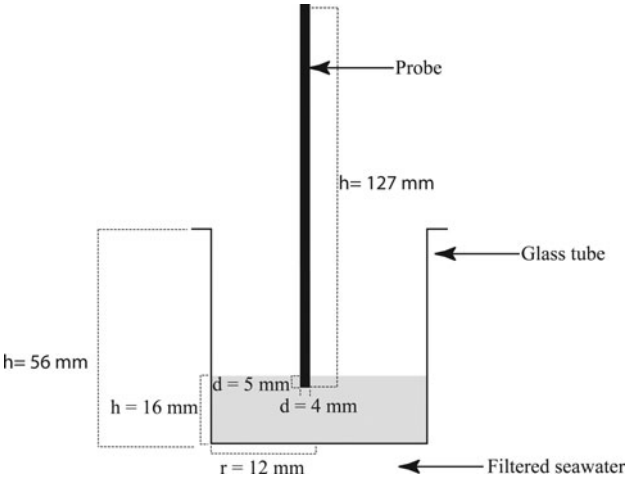

Fig. 1. The sample tube and ultrasonic probe arrangement during the treatment.

Scanning electron microscopy (SEM)

Each collected sample for SEM analysis was preserved in 2% glutaraldehyde. The samples were first fixed in 2% osmium tetroxide for 30 minutes followed by dehydration using ethanol series. Thereafter the samples were exposed to tertiary butyl alcohol for an hour (Truby, 1997). They were then transferred onto gold coated brass stubs. The samples were then freeze dried for 20 minutes, sputter coated and viewed under SEM.

Modelling of the ultrasonic probe output

The output of the probe consists of ultrasonic waves at 20 kHz directed downward in the form of a beam. In order to understand the forces that the larvae were subjected to, a few barnacle larvae were stained violet with a prominent dye, rose Bengal, and their movement was monitored visually during the ultrasonic irradiation treatment. The probe tip was immersed at different liquid depths within the test tubes and the effectiveness of these conditions on larval disruption was studied.

Standardization of the treatment

The treatment procedure was standardized based on numerous trial runs by varying different parameters such as power level, treatment time and immersion depth. The parameters involved have been discussed individually and the values selected for standardization have been specified and justified below.

Depth of immersion

Arrangement of the sample tube and ultrasonic probe during the treatment is shown in Figure 1. In the present investigations the depth of immersion was 5 mm (Figure 2), as it was found to cover almost the entire sample volume

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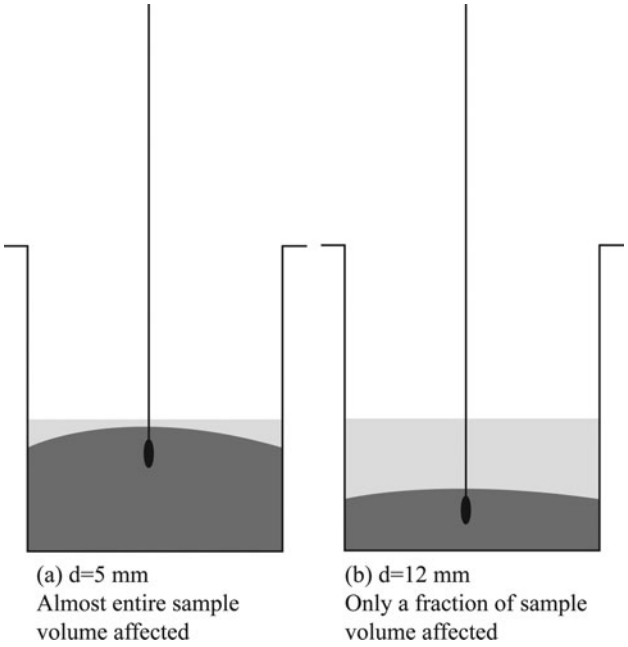

Fig. 2. Effect of the depth of the probe tip 'd' on the sample volume affected (volume affected is depicted in dark shade and the unaffected volume is in light shade).

(qualitative). Since a larger volume is covered, there is a higher probability of the larva being caught in the acoustic streaming during irradiation and thus, a higher consistency in the resultant data.

Sample volume and larval density

A sample volume of 5 ml was used so that the water would be at a sufficient height (~16 mm) above the tube-bottom and is capable of dipping the ultrasonic horn probe. Keeping the volume minimal, higher power densities could be achieved at lower overall dissipation of probe power levels. The requirement of the present study being quantification of energy required to disrupt a single larva, it was ensured that only a single larva was transferred into the tube, so as to avoid the cushioning of one larva by another (shadow effect).

Filter size for sample water

Filtered seawater was used in the samples so as to minimize the number of particles. A 0.22 μm filter was employed so that there were no foreign particles of comparable size (to that of larvae) which could be confused with the larva or its debris.

Power output

The output power of the generator (of the ultrasonic homogenizer) could be varied from 30W to 110W, in steps of 1W. Therefore, the output of the transducer could be varied from 0.4W to 2.25W using continuous duty cycle. Acoustic power and sample volume are the parameters that have the greatest influence on disruption level (Feliu *et al.*, 1998). Since constant sample volume was used, the power was considered as the most influential parameter.

Treatment time

A treatment time of ≤120 seconds was used in the present investigation. The power level and the treatment time are the most important practical parameters in disinfection technologies as they determine the treatment costs and efficiencies, and hence, most of the present work deals with the variations in power and time, indicating the total energy requirement. Two experiments were carried out and are described with the help of schematic representations 1 and 2.

Experiment 1: influence of power level and treatment time on pulverization

The power levels and treatment time were altered, while keeping the pulverization efficiency as a constant. ('Pulverization efficiency' was quantitatively defined as a condition of the larva of, *Balanus amphitrite*, in which it had been crushed to pieces of an average size ≤30 microns, with no one piece >30 μm in size.)

The treatment time required for pulverization was determined for 4 different power levels (30W, 50W, 80W and 110W). Trials carried out at each of the power levels helped to ascertain the values of treatment time (t). A triplicate (set of 3 samples of live moving larvae) was subjected to ultrasonic treatment at a power level of p = 110W, for an initial treatment time, t = 35 seconds. The samples were observed under the microscope and if all 3 samples did not show pulverization, treatment time was incremented by 5 seconds and the sample was sonicated with the new irradiated time 't'. The vials of larvae exposed for any given treatment were discarded after the observations so that the larvae were treated only once.

For power levels of 30W, 50W and 80W, the initial treatment times used were 90 seconds, 150 seconds, and 375 seconds respectively. Increments were made in steps of 10 seconds for 80W, 30 seconds for 50W and in steps of 45 seconds for 30W. Temperatures were recorded immediately before and after the treatment of each sample using a digital thermometer.

Pulverized samples were photographed wherever necessary and also preserved in 2% glutaraldehyde for subsequent observation through SEM (Plates 1 & 2). A graph was plotted between the power and the time required for pulverization. The energy supplied to the larva is plotted against the acoustic power density (Figures 3 & 4).

Experiment 2: influence of varying treatment time, at constant power level on pulverization

The variation in the pulverization efficiency was observed with varying treatment time, at a constant power level of 80W. The treatment time was varied as 30 seconds, 60 seconds and 90 seconds based on the results from experiment 1. Samples were taken in sets of 3 (triplicates) and subjected to ultrasonic treatment at a power level of 80W, for time t. The value of t was varied as; 30 seconds, 60 seconds and 90 seconds. Temperatures were recorded before, and after the treatment of each sample. After the treatment of each triplicate, the samples were observed under the microscope and photographed wherever necessary.

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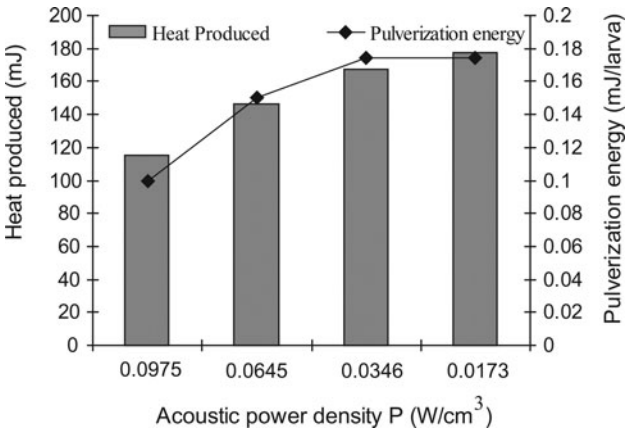

Fig. 3. The pulverization energy/heat produced versus Acoustic power density.

Quantification of bacteria

In another set of experiments the larvae were sonicated using a range of mid-power levels used in the above experiment (i.e. 50, 60, 70 and 80W) upto 180 seconds. The water from the sample tube was then fixed with formaldehyde (final concentration 1 to 2%; v/v) to quantify the bacterial numbers. The quantification of bacteria was carried out by using acridine orange and epifluorescence microscopy (Daley & Hobbie, 1975).

Statistical analysis

The data on bacterial abundance were log-transformed to ensure normality and homogeneity of variances before being subjected to statistical analysis. The pattern seen in the bacterial abundance with increasing input power and exposure time was subjected to one-way ANOVA followed by a post-ANOVA Scheffé's test (Sokal & Rohlf, 1981).

RESULTS

After standardizing the depth of probe immersion for effective disruption to 5 mm, numerous trials with the rose Bengal preserved and live larvae were carried out. Since the stain used had

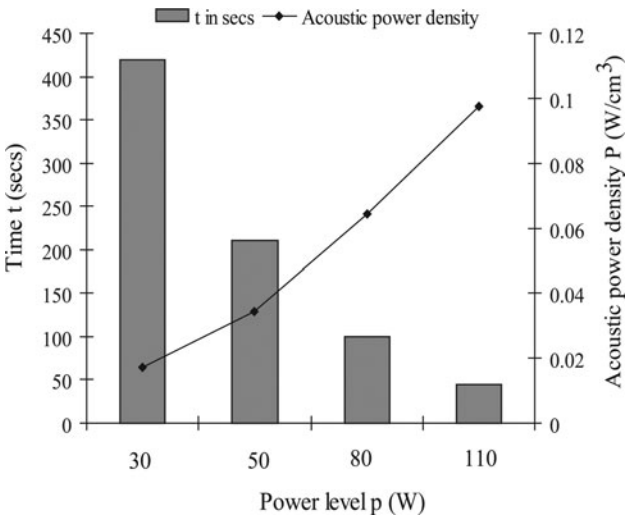

Fig. 4. Pulverization time/acoustic power density versus Power level.

preserved the larvae, the movement shown by them was purely due to the acoustic streaming generated by the passage of ultrasound. Trials with, live, larvae also revealed, that larvae close to, or anywhere below the probe tip were immediately sucked into the liquid jet created by the oscillation of the horn, while those that were well above the probe tip, were relatively unaffected, and hence, much more independent in their movement.

In conclusion, almost all the ultrasonic energy is concentrated in the volume of water adjacent to, and/or below the probe tip. This can be called the 'processed volume'. Since the exact ultrasonic power distribution in the processed volume was unknown, the power density that the larva is subjected to is approximated, as the average power density over the processed volume, i.e. acoustic power density = (transducer power output, watts/processed volume, cm^3).

The 'acoustic power density' is a more universal treatment parameter than the 'power level' as it is independent of the methods, equipment, sample volume, etc. However, it suffers from inaccuracy due to the approximation of the transducer conversion efficiency. Hence, throughout this report both parameters have been used.

So, given a treatment at a particular power level '**p**', watt (W) for a treatment time of '**t**', sec, with the depth of the probe as '**d**', mm, the height of water above the tube-bottom as '**h**', mm and the radius of the tube as '**r**', mm (Figure 1), the acoustic power density '**P**' and the acoustic energy density '**E**' in the processed volume was calculated using the following equations (it inherently assumes that no significant energy dissipation takes place in the liquid above the lower tip area of probe):

$$P = (p)/[\Pi \times r^2 \times (h - d)],\ W/cm^3 \qquad \text{(Equation. 1)}$$

$$E = P \times t\ J/ml \qquad \text{(Equation. 2)}$$

$$0)\ 100s \times 0.0645 \frac{J}{s.ml} = 6.45 J/ml$$

$$1)\ 420\,s \times 0.0173 \frac{J}{s.ml} = 7.266 J/ml$$

$$2)\ 210\,s \times 0.0346 \frac{J}{s.ml} = 7.266 J/ml$$

$$3)\ 45\,s \times 0.0975 \frac{J}{s.ml} = 4.38 J/ml$$

Then, the energy supplied to a nauplius of volume 'v', is given by:

$$N = E \times v\ mJ/larva \qquad \text{(Equation. 3)}$$

The energy supplied to the larva for its pulverization (i.e. pulverization energy) was calculated for each of the acoustic power densities using equations 2 & 3. An average volume of 0.4 mm*0.3 mm*0.2 mm = 0.024 mm^3 (for each larva) was used for each of the samples.

Also, the ultrasonic energy converted to heat energy (heat produced) was independently estimated as

$$H = m \times c \times \Delta t \qquad \text{(Equation. 4)}$$

where 'm' is the mass of 5 ml of seawater, g, 'c' is its specific heat (~cal/gm °C) and 'Δt', (°C) the rise in temperature

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observed during the treatment period. The heat energy represents the ultrasonic energy retained by the media and is a function of only the equipment used and the acoustic power, not of the sample-volume. The pulverization energy and the heat produced at each acoustic power density are shown in Figure 3.

The results of experiment 1 are shown in Figure 4, plotted between the time required for pulverization (i.e. pulverization time) of the sample, and the power used. The 'pulverization time', expressed as (seconds), and 'acoustic power density' values, calculated using equations 2 & 3 expressed as W/cm^3 are plotted on the Y-axis and the corresponding power levels, expressed as watts (W), are plotted along the X-axis. The results indicated that from a pulverization time of 420 seconds at 0.0173 W/cm^3 the curve drops to half its value; 210 seconds at 0.0346 W/cm^3, again to 100 seconds at 0.0645 W/cm^3, and then again to 45 seconds at 0.0975 W/cm^3. The rise in temperature at higher power density is low, i.e. 5.5°C at 0.0975 W/cm^3 when compared at 0.0173 W/cm^3 (8.5°C). The results of experiment 2 are shown in Figure 5. The pulverization energies expressed as J/ml are plotted against the acoustic power densities. The heat produced during a pulverization treatment is seen to decrease, approximately linearly, with increasing acoustic power. This clearly indicates that at higher power density, pulverization is more energy efficient, i.e. requires less total energy.

The bacterial abundance after the pulverization of larvae using different power levels and time is shown in Figure 6. When the larvae were sonicated at lower power levels, an increase in the bacterial numbers was evident up to 90 seconds after which there was a slow decline (17.79%). When a comparison was made across the power treatments, a significant difference in bacterial abundance was observed at 30 and 90 seconds ($P \leq 0.001$, one-way ANOVA; $P \leq 0.05$, Scheffé's test). At higher power levels, less time was required to obtain the highest bacterial numbers followed by a faster decline in their numbers subsequent to further sonication until 180 seconds.

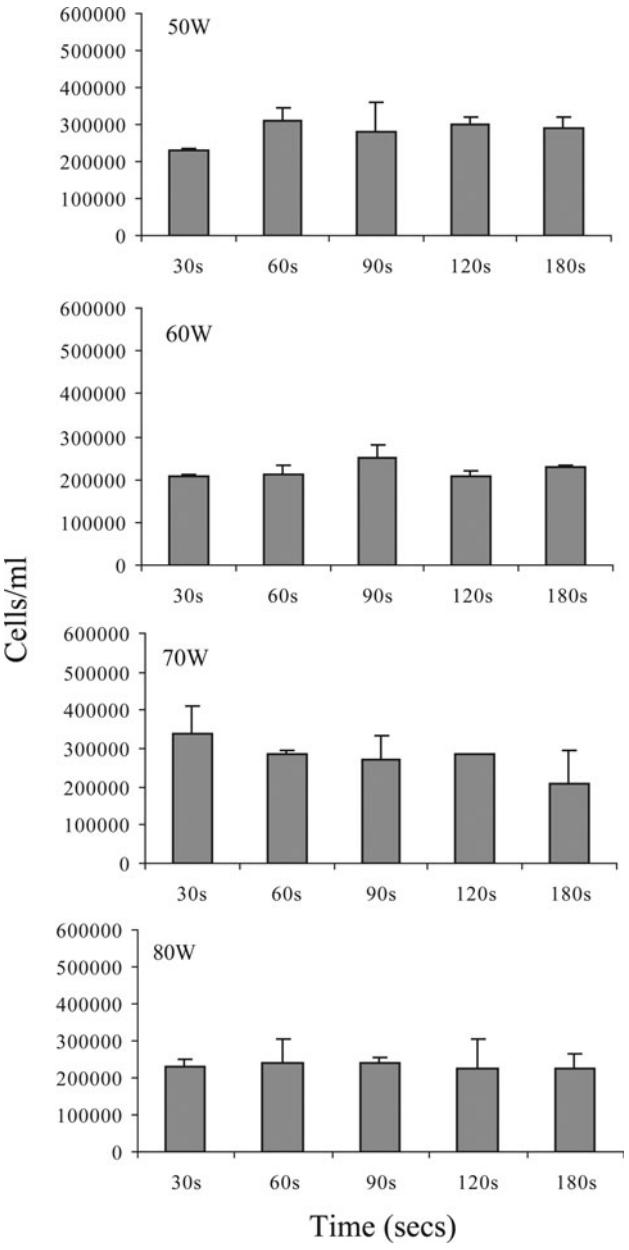

Fig. 6. Effect of four different acoustic power levels using different treatment times on the bacterial abundance.

DISCUSSION

Ultrasonic liquid treatment uses high frequency wave energy to cause vibrations in liquids resulting in a phenomenon called 'acoustic cavitation', which is the formation, growth, and implosive collapse of microscopic gas and/or vapour bubbles that are generated when ultrasonic waves are propagated through a liquid medium. Thus, acoustic cavitation results from the mechanical interaction between sound waves and bubbles in liquids (Price, 1992; Crum *et al.*, 1999; Mason, 1999; Young, 1999; Ashokkumar *et al.*, 2007). When these cavitation bubbles collapse, a series of mechanical and physical effects such as shear forces and shock waves are generated. The collapse of cavitation bubbles also results in the generation of heat within the bubbles for which these bubbles are also referred to as micro-reactors or hot spots (Suslick *et al.*, 1986; Ashokkumar *et al.*, 2007). The chemical changes that take place due to the cavitation induced by the passage of sound waves are commonly known as sonochemistry (Gogate, 2002). The present study revealed that when the

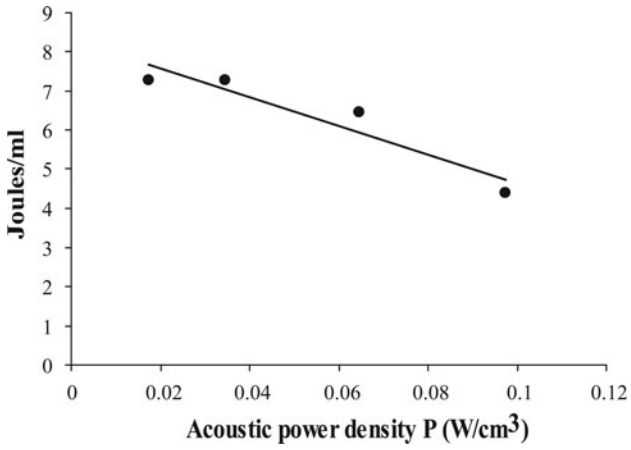

Fig. 5. Pulverization energy versus Acoustic power density.

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larvae were sonicated using ultrasound energy, they were caught in violent agitations that forced them into moving along random, looped pathways. This observation agrees with the mechanism proposed earlier (Doulah, 1977), that an ultrasound results in the formation of eddies (acoustic streaming). Also, it was observed that all the ultrasonic energy is concentrated in the volume of water adjacent to, and/or below the probe tip referred to as 'processed volume'. Given that the larva is within this processed volume just before the treatment, it is necessarily subjected to movement within it, assuming that the larva had equal probability of being at any position within the processed volume. But, the total number of cavitation events per unit time is independent of the volume of the suspension irradiated and is a function only of the power input to the transducer (Davies, 1959). A 1970s study on the treatment of shipboard waste-water demonstrated that effective cavitation could not be attained for the volumes of water being treated (NRC, 1996). Consequently, sufficient sterilization of the wastewater was not achieved. Traditional methods of ultrasonic treatments were reviewed in *Stemming the tide* (NRC, 1996), which states that effectiveness is a variable when treatment is applied to higher organisms such as zebra mussel veligers and fish. NRC concluded however, that such ultrasound systems could destroy fungi, yeasts, and pathogenic bacteria. The energy that exists within the cavity, at the time of collapse, causes both physical and chemical effects that are responsible for the rupture of organisms during cavitation. The conditions immediately preceding collapse of a cavitation bubble are similar in magnitude to ultra-high energy combustion conditions. Within the cavitation bubble and the immediate surround area, temperatures range from 2000 to 5000°C, and pressure reaches 1800 atmospheres (Buchholz *et al.*, 1998). Higher frequencies, warmer temperatures and lower concentrations of dissolved matter have been found to increase the effect of ultrasound pulses (Mesbahi, 2004). Evaluating the effect of ultrasound at a frequency similar to that employed in the present investigation, i.e. 20 kHz, it was thought that stand-alone ultrasonic treatment systems for ballast water may be effective for planktonic organisms >100 μm in size but smaller planktonic forms such as phytoplankton and bacteria will require an additional or alternative treatment system (Holm *et al.*, 2008). It was also observed that zooplankton tested all suffered 90% mortality after exposure times <10 seconds and at low energy densities <20 J/ml. In the present investigation 0.1 mJ/larva of pulverization energy at power density of 0.0975 W/cm^3 was efficient in pulverization of barnacle larva with slow rise in temperature. Thus, temperature can be considered to have little effect on the rate of killing in such organisms and therefore, it can be concluded that mechanical disruption was the main mechanism of pulverization. Previous reports also suggest that physical processes could be more important at the frequencies employed in the present investigation (Tiehm, 2001). Thus, under such conditions, the primary means for biological eradication are the mechanical effects rather than chemical reactions that result from cavitation. Cell membranes and organisms are literally ruptured or blown apart from the intense energy delivered in the form of shock waves. These (mechanical) effects include: (1) complete destruction of larger biota; and (2) disturbance or rupture of biological cell membranes, leading to subsequent death of an organism.

The cell breakage by ultrasound is a single hit type of phenomenon, such as the occurrence of a cavitation (implosion) in close proximity to a cell (Anand *et al.*, 2007). The shock waves generated by transient cavitation are the main physical force responsible for mechanical disruption of the microbe. The strength of the exoskeleton of the organisms subjected to cavitation also plays a significant role in its effective disruption. It has been shown earlier that microstreaming resulting from stable cavitation produces stresses that are sufficient to disrupt cell membranes (Scherba *et al.*, 1991). The damage caused by fluid shear stress is thought to depend on the erosion of the outer cell wall polymers, particularly at weakened places such as division or budding scars (Anand *et al.*, 2007). Most of the zooplankton develops either external or internal skeletons (Hardy, 1956; Pennington & Hadfield, 1989) for support or protection from predators in the aquatic environments. The exoskeletons are either chitinous as in the crustacea or calcareous as in the larval molluscs or brachiopods (Hardy, 1956). The major component of the cuticles and exoskeletons of worms, molluscs and arthropods consists of chitin, which is one of the most abundant polysaccharides in nature (Jeuniaux, 1982). Crustaceans and millipedes have calcium as a major component of cuticle and play a significant role in cuticle hardness (Willis, 1999). The gradient in the stiffness and hardness through the cuticle thickness is related to a honeycomb mechanism of the twisted plywood structure which is formed by the helicoidal stacking sequence of the fibrous chitin–protein layers (Raabe *et al.*, 2005). Once the cell membrane is sheared (a physical consequence of cavitation), chemical oxidants can then enter the cell attacking internal structures (Anand *et al.*, 2007). Chemical oxidants produced as a result of ultrasonic irradiation include free radicals like the hydroxyl radicals. One of the recent studies, by Gavand *et al.* (2007) reported that a combination of sonication and advanced chemical oxidants could be a more promising method to eradicate aquatic algae and macroinvertebrates in ballast water. The biochemical composition of the exoskeleton and its strength differs for different invertebrate larvae, thus the energy required to pulverize or sonicate different sizes of larval forms would be different (Holm *et al.*, 2008). Thus the present study aimed at elucidating the energy required for the pulverization of barnacle larva, as well as, the heat produced in the process using four different acoustic power densities. The dimension of the larvae subjected to pulverization and the level of disruption as quantified by the size of pulverized fragments were kept constant. Some preliminary experiments carried out on different larval stages of barnacles and copepods of different sizes indicated variations in the energies required for disruption, copepods requiring higher energies than barnacle larvae (personal observation). In real-life there will be a gamut of organisms belonging to different taxa and thus energy required to treat/disrupt these organisms would be different along with the shadow effect (shock wave impact attenuated by physical obstruction) and this needs further investigation.

For the range of acoustic power densities used, the following conclusions can be drawn about the requirements of time and energy, trends shown by the heat produced and the extent of pulverization. From Figure 4 it is evident that pulverization time showed an exponential decrease with an increase in the acoustic power density, such that, at very low power densities, the time required tends toward infinity. The temperature rise

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during a pulverization treatment is seen to marginally decrease, approximately linearly, with increasing acoustic power.

Treatment time is of critical importance, especially, in high flow systems that are used in large-scale treatments. So, higher acoustic power densities can be used to achieve exponentially lower treatment times, so as to make ultrasonic treatment feasible for use in such systems.

Pulverization energy is the energy that must be supplied to the larva to pulverize it, and not the total input energy by the transducer. From Figure 5 it is observed that the exponential decrease in treatment time has resulted in an almost linear decrease in required pulverization energy with increasing acoustic power densities. The four points in the graph in Figure 5, representing combinations of energy and acoustic power densities, can be utilized to design disinfection systems for pulverizing larvae of *Balanus amphitrite* or similar barnacle species. However, the data provided have limited application in that they cover only a narrow range of acoustic power densities, viz. '0.0173' to '0.0975' W/cm^3.

It was observed that when the organism is pulverized, various microbes embedded in its exoskeleton, as well as, those within its gut, are dislodged and thus, become vulnerable to subsequent treatment. Most disinfection treatments use a combination of treatment technologies in stages. Experimental results have also shown that hydrodynamic cavitation and or turbulent shear dominantly originating from cavitation are effective tools and could kill 80% zooplankton present in seawater (Sawant *et al.*, 2008). Jyoti & Pandit (2004) reported that a hybrid technique which combines hydrodynamic cavitation, acoustic cavitation and hydrogen peroxide appears to be an attractive alternative to any one technique on its own for the reduction in the heterotrophic plate count bacteria as well as indicator microorganisms such as the total coliforms, faecal coliforms and faecal streptococci. Thus, while treating macro-organisms, it becomes important, not only to destroy them, but also kill or at least, make vulnerable, the microbes harboured within them.

Our observations indicated that larvae were split into pieces measuring $\leq$30 μm. The surfaces of the naupliar debris showed numerous perforations and indentations (0.2 to 3 μm), however there was no peculiar pattern in the way they were destroyed. When low power levels were used, the numbers of bacteria released in the water column following pulverization increased and was evident up to 90 seconds followed by a slow decline, whereas at higher power density (0.0975 W/cm^3) this could be achieved in 45 seconds of exposure time and pulverization energy of 0.1 mJ/larva. The destruction of bacteria (size approximately 5 μm) is brought about only after they are released following the disruption of the bacteria bearing organism. Earlier work (Mahulkar *et al.*, 2009), clearly shows that continued irradiation with ultrasound is capable of destroying the bacteria. The bacterial population plotted versus time goes through maxima, which more or less coincides with the complete disruption of the main (target), bacteria bearing larva. The ultrasonic irradiation, in the initial stages disrupts the larva and also the bacteria, but the rate of release of the bacteria in the initial stages is significantly higher than its destruction and hence it goes through a maxima. After this, the bacterial population shows a steady decrease with continued irradiation using ultrasound. The effects related to the microbial/larval concentration do show an optimum and it has been observed to be in the range of 1% wt/volume (Anand *et al.*, 2007). The fragmentation size analysis is necessary as it will be useful for subsequent solid–liquid separation requirements. Once, the time of exposure versus fragmentation rate is known, a flow through system can be designed. The following two possible strategies are suggested: (a) multiple circulation of the suspension through the cavitating zone, where the cumulative exposure time matches the ultrasound exposure time obtained from the batch studies; or (b) continuous flow through system, having a circulation rate, much higher than the addition and withdrawal rate, again matching the required exposure time.

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