# USES OF ULTRASOUND IN THE BIOLOGICAL DECONTAMINATION OF WATER

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**Abstract**
The aim of these studies was to investigate the effect of ultrasound at different frequencies, in the presence and absence of chlorine as a biocide, on bacterial suspensions. An increase in percent kill for *Bacillus subtilis* occurred with increasing duration of exposure and intensity of ultrasound in the low-kilohertz range (20kHz & 38kHz) but at higher frequencies (512kHz & 850kHz) significant bacterial declumping occurred. Using sodium hypochlorite as a bactericide for *Escherchia coli* suspensions the timing of the ultrasonic treatment proved important. At 20kHz the improvement in biocidal activity was greatest when the ultrasound was applied at the same time as the hypochlorite whereas at 850kHz the improvement was best when ultrasound was used as a pre-treatment immediately followed by hypochlorite addition under silent conditions.

Scale-up has been achieved in the commercial exploitation of ultrasonic disinfection using equipment operating at both low and high frequencies.

## Introduction

The destruction of microorganisms by power ultrasound has been of considerable interest in recent years. Early research in the field can be traced back to the work of Harvey and Loomis in 1929 which examined the reduction in light emission (related to bacterial kill) from a seawater suspension of rod shaped *Bacillus Fisheri* caused by sonication at 375kHz at 19 $^{o}$C [1]. The final sentence of the paper predicted a poor future for the commercial exploitation of sonication (which they referred to as "raying") and this read:

*"In conclusion we can state that, under proper conditions of raying, luminous bacteria can be broken up and killed by sound waves of approximately 400,000 frequency and the solutions sterilized, but that the method is not one of any practical or commercial importance because of the expense of the process."*

Today that situation has changed, ultrasonic technology is more commonplace, costs have been reduced and applications are more economic. Power ultrasound can now be considered to be a viable alternative to conventional bactericidal techniques [2] .

Ultrasound is able to inactivate bacteria and deagglomerate bacterial clusters or flocs through a number of physical, mechanical and chemical effects arising from acoustic cavitation. On collapse, cavitation bubbles produce enough energy to mechanically weaken or disrupt bacteria or biological cells via a number of processes.

- Forces due to surface resonance of the bacterial cell are induced by cavitation. Pressures and pressure gradients resulting from the collapse of gas bubbles which enter the bacterial solution on or near the bacterial cell wall. Bacterial cell damage results from mechanical fatigue, over a period of time, which depends on frequency.
- Shear forces induced by microstreaming occurs within bacterial cells.
- Chemical attack due to the formation of radicals (H• and OH•) during cavitation in the aqueous medium. These radicals attack the chemical structure of the bacterial cell wall and weaken the cell wall to the point of disintegration.
- Amongst the final products of this sonochemical degradation of water is hydrogen peroxide ($H_2O_2$), which is a strong bactericide.

Sonication alone can provide powerful disinfection. However, to achieve 100% kill rates using only ultrasound it is necessary to use high ultrasonic intensities. This makes the technique expensive to use for general large-scale decontamination but nevertheless there is a drive towards the use of ultrasound in decontamination as an adjunct to a bactericide and in conjunction with other techniques [3].

In this paper we will explore the effect of different ultrasonic frequencies on bacteria kill both alone and with a bactericide and examine recent attempts at the scale up of ultrasonic dsinfection at both low and high frequencies.

## Experimental Notes

In order to obtain comparable results the initial concentrations of bacteria suspensions used in these experiments were made up to the same initial Optical Density. The effects of ultrasound on cell destruction and inactivation were monitored using standard plating out techniques. The plate counts revealed the viable Colony Forming Units (CFU's) in the sample however the CFU can be a single cell or a group of cells.

In the absence of bactericide a low kill rate was obtained and these results are quoted as % remaining CFU. In the presence of a bactericide substantially higher kill rates result and in these cases the results are quoted as log[CFU/ml]. A reduction of viable cells from 100% to 10% is equivalent to a single log reduction and industry normally considers that a five log reduction is an acceptable level of bacterial kill.

Throughout this paper the terms "low" and "high" frequencies when applied to laboratory equipment refer to ultrasound at 20-40 kHz and 0.5-0.85 MHz respectively.

## Results and Discussion

*The effect of ultrasound alone on suspensions of Bacillus subtilis [4].*

The results obtained (Figure 1) indicate that at both 20 and 38kHz there appears to be no dramatic effect on the viability of the bacteria except that there is a small but detectable drop after 15 minutes sonication. In contrast the higher frequencies produce an immediate rise in the CFU over the first five minutes followed by a steady fall, but the level remains above the original concentration even after 15 minutes sonication. These results suggest that the major effect of high frequency ultrasound is the declumping of bacterial agglomerates with little deactivation. This might also indicate that the declumping effect at the lower frequencies (to produce a greater number of CFU's) masks the actual deactivation.

When the same source of 20 kHz ultrasound is used to sonicate a smaller volume of bacterial suspension there is a resultant increase in the intensity of ultrasound entering the system (Figure 1). This is to be expected in that the acoustic power entering the system is increased with a consequent increase in cavitation.

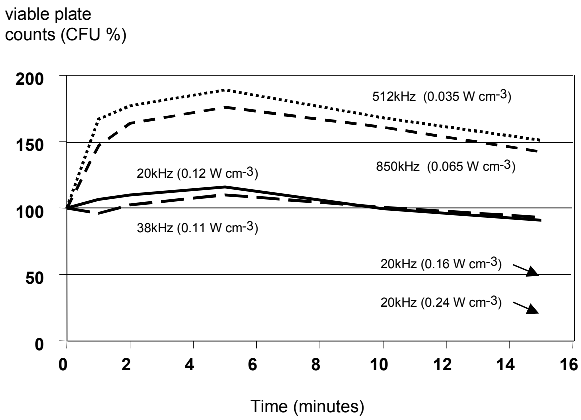


Figure 1: The effects of sonication of a suspension of *Bacillus subtilis* at different ultrasonic frequencies.

Sonication has two effects on *Bacillus subtilis* suspensions. The first is bacterial declumping which breaks up bacterial clumps into a greater number of individual bacteria in a suspension, and the second bacterial killing which results in less individual viable bacteria present in a suspension. The overall effect of applying ultrasound is thus the result of a competition between killing and declumping bacteria in solution. The net effects of such a competition can be classified into three apparently different shapes for curves representing bacterial survival against time (Figure 2).

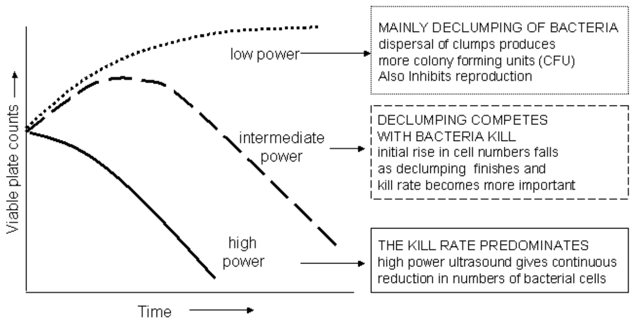


Figure 2: The effects of sonication alone on the survival of bacteria in water.

*Effect of ultrasound with a bactericide on suspensions of Escherchia coli [5]*

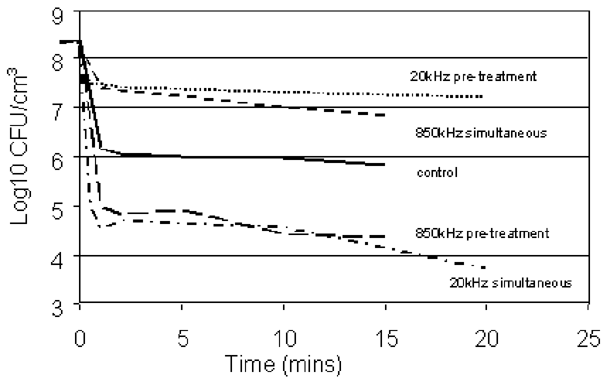


Figure 3: Comparison of 1 minute sonication with chlorination *Escherchia coli* at different frequencies.

Pre-treatment using 850kHz proved to be very effective at 1minute exposure with an increase of kill by about 2-log reduction in comparison with the control experiment (Figure 3). In contrast to this pre-treatment for 1 minute at 20kHz had a small adverse effect on the rate of kill. A possible explanation for this is that sonication at the higher frequency results in the deagglomeration of cells making them more susceptible to the biocide but that this process does not predominate at 20 kHz.

Simultaneous chlorination and sonication at 20 kHz for 1 minute resulted in almost a 2-log reduction in kill but an adverse effect was observed using 850 kHz. Here the positive effect can be ascribed to cavitation that will affect the uptake of biocide into the cell. It is possible that at 850kHz the dead cells present in the suspension (produced via normal biocidal kill) may be agglomerated into protective clumps at high frequency.

To improve the biocidal effects of chlorination on suspensions of *Escherchia coli* pre-treatment with ultrasound is better using a short period of sonication at 850kHz whereas sonication simultaneously applied with chlorination is better using a short period at 20kHz. Either option gives a similar improvement in kill but the pre-treatment is the more effective in terms of acoustic energy input which may make it more appealing for industrial exploitation.

*The scale-up of ultrasonic disinfection at different frequencies.*

The experimental results above suggest that there are two different types of effect on suspensions of bacteria depending upon the frequency of ultrasound applied to the system. The question then becomes whether either or both types of effects can be harnessed for large-scale commercial exploitation. Some of the pros and cons of the scale-up of each type are listed in Table 1.

Table 1: Possible effects of ultrasonic frequency on scale-up

| **Low frequency range** (ca. 20 kHz) | **High frequency range** (ca. 1 MHz) |
|---|---|
| good penetration through clean and polluted water | poor penetration |
| High powers available from commercial equipment | generally only low powers available |
| High power input can give cell disruption and direct bacteria kill | normally deagglomeration of clumps of bacteria and deactivation possible |
| Large scale applications exist for general processing applications | large scale applications are not common |

*Bio-effects of sonication using a Push-Pull™ system [6].*

A useful method of introducing ultrasound into a medium flowing through a tube is via the coaxial insertion of a radially emitting bar into a pipe containing the flowing liquid. The vibrational energy is transferred from the longitudinal mode oscillations of a transducer at one end to vibrational motion directed perpendicular to the surface of the tube (radial). A number of systems are available for this purpose one of which is a solid titanium tube driven by a transducer at each end (Martin Walter Push-Pull system, 27kHz). The tube responds to the transducers by emitting radial ultrasound at half-wavelength distances along its length. This device was originally developed as an alternative to submersible assemblies for cleaning purposes.

A Push-Pull reactor in a glass vessel of volume 5 litres was used to treat 20 litres of *Bacillus subtilis* suspension (O.D. 0.3 @ 440nm) in a flow loop system at a flow rate of 10 $lmin^{-1}$. The effect of treatment was monitored for 60 minutes using viable counts [Figure 4]. When the initial viable count is normalised to 100% it can be seen that sonication produces an initial but brief increase in % Colony Forming Units (CFU), indicating bacterial declumping had occurred. The declumping effect only takes place over the first 2 minutes of sonication. After this period there is a steady decrease in % CFU. After 60-minute sonication approximately 73% of the viable bacteria are inactivated or destroyed.

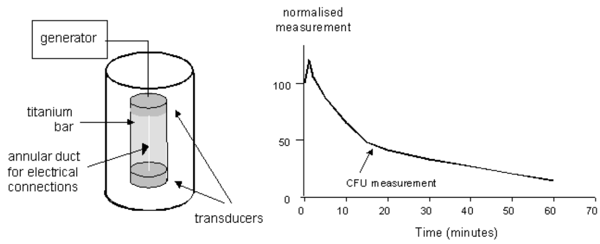


Figure 4: The bio-effects of 60 minute sonication of a suspension of Bacillus subtilis using a Push-Pull reactor (shown as a schematic left).

An example of the scale-up for sonication at low frequency using a radial emitting insert is the system developed by FFR Ultrasonics [7]. Using a series of sonicated disc inserts (3kW) within a pipe carrying the water to be treated a process rate of 33 $lsec^{-1}$ has been attained using ten discs in series give a 30kW system.

*Bio-effects of sonication using a Sonoxide™ system [6]*

A Sonoxide unit similar to the system used for cooling tower water treatment [8] but an earlier model was used to treat 20 litres *Bacillus subtilis* suspension (O.D. 0.3 @ 440nm). Sonication was carried out at a flow rate of 10 $lmin^{-1}$ in a flow loop system using a Sonoxide unit operating at 300W. Since this system operated at a much smaller power than the Push-Pull the monitoring time for the observation of the effects of treatment was extended to 120 hours [Figure 5]. The initial effects of sonication once again showed an initial rise in CFU over a 2-hour period followed by a steady decrease. After 5 days sonication around 85% of the viable bacteria were inactivated or destroyed.

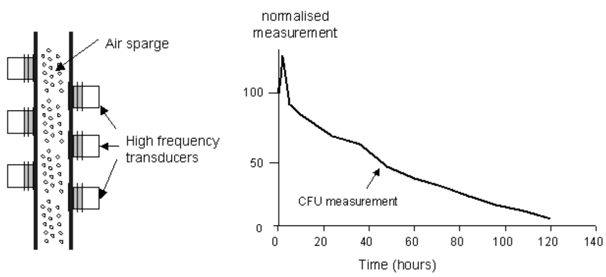


Figure 5: The bio-effects of 120 hours sonication of a suspension of Bacillus subtilis using a Sonoxide reactor (shown as a schematic left).

The advantage of this unit is that it has a low enough power rating for continuous use. In a closed loop it will only reduce bacterial contamination slowly but having done so it will then keep the contamination at a low level during subsequent continuous operation.

An example of a scale-up system for sonication at high frequency is the Sonoxide process for purifying cooling tower water. In a loop treatment system the process is active against general water contamination and against legionella and biofilm. The system also prevents scaling (no water softening agents are required). When installed in a badly contaminated system the process can take up to 1-2 weeks to get the contamination reduced to acceptable levels but after that the low levels are continuously maintained. This is because the process reduces reproduction rather than killing the biological material outright. The volumes that can be treated range from $8m^3h^{-1}$ to $56m^3h^{-1}$.

**Conclusions**

Sonication has two general effects on suspensions of bacteria. The first is bacterial declumping which breaks up bacterial agglommerates into a greater number of individual bacteria in a suspension, and the second is bacterial killing (or deactivation) which results in less individual bacteria capable of reproduction being present in a suspension. The overall effect of applying ultrasound is thus the result of a competition between declumping and deactivation of bacteria in solution. To improve the biocidal effects of sonication alone a biocide can be used. In the case of chlorination, the biocidal effect of high frequency (850kHz) ultrasound on suspensions of Escherchia coli is better when a short period of sonication is applied followed by normal biocidal treatment. On the other hand at lower frequencies (20kHz) better results are obtained using a short period of sonication at applied at the same time as chlorination. Either option gives a similar improvement in kill but the pre-treatment at low power and high frequency is the more effective in terms of acoustic energy input.

For effective decontamination using a single pass of the contaminated system through a sonicated processor, in the presence of a biocide, large energies at lower frequencies (20 to 40kHz) would be required. In the presence of a biocide high energies would also be required for large throughputs.

It should be noted however that in a closed loop system rapid kill is not so necessary and a slow rate of deactivation can bring down contamination to a low level over a period of time. This is acceptable since continued low-energy sonication will then maintain that level without the need for further additions of biocide.

**References**

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[7] Information supplied by FFR Ultrasonics, Queniborough, Leicestershire, UK.

[8] The Sonoxide system was developed by Undatim Ultrasonics in Belgium and is now owned by Ashland.