Print ISSN 2094-1064
Online ISSN 2244-0437

Liceo Journal of Higher Education Research
doi: http://dx.doi.org/10.7828/ljher.v19i1.1623

Bacterial Fabricated Ultrasonic Sterilization of Culture Media

EMMALYN B. CUTAMORA
ORCID NO. 0000-0002-2799-0150
ecutamora@cebudoctorsuniversity.edu

Cebu Doctors' University
Cebu City, Philippines

ABSTRACT

The study aimed to determine the effect of the fabricated ultrasonic device in sterilizing culture media and inactivating *Escherichia coli*. The study was conducted using a randomized experimental design. Thioglycollate broth applied with sonic frequencies at 50,80, and 126 kHz for 15 minutes shows the variability of absorbance readings. The findings revealed that after 15 minutes of treatment, Thioglycolate broth exposed at 80 and 126 kHz is viable to be used as a culture medium to inoculate the organism for microbial analysis, as evidenced by the comparable results of the autoclaved sample. The medium was treated at 50 kHz, and those untreated samples had higher absorbance readings than the autoclave. Sonolysis at 15 minutes of exposure impacts the inactivation of *Escherichia coli* at 50 kHz, 80, and 126 kHz, as seen in the decrease of the colony count at increasing sonic frequencies. The results revealed a higher mean on unsterilized plates with 126 kHz treatment, with a lesser colony count. Colony count reductions of 6%, 14%, and 29% were obtained at ultrasonic frequencies of 50 kHz, 80 kHz, and 126 kHz, respectively. The majority of the inactivation occurred at 126 kHz exposure.

Keywords: Sonolysis, Thioglycolate, turbidity, kHZ, absorbance, CFU/ml

INTRODUCTION

Bacteria have to be cultured in the laboratory on artificial media, and culture media are typically sterilized to destroy the viability of the indigenous microbial population before inoculation with the desired organisms (Henry, 2001). The sterilization method most employed is heating with pressurized steam or autoclave,

which is necessary for the destruction or complete removal of all microorganisms, including spore-forming and non-spore-forming bacteria, viruses, fungi, and protozoans. With this, ultrasonic devices are used for microbial control and treatment in aquaculture facilities, ornamental water bodies, recreational waters, and drinking water reservoirs. However, scientific literature has limited data on microbial inactivation on culture media treated with ultrasound.

Ultrasonic processing is still in its infancy and requires much future research to develop the technology on an industrial scale. According to Piyasena et al. (2003), ultrasound processing or sonication is one of the alternative technologies that has shown promise in the food industry. Sonication alone is ineffective in killing bacteria in food; however, using ultrasound coupled with pressure and heat is promising. Thermosonic (heat plus sonication), manosonic (pressure plus sonication), and manothermosonic (heat and pressure plus sonication) treatments are likely the best methods to inactivate microbes, as they are more energy-efficient and effective in killing microorganisms.

The ultrasound is generated from mechanical or electrical energy via an ultrasonic transducer (Zheng et al., 2017). Frequencies above 18 KHz are usually considered ultrasonic. The frequencies used for ultrasonic cleaning range from 20 kHz to over 100 kHz. The most commonly used frequencies for industrial cleaning are those between 20 and 50 kHz (Suslick and Price, 1999, as cited by Mahvi, 2009).

According to some studies, ultrasound-induced cavitation is a handy and versatile tool to carry out chemical reactions. Leong et al. (2011) described power ultrasound as the section of the sound spectrum from 20 kHz up to around 1 MHz.

The basis of many ultrasound applications in this frequency range is acoustic cavitation, which is the formation, growth, and collapse of microbubbles within an aqueous solution resulting from pressure fluctuations in the applied sound field. The event of a collapsing bubble is a microscopic implosion that generates high local turbulence and the release of heat energy.

Leong et al. (2011) referenced Matula (1999) and suggested that the illumination during acoustic cavitation is attributed to gas bubbles. Inertial cavitation leads to elevated temperatures and pressures, initiating chemical reactions within and around the bubbles. Consequently, the use of power ultrasound for microbial destruction has gained significant attention as an alternative to traditional bactericidal methods. While heating damages bacteria, ultrasonic treatment has a more pronounced impact, as Harvey & Loomis (1928) and Mahvi (2009) noted.

Suslick and Price (1999) emphasized the role of cavitation, shear disruption, localized heating, and free radical formation in causing bacterial cell damage, with mechanical fatigue occurring over a frequency-dependent duration (Mahvi, 2009). According to Mason & Joyce (2008), as cited by Mahvi et al. (2005), microstreaming-induced shear forces within bacterial cells result in the formation of radicals, attacking the chemical structure of the cell wall and eventually leading to its disintegration.

This is why brief exposure to ultrasound reduces cell wall thickness, attributed to the release of the cytoplasm membrane. The deactivation of fecal coliforms is likely due to a combination of physical and chemical processes occurring during acoustic cavitation. Sound wave-induced vibrations cause gas vesicles within cells to resonate, leading to bubbles' formation, expansion, and contraction in a phenomenon known as cavitation. Ultimately, the ultrasound disrupts these bubbles, causing damage to the cells. The extent of cavitation and its impact on the cells is regulated by the sound waves' frequency, intensity, and duration, as noted by Rajasekhar et al. (2012), as cited by Laliberte & Haber (2014).

Sterilizing culture media is a growing concern in hospitals, especially in the Microbiology laboratory. Autoclave treatment is widely used for this purpose. However, the rising daily volume of media to be sterilized and the limited availability of autoclave machines in some hospitals have increased the importance of ultrasonic devices. This shift is driven by the growing demand for alternative methods in culture media processing.

FRAMEWORK

The study utilized the fabricated ultrasonic device and evaluated the effect of sonication in sterilizing Thioglycollate broth before and after the inoculation of the organism. Thioglycollate broth was sterilized at 50, 80, and 126 kHz, and the absorbance readings were determined after 15 minutes of sonication and 24 to 48 hours of incubation. The growth in the Nutrient agar was also evaluated. Thioglycollate broth with *Escherichia coli* was subjected to autoclave and ultrasonic treatment at 50,80, and 126 kHz for 15 minutes. The colony count was determined using the pour plate method. Another set of Thioglycollate medium inoculated with the bacterial suspension was treated at 126 kHz at different time intervals, and the colony count was evaluated after 24 hours of incubation (Cao et al., 2018). The constructed ultrasonic wave emitter was first introduced in the study of Butao et al. (2013) and was proven effective as a non-chemical approach for water decontamination. The results revealed that

ultrasonic waves can deactivate microbes significantly in wastewater systems as a function of frequency and time.

The study uses microbiological culture media, a solid, liquid, or semi-solid composite designed to support the growth of microorganisms. It is represented as the commonly seen solid agar in a dish (the Petri dish) or liquid broth in a bottle (Sandle, 2023). The bacterium inoculated and used in the study is *Escherichia coli* because it can be grown and cultured efficiently and expensively. According to the World Health Organization (WHO) report (2018, February 17), most *Escherichia coli* strains are harmless, but some can cause severe food poisoning, a foodborne disease. This is transmitted to humans primarily through the consumption of contaminated foods.

The thermal effect of ultrasound has also been studied extensively (Suslick & Nyborg, 1990). The study found that this thermal effect is primarily due to cavitation, or the heat accumulation generated by high ultrasonic power and signal duty ratio (Shi & Wang, 2012). However, the thermal effect may also bring some security concerns. However, the thermal effect may also bring some security concerns.

As is commonly understood, culture media are typically sterilized to eliminate the existing microbial population before introducing the desired culture (Boeck et al., 1989). The prevalent method for sterilization involves heating with pressurized steam or using an autoclave. There is a smooth transition in elastic mediums like air and most solids as sound waves propagate (Chen, et al., 2017). The transition remains continuous in non-elastic mediums such as water and most liquids, provided the sound's amplitude or loudness is relatively low. However, as the amplitude increases, the negative pressure in rarefaction areas eventually reaches a point where it causes the liquid to fracture due to this negative pressure, resulting in a phenomenon known as cavitation.

OBJECTIVES OF THE STUDY

The study aimed to determine the effect of the fabricated ultrasonic device in sterilizing the culture media and inactivating *Escherichia coli*. Specifically, the study attempted to address the following inquiries to determine: (1) the shelf life of the Thioglycollate broth after the 15-minute application of sterilization and after the 24 to 48 hours incubation of the following treatments: a) ultrasound at 50, 80, and 126 kHz for 15 minutes, b) unsterilized and c) autoclaving; (2) the mean colony count of the nutrient agar inoculated with bacterial suspension of *Escherichia coli* of the following treatments: a) ultrasound at 50, 80 and 126 kHz,

and b) unsterilized and c) autoclaving; (3) the mean colony count of the Nutrient agar after ultrasonic treatment (4) the percentage decreased of colony count on Nutrient agar treated with ultrasonic exposures at 126kHz and different time intervals, and (5) Is there a significant difference in the colony count of *Escherichia coli* on Nutrient agar between untreated and the ultrasonically treated groups.

Hypothesis

H_O: There is no significant difference in the colony count of *Escherichia coli* on the nutrient agar between untreated and the ultrasonically treated groups.

RESULTS AND DISCUSSION

This portion presents and analyzes the data gathered throughout the entire course of the experiment. The findings of the study were presented, analyzed, and interpreted in five sections, namely, the Shelf life of the Thioglycollate broth as reflected by its optical density, Growth on the Nutrient Agar after 24 and 48 hours of incubation, mean colony count of the Nutrient agar after ultrasonic treatment, Significant difference of the colony count at different treatment conditions. The percentage of the decrease in colony count treated at 126 kHz at different time intervals of ultrasonic exposures.

The Shelf Life of the Sterilized Thioglycollate Culture Medium

Ultrasound at 50, 80, and 126 kHz for 15 minutes. Table 1 illustrates the shelf life of the sterilized thioglycollate culture medium treated with sonication at 50, 80, and 126 kHz for 15 minutes with the untreated and autoclaved samples. After 15 minutes and 24 to 48 hours of incubation, the optical density was determined using the spectrophotometer read at 610 nm.

Table 1

Absorbance Reading at Different Treatment Conditions on the Shelf Life with Ultrasound at 50, 80, and 126 kHz for 15 minutes

Sonication Frequencies (kHz)	Absorbance Reading		
	after 15 minutes	after 24 hours of incubation	after 48 hours of incubation
50	0.083	0.672	0.974
80	0.051	0.356	0.655
126	0.049	0.070	0.169
Control (unsterilized)	0.091	0.701	0.998
Control (Autoclaving)	0.048	0.059	0.089

Results in Table 1 reveal an absorbance of 0.083 at 50 kHz, 0.051 at 80 kHz, 0.049 at 126 kHz, 0.048 when autoclave, and 0.091 for untreated samples. After 24 hours of incubation, absorbance readings at 50 kHz increased to 0.672, 0.356 at 80 kHz, 0.070 at 126 kHz, 0.059 for autoclave, and 0.701 for untreated samples. Absorbance reading further increased after 48 hours of incubation. At 50 kHz, the absorbance reading was 0.974, 0.655 at 80 kHz, and 0.169 at 126 KHz. The untreated sample has a 0.998 absorbance reading, while 0.089 for the autoclave.

The findings reveal that after 15 minutes of treatment, Thioglycollate broth exposed at 80 and 126 kHz (a difference of 0.001 to 0.003) had a comparable result with the autoclaved sample. Organisms can be inoculated for growth because sterility is ensured. The medium treated at 50 kHz and those untreated samples have higher absorbance readings with a difference of 0.035 and 0.043, respectively, compared to the autoclave. Shareef, Hamasaeed, & Ismaeil (2019) upheld that a few minutes of heating by microwave oven irradiation is a practical, accessible, rapid, and economical (energy-saving) way to sterilize different sizes of different types of culture media with no effects on the quality of culture media and microbial growth after incubation.

After 24 hours of incubation, there was evidence of turbidity on solutions treated with 50 kHz sonication and the unsterilized medium with a cloudy appearance at 80 kHz. There is a more significant increase in the absorbance reading for solutions treated at 50 kHz and those untreated. At 80 kHz and

126 kHz, there was a 0.297 and 0.011 difference from the autoclave sample. Thioglycollate broth remains clear at 126 kHz, comparable with the autoclave treatment. When culture specimens are taken, swabs should be moistened (with saline solution), and the sample should be plated onto blood agar or into nutrient and Thioglycollate broth as soon as possible (Maggs et al., 2008).

Incubating the medium for 48 hours increased the absorbance reading of those treated at 50 and 80 kHz, as reflected by its turbid and cloudy appearance. There is an increase of absorbance on samples treated at 126 kHz and autoclave with a difference of 0.08. At 48 hours of incubation, Thioglycollate medium showed a slightly cloudy appearance.

The absorbance reading or optical density indirectly reflects the number of bacteria in a solution. This determines the number of bacteria growing in a culture at certain times based on the absorbance of the suspension. Using a spectrophotometer increases the amount of absorbed light as the cell population increases. Optical density (OD) as an indication of biomass density is readily applicable to microalgae cultivation and has been used on a laboratory scale to significant levels of success. Biomass can be measured online using OD measurements at various wavelengths calibrated by offline dry weight and cell counts (Chuka-Ogwude et al., 2020; Havlik, 2013). OD measurements can be based on absorption, transmission, reflection, and scattering properties of the culture broth, and several inline optic fiber probes have been designed based on these and are commercially available.

In the laboratory, it is essential to sterilize culture media before inoculating bacteria and incubating at 37 degrees Celsius. Research indicates that exposing Thioglycolate medium to 126 kHz for 15 minutes results in a clear solution comparable to autoclaving, with viability lasting up to 24 hours. The solution remains clear, showing a slight absorbance difference of 0.011. Mahvi (2009) notes that ultrasonic cleaning frequencies typically range from 20 to over 100 kHz.

In non-elastic media like water and most liquids, increasing the amplitude causes negative pressure in rarefaction areas, leading to liquid fracture—a phenomenon known as cavitation (Zupanc et al. (2019). According to Erriu et al. (2014) and Baker et al., (2001) that the effects of cavitation on bacteria can be divided into several stages: (a) Under the action of ultrasound, the micro-bubbles will periodically expand and contract and the nearby bacteria and bacterial film will also scale accordingly; (b) If the ultrasonic intensity is high enough, the generated pressure gradient will burst the bubbles instantaneously, and the resulting instantaneous shearing force may have a shearing effect on the

bacterial membrane, thereby destroying the original structure of the bacterial membrane (Guzman et al., 2003; Suslick & Nyborg, 1990); (c) Cavitation may cause the degradation of local water, thereby deriving the antibacterial ammonia peroxide (Fedorov et al., 2022). At present, the cavitation effect of ultrasound has been extensively studied. Regarding bacterial inhibition, newer studies have paid more attention to the effects generated after the bubble burst, such as microjets. Cavitation bubbles form at these sites, oscillating under positive pressure influence and growing to an unstable size. The violent collapse of these bubbles results in implosions, emitting shock waves from the collapse sites (Mahvi, 2009).

Bacterial Growth on the Nutrient Agar After Sonication and Autoclaving

Observation of Colonies in Incubated Plates after 24 to 48 hours. After the application of the different treatment conditions for 15 minutes, 1 ml. of Thioglycollate broth was poured into the empty Petri Dishes together with the melted Nutrient agar in replicates. Plates were incubated for 24 to 48 hours, and the growth of colonies were observed. Table 2 shows the growth of organisms after each treatment together with the unsterilized sample.

Table 2

Incubation Periods in 24 and 48 Hours on the Shelf-Life Bacterial Growth on the Nutrient Agar After Sonication and Autoclaving

SONICATION FREQUENCIES (kHz)	INCUBATION PERIODS (Hrs.)	
	24	48
50	3 plates with growth	3 plates with growth
80	2 plates with growth, 1 plate without growth	3 plates with growth
126	3 plates without growth	2 plates with growth, 1 plate without growth
Control (Unsterilized)	3 plates with growth	3 plates with growth
Control (Autoclave)	3 plates without growth	3 plates without growth

As seen in Table 2, colonial growth was observed at 50 and 80 kHz in the unsterilized samples after 24 and 48 hours of incubation. Considerable colonial growth was observed at 50 kHz, while a combination of small and medium-sized

colonies was observed at 80 kHz. Petri plates exposed to autoclave and 126 kHz treatment showed no growth in all the replicates after 24 hours of incubation. However, after 48 hours, two of the three plates showed growth for plates with 126 kHz sonication, while no growth was observed in the autoclaved plates. This indicates that the culture media was sterilized at 126 kHz and is viable until 24 hours of incubation. It is further observed that ultrasound treatment at 126 kHz is more effective than at 50 and 80 kHz. Stable and inertial cavitation thresholds for combinations are reported here for 120-kHz ultrasound. The mass loss was also measured for exposure to 120 kHz, 80% duty cycle ultrasound without any cavitation present, with stable cavitation alone, or with both stable and inertial cavitation (Datta et al., 2006).

It is often necessary to determine how many live bacteria are seen in a sample, especially when measuring growth rates or determining disinfectant effectiveness. This involves plating them on suitable growth media, particularly the Nutrient Agar. The plates are incubated until visible colonies are seen, usually 24 to 48 hours. The colonies growing on the plate are considered to have started from one viable bacterial unit. As in the case of this experiment, ultrasound creates large cavitation bubbles at 126 kHz, which collapse upon and initiate powerful jet streams exerting shear solid forces in the liquid medium. However, the formation of a colony observed after 48 hours of incubation may be prevented by increasing the time of sonication application.

The growth of colonies on the agar plates (Cooper et al., 1968) after 48 hours of incubation could be attributed to the spore-forming ability of the Gram-positive rod bacteria. Spores could be resistant to ultrasound waves at these frequencies. After the ultrasonic treatment, the non-spore-forming bacteria could have been killed, and what remains are those that were spore-forming rods, which were temporarily inactivated and started to germinate during the 48 hours of incubation.

The findings also reveal that saturated steam under pressure is the most practical and dependable agent for sterilization. The autoclave sterilizes anything that is not injured by steam and high temperature. This includes most solid and liquid media types, solutions, rubber tubing and stoppers, discarded cultures, and contaminated media before washing. This finding supports the works of Rutala & Weber (2015), who claimed that medical devices that have contact with sterile body tissues or fluids are considered critical items. When used, these items should be sterile because any microbial contamination could result in disease transmission. Such items include surgical instruments, biopsy forceps, and implanted medical devices.

Mean Colony Count of *Escherichia coli* on Nutrient Agar Treated at Various Ultrasonic Frequencies

Inoculation of *Escherichia coli* and Exposure to Different Ultrasonic Treatments for 15 minutes. *Escherichia coli* was inoculated on nutrient agar and exposed to ultrasonic treatment for 15 minutes to detect the bacterial content of the specimens adequately. Table 3 reveals the mean colony count of the culture medium at different treatment conditions.

Table 3

Mean Colony Count of the Nutrient Agar Inoculated with Escherichia coli at Different Treatment Conditions for 15 minutes

SONICATION FREQUENCIES (kHz)	MEAN COLONY COUNT (CFU/ml)
Unsterilized	360,000
50	338,750
80	312,500
126	255,833

Results reveal a mean of 360,000 CFU/ml on unsterilized plates. At 50 kHz, the mean colony count was 338,750, while 312,500 CFU/ml was at 80 kHz. Plates with 126 kHz treatment have a mean colony count of 255,833 CFU/ml. Comparing the results with the unsterilized, it is evident that colony count decreased when ultrasonic treatment was applied to the culture medium inoculated with E. coli. Colony count reductions of 6%, 14%, and 29% were obtained at ultrasonic frequencies of 50 kHz, 80 kHz, and 126 kHz, respectively. The majority of the inactivation occurred at 126 kHz exposure.

The findings show that ultrasonic treatment can reduce colony count when sonication is applied at different frequencies within 15 minutes of exposure. It indicates that as the sonic frequencies increased, the bacterial counts decreased, which is supported by the works of Dodge and Ludington (2023), who drew a boundary box of the area for which counting is to be done. Adjust the size and proportion until all the spots are within their sonication of cells.

Cavitation, induced by ultrasound at low frequencies, is an effective means for the disintegration of bacterial cells. According to Neis and Blume (2003), at low ultrasound doses bacteria, and flocs can be decamped by mechanical

shear stresses, and at increased doses ultrasound cavitation has an impact on the cell walls such that they are broken. These findings align with the study made by Dehgahani (2019) on the inactivation of Clostridium perfringes causing sonolysis as a result of acoustic cavitation on the bacterial cells. Accordingly, the cells are rendered inviable during ultrasound waves due to free-radical attacks, including hydroxyl radical attack and physical disruption of the cell membranes. The findings made by Allison et al (2007) show that cell viability on *Escherichia coli* decreased exponentially with time at different intensities of ultrasound due more to mechanical effects.

Colony Count at 126 kHz Ultrasonic Treatment at Different Time Exposures

Ultrasonic Treatment at Different Time Exposures. Table 4 presents the mean and the percentage decrease of colony count sonicated at 126 kHz exposed at different intervals of time exposure.

Table 4

Colony Count at 126 kHz at Different Time Intervals

Treatment Time Interval	Colony Count (CFU/ml)	Percentage Decreased Against Unsterilized Sample
Control (Unsterilized)	360,000	
15 minutes	255,833	28.94%
45 minutes	212,500	40.98%
60 minutes	160,416	55.44%
90 minutes	107,916	70.03%
105 minutes	4,200	99.61%
120 minutes	0	0%

The findings in Table 4 show the decreasing effect of ultrasonic treatment at 126 kHz on colony count. From a mean count of 360,000 CFU/ml for the unsterilized samples, there was evidence of a 28.94 % decrease at 15 minutes of exposure, 40.98 % at 45 minutes, 55.44% at 60 minutes, 70.03% decrease at 90 minutes and 99.61% at 105 minutes exposure. *Escherichia coli* was deactivated at 120 minutes of exposure, as evidenced by the 0% growth in the Nutrient Agar. The results affirmed the study of Meena, Byresh, Sunil, Rawson,

and Venkatachalapathy (2023) that treatment significantly enhanced microbial growth and reduced the fermentation time. Sonication increased the total phenol content, total flavonoid content, antioxidant activity, and reduced sugar content. The particle size was significantly reduced for sonicated samples, which could also be responsible for the enhancement of microbial growth.

Additionally, the results show that increasing sonication time significantly affects bacterial kill. It is anticipated that ultrasonic waves hit the microbial cell walls. Bacteria are susceptible to low-frequency and low-intensity ultrasound. Part of them are neutralized, which has been proved by a lower number of CFUs in sonication groups than in the control group (Dudek et al., 2020). The more giant bubbles upon implosion give a high mechanical effect, leading to microbial cell disintegration. The temperature rises in each sample with the increase in treatment time. At low treatment time, less ultrasonic energy is supplied to the culture medium, resulting in bacteria deagglomeration but without bacterial cells' disintegration. Thus, to damage the organism's higher ultrasound energy input, it is necessary.

Significant Difference in the Colony Count Inoculated with Escherichia coli.

Colony Count Inoculated with *Escherichia coli*. Table 5 shows the significant Difference test in the mean colony count between the unsterilized and the ultrasonic-treated groups.

Table 5

Test of Significance Using One-Way ANOVA

Variables	df	Computed ANOVA F-value	p-value	Analysis at 0.05	Decision
Colony Count Untreated 50 kHz 80 kHz 126 kHz	11	138.638	0.000	Significant	Reject H_0

Colony count significantly differs at a 0.05 significance level, with a p-value of 0.000 and an F value of 138.638. This proves that ultrasonic treatment on culture media can decrease colony-forming units per milliliter of the sample. Ultrasonic treatment increasing the magnitude of sonic frequency leads to an improvement

of microbial inactivation on culture media inoculated with *Escherichia coli*. (Nunes, da Silva, Bastos, & de Souza, 2022). Based on the results of Table 4, the null hypothesis is rejected. The improvement of mechanical properties for the ultrasonic melt-treated alloy is attributed to grain refinement and texture evolution. Choosing the appropriate input acoustic pressure ratio is essential to optimize the grain refinement of DUF melt treatment. Under the premise of relevant symmetrical distribution of the cavitation area, increasing the acoustic pressure input for 15 kHz ultrasonic is beneficial to improve the grain refinement efficiency. (Chen, Jia, Le, Ning, Yin, Hu, & Yu, 2021).

Significant Difference Using Multiple Comparisons

Multiple Comparisons of Three Treatment Groups. Table 6 presents the significant differences in the colony count among the three treatment groups at a 0.05 significance level.

Table 6

P-values Using Multiple Comparisons

Groups	p-values	Analysis at 0.05	Decision
A. Unsterilized against			
50 kHz	0.004	Significant	Reject H_0
80 kHz	0.000	Significant	Reject H_0
126 kHz	0.000	Significant	Reject H_0
B. 50 kHz against			
80 kHz	0.001	Significant	Reject H_0
C. 80 kHz against			
126 kHz	0.000	Significant	Reject H_0

Comparing the results with the unsterilized group, ultrasonic treatment at 50,80 and 126 kHz frequencies significantly reduced the colony count, having a p-value of 0.004 and 0.000, respectively. Ultrasonic treatment at 50 kHz against 80 kHz revealed a significant difference in the colony count with a p-value of 0.001. However, ultrasonic treatment at 80 kHz against 126 kHz depicted a significant difference in the colony count with a p-value of 0.000. As reflected in the results, the null hypothesis is rejected. The findings validate the study

of Butao et al. (2013) that ultrasonic application can have a viable effect on decreasing bacterial growth. In connection with microbial deactivation, the large, suspended matter hindered the direct contact of the sound waves with the target organisms. As matter may absorb, reflect, or scatter some of these waves and change their basic properties (such as wavelength, speed, and amplitude) before hitting the target, it may act as a shield against the biocide effects of the waves.

However, with increasing frequency and exposure time, more suspended solids will be deagglomerated to finer particles. Hence, microbes will be in direct contact with ultrasonic waves and are effectively deactivated. The high-intensity ultrasound is a versatile technology with different applications in food safety and product development. However, the effects of this technology are still varied and very dependent on the processing parameters, which affect the acoustic intensity or the total acoustic energy received by the food. Due to other factors, there is no ideal ultrasonic power or treatment time for each ultrasound application (Guimarães et al., 2021).

CONCLUSIONS

Based on the findings, the fabricated ultrasonic treatment can be used as a sterilization medium for culture media. Ultrasonic waves ranging from 50 to 126 kHz significantly decrease bacterial growth in the culture media. However, complete inactivation of *Escherichia coli* should be done at a sonic frequency of 126 kHz for 120 minutes of exposure. The results showed that the colony count decreased as exposure time was increased. Therefore, a recommendation was formulated for future studies to optimize the use of higher frequencies and add exposure time to attain maximum deactivation performance of the ultrasonic treatment, utilize other bacterial pathogens with clinical importance, and utilize other culture media to evaluate the ultrasonic effect and examine its viability.

LITERATURE CITED

Allison, V. J., Condron, L. M., Peltzer, D. A., Richardson, S. J., & Turner, B. L. (2007). Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. *Soil Biology & Biochemistry*, *39*, 1770-1781.

Baker, K., Robertson, V., & Duck, F.A. (2001). A review of therapeutic ultrasound: biophysical effects. *Physical Therapy, 81*, 1351-1358.

Boeck, L. D., Alford, J. S., Pieper, R. L., et al. (1989). Interaction of media components during bioreactor sterilization: definition and importance of R0. *Journal of Industrial Microbiology, 4*, 247–252. https://doi.org/10.1007/BF01574082

Butao, C., Baje, B., Judas, M., Ochavo, R., Maglasang, K., & Duhaylungsod, K. (2013). Microbial Inactivation of Wastewater via Ultrasonication. *Asia-Pacific Journal of Science, Mathematics and Engineering (APJSME), 1*(1), 15-17. ISSN: 2244-5471.

Cao, X., Zhang, M., Mujumdar, A. S., Zhong, Q., & Wang, Z. (2018). Effects of ultrasonic pretreatments on quality, energy consumption and sterilization of barley grass in freeze drying. *Ultrasonics Sonochemistry, 40*(Part A), 333-340. https://doi.org/10.1016/j.ultsonch.2017.06.014

Chen, F., Zhang, J., Liu, Q., Gu, H., & Yang, L. (2017). An efficient approach for the extraction of orientin and vitexin from Trollius chinensis flowers using ultrasonic circulating technique. *Ultrasonics Sonochemistry, 37*, 267-278.

Chen, X., Jia, Y., Le, Q., Ning, S., Yin, Z., Hu, C., & Yu, F. (2021). Understanding dual-frequency ultrasonic melt treatment on grain refinement and mechanical properties improvement of AZ80 magnesium alloy: Experiment and simulation. *Journal of Materials Research and Technology, 15*, 4758-4767.

Chuka-Ogwude, D., Ogbonna, J., & Moheimani, N. R. (2020). A review on microalgal culture to treat anaerobic digestate food waste effluent. *Algal Research, 47*, 101841. https://doi.org/10.1016/j.algal.2020.101841

Cooper, A. L., Dean, A. C. R., & Hinshelwood, C. N. (1968). Factors affecting the growth of bacterial colonies on agar plates. Proceedings of the Royal Society of London. *Series B. Biological Sciences, 171*(1023), 175-199.

Datta, S., Coussios, C. C., McAdory, L. E., Tan, J., Porter, T., De Courten-Myers, G., & Holland, C. K. (2006). Correlation of cavitation with ultrasound enhancement of thrombolysis. *Ultrasound in Medicine & Biology, 32*(8), 1257-1267.

Dehghani, M. H., Zarei, A., & Yousefi, M. (2019). Efficiency of ultrasound for degradation of an anionic surfactant from water: Surfactant determination using methylene blue active substances method. *MethodsX, 6*, 805-814. https://doi.org/10.1016/j.mex.2019.03.028

Dodge, R., & Ludington, W. B. (2023). Fast Colony Forming Unit Counting in 96-Well Plate Format Applied to the Drosophila Microbiome. *JoVE (Journal of Visualized Experiments)*, (191), e64298.

Dudek, P., Grajek, A., Kowalczewski, J., Madycki, G., & Marczak, D. (2020). Ultrasound frequency of sonication applied in microbiological diagnostics has a major impact on viability of bacteria causing PJI. *International Journal of Infectious Diseases, 100*, 158-163.

Erriu, M., Blus, C., Szmukler-Moncler, S., Buogo, S., Levi, R., Barbato, G., Ripa, D. M., Denotti, G., Piras, V., & Orrù, G. (2014). Microbial biofilm modulation by ultrasound: Current concepts and controversies. *Ultrasonics Sonochemistry, 21*, 15-22. https://doi.org/10.1016/j.ultsonch.2013.05.011

Fedorov, K., Cako, E., Dinesh, K., Cheshmeh Soltani, R. D., Wang, Z., Khan, J. A., & Boczkaj, G. (2022). *Cavitation-Based Processes for Water and Wastewater Treatment*. In M. Nasr & A. M. Negm (Eds.), Cost-efficient Wastewater Treatment Technologies (The Handbook of Environmental Chemistry, Vol. 118). Springer. https://doi.org/10.1007/698_2022_866

Guimarães, J. T., Scudino, H., Ramos, G. L., Oliveira, G. A., Margalho, L. P., Costa, L. E., & Cruz, A. G. (2021). Current applications of high-intensity ultrasound with microbial inactivation or stimulation purposes in dairy products. *Current Opinion in Food Science, 42*, 140-147.

Guzman, H. R., Nguyen, D. X., Khan, S., & Prausnitz, M. R. (2003). Ultrasound-mediated disruption of cell membranes. I. Quantification of molecular uptake and cell viability. *Journal of Acoustic Society of America, 113*(1), 643-651.

Harvey, E. N., & Loomis, L. (1928). High Frequency Sound Waves of Small Intensity and Their Biological Effects. *Nature, 121*, 622.

Havlik, I., Hlaváč, P., & Turonová, H. (2013). Optical density measurement as a tool for microalgae cultivation monitoring. *Agriculture (Poľnohospodárstvo), 59*(3), 120-130.

Henry, J. B. (2001). *Clinical Diagnosis and Management by Laboratory Methods* (20th ed.). Saunders.

LaLiberte, G., & Haber, E. (2014). *Literature Review of the Effects of Ultrasonic Waves on Cyanobacteria, Other Aquatic Organisms, and Water Quality*. Wisconsin Department of Natural Resources. Research Report.

Leong, T., Ashokkumar, M., & Kentish, S. (2011). The Fundamentals of Power Ultrasound - A Review. *Acoustics Australia, 39*, 2-5.

Maggs, D. J., Miller, P. E., & Ofri, R. (2008). *Slatter's Fundamentals of Veterinary Ophthalmology (Fourth Edition)*. W.B. Saunders. ISBN 9780721605616.

Mahvi, A. H. (2009). Application of ultrasonic technology for water and wastewater treatment. *Iranian Journal of Public Health, 38*(2), 1-17.

Mahvi, A. H., Dehghani, M. H., & Vaezi, F. (2005). Ultrasonic technology effectiveness in total coliforms disinfection of water. *Journal of Applied Sciences, 5*, 856-858.

Mason, T. J., & Joyce, E. (2008). Sonication used as a biocide. A review: Ultrasound a greener alternative to chemical biocides? *Chemistry Today, 26*(6). http://chemistry-today.teknoscienze.com/testata.asp?id_testata=154&folder=backissue

Meena, L., Buvaneswaran, M., Byresh, T. S., Sunil, C. K., Rawson, A., & Venkatachalapathy, N. (2023). Effect of ultrasound treatment on white finger millet-based probiotic beverage. *Measurement: Food, 10*, 100090.

Neis, U., & Blume, T. (2003). Ultrasonic disinfection of wastewater effluents for high-quality reuse. *Water Science and Technology: Water Supply, 3*(4), 261–267.

Nunes, B. V., da Silva, C. N., Bastos, S. C., & de Souza, V. R. (2022). Microbiological inactivation by ultrasound in liquid products. *Food and Bioprocess Technology, 15*(10), 2185-2209.

Piyasena, P., Mohareb, E., & McKellar, R. C. (2003). Inactivation of microbes using ultrasound: A review. *International Journal of Food Microbiology, 87*(3), 207-216. https://doi.org/10.1016/s0168-1605(03)00075-8

Rajasekhar, P., Fan, L., Nguyen, T., & Roddick, F. (2012b). A Review of the Use of Sonication to Control Cyanobacterial Blooms. *Water Research, 46*, 4319-4329. https://doi.org/10.1016/j.watres.2012.05.054

Rutala, W. A., & Weber, D. J. (2015). *Disinfection, sterilization, and control of hospital waste*. In J. E. Bennett, R. Dolin, & M. J. Blaser (Eds.), Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (pp. 3294–3309.e4). https://doi.org/10.1016/B978-1-4557-4801-3.00301-5

Sandle, T. (2023). Lifting the lid of the Petri dish: The science of microbiological culture media. *IST Journal,* 17-21.

Shareef, S. A., Hamasaeed, P. A., & Ismaeil, A. S. (2019). Sterilization of culture media for microorganisms using a microwave oven instead of autoclave. *Raf. J. Sci., 28*(1), 1-6.

Shi, S., & Wang, G. (2012). Numerical Calculation of Thermal Effect on Cavitation in Cryogenic Fluids. *Chinese Journal of Mechanical Engineering, 25*(6), 176. DOI: 10.3901/CJME.2012.06.1176.

Suslick, K. S., & Nyborg, W. L. (1990). Ultrasound: Its Chemical, Physical and Biological Effects. *Journal of the Acoustical Society of America, 87*(2), 919–920. https://doi.org/10.1121/1.398864

Suslick, K. S., & Price, G. J. (1999). Applications of Ultrasound to Materials Chemistry. *Annual Review of Materials Science, 29*(1), 295-326. https://doi.org/10.1146/annurev.matsci.29.1.295

World Health Organization (WHO). (2018, February 17). *E. coli*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/e-coli

Zheng, J., Su, C., Zhou, J., Xu, L., Qian, Y., & Chen, H. (2017). Effects and mechanisms of ultraviolet, chlorination, and ozone disinfection on antibiotic resistance genes in secondary effluents of municipal wastewater treatment plants. *Chemical Engineering Journal, 317*, 309-316. https://doi.org/10.1016/j.cej.2017.02.076

Zupanc, M., Pandur, Ž., Stepisnik Perdih, T., Stopar, D., Petkovšek, M., & Dular, M. (2019). Effects of cavitation on different microorganisms: The current understanding of the mechanisms taking place behind the phenomenon. A review and proposals for further research. *Ultrasonics Sonochemistry, 57*, 147-165. https://doi.org/10.1016/j.ultsonch.2019.05.009