JURNAL UPI

Indonesian Journal of Science & Technology

Journal homepage: http://ejournal.upi.edu/index.php/ijost/

IJoST Indonesian Journal of Science & Technology

Bacterial Cell Inactivation Using a Single-Frequency Batch-Type Ultrasound Device

Poetro Sambegoro[1†], Maya Fitriyanti[2†], Bentang Arief Budiman[1], Kamarisima[2], Sekar Wangi Arraudah Baliwangi[1], Calvin Alverian[1], Saeed Bagherzadeh[3], Ganesan Narsimhan[4], Pingkan Aditiawati[2], Ignatius Pulung Nurprasetio[1*]*

[1]Faculty of Mechanical and Aerospace Engineering, Institut Teknologi Bandung, Indonesia
[2]School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia
[3]School of Materials Engineering, Purdue University, Indiana, USA
[4]Department of Agricultural and Biological Engineering, Purdue University, Indiana, USA
Correspondence: E-mail: poetrols@itb.ac.id, ipn@ftmd.itb.ac.id

ABSTRACTS

Ultrasound technology employs cavitation to generate high-pressure soundwaves to disrupt bacterial cells. This study reveals the effectiveness of a single frequency ultrasound device for bacterial cell inactivation. A low-cost ultrasound device having a single frequency, i.e. 22 kHz for lab-scale application, was developed first, and the prototype was mechanically designed and analyzed using the finite-element method to assure the targeted natural frequency could be achieved. The prototype was then tested inactivating bacterial cells, Escherichia coli (E. coli) and Bacillus subtilis (B. subtilis), in a simple medium and a food system, and the results were then compared to a commercial system. A treatment time of up to 15 minutes was able to reduce E. coli and B. subtilis cells by 3.3 log and 2.8 log, respectively, and these results were similar to those of the commercial system. The effectiveness of bacterial cell inactivation using the developed single-frequency ultrasound device is then discussed. The findings are useful for designing low-cost ultrasound devices for application in the food industry.

ARTICLE INFO

Article History:
Received 28 Nov 2020
Revised 28 Des 2020
Accepted 13 Jan 2021
Available online 20 Jan 2021

Keyword:
Ultrasonication,
Cavitation,
Bubble,
Bacterial inactivation

1. INTRODUCTION

Ultrasound technology has been developed for various applications, among which are microbial cell inactivation and DNA extraction (Chemat *et al.,* 2011). Cavitation occurs when vapor bubbles form in a fluid due to a drop-in pressure below the saturated pressure (Triawan *et al.*, 2019). Using ultrasonic technology, this pressure drop is provided by a fluctuating transducer. After the bubbles are formed, they undergo an expansion and compression phases and collapse. The collapse of the cavitation bubbles can exert a high pressure due to the shock wave and microjet effects. The bursting cavitation bubbles have a highly destructive effect that can be used to damage bacterial cell walls. Water molecules in the vicinity of the bubbles also exert a shear stress effect and cause deformation of the cell membrane. At the same time, the local temperature also rises sharply. These complex phenomena contribute to the overall bacterial inactivation.

Several independent studies on bacterial inactivation using ultrasound have been performed. Ultrasonic waves generate the bubbles and drive them to explode, resulting in high pressure and temperature, and active species in some cases (Wu & Nyborg, 2008). Shear force due to an ultrasonic pressure wave is commonly known to be the primary cell-inactivation mechanism leading to cell disruption and death (Shengpu Gao *et al.,* 2014a; Liao *et al.,* 2018; Piyasena *et al.,* 2003). At higher frequency ranges, this physical effect may be accompanied by a sonochemical reaction in the surrounding medium triggered by the increasing temperature and pressure from the collapsing bubbles (Ashokkumar, 2011).

Heat treatment is the most conventional method applied to bacterial cell inactivation in the food industry (Abdurrahman *et al.,* 2019; Chemat *et al.,* 2011; Piyasena *et al.,* 2003; Raso & Barbosa-Cánovas, 2003). However, the intensity of the heat treatment may have several negative impacts on food quality and nutrition (Chemat *et al.,* 2011). Another common method to inactivate bacteria is by adding chemical preservatives. This method may be effective but some adverse side effects might not be tolerable in the long term. Alternative approaches such as application of natural antimicrobial molecules (Brogden, 2005; Lyu *et al.*, 2019; Xiang *et al.,* 2016) and non-thermal technologies (pulsed electric fields, UV light, high hydrostatic pressure, microwave, ultrasound) have been explored to inactivate cells (Butz & Tauscher, 2002; Leonelli & Mason, 2010; Raso & Barbosa-Cánovas, 2003).

Unlike heat or chemical treatment, mechanical treatments by using membrane filter (Bilad, 2017; Gao *et al.,* 2018; Sharpe *et al.,* 1979) and ultrasound can minimize negative impacts in food quality and prevent harmful health effects (Chemat *et al.,* 2011). While the membrane filter requires high maintenance and does not inactivate the bacteria, the ultrasound treatment has proven to inactivate the bacteria effectively. Ultrasound treatment especially for food preservation can also be assisted with other physical methods such as high pressure (manosonication), heat (thermosonication) and combination of both (manothermosonication) to enhance its effectiveness (Piyasena *et al.,* 2003; Sango *et al.,* 2014; Zupanc *et al.,* 2019). Although, these ultrasounds assisted technology is not within our scope of experiment, but several studies have mentioned their promising results for milk and juices (Bermúdez-Aguirre *et al.,* 2009; Walkling-Ribeiro *et al.,* 2009).

The physical parameters of the ultrasound device are important to achieve maximum inactivation effect. Working frequency and intensity are important parameters that can influence the cavitation effect in the propagating media (Brotchie *et al.,* 2009).

The readily available commercial ultrasound device for cell disruption is normally operated at a single frequency of 20 kHz. However, several studies showed that

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

frequencies higher than 20 kHz cause more effective biocidal effects (Shengpu Gao *et al*., 2014a; Shengpu Gao *et al.*, 2014b). This motivated us to develop a low-cost ultrasound device prototype with a configuration that allows for different frequency ranges. We designed a batch-type ultrasound device installed with a piezoelectric of 22 kHz.

The batch was designed to have a natural frequency of 22 kHz to ensure that the resonance phenomenon occurs. The prototype was then tested on *Escherichia coli* and *Bacillus subtilis* in a simple medium (e.g., phosphate buffer) and a more complex medium (e.g., milk). The results were also compared to those from another experiment using a commercial-probe ultrasound device. Parameters affecting the bacterial cell inactivation were comprehensively discussed.

2. METHODS

2.1. Ultrasound prototype design and manufacture

The ultrasound prototype presented in this study was of a tubular configuration that had been developed previously (Borthwick *et al.,* 2005). Prior to manufacturing, vibration analysis had to be conducted to assure the prototype could vibrate with the maximum amplitude at the designated frequency, which directly corellated with the prototype efficiency.

The vibration analyses, consisting of modal analysis and harmonic analysis, were performed using the finite element method (FEM). Modal analysis was conducted to get the characteristics of the system, including its natural frequency and corresponding vibration modes (Eslaminejad *et al*., 2019). These parameters are the foundation for harmonic response analysis, which results in a response at every determined frequency. The peak response corresponds to the natural frequency of the system.

The system was a cylinder with one closed end designed using the ANSYS Design Modeler. The cylinder tank had an outer diameter 45 mm, inner diameter (Di) of 25 mm, height of 22 mm, and hole depth of 18 mm **Figure 1**. The tank, which was made of steel, was then fitted to a 22 kHz piezoelectric transducer (Steiner & Martins Inc, Doral, FL) and had the mechanical properties presented in **Table 1**. The steel material was selected because it can convert mechanical vibration generated by the transducer to be pressure waves in liquid inside the tank with low damping (Nurprasetio *et al*., 2018).

A pressure load to substitute the transducer contact was applied to the outer perimeter of the cylinder surface as the loading condition, which can be seen in **Figure 2**. The magnitude of the pressure was obtained by dividing the maximum force of the piezoelectric with its surface area; in this case, 0.25 MPa was input into the system. The settings used for harmonic response analysis are shown in **Table 2**. The overall system consisted of a signal generator (Rigol DG 812), a power amplifier (PCA MOS 4175), an oscilloscope (GW Instek GDS 1054B), and a transformer (ING 10A 110 240). **Figure 3** is a schematic of the circuit of the experimental apparatus.

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

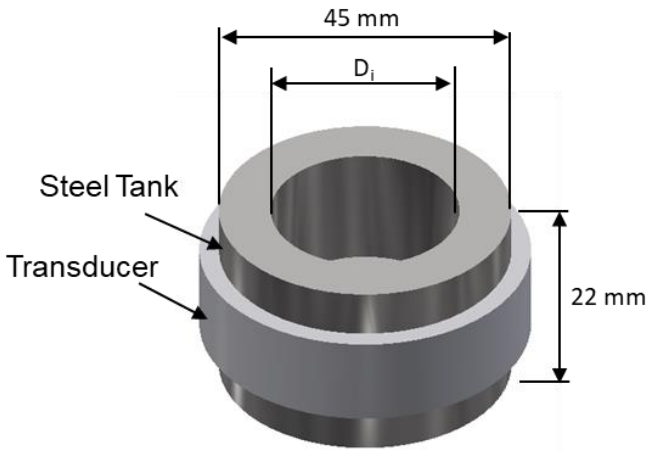

Figure 1. CAD model of batch type ultrasound prototype

Table 1. Mechanical properties of steel used for ultrasound prototype material

Mechanical Properties	Value
Density	7800 kg/m^3
Young's Modulus	200 GPa
Poisson Ratio	0.3
Bulk Modulus	166.7 GPa
Shear Modulus	76.9 GPa

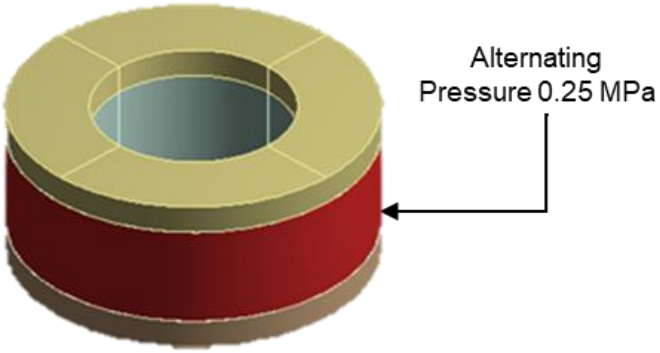

Figure2. Boundary condition for harmonic response simulation

Table 2. Input data of harmonic response analysis

Parameter	Value(s)
Frequency Spacing	Linear
Minimum testing frequency	0 Hz
Maximum testing frequency	40000 Hz
Solution Intervals	40 (0-40 kHz) 200 (36-38 kHz)
Solution Method	Mode Superposition

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

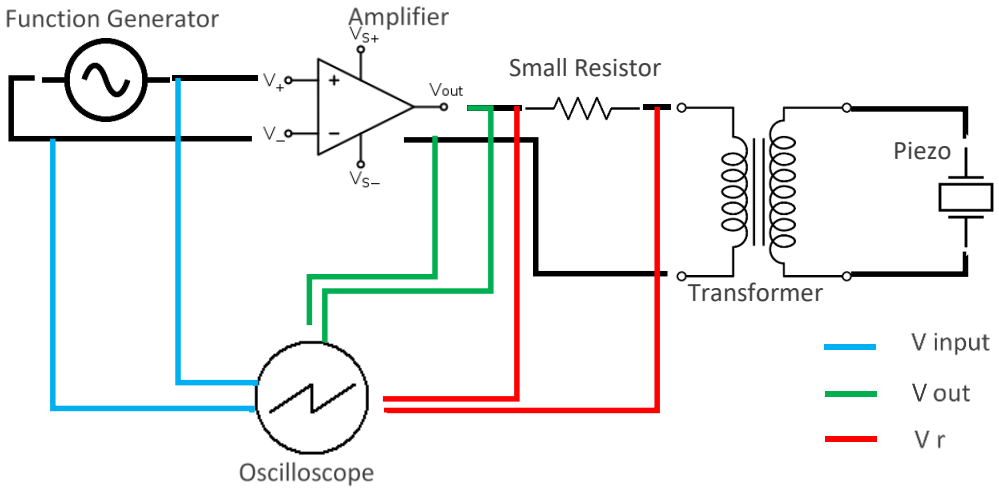

Figure 3. Electrical resonance circuit of the experimental apparatus

2.2 Bacterial cell preparation

E. coli and *B. subtilis* cultures were provided by the Microbiology Laboratory at the School of Life Sciences and Technology at ITB. Each culture was grown overnight in Luria Broth media (HiMedia, Mumbai, India) at 37 °C, and it reached 6.5-7 log colony-forming unit per mililiter (CFU/ml). The culture was then harvested and centrifuged down at 10,000 g for 5 minutes. The pellet was washed twice using a phosphate buffer saline solution at a pH of 7.4. The final suspension with the same buffer was used for the ultrasound treatment.

2.3 Food sample preparation

Commercial pasteurized cow milk (full cream) was used as a sample. The milk, with a pH of 6.5, was obtained from a local supermarket. Prior to ultrasound treatment, two batches of pasteurized milk were each inoculated with *E. coli* and *B. subtilis* and incubated overnight at 37 °C until they reached 6.5-7 log CFU/ml. The inoculated milk was stored at 4°C before the experiment.

2.4. Ultrasound experiment

Ultrasound treatment was carried out using the 20 kHz prototype and a 20 kHz probe cell disruptor (Omni Ruptor 4000, GA, USA) for comparison. Five ml of bacterial suspension in pH 7.4 PBS and inoculated milk were exposed to ultrasound for 1, 5, 10, and 15 minutes. The probe cell disruptor was set at 20% and 40% power output (equal to 80W and 160W) with constant sonication. To determine number of surviving cells after treatment, 0.1 ml of each treated sample was spread in Luria Agar (HiMedia, Mumbai, India) and grown at 37 °C for 12 – 16 hours until the colony was viable.

2.5. Microscopic analysis

To study cell agglomeration of *B. subtilis* before and after ultrasound treatment, cell suspension was analyzed under an inverted phase contrast microscope (Nikon Eclipse TE 300, NY, USA). The culture suspension was dropped onto a microscope slide (10-20 □l) using a glass slide cover. Visualization was performed at 10x, 20x, and 40x magnification.

2.6. Genomic analysis

Extraction of genomic DNA of *E. coli* and *B. subtilis* after ultrasound treatment was performed according to Rapley. The extracted DNA was then amplified using a mini8 thermal cycler (miniPCR, MA, USA) with a temperature cycling consisting of a

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

denaturation stage at 94 °C for 5 min, followed by 30 cycles of 94 °C for 20s, 60 °C for 30s and 72 °C for 30s, and a final stage at 72 °C for 10 min (González-González *et al.*, 2019). After amplification, the genomic DNA was loaded onto a 1.5 % agarose gel with 100 V for 25 min. The DNA bands were visualized with long-wave UV light.

3. RESULTS AND DISCUSSION

3.1. Modal analysis of ultrasound prototype

Natural frequencies of modal analysis obtained from finite-element analysis are presented in **Table 3**. The first six modes were rigid body movement, which means the tank did not undergo any significant internal deformation. The rest of the modes were the bending modes. Two pairs of modes, 7^{th} and 8^{th}, as well as 9^{th} and 10^{th}, each had the same natural frequencies, however they had different phases. This was due to the symmetric geometry of the tank.

The 7^{th} and 11^{th} modes show bending oscillation in axial and radial movement as presented in **Figure 4**. These modes follow the loading condition from the piezoelectric (cyclic radial force on the outer surface of the tank).

Modal analysis **Figure 5** of a tank with a 25 mm inner diameter (D_i) shows the maximum radial displacement (7.8×10^{-3} mm) that occurs at 37 kHz. Ideally, the prototype should be operated at this frequency to ensure high-power conversion efficiency. However, one of the constraints in the design was the availability of the commercial piezoelectric transducer.

In this work, we limited our work by designing the prototype using a piezoelectric transducer with a working frequency of 22 kHz. Due to this constraint, the radial mode needed to be lowered from 37 kHz to 22 kHz. This could be achieved either by lowering the stiffness or by adding mass. The first option proved to be simpler. The stiffness of the tank was lowered by decreasing the wall thickness between the inner and outer surfaces. This parametric study was performed by increasing the inner diameter (D_i) by 1 mm increments until the natural frequency was found to be 22 kHz.

The natural frequency of 22 kHz was obtained when the inner diameter of the tank was 35 mm (23kHz) to 37 mm (21 kHz). For higher frequency accuracy, the resolution of the harmonic response was reduced to 100 Hz, and the harmonic response was conducted between 20 kHz to 25 kHz to shorten processing time. The increment of inner diameter (D_i) was reduced to 0.1 mm to improve accuracy. The parametric study is presented in **Figure 6**. The results show that an inner diameter of 35.7 mm gives a natural frequency of 22 kHz; consequently, 35.7 mm was chosen as the inner diameter of the tank. A linear relationship was observed between the natural frequency and inner diameter for the range of 25 to 38 mm. This indicates that a typical batch can be designed and manufactured for different natural frequencies by simply changing the inner diameter.

Harmonic response analysis was repeated to analyze the tank displacement response between 0 and 40 kHz using the new inner diameter. The resolution was 1 kHz, but near the resonance frequency area, the resolution was lowered to 10 Hz. Moreover, the frequency response of tank stress (normal stress in the radial direction) was also conducted to evaluate the ability of the tank to withstand vibration at the resonance frequency.

The displacement and stress at 22 kHz were 1.7×10^{-3} mm and 5.7 MPa, respectively. The displacement was 100 times higher than that of the first design. Maximum displacement and stress occurred at 22.04 kHz with 5.5×10^{-3} mm displacement and 18.5 MPa. Maximum stress was found to be only 18.5 MPa, which was lower than the fatigue strength; thus, the tank could have an infinite lifetime. The FEM analysis ensures that the prototype can be built and used for the experiment.

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

Table 3. Natural frequencies and their modes

Mode	Frequency (Hz)	Type of Mode
1	0	Rigid body movement
2	1.47e-2	Rigid body movement
3	2.11e-2	Rigid body movement
4	0.14024	Rigid body movement
5	0.14754	Rigid body movement
6	0.15828	Rigid body movement
7	19999	Bending
8	19999	Bending
9	34175	Bending
10	34175	Bending
11	37634	Bending
12	40436	Bending

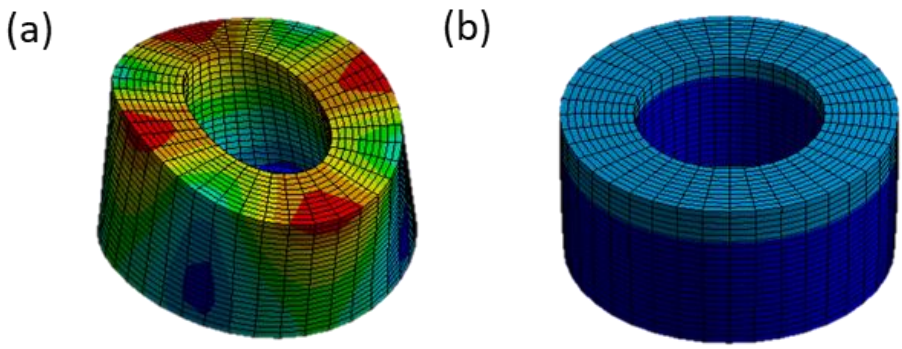

Figure 4. Bending mode shapes of the tank under (a) 20000 Hz (7th mode) and (b) 37540 Hz (11th mode)

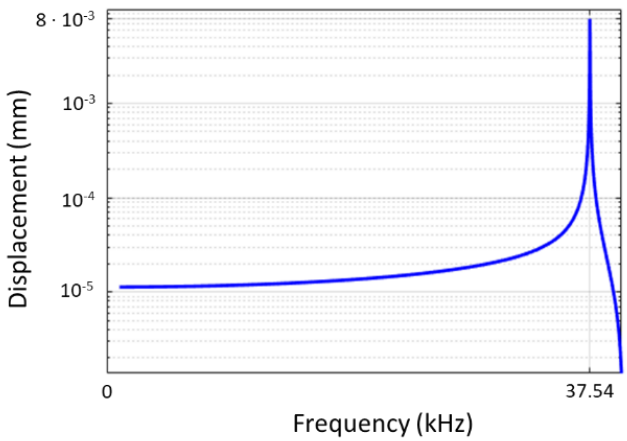

Figure 5. Maximum displacement of the tank for all tested frequencies

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

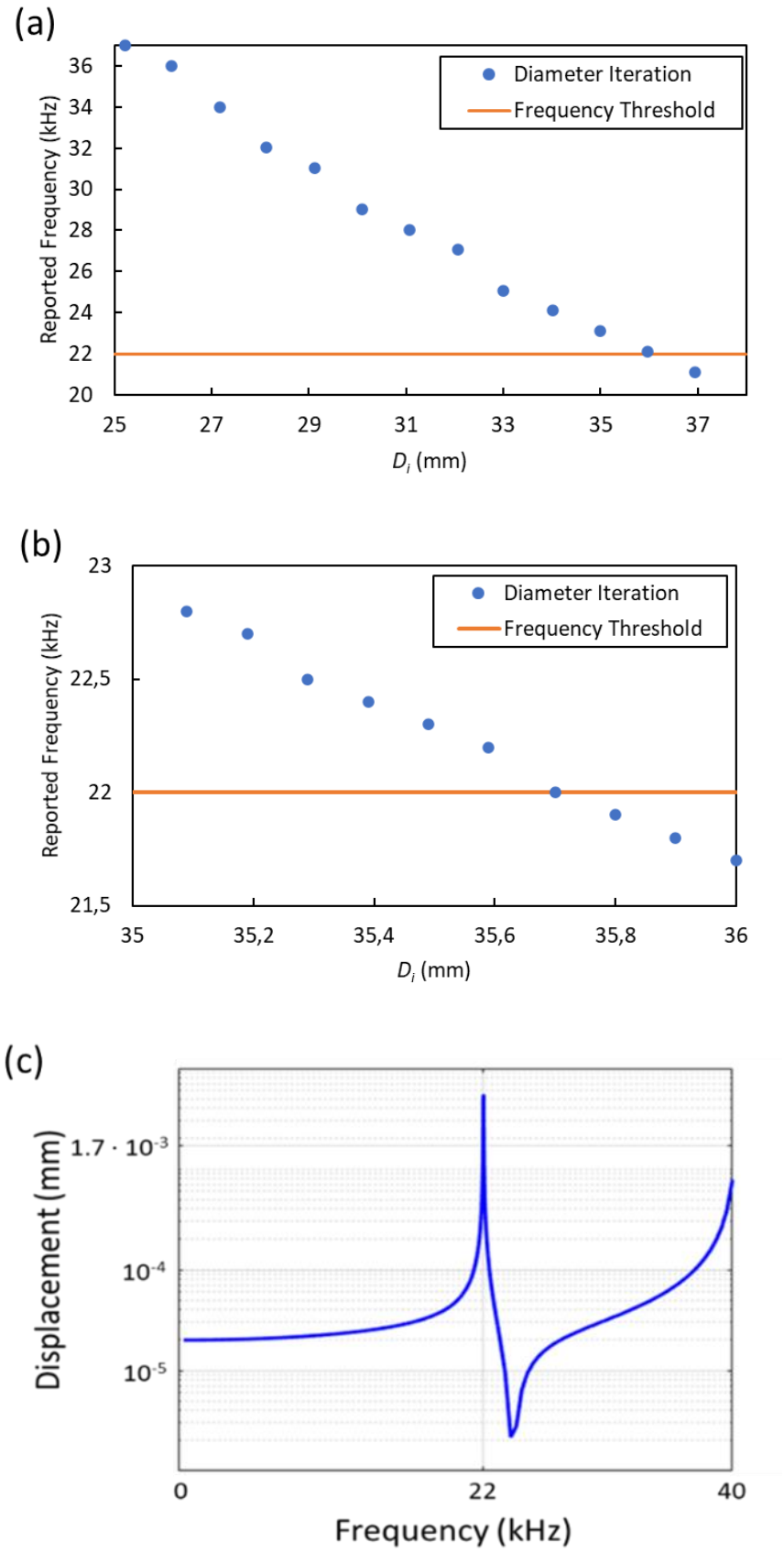

Figure 6. (a) The natural frequency alteration due to inner diameter changes. (b) A natural frequency of 22 kHz when the inner diameter was changed to 35.7 mm. (c) The maximum displacement of the tank for a frequency of 22 kHz.

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

3.2. Manufacturing and prototyping

The cylinder was machined using a standard milling and drilling machine with 100 ?m accuracy. This accuracy is required to ensure a natural frequency of the cylinder of around 22 kHz. The piezoelectric transducer was force fitted on the outer surface of the cylinder. Since the piezoelectric has an excited frequency of 22 kHz, the cylinder resonates and causes abundant bubble generation. The system was then connected to a function generator and an amplifier to enhance the signal.

3.3 Microbiological and genomic DNA analysis

Figure 7 shows the reduction of viable cell numbers (cell densities) after being treated with a probe cell disruptor. The treatment was carried out at different ultrasonication time periods and power levels. In general, the results indicate a decline in cell numbers, with *E. coli* being more sensitive to ultrasonication compared to *B. subtilis*. After a 15-minute sonication at 160 W **Figure 7**a, the cell density was reduced by 3.6 log for *E. coli* while *B. subtilis* was reduced by 3.4 log. At 80 W for 15 minutes, the cell density was reduced by 3.4 log for *E. coli* and 3.0 log for *B. subtilis* **Figure 7**b. The prototype, with a lower power level of 8 W and a 15-minute exposure time, reduced *E. coli* and *B. subtilis* cell densities by 3.3 log and 2.8 log, respectively **Figure 8**. This result was comparable to treatment with a probe cell disruptor at 80 W. The prototype succesfully replicated the tendency of experimental results from commercial probes.

For treatment of bacterial cells in a more complex matrix such as milk, both commercial probes and our prototype show less inactivation sensitivity. For the commercial probe, cell density was reduced by up to 3.0, whereas for our prototype, the cell density reduction was up to 2.5 log. The different inactivation sensitivities with respect to the medium compositions and viscosities were ultimately influenced by acoustic transfer and distribution of power within the reaction solution (Bermúdez-Aguirre *et al.,* 2009; Fitriyanti & Narsimhan, 2018; Joyce *et al.*, 2011). In the case of milk composition, the lower efficiency at the current treatment intensity might have been due to milk proteins and cell agglomeration, which provided protection against biocidal attack.

It can be seen **in Figure 7a** and **7b** that both power level and exposure time increase cell inactivation. This has also been widely observed in other studies (Bermúdez-Aguirre *et al.,* 2009; Shengpu Gao *et al.,* 2014; Joyce *et al.,* 2011; Joyce *et al.*, 2003; Sango *et al.,* 2014; Tiwari *et al.,* 2009). A study conducted by (Joyce *et al*., 2011; Fülscher & Roos, 1994) observed that ultrasound treatment with an intensity of 0.24 W/cm^3 (20 kHz probe sonicator) is more effective in reducing *B. subtilis* cells than treatment with an intensity of 0.18 W/cm^3 (38 kHz batch sonicator). Frequency and power level are two important factors in determining the effect of ultrasound because they influence the size and number of cavitation bubbles (Joyce *et al.,* 2011). It has been found that at higher frequencies (>500 kHz) and lower power levels, cavitation bubbles are smaller and collapse with less energy due to a shorter acoustic cycle, which means a shorter time for bubble formation. As a consequence of this phenomenon, a bacterial suspension treated with a higher frequency and a lower power level experiences a significantly lower mechanical effect (Joyce *et al.,* 2011).

The difference in sensitivity between the two tested bacterial cells, *E. coli* and *B. subtilis*, may be due to differences in their cell wall characteristics. As mentioned in previous studies, gram-negative bacteria with thinner cell walls such as *E. coli, E. aerogenes*, and *K. pneumoniae* are more sensitive to ultrasonication than gram-positive bacteria with thicker cell walls such

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

as *B. subtilis* and *A. pullulans* (Fitriyanti & Narsimhan, 2018; Shengpu Gao *et al.*, 2014; Joyce *et al.*, 2011). It has also been observed that cocci, or spherical bacteria, are more resistant than bacilli, or rod shaped bacteria (Joyce *et al.*, 2011). This corresponds to our experimental results, where *E. coli* cell reduction was higher than that of *B. subtilis.* While **Figure 7** shows the predicted trend in terms of treatment time, the inactivation rate seems to vary with differing time periods. This may be due to the agglomeration of the bacterial cells. Fülscher reported that ultrasound treatment (20 and 38 kHz) for 1 to 10 minutes did not have a dramatic effect on the cell viability, except for a small drop after 15 minutes of treatment. This insignificant result suggested a declumping of bacterial agglomerates with little deactivation. We also observed similar agglomeration as shown in **Figure 9**. Agglomeration of cells or biofilm formation were indicated in untreated *B. subtilis* suspension. After 15 minutes of treatment, a declumping of bacterial cells occurred, resulting in distinct individual cells.

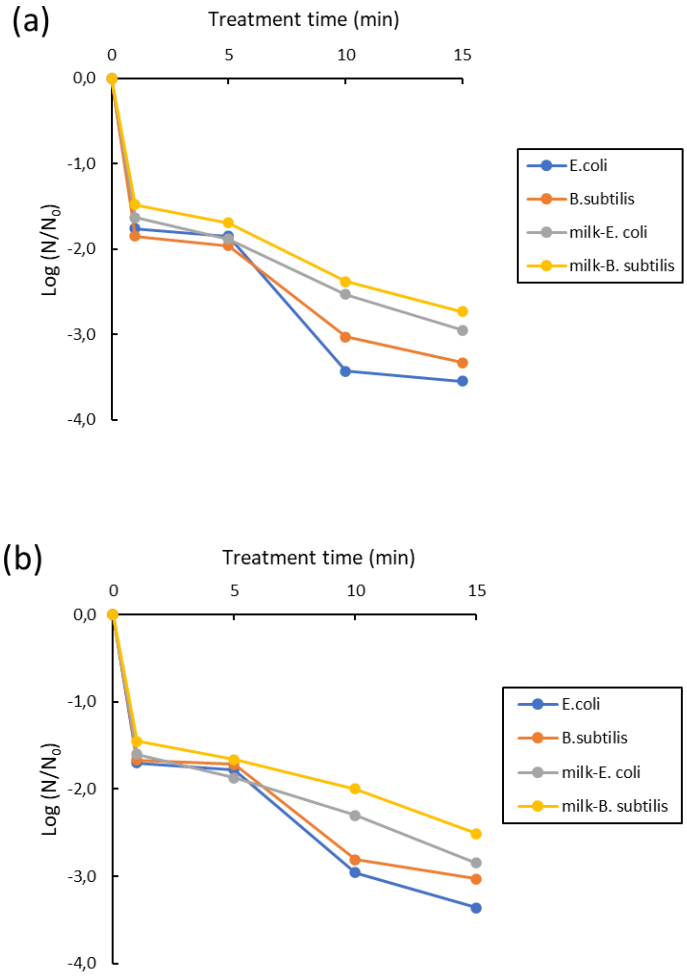

Figure 7. Viable cell reduction of *E. coli* and *B. subtilis* after treatment with probe ultrasound (20 kHz) using different input power levels: (a) 80 watt and (b) 160 watt. N = cell number after treatment, N_0 = initial cell number. Experiment kept at room temperature with constant sonication

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

Outside the conventional power ultrasound frequency range (100 kHz < frequency < 2 MHz), acoustic cavitation may induce mechanical effects and sonochemical reactions (production of free radicals) that can damage DNA, enzymes, liposomes, and membranes (Butz & Tauscher, 2002; Shengpu Gao, Hemar, *et al.*, 2014; Wu & Nyborg, 2008). As observed by Gao et al. (Shengpu Gao *et al.*, 2014) ultrasound at 850 kHz was highly efficient in inactivating bacteria such as (*B. subtilis*, *Enterobacter aerogenes*, *Staphylococcus epidermidis*) and yeast (*Aureobasidium pullulans*) with inactivation rates of up to 99%. The inactivation is due to mechanical effects and generation of hydroxy radicals that cause damage to DNA and proteins. Based on these studies, we performed a genomic DNA analysis to examine the effect of prototype power intensity on bacterial cell DNA. **Figure 9** presents genomic DNA bands of *E. coli* (B and C) and *B. subtilis* (D and E) after treatment with our prototype. The fluorescent density of DNA bands decreased with longer ultrasound treatment time. Based on these results, the cells disintegrated, causing DNA molecules to be detected in the surrounding medium using gel electrophoresis. The results were consistent with another report which also observed a decrease in DNA bands for cells treated with longer sonication (Liao *et al.*, 2018).

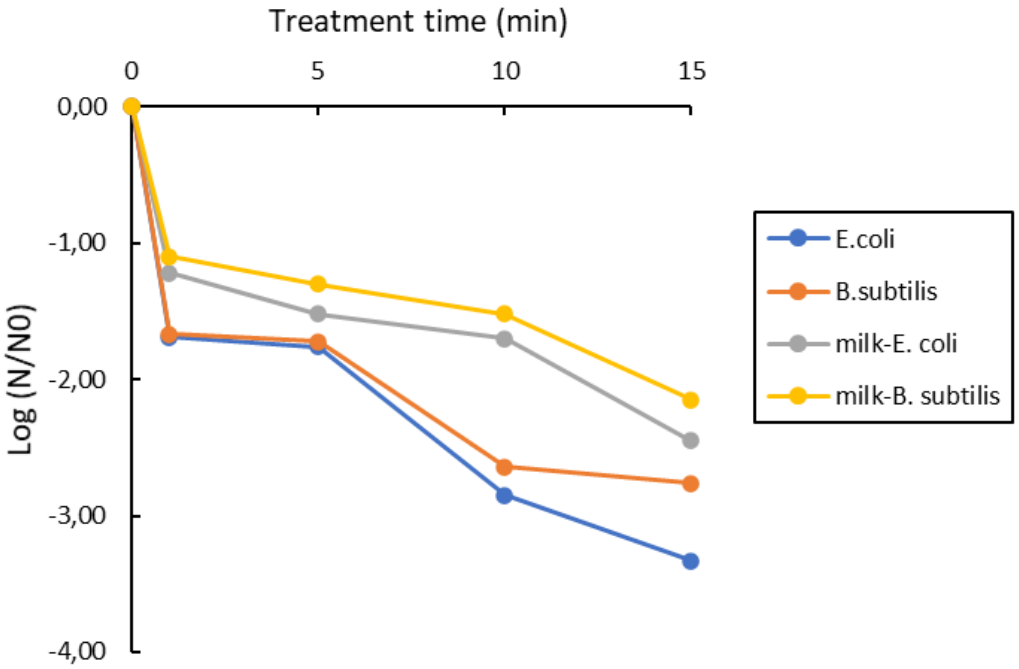

Figure 8. Viable cell reduction of *E. coli* and *B. subtilis* after treatment with prototype ultrasound (22 kHz). N = cell number after treatment, N_0 = initial cell number. Experiment kept at room temperature with constant sonication

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

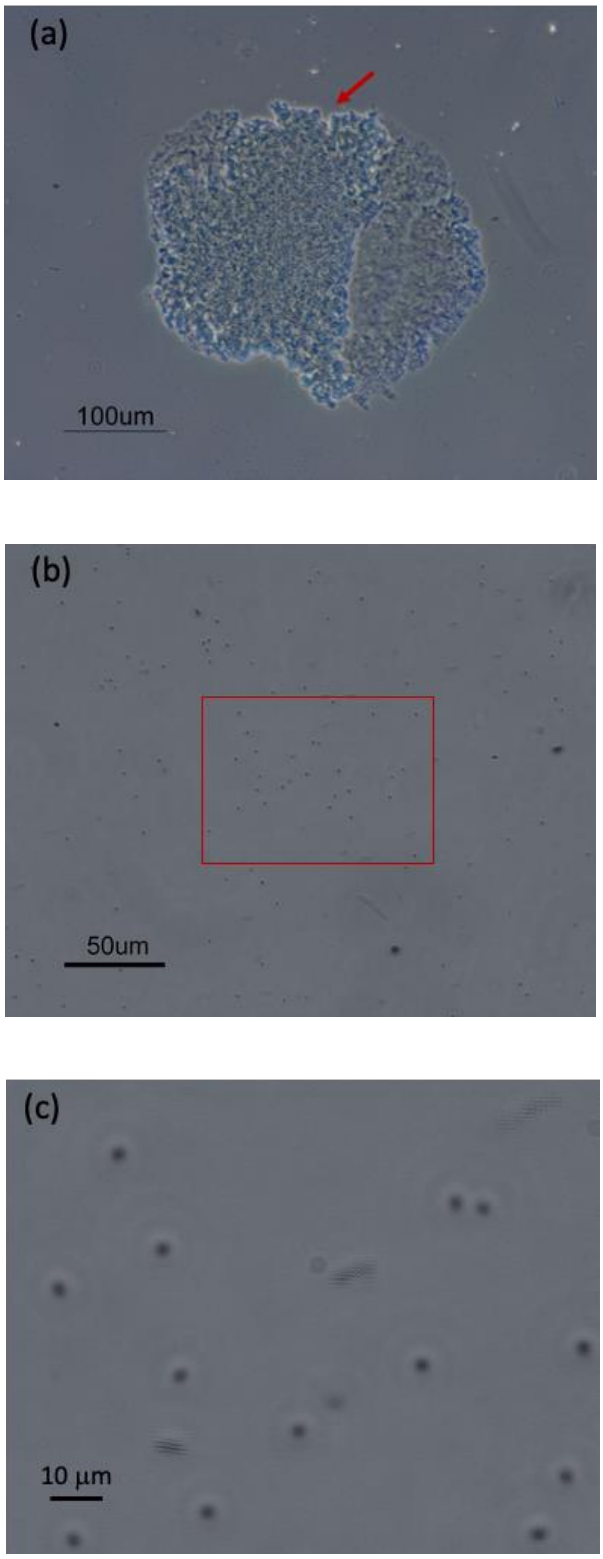

Figure 8. Phase contrast microscopy of *B. subtilis*. (a) Untreated culture. Red arrow shows agglomeration/biofilm formation. (b) After treatment with the ultrasound prototype for 15 minutes. Red box shows declumping of cells. (c) Closer look at cells from panel B shows distinct cells.

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

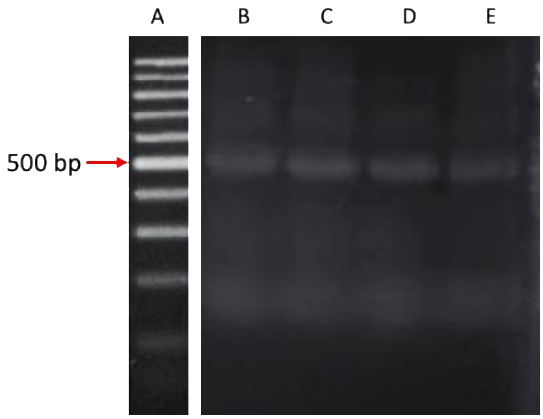

Figure 9. Agarose gel electrophoresis of genomic DNA from bacterial cells treated with prototype ultrasound for 5 and 10 minutes. A = marker, B = *B. subtilis* (5 min), C = *B. subtilis* (10 min), D = *E. coli* (5 min), E = *E. coli* (10 min)

4. CONCLUSION

The readily available ultrasound probe used in the lab for cell disruption works mainly at 20 kHz. This limitation creates an opportunity for exploring a low-cost prototype. Due to the constraints imposed by the availability of piezoelectric transducers, the prototype geometry was optimized by changing the tank's inner diameter. The peak resonance with a frequency of 22 kHz was obtained for a diameter of 35.7 mm. The relationship between the natural frequency and the inner diameter of the tank was revealed. This current prototype design will later allow further modifications such as the use of transducers of different frequencies and a continuous flow-through system.

The current ultrasound prototype was able to inactivate bacterial cells, *E. coli* and *B. subtilis* in a simple medium (PBS, pH 7.4) and in a food system (milk, pH 6.5). The results were comparable to treatment with a commercial probe cell disruptor. From the experiment, it was confirmed that *B. subtilis* has more resistance to cavitation bubbles generated by an ultrasonicator than *E. coli*. The experiment also confirmed that the cell density reduction in milk is lower. Further biochemical analysis and ultrasound power intensity variations need to be performed to understand this phenomenon better.

5. ACKNOWLEDGEMENT

This research was supported by the Institute of Research and Community Service, Institut Teknologi Bandung, under the 2020 Multidisciplinary Research Program.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

7. REFERENCES

Abdurrahman, Umam, R., Irzaman, Palupi, E. K., Saregar, A., Syazali, M., Adi, L. C. (2019). Optimization and interpretation of heat distribution in sterilization room using convection pipe. *Indonesian Journal of Science and Technology*, *4*(2), 204–219.

DOI: http://dx.doi.org/10.17509/ijost.v6i1
p- ISSN 2528-1410 e- ISSN 2527-8045

Ashokkumar, M. (2011). The characterization of acoustic cavitation bubbles - An overview. *Ultrasonics Sonochemistry*, *18*(4), 864–872.

Bermúdez-Aguirre, D., Corradini, M. G., Mawson, R., and Barbosa-Cánovas, G. V. (2009). Modeling the inactivation of Listeria innocua in raw whole milk treated under thermo-sonication. *Innovative Food Science and Emerging Technologies*, *10*(2), 172–178.

Bilad, M. R. (2017). Membrane bioreactor for domestic wastewater treatment: Principles, challanges and future research directions. *Indonesian Journal of Science and Technology*, *2*(1), 97–123.

Borthwick, K. A. J., Coakley, W. T., McDonnell, M. B., Nowotny, H., Benes, E., and Gröschl, M. (2005). Development of a novel compact sonicator for cell disruption. *Journal of Microbiological Methods*, *60*(2), 207–216.

Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. *Nature Reviews Microbiology*, *3*(3), 238–250.

Brotchie, A., Grieser, F., and Ashokkumar, M. (2009). Effect of power and frequency on bubble-size distributions in acoustic cavitation. *Physical Review Letters*, *102*(8), 1–4.

Butz, P., and Tauscher, B. (2002). Emerging technologies: Chemical aspects. *Food Research International*, *35*(2–3), 279–284.

Chemat, F., Zill-e-Huma, and Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, *18*(4), 813–835.

Eslaminejad, A., Ziejewski, M., and Karami, G. (2019). An experimental–numerical modal analysis for the study of shell-fluid interactions in a clamped hemispherical shell. *Applied Acoustics*, *152*, 110–117.

Fitriyanti, M., and Narsimhan, G. (2018). Synergistic effect of low power ultrasonication on antimicrobial activity of cecropin P1 against E. coli in food systems. *Lebensmittel-Wissenschaft and Technologie*, *96*, 175–181.

Fülscher, M. P., and Roos, B. O. (1994). The excited states of pyrazine: A basis set study. *Theoretica Chimica Acta*, *87*(4–5), 403–413.

Gao, Shengpu, Hemar, Y., Ashokkumar, M., Paturel, S., and Lewis, G. D. (2014). Inactivation of bacteria and yeast using high-frequency ultrasound treatment. *Water Research*, *60*, 93–104.

Gao, Shengpu, Lewis, G. D., Ashokkumar, M., and Hemar, Y. (2014a). Inactivation of microorganisms by low-frequency high-power ultrasound: 1. Effect of growth phase and capsule properties of the bacteria. *Ultrasonics Sonochemistry*, *21*(1), 446–453.

Gao, Shengpu, Lewis, G. D., Ashokkumar, M., and Hemar, Y. (2014b). Inactivation of microorganisms by low-frequency high-power ultrasound: 2. A simple model for the inactivation mechanism. *Ultrasonics Sonochemistry*, *21*(1), 454–460.

Gao, Siyue, Pearson, B., and He, L. (2018). Mapping bacteria on filter membranes, an innovative SERS approach. *Journal of Microbiological Methods*, *147*(March), 69–75.

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045

González-González, E., Mendoza-Ramos, J. L., Pedroza, S. C., Cuellar-Monterrubio, A. A., Márquez-Ipiña, A. R., Lira-Serhan, D., Alvarez, M. M. (2019). Validation of use of the miniPCR thermocycler for Ebola and Zika virus detection. *PLoS ONE*, *14*(5), 13–16.

Joyce, E., Al-Hashimi, A., and Mason, T. J. (2011). Assessing the effect of different ultrasonic frequencies on bacterial viability using flow cytometry. *Journal of Applied Microbiology*, *110*(4), 862–870.

Joyce, E., Mason, T. J., Phull, S. S., and Lorimer, J. P. (2003). The development and evaluation of electrolysis in conjunction with power ultrasound for the disinfection of bacterial suspensions. *Ultrasonics Sonochemistry*, *10*(4–5), 231–234.

Leonelli, C., and Mason, T. J. (2010). Microwave and ultrasonic processing: Now a realistic option for industry. *Chemical Engineering and Processing: Process Intensification*, *49*(9), 885–900.

Liao, X., Li, J., Suo, Y., Chen, S., Ye, X., Liu, D., and Ding, T. (2018). Multiple action sites of ultrasound on Escherichia coli and Staphylococcus aureus. *Food Science and Human Wellness*, *7*(1), 102–109.

Lyu, Y., Fitriyanti, M., and Narsimhan, G. (2019). Nucleation and growth of pores in 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) / cholesterol bilayer by antimicrobial peptides melittin, its mutants and cecropin P1. *Colloids and Surfaces B: Biointerfaces*, *173*, 121–127.

Nurprasetio, I. P., Budiman, B. A., Triawan, F., and Hafid, M. (2018). Measurement of pressure wave speed in stainless-steel pipe generated by water hammer. *MATEC Web of Conferences*, *197*, 4–7.

Piyasena, P., Mohareb, E., and McKellar, R. C. (2003). Inactivation of microbes using ultrasound: A review. *International Journal of Food Microbiology*, *87*(3), 207–216.

Raso, J., and Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. *Critical Reviews in Food Science and Nutrition*, *43*(3), 265–285.

Sango, D. M., Abela, D., Mcelhatton, A., and Valdramidis, V. P. (2014). Assisted ultrasound applications for the production of safe foods. *Journal of Applied Microbiology*, *116*(5), 1067–1083.

Sharpe, A. N., Peterkin, P. I., and Dudas, I. (1979). Membrane filtration of food suspensions. *Applied and Environmental Microbiology*, *37*(1), 21–35.

Tiwari, B. K., O'Donnell, C. P., Muthukumarappan, K., and Cullen, P. J. (2009). Effect of sonication on orange juice quality parameters during storage. *International Journal of Food Science and Technology*, *44*(3), 586–595.

Triawan, F., Budiman, B. A., Nurprasetio, I. P., and Sunnardianto, G. K. (2019). Direct and indirect measurement techniques of cavitation intensity: A brief review. *Journal of Physics: Conference Series*, *1402*(4), 1-6.

Walkling-Ribeiro, M., Noci, F., Riener, J., Cronin, D. A., Lyng, J. G., and Morgan, D. J. (2009). The impact of thermosonication and pulsed electric fields on Staphylococcus aureus

DOI: http://dx.doi.org/10.17509/ijost.v6i1
p- ISSN 2528-1410 e- ISSN 2527-8045

inactivation and selected quality parameters in orange juice. *Food and Bioprocess Technology*, *2*(4), 422–430.

Wu, J., and Nyborg, W. L. (2008). Ultrasound, cavitation bubbles and their interaction with cells. *Advanced Drug Delivery Reviews*, *60*(10), 1103–1116.

Xiang, N., Lyu, Y., Zhu, X., Bhunia, A. K., and Narsimhan, G. (2016). Methodology for identification of pore forming antimicrobial peptides from soy protein subunits β-conglycinin and glycinin. *Peptides*, *85*, 27–40.

Zupanc, M., Pandur, Ž., Stepišnik Perdih, T., Stopar, D., Petkovšek, M., and 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.

DOI: https://doi.org/10.17509/ijost.v6i1.31516
p- ISSN 2528-1410 e- ISSN 2527-8045