ACTA MICROBIOLOGICA BULGARICA Volume 39 / 1 (2023)

Effects of Ultrasonic Specific Energy on Time-Dependent Inactivation Rate of *Proteus mirabilis* and *Streptococcus sanguinis*

Özge Kekeç[1], Barış Gökalsın[1], Birkan Açıkgöz[1], Didem Öner Özdaş[2], Cenk Sesal[1*]

[1]*Department of Biology, Faculty of Science, The University of Marmara, İstanbul, Turkey*
[2]*Department of Pedodontics, Faculty of Dentistry, The University of Aydın, İstanbul, Turkey*

Abstract

Ultrasound has been used as an alternative and enhancer approach for the inhibition of microorganisms in recent years. The purpose of the current study was to determine the inactivation of *Streptococcus sanguinis* and *Proteus mirabilis* bacteria species via ultrasound. For this purpose, bacterial suspensions with $5x10^3$, $1.5x10^4$, and $3x10^4$ colony forming unit (cfu/ml) concentrations were ultra-sonicated with 30 kHz frequency and 100 W power ultrasound for various sonication time periods. As a result, time-dependent inhibition rates were observed with bacteria concentration differences. The specific energy (γ) of different concentrations of *S. sanguinis* and *P. mirabilis* was also calculated. The rate constant of ultrasonic inhibition was evaluated in the linear region against sonication time. Then, the t-test was applied to all of the data. The results of $5x10^3$, $1.5x10^4$, and $3x10^4$ cfu/ml for *S. sanguinis* and *P. mirabilis* were statistically significant at $<p= 0.02, 0.02, 0.006$ for *S. sanguinis*, and at $<p=0.003, 0.004, 0.003$ for *P. mirabilis*, respectively.
Keywords: ultrasound, bacteria, time-dependent inactivation, specific energy, *Proteus mirabilis*, *Streptococcus sanguinis.*

Резюме

През последните години ултразвукът се използва като алтернативен и усилващ подход за инхибиране развитието на микроорганизми. Целта на настоящото изследване е да се ефекта на ултразвука върху развитието на бактериите *Streptococcus sanguinis* и *Proteus mirabilis*. За тази цел, бактериални суспензии с концентрации $5x10^3$, $1.5x10^4$ и $3x10^4$ единици образуващи колонии (cfu/ml) са обработени с ултразвук с 30 kHz честота и 100 W за различни периоди от време на обработка. Наблюдава се инхибиране в зависимост от времето на обработка и концентрацията на бактериите. Изчислена е също специфичната енергия (γ) на различни концентрации на *S. sanguinis* и *P. mirabilis*. Скоростната константа на инхибирането с ултразвук е оценена в линейната област спрямо времето на обработка. Към всички данни е приложен t-тестът. Резултатите от $5x10^3$, $1.5x10^4$ и $3x10^4$ cfu/мл за *S. sanguinis* и *P. mirabilis* са определени като статистически значими съответно при $<p= 0.02, 0.02, 0.006$ за *S. sanguinis* и при $<p=0.003, 0.004, 0.003$ за *P. mirabilis*.

Introduction

Most microorganisms cause many problems so inactivation of microorganisms is of significant importance (Piyasena *et al*., 2003). Over the years, researchers have studied to develop effective methods. Generally, ultraviolet (UV) light, electrical, mechanical, chemical (chlorination, ozonation), thermal (pasteurization), and ultrasound treatments have been tested for effective disinfection (Piyasena *et al*., 2003; Schwartz *et al*., 2003; Gibson *et al*., 2008; Hulsmans *et al*., 2010; Madron *et al*., 2012; Jin *et al*., 2013). Some microorganisms become resistant to available disinfection techniques, such as products, ultraviolet light, and heat treatment. For this reason, ultrasound applications have been added to ozonation or UV irradiation for the disinfection of bacteria (Piyasena *et al*., 2003). To this end, various ultrasound equipment has been developed and improved.

Known as one of the four sound categories, ultrasound refers to frequencies greater than 20 kHz, that is, sounds higher than the upper limit of human hearing (Thompson and Doraiswamy, 1999). For many years, ultrasound has been used as a standard method in microbiology. When ultra-

* Corresponding author: csesal@marmara.edu.tr

sound is applied to a liquid medium, bubbles are produced by the ultrasonically induced cavitations in the solution. Cavitation bubbles in liquid media generate physical effects, such as microstreaming, high shear force, shock waves, and in addition, chemical reactions such as free radicals, and hydrogen peroxide (Li *et al*., 2016). By acting on the cell wall of bacteria, radicals weaken its structure and bring it to the level of disintegration (Piyasena *et al*., 2003).

The collapse of bubbles during cavitation generates localized hot spots of high temperature and pressure. In the implosion process, when the vapor contained in the bubble is compressed rapidly, it causes heat generation due to thermodynamics. As a result of the swift implosion, a localized hot spot occurs and then a little heat is transferred to the surrounding liquid. In these hot spots, it is estimated that temperatures equivalent to about 5000°C, and a pressure of about 1000 atm are reached. As a result of the interactions between the microorganisms and cavitation bubbles, microorganisms are disintegrated and destroyed by ultrasonic waves (Arrojo and Benito, 2008; Gogate and Kabadi, 2009).

Sonication or ultrasound irradiation alone has become a preferred method in recent years as it can provide powerful disinfection under suitable ambient pressure and temperature conditions without the need for any chemical compound. If ultrasound is utilized alone, however, significant ultrasonic intensities are required to achieve 100% inhibition rates. The inhibition process of microorganisms by ultrasound has been explained by the physical and/or chemical changes that occur during ultrasonic irradiation (Zupanc *et al.,* 2019). While the mechanism is not clear, according to Pandur *et al*. (2022), the cell wall structure and bacterial physiological conditions are the key factors that affect the efficiency of bacterial sonolysis by ultrasound. Furthermore, Ananta *et al*. (2005) declared that the effects of ultrasound can be different on Gram-negative and Gram-positive bacteria owing to the morphological characteristics of the cell. Also, Koda *et al*. (2009) tested the inhibition rate of ultrasound at 20 and 500 kHz on *Escherichia coli* (Gram-negative) and *Streptococcus mutans* (Gram-positive) bacteria and observed different survival curves between *E. coli* and *S. mutans* at the highest power sonication. Loske *et al*. (2002) studied bacterial species that have different cell walls by using underwater shock waves and offered a similar suggestion.

In this study, a Gram-negative bacterium, *Proteus mirabilis,* and a Gram-positive bacterium, *Streptococcus sanguinis,* were selected. *S. sanguinis* is a key factor in the attachment of other oral microorganisms which colonize the oral cavity and tooth surface, pioneering the formation of dental plaque, development of caries, and periodontal diseases (Caufield *et al*., 2000; Zhu *et al*., 2018). *P. mirabilis,* a Gram-negative bacterium, part of the Enterobacteriaceae family and widely found in soil and water, can cause infections of the urinary tract of humans (Jansen *et al*., 2003). The purpose of the current study was to determine the effects of ultrasound treatment with different sonication times on the viability of different concentrations of the Gram-negative bacterium *P. mirabilis* and Gram-positive bacterium *S. sanguinis*. Following this purpose, the new aspect of the present study is the use of a 30 kHz and 100 W horn-type sonicator for logarithmic inactivation, especially for oral bacteria: *P. mirabilis* and *S. sanguinis*. Also, it examines the effects of time and specific energy-dependent ultrasonic irradiation on the survival curves of *P. mirabilis* and *S. sanguinis*.

Material and Methods

Bacterial cultivation and preparation of bacterial suspensions

S. sanguinis and *P. mirabilis* bacterial strains were obtained from Yeditepe University, Microbiology Lab., and were incubated in Luria-Bertani (LB) broth for 24 h at 37°C. The incubated bacterial suspensions were prepared for $5x10^3$, $1.5x10^4$, and $3x10^4$ colony forming units (cfu/ml) following the McFarland standards in physiological distilled water at 625 nm wavelength optical density (OD) by spectrometric measurements (Beckman Coulter, Germany).

Ultrasound treatment setup

Ultrasonic irradiation was applied using a horn-type sonicator with 30 kHz frequency and 100 W power, according to Sesal and Kekec (2014a). The following equations were used to calculate the values (1-4). R: diameter of sonotrode probe, P: power (Watt), BC: bacteria concentration, V: sample volume, t: time. According to Equation (4), the specific energy increases as the bacterial population decreases.

$$\text{Specific area of sonotrode } (A) = \frac{\pi R^2}{4} \quad (1)$$

$$\text{Ultrasonic power } (\sigma) = \frac{P}{A} \quad (2)$$

$$\text{Ultrasonic density (d)} = \frac{P}{V} \quad (3)$$

$$\text{Specific energy } (\gamma) = \frac{P\,t}{B_C\,V} \quad (4)$$

Treatment on bacteria

Bacterial suspensions (100 mL) with different concentrations of *S. sanguinis* and *P. mirabilis* were ultra-sonicated for the 5, 10, 15, 20, and 30 minutes using an ultrasonic probe submerged into the bacterial suspensions as explained in the setup section, in triplicate. After sonication, the samples were inoculated on growth media and incubated overnight at 37°C. Incubated colonies were counted by the colony counter according to the colony counting method. The number of cfu after the ultrasonic irradiation treatment of *P. mirabilis* and *S. sanguinis* bacteria was calculated. The average survival ratio measured from each run was given as (N/N0). The average of trials was calculated. The data were standardized to a value of 100 percent cfu for the graphical display. The findings of each experiment were compared to those of the control group. In the linear area of logarithmic bacterial populations against sonication time, the rate constant of ultrasonic inactivation was calculated using Equation (5) below, where ku is the ultrasound equation constant, t is time, N0 is the starting, and N is the final bacterial population (Thompson and Doraiswamy, 1999).

$$\text{Log } (N/N0)av = -2.303kut \qquad (5)$$

Statistical analysis

The time-dependent inhibition rate of the samples was analyzed by using a 1-Tailed t-test in SPSS 15.0 for Microsoft Windows.

Results

The inactivation of bacteria was anticipated to be more effective as the specific energy increased. The time-dependent specific energy levels from high to low were determined at concentrations of $5x10^3$, $1.5x10^4$, and $3x10^4$ cfu/ml, respectively (Fig. 1). The specific energy (γ) W.sec/cfu in different concentrations of *S. sanguinis* and *P. mirabilis* is shown in Fig. 1. Specific energy levels in all concentrations were observed to reach their maximum between the 20th and 30th minutes. The specific energy of the lowest bacterial concentration ($5x10^3$cfu/ml) was around eight-fold greater than that of $3x10^4$ cfu/ml, and roughly four times greater than that of $1.5x10^4$ cfu/ml, respectively.

The survival ratio of $5x10^3$, $1.5x10^4$, and $3x10^4$ cfu/ml concentrations of *P. mirabilis* and *S. sanguinis* against sonication time at the constant frequency and power are shown in Fig. 2-4. The logarithmic bacterial death rate increased for *P. mirabilis* depending on sonication time. After the 20th minute, a significant increase in death rates was

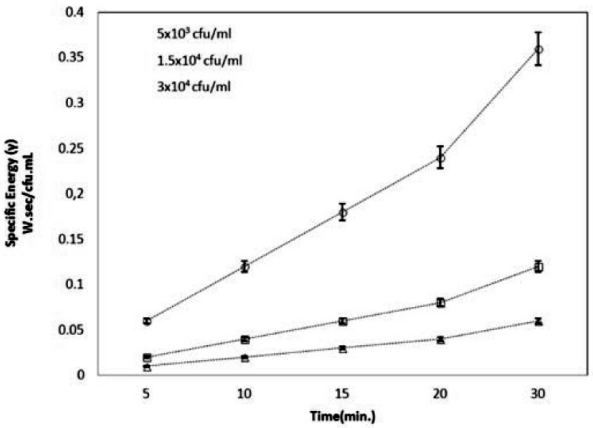

Fig. 1. The specific energy values observed at different bacterial concentrations depending on time

detected for the $5x10^3$ and $1.5x10^4$ cfu/ml samples. On the other hand, in samples with a higher concentration of bacteria, the mortality rate decreased. When the sonication time ended at the 30th minute, changes in the population were observed from 3.70 to 2.93 log for $5x10^3$ cfu/ml, 4.18 to 3.54 log for $1.5x10^4$ cfu/ml, and 4.48 to 3.91 log for $3x10^4$ cfu/ml for each concentration of *P. mirabilis* bacterium. In addition, the decreases were calculated as -0.77 log, -0.64 log, and -0.57 log, respectively. In comparison to *P. mirabilis*, *S. sanguinis* showed a lower rise in mortality rates in all samples. When the sonication time ended at the 30th minute, changes in the population were observed from 3.70 to 3.45 log for $5x10^3$ cfu/ml, 4.18 to 4.03 log for $1.5x10^4$ cfu/ml, and 4.48 to 4.36 log for $3x10^4$ cfu/ml. When this change was analyzed, the decrease rates were calculated as -0.25, -0.15, -0.12 log for the $5x10^3$, $1.5x10^4$, and $3x10^4$ cfu/ml samples, respectively.

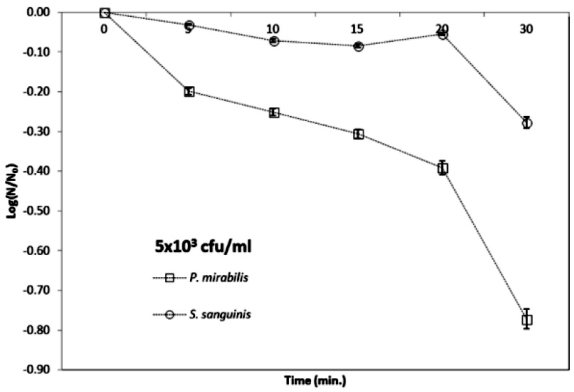

Fig. 2. The time-dependent logarithmic inactivation rate of *S. sanguinis* and *P. mirabilis* at a concentration of $5x10^3$ cfu/ml

The T-test was carried out by using all data. Test results for $5x10^3$, $1.5x10^4$ and $3x10^4$ cfu/ml concentrations of *S. sanguinis* were determined statistically significant based on the $< p = 0.02$, $< p =$

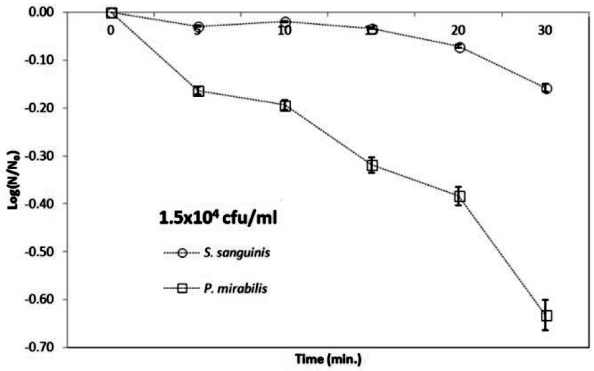

Fig. 3. The time-dependent logarithmic inactivation rate of *S. sanguinis* and *P. mirabilis* at a concentration of 1.5x10^4 cfu/ml

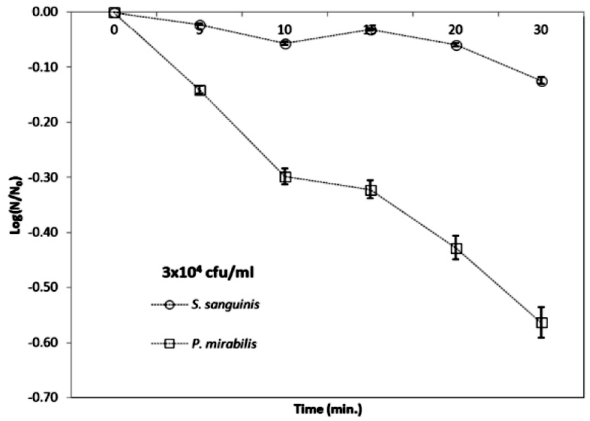

Fig. 4. The time-dependent logarithmic inactivation rate of *S. sanguinis* and *P. mirabilis* at a concentration of 3x10^4 cfu/ml

0.02 and < p = 0.006 values, respectively. Similarly, the results of 5x10^3, 1,5x10^4, and 3x10^4 cfu/ml samples of *P. mirabilis* were obtained statistically significant with < p = 0.003, < p = 0.004, and < p = 0.003 values.

Discussion

Due to its environmentally friendly effect, ultrasound is preferred in disinfection, especially in the 20 kHz and 500 kHz range (Thompson and Doraiswamy, 1999; Joyce *et al*., 2003; Gogate and Kabadi, 2009; Zhu *et al*., 2018; Zhang *et al*., 2021). In this regard, in the current study, the bacterial suspensions with 5x10^3, 1.5x10^4, and 3x10^4 cfu/ml concentrations of *P. mirabilis* and *S. sanguinis* bacterial species were ultra-sonicated with a frequency of 30 kHz and 100 W power under different sonication time conditions. For the ultrasonic treatment, an ultrasonic probe was used, which is more commonly preferred in industrial applications owing to its ability to produce much more acoustic power than an ultrasonic bath (Hoo *et al*., 2022). On the other hand, high-intensity ultrasound, with its low frequency (20–100 kHz) and high power density (10–1000 W/cm^2), is more destructive and has an important place in cell disruption in the industry (Hoo *et al*., 2022). Hence, ultrasound of 30 kHz and 100 W was applied. Additionally, Koda *et al*. (2009) showed that the logarithm of the survival ratio of *E. coli* and *S. mutans* against irradiation time at the frequency of 500 kHz decreased linearly, whereas, in our previous study by Sesal and Kekec (2014b), we observed a similar time-dependent effect for *E. coli* at the frequency of 30 kHz. At the same time, low-frequency ultrasound was observed to have a stronger effect on the removal of *P. mirabilis* biofilms (Mott *et al.*, 1998). Consequently, a statistically significant time-dependent increase in the mortality rate of *S. sanguinis* and *P. mirabilis* after ultrasonic irradiation with 30 kHz frequency and 100 W power was observed for all concentrations.

Also, the effect of specific energy-dependent ultrasonic irradiation on the survival curves of *P. mirabilis* and *S. sanguinis* bacteria was evaluated. The results revealed that the specific energy increased depending on the time and varied depending on the bacterial concentration of the sample. The specific energy was analyzed according to the time graphs for all concentrations: 5x10^{3}cfu/ml had eight-fold greater specific energy than 3x10^4 cfu/ml, and roughly four times greater than 1.5x10^4 cfu/ml. The concentration of bacteria decreased due to death, and the rate of increase of specific energy increased even more. Therefore, the specific energy increased much more at all concentrations between 20-30 minutes.

The logarithmic bacterial death rates increased time-dependently due to the decrease in concentration for both bacteria. In other words, the lower the bacteria density, the more effective ultrasound irradiation was. The increase in specific energy, which is directly proportional to applied power and the duration of the application, causes cavitation collapse induced ultrasonically, damaging the bacterial membrane. Therefore, the membrane fails to function and/or diffuse out its cellular content, and thus the cellular content increases in the solution (Yan *et al.,* 2010; Huang *et al*., 2017). Depending on the increase in bacterial aggregates, a time-dependent de-clumping process was observed after applying ultrasonic irradiation. Also, it was observed that the mortality rate for both bacteria dropped between the 10th and 20th minutes. Additionally, although the death rate at 3x10^4 cfu/ml concentration at the 20th minute was higher than that of the 1.5x10^4 concentration, it showed a 1.5x10^4 higher mortality rate at

the 30th minute. According to Joyce *et al*. (2003), sonication has two different effects in this case. Firstly, the bacterial clusters in the suspension are dispersed and turned into single bacteria; secondly, the killing of bacteria occurs with the breakdown of single bacteria, which are separated. Therefore, a competition between both killing and de-clumping bacteria in the solution brings about the overall effect of ultrasound.

The mortality rate increased between the20th and 30th minutes in both bacteria at all concentrations. When the sonication time ended at the 30th minute, the decreases were calculated as -0.77 log, -0.64 log, and -0.57 log for the $5x10^3$, $1.5x10^4$, and $3x10^4$ cfu/ml samples of *P. mirabilis*; -0.25, -0.15, -0.12 log for the $5x10^3$, $1.5x10^4$, and $3x10^4$ cfu/ml samples of *S. sanguinis*, respectively. *S. sanguinis* showed a lower rise in mortality rates in all samples compared with *P. mirabilis*. Chu *et al*., (2001) observed that after the 30th minute of sonication, the cell walls were almost completely damaged and that longer sonication times resulted in a total cell wall collapse. Furthermore, it is stated that cavitation affects inactivation by causing permanent damage to the cell wall, and thus, despite the use of high bacterial densities, inactivation percentages of > 99 percent were achieved after 45 minutes (Amabilis-Sosa *et al*., 2018). For this reason, mortality rates can be impacted by the cell wall structure of Gram-positive organisms, which normally has a thicker and more tightly adherent layer of peptidoglycan than Gram-negative organisms (Drakopoulou *et al*., 2009). Herein, when mortality rates were compared by Gram-status, it was determined that Gram-positive *S. sanguinis* had a lesser increase in death rates in all samples in comparison with Gram-negative *P. mirabilis*. In particular, the strong effect increased after 10 minutes in *P. mirabilis*, while it increased after 20 minutes in *S. sanguinis.* Amabilis-Sosa *et al*. (2018) attributed the higher mortality rate of Gram-positive *Bacillus subtilis* than the Gram-negative coliforms to the strong effect of cavitation due to the spacing between membrane layers of Gram-positive bacteria. Besides, in our previous study, Gram-negative *E. coli* were inactivated by time-dependent ultrasound, whereas Gram-positive *S. aureus* were not affected at all (Sesal and Kekec, 2014a). Similarly, Drakopoulou *et al*. (2009) found that Gram-negative bacteria, such as total coliforms, fecal coliforms, and *Pseudomonas* spp. were more affected by sonication than Gram-positive *Clostridium perfringens* and fecal streptococci. In addition, it has been observed that cavitation is more effective in cases of remodeling, weakening, or absence of the peptidoglycan structure (Pandur *et al*., 2022). On the other hand, according to Gao *et al*. (2014), microbes with a thicker and softer capsule, rather than the Gram-status, are more resistant to ultrasonic deactivation. Additionally, when the kill rates of Gram-negative bacteria *P. gingivalis* and Gram-positive bacteria *S. aureus* were compared, no significant relationship to the Gram-status was discovered (Kamineni and Huang, 2019). Some studies claimed that Gram-negative bacteria were more susceptible to ultrasound inhibition than Gram-positive bacteria, whereas others found no relationship between the Gram-status and ultrasonic inactivation. Further studies are needed to elucidate the reasons.

Conclusion

In conclusion, the sonication parameters utilized in the current paper are likely to be excellent for Gram-negative bacteria disinfection, especially when utilized for long periods. Nowadays, ultra-sonication is widely used in hospital sterilization. Ultrasonic cleaning units are common in clinical facilities, and are used on a variety of equipment, particularly dental and surgical instruments. Therefore, it is necessary to determine the process that will inactivate Gram-positive bacteria for more effective sterilization of devices used for such purposes. However, the effect of the different specific energy levels generated by ultra-sonication and the duration of sonication on microbial degradation needs to be studied more extensively.

Acknowledgments

This work was supported by Marmara University BAPKO Project No: Fen-B-130612-0222. We thank Yeditepe University, Microbiology Lab. for providing the strains.

References

Amabilis-Sosa, L. E., M. Vázquez-López, J. L. G. Rojas, A. Roé-Sosa, G. E. Moeller-Chávez (2018). Efficient bacteria inactivation by ultrasound in municipal wastewater. *Environments* **5**: 47.

Ananta, E., D. Voigt, M. Zenker, V. Heinz, D. Knorr (2005). Cellular injuries upon exposure of *Escherichia coli* and *Lactobacillus rhamnosus* to high-intensity ultrasound. *J. Appl. Microbiol.* **99**: 271-278.

Arrojo, S., Y. Benito (2008). A theoretical study of hydrodynamic cavitation. *Ultrason. Sonochem*. **15**: 203-211.

Caufield, P. W., A. P. Dasanayake, Y. Li, Y. Pan, J. Hsu, J. M. Hardin (2000). Natural history of *Streptococcus sanguinis* in the oral cavity of infants: evidence for a discrete window of infectivity. *Infect. Immun.* **68**: 4018-4023.

Chu, C. P., B. V. Chang, G. S. Liao, D. S. Jean, D. J. Lee (2001). Observations on changes in ultrasonically treated

waste-activated sludge. *Water Res.* **35**: 1038-1046.

Drakopoulou, S., S. Terzakis, M. S. Fountoulakis, D. Mantzavinos, T. Manios (2009). Ultrasound-induced inactivation of Gram-negative and Gram-positive bacteria in secondary treated municipal wastewater. *Ultrason. Sonochem*. **16**: 629-634.

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

Gibson, J. H., D. H. N. Yong, R. R. Farnood, P. Seto (2008). A Literature review of ultrasound technology and its application in wastewater disinfection. *Water Qual. Res. J. Can*. **43**: 23–35.

Gogate, P. R., A. M. Kabadi (2009). A review of applications of cavitation in biochemical engineering/biotechnology. *Biochem. Eng. J.* **44**: 60-72.

Huang, G., S. Chen, C. Dai, L. Sun, W. Sun, Y. Tang, F. Xiong, R. He, H. Ma (2017). Effects of ultrasound on microbial growth and enzyme activity. *Ultrason. Sonochem*. **37**: 144-149.

Hulsmans, A., K. Joris, N. Lambert, H. Rediers, P. Declerck, Y. Delaedt, F. Ollevier, S. Liers (2010). Evaluation of process parameters of ultrasonic treatment of bacterial suspensions in a pilot scale water disinfection system. *Ultrason. Sonochem*. **17**:1004–1009.

Hoo D. Y., Z. L. Low, D. Y. S. Low, S. Y. Tang, S. Manickam, K. W. Tan, Z. H. Ban (2022). Ultrasonic cavitation: An effective cleaner and greener intensification technology in the extraction and surface modification of nanocellulose. *Ultrason. Sonochem*. **90**: 106176.

Jansen, A. M., C. V. Lockatell, D. E. Johnson, H. L. Mobley (2003). Visualization of *Proteus mirabilis* morphotypes in the urinary tract: the elongated swarmer cell is rarely observed in ascending urinary tract infection. *Infect. Immun.* **71**: 3607-3613.

Jin, X., Z. Li, L. Xie, Y. Zhao, T. Wang (2013). Synergistic effect of ultrasonic pre-treatment combined with UV irradiation for secondary effluent disinfection. *Ultrason. Sonochem*. **20**: 1384-1389.

Joyce, E., S. S. Phull, J. P. Lorimer, T. J. Mason (2003). The development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power and sonication time on cultured *Bacillus* species. *Ultrason. Sonochem*. **10**: 315-318.

Kamineni, S., C. Huang (2019). The antibacterial effect of sonication and its potential medical application. *Sicot. J.* **5**: 19.

Koda, S., M. Miyamoto, M. Toma, T. Matsuoka, M. Maebayashi (2009). Inactivation of *Escherichia coli* and *Streptococcus mutans* by ultrasound at 500 kHz. *Ultrason. Sonochem*. **16**: 655-659.

Li J., J. Ahn, D. Liu, S. Chen, X. Ye, T. Ding (2016). Evaluation of ultrasound-induced damage to *Escherichia coli* and *Staphylococcus aureus* by flow cytometry and transmission electron microscopy. *Appl. Environ. Microbiol*. **82**: 1828–1837.

Loske, A. M., U. M. Alvarez, C. Hernández-Galicia, E. Castaño-Tostado, F. E. Prieto (2002). Bactericidal effect of underwater shock waves on *Escherichia coli* ATCC 10536 suspensions. *Innov. Food Sci. Emerg. Technol*. **3**: 321-327.

Madron, M. S., S. R. McClure, R. W. Griffith, C. Wang (2012). Absence of bactericidal effect of focused shock waves on an *in-vitro* biofilm model of an implant. *Can. J. Vet. Res.***76**: 129-135.

Mott, I. E. C., D. J. Stickler, W. T. Coakley, T. R. Bott (1998). The removal of bacterial biofilm from water-filled tubes using axially propagated ultrasound. *J. Appl. Microbiol*. **84**: 509-514.

Pandur, Ž., M. Dular, D. Stopar (2022). Bacterial cell wall material properties determine *E. coli* resistance to sonolysis. *Ultrason. Sonochem.* **83**: 105919.

Piyasena, P., E., Mohareb, R. Mckellar (2003). Inactivation of Microbes Using Ultrasound: A Review. *Int. J. Food Microbiol.* **87**: 207–216.

Schwartz, T., S. Hoffmann, U. Obst (2003). Formation of natural biofilms during chlorine dioxide and UV disinfection in a public drinking water distribution system. *J. Appl. Microbiol.* **95**: 591-601.

Sesal, N. C., Ö. Kekeç (2014a). Inactivation of *Escherichia coli* and *Staphylococcus aureus* by ultrasound. *J. Ultrasound. Med*. **33**: 1663-1668.

Sesal, N. C., Ö. Kekeç (2014b). Effects of pulsed ultrasound on *Escherichia coli* and *Staphylococcus aureus*. *Trans. R. Soc. Trop. Med. Hyg.* **108**: 348-353.

Thompson, L. H., L. K. Doraiswamy (1999). Sonochemistry: science and engineering. *Ind. Eng. Chem. Res.* **38**: 1215-1249.

Yan, Y., L. Feng, C. Zhang, H. Zhu, Q. Zhou (2010). Effect of ultrasonic specific energy on waste activated sludge solubilization and enzyme activity. *Afr. J. Biotechnol.* **9**: 1776-1782.

Zhang, H., S. Tsai, R. V. Tikekar (2021). Inactivation of *Listeria innocua* on blueberries by novel ultrasound washing processes and their impact on quality during storage. *Food Control*. **121:** 107580.

Zhu, B., L. C. Macleod, T. Kitten, P. Xu (2018). *Streptococcus sanguinis* biofilm formation and interaction with oral pathogens. *Future Microbiol.* **13**: 915-932.

Zupanc, M., Ž. Pandur, T. S. Perdih, D. Stopar, M. Petkovšek, M. Dular (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. *Ultrason. Sonochem.* **57**: 147-165.