

Effectiveness of Ultrasound on the Destruction of *E. coli*

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Abstract: The aim of this study was to investigate the impact of sonication as a disinfection method for determining the effectiveness of ultrasound waves on the inactivation of *E. coli*. Ultrasound waves at a frequency of 42 kHz were used to expose aqueous suspension of *E. coli*. Ultrasound waves display a strong influence on the rate of *E. coli* disruption in water. Inactivation occurs most at the highest sonication time. This study show that sonication in 42 kHz is capable to some degree of inactivating *E. coli*.

Key words: *E. coli*, sonication, disinfection, ultrasound wave, frequency

INTRODUCTION

Escherichia coli or *E. coli* is a type of fecal coliform bacteria commonly found in the intestines of animals and humans. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage may contain many types of disease-causing organisms. Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7. *E. coli* is a normal inhabitant of the intestines of all animals, including humans. When aerobic culture methods are used, *E. coli* is the dominant species found in feces. Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. A minority of *E. coli* strains is capable of causing human illness by several different mechanisms. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins [verotoxin (VT), shiga-like toxin] are closely related or identical to the toxin produced by *Shigella dysenteriae*^[1,2].

The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage may contain many types of disease-causing organisms. Water can be contaminated in a variety of ways. Main sources of *E. coli* are municipal sewage discharges or runoff from failing septic systems, animal feed operations, farms and faeces deposited in woodlands from warm blooded animals. In urban areas, the *E. coli* from the excrement of warm blooded animals (such as pets in a park or on the street) may be washed into creeks, rivers, streams,

lakes, or groundwater during rainfalls or snow melts. The contamination in water is often highest immediately following a storm, because of the runoff. In addition, infected bathers can unknowingly contaminate water, or contamination can occur from boaters discharging wastes directly into the water. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E. coli* may end up in drinking water^[1,3].

The water can be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate *E. coli*. Systems using surface water sources are required to disinfect to ensure that all bacterial contamination is inactivated, such as *E. coli*. Systems using ground water sources are not required to disinfect, although many of them do^[4].

Several studies have shown that the efficiency of disinfection technique is dependant on the concentration of suspended solids. Because suspended solids can protect bacteria from being destroyed by disinfectants. For example, the efficiency of ultraviolet irradiation is affected by high concentrations of suspended matter.

Also, chlorine is traditionally used for disinfection. With the use of chlorine a possibility exists that by-products may form, which are potentially toxic and carcinogenic. Due to these problems alternative disinfection techniques are being evaluated and the benefits of the use of ultrasound in the water industry are now of considerable interest^[5,6].

The one way to inactivate *E. coli* is with ultrasound. When liquids are exposed to these vibrations, both physical and chemical changes occur as a result of a physical phenomenon, known as cavitation. Cavitation is the formation, expansion and implosion of microscopic gas bubbles in liquid as the molecules in the liquid absorb ultrasound energy. Compression and

rarefaction waves rapidly move through the liquid media. If the waves are sufficiently intense they will break the attractive forces in the existing molecules and create gas bubbles. As additional ultrasound energy enters the liquid, the gas bubbles grow until they reach a critical size. On reaching a critical size, the gas bubbles implode or collapse. The energy that exists within the cavity and in the immediate vicinity of the gas bubbles just before collapse causes both physical and chemical effects in the liquid. Physical effects result when cavitation is intense enough to rupture cell membranes, free particulates from solid surfaces and destroy particles and organisms through particulate collisions or by forcing them apart^[6-8].

In this study, the major objective was determining the effectiveness of ultrasound waves on the destruction of *E. coli* in water.

MATERIALS AND METHODS

Ultrasound Batch Reactor: Ultrasound was applied to samples using a Laboratory cleaning bath with the following characteristics:

Input: 220-230V 155W
Output: 70W 42 kHz

Experiments: Microbiological experiments involved sonicating of *E. coli* and observing the effects of ultrasound upon its growth. Before sonication, the concentration of *E. coli* in water was adjusted to as high as 1600 (MPN 100 mL⁻¹). This sample was added to the batch reactor in which sonication could be performed. For micro-organisms destruction investigation in ultrasound bath, small volumes (200, 400, 600 mL) of water have been used. All components in laboratory placed in an autoclave for disinfection before each test. The effect of sonicating different volumes of water was measured for the same time intervals. The samples were sonicated in periods of 1, 15, 30, 45, 60, 75 and 90 min. For each trial namely, each sample was exposed to all of the durations.

The standard test for *E. coli* carried out by the multiple – tube fermentation technique in research. In this test, results of the examination of replicate tube and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. The precision of each test depends on the number of tubes used. When drinking water is analyzed to determine, use the fermentation technique with 10 replicate tubes each containing 10 mL, 5 replicate tubes each containing 20 mL, or a single bottle containing a 100 mL sample portion. *E. coli* test (using EC medium) is applicable to investigations of drinking water, stream pollution, raw water sources, wastewater treatment systems and general water quality monitor. Submit all presumptive fermentation tubes showing any amount of gas, growth, or acidity within 48 h of incubation to the *E. coli* test.

Incubate inoculated EC broth tubes in a water bath a 44.5±0.2°C for 24±2h. Place all EC tubes in water bath within 30 min after inoculation. Maintain a sufficient water depth in water bath incubator to immerse tubes to upper level of the medium. Gas production with growth in an EC broth culture within 24 ± 2h or less is considered a positive *E. coli* reaction. Failure to produce gas constitutes a negative reaction. If multiple tubes are used, calculate MPN from the number of positive EC broth tubes as described in standard methods book^[9].

RESULTS AND DISCUSSION

The biocidal effects of ultrasound are showed in Table 1. As it is considered by 1, 15, 30, 45, 60, 75 and 90 min of sonication about 0.00, 78.30, 87.00, 98.50, 99.60, 99.70 and 99.80% of the *E. coli* present are destroyed respectively. Besides, the results show that increasing the sonication time has a significant effect on bacterial kill. The results in Table 1 indicate that considerable levels all in activation can be expected at higher periods. Also, analyses showing that there was no significant difference between mean of different volumes.

Table 1: The Effect of Ultrasound on Removal of *E. coli*

Ultrasound time (min)	Removal efficiency			Average (%)
	200 mL	400 mL	600 mL	
1	0.00	0.00	0.00	0.00
15	79.50	79.50	76.00	78.30
30	90.00	86.00	85.00	87.00
45	99.00	98.50	98.00	98.50
60	99.70	99.50	99.50	99.60
75	99.80	99.70	99.50	99.70
90	99.90	99.90	99.50	99.80

CONCLUSION

This study indicates that ultrasound waves for water disinfection are suitable. Because ultrasound reduces the amount of chlorine required for disinfection. Also, leads to greater efficiency in destruction of bacterial cells. Ultrasound leads to the formation of dead bacterial cells. These preliminary experiments indicate that ultrasonic in the low-kHz frequency range has some efficacy in inactivating some bacterial agents that may present in water. Also, experiments show that it is possible to decrease the number of *E. coli* present in the water and that the process depends on sonication time, frequency and intensity of the ultrasound waves.

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