Feasibilities of foam-forming bacteria disintegration by ultrasonic atomization

Petr Schovanec[1,*], *Darina* Jasikova[1], *Michal* Kotek[1], *Karel* Havlicek[2], and *Jakub* Eichler[3]

[1] Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Institute of New Technologies and Applied Informatics, Studentska 1402/2, Liberec 1, 461 17, Czech Republic
[2] Department of Environmental Technology, Technical University of Liberec, Institute for Nanomaterials, Advanced Technology and Innovation, Studentska 1402/2, Liberec 1, 461 17, Czech Republic
[3] Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Institute of Mechatronics and Computer Engineering, Studentska 1402/2, Liberec 1, 461 17, Czech Republic

Abstract. This article presents the feasibilities of bacterial disintegration using ultrasonic atomization. Ultrasound creates acoustic cavitation, which by its effects represents a high gradient of pressure in the form of a shock wave and a local force effect. In our case, the ultrasonic excitation produces a fine micron mist of 3 micrometers. The generated microcavities create a cavitation cloud that is able to disintegrate the foam-forming bacteria or even inactivate bacterial cells. The main goal of the research is the use of ultrasound to disintegrate the foam-forming bacteria during the impact of acoustic cavitation on living cells.

1 Introduction

The cavitation phenomena can be defined as a collection of effects connected to the origin, activities, and collapse of macroscopic bubbles in a liquid [7-10]. There are many ways to generate bubbles. For example, a rotating propeller, a vibrating surface, local deposition of energy, electrical discharge, or ultrasound. In our case, the cavitation bubbles are generated by ultrasound. Ultrasound usually leads to forming a cavitation cloud where the bubbles are not separated. These bubbles create structures that react collectively; however, individual bubbles are fundamental elements of these structures [3,4].

The research in the field of cavitation previously focused mainly on the investigation of shock waves, bubble behavior in the surrounding of solid surface, and the bubble behavior in variously viscous liquids [1-3]. However, the current investigations also focused on the use in the field of foam-forming bacteria disintegration to be included.

The advantage of using ultrasound is the absence of other chemicals that otherwise need to be added to the system. Foam-forming bacteria is disintegrated in a physicomechanical way, and with suitable settings of operating frequencies and ultrasound intensities, it is a very gentle method.

2 Experimental

The experimental part is based on previous research [5, 6], which contains bubble interaction surrounding of solid surface.

In this experiment, we used the fine micron mist and its microcavities phenomena to disintegrate foam-forming bacteria. The main potential is in use in wastewater treatment plants. Previously, bacteria sterilization research focused on using cavitation to defoam and sterilize the environment [14].

The experiment contains a laboratory power supply, oscillator, and piezoelectric transducers. The laboratory power supply powers the oscillator, which generates a signal with a frequency of 1.65 MHz. This signal is conducted to piezoelectric transducers, which generate ultrasonic waves. Piezoelectric transducers are placed at the bottom in a vessel containing 1.4 liters of water and foam-forming bacteria from a wastewater treatment plant. The setup of the whole experiment is seen in Fig. 1.

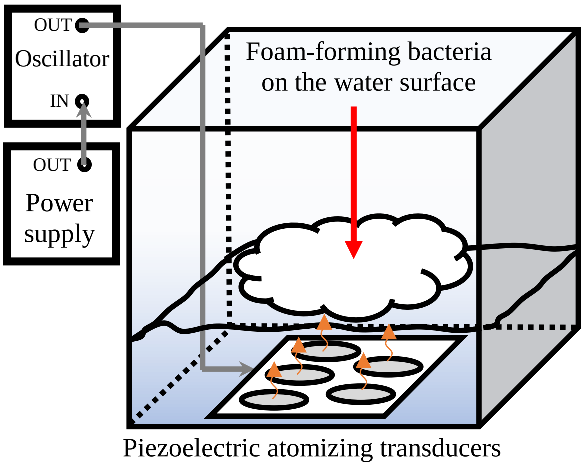

Fig. 1. The experimental setup.

---

[*] Corresponding author: petr.schovanec@tul.cz

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (http://creativecommons.org/licenses/by/4.0/).

2.1 Effects of ultrasonic

Thermal, mechanical, or physicochemical effects occur during the ultrasound. The thermal effects occur when ultrasound passes through living tissue due to energy absorption, leading to the tissue's heating. It happens mainly at the tissue interface but also when passing through homogeneous tissue. The level of the energy absorption depends on the frequency of the ultrasound. Absorption and dispersion in tissues increase with increasing frequency [10].

The mechanical effects of ultrasound can include cavitation, in which rapid pressure changes occur due to the compaction and dilution of the environment, i.e., the oscillation of molecules. During this physical phenomenon, vacuum cavities are formed in flowing fluids or places of fluids with rapid pressure changes, which by their extinction damage cell structures.

While during the physicochemical effects of ultrasound are accelerated chemical reactions due to the excitation of molecules. Physicochemical effects in combination with thermal effects cause local heating of the tissues and thus lead to increased blood flow to the tissues and accelerated metabolism.

2.2 Underwater ultrasonic transducer

The behavior of the ultrasonic transducer located underwater at the bottom of the vessel is seen in Fig. 2. The behavior of the transducer can be easily influenced by two parameters, the power input of the transducer or the height of the water level. Here we have chosen several different water level heights. The ultrasonic wave directed at the water surface causes a water column to disintegrate into water particles and mist.

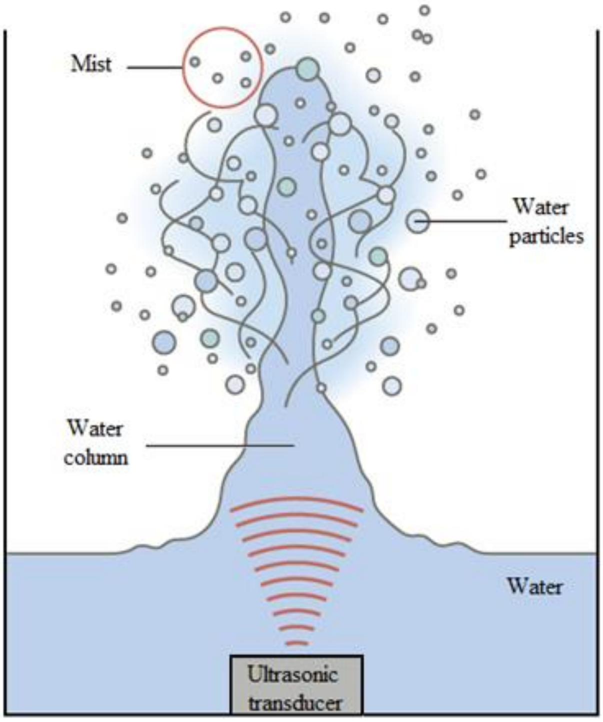

Fig. 2. The behavior of the ultrasonic underwater.

2.3 Parameters of ultrasonic transducers

In addition to their size and diameter, the main parameters of ultrasonic transducers include resonant frequency, resonant impedance, capacitance, average water droplet size, and maximum input power can be seen in Table 1.

Table 1. Basic parameters of ultrasonic transducers.

Producer and Type	Midas Com., Ltd. M165D25	APC Int., Ltd. #50-1014
Resonance Frequency	1.65 MHz ± 3%	1.65 MHz ± 10%
Resonance Impedance	2 Ω	< 5 Ω
Capacitance @ 1kHz	2 nF ± 20%	1.9 nF ± 30%
Dissipation Factor @ 1kHz	0.5% max.	< 0.5%
Input Power (maximum)	30 Watt	29 ± 1 Watt
Average Water Droplet Size	3 µm	2 µm

2.4 Foam-forming bacteria

Foam at wastewater treatment plants containing a mixture of fibrous bacteria (Microthrix, Gordonia, Skermania) was used to test the effect of ultrasonic micro-cavitation on the vitality of these bacteria. Fibrous bacteria in the foam are highly resistant microorganisms, where their removal at wastewater treatment plants is necessary. Monitoring the influence of various parameters (settings) of generated a fine micron mist on these bacteria gives an idea of their viability under given conditions in a particular environment [11-13].

The foam composed of bacteria and resistant interconnected structures easily creates a clearly defined transition with the aqueous environment. This complex structure behaves similarly to a partially solid surface. In view of this fact, the behavior of ultrasonic micro cavitation bubbles can be expected to impact a solid surface described in more detail by [5].

We focused on the behavior of the ultrasonic micro cavitation concerning the disintegration of foam-forming bacteria on the water surface, both in terms of visualization and the study of the behavior of ultrasonic transducer submerged in the liquid, which causes locally accelerated by vibration.

3 Results

Several different water level heights were tested. Ultrasound was generated towards the foam-forming bacteria placed on the water surface. Ultrasound creates acoustic shock waves followed by an increasing cavitation bubble. The action of ultrasound leads to the disintegration of the foam-forming bacteria. The magnitude of the impact of ultrasound on the foam-forming bacteria depends on the distance from the water surface.

Ultrasound has minimal effect on foam-forming bacteria over long distances because the foam has only been moved away from the action of ultrasound. When ultrasound acts on foam-forming bacteria over shorter distances, it was disintegrated to a certain extent. The most significant disintegration effects were recorded at a length of two centimeters. We had performed PIV measurements for this distance, as shown in Fig. 3 and Fig. 4.

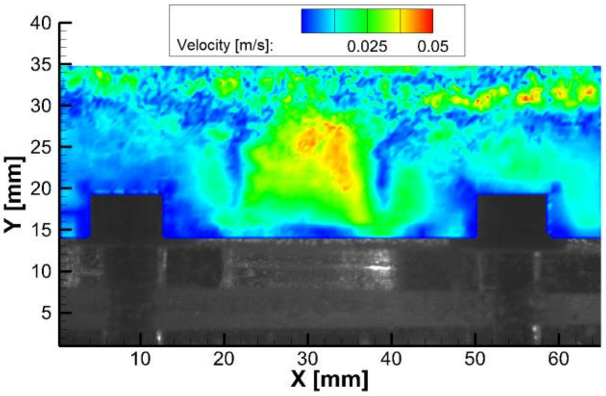

Fig. 3. Scalar map of velocities obtained using the PIV method.

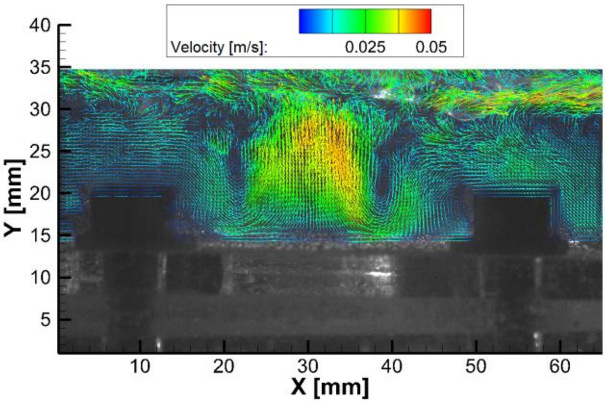

Fig. 4. Vector map of velocities obtained using the PIV method.

Fig. 3 and Fig. 4 shows the liquid's flow velocities from the one ultrasonic transducer towards the water surface on which the bacterial foam is spread. We can infer that the flow velocities between the ultrasonic transducer and the water surface reach values of about 0.04 $m \cdot s^{-1}$. It is important to emphasize that the device contains five piezoelectric atomizing transducers located at the vessel's bottom.

Fig. 5 shows the foam-forming bacteria on the water surface before the ultrasonic cavitation. The ultrasound transducer was set at a frequency of 1.65 MHz and was applied to the foam-forming bacteria for one minute. Fig. 6 shows foam-forming bacteria after ultrasound application, which can be observed as affecting fibrous bacteria's viability.

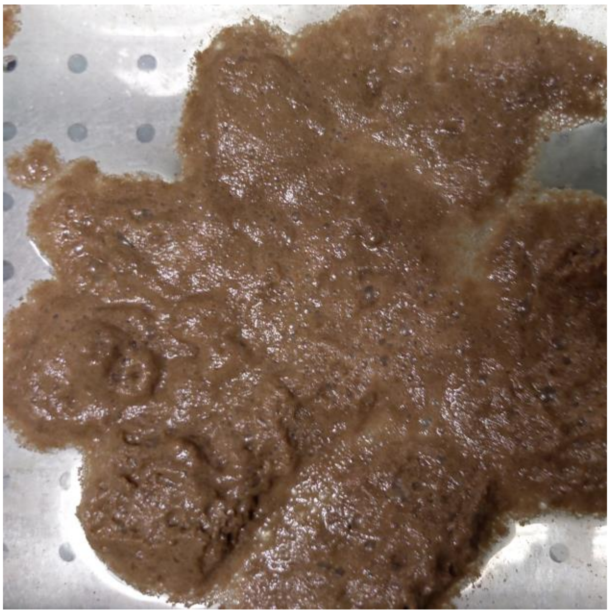

Fig. 5. The photograph was taken before ultrasonic cavitation.

Fig. 6. The photograph was taken after ultrasonic cavitation.

Despite these results in Fig. 6, whether the bacteria were wholly eliminated was uncleared. We evaluated live and dead bacteria for this reason. The evaluation was done using confocal microscopy of foam-forming bacteria. The green color indicates live and red color dead bacteria (Microthrix, Gordonia, Skermania) contained in the foam-forming bacteria in Fig. 7 and Fig. 8.

Fig. 7 shows the record of live/dead confocal microscopy. It's a reference sample of a foam-forming bacteria that was not affected by ultrasound. While Fig. 8 shows the effects of five ultrasonic transducers after one minute of operation, and as can be seen, many bacteria have been eliminated.

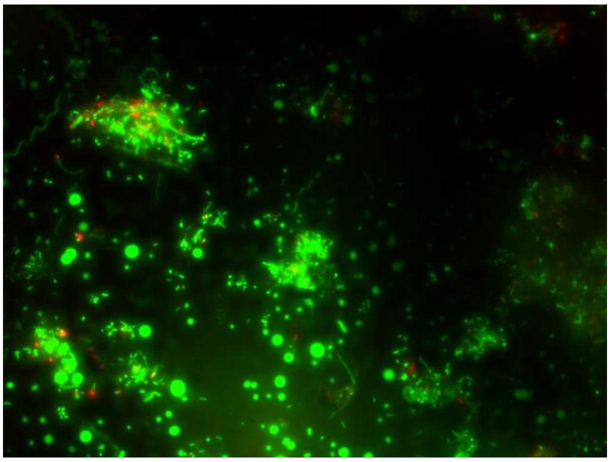

Fig. 7. The evaluation of live/dead bacteria, reference image before the ultrasound.

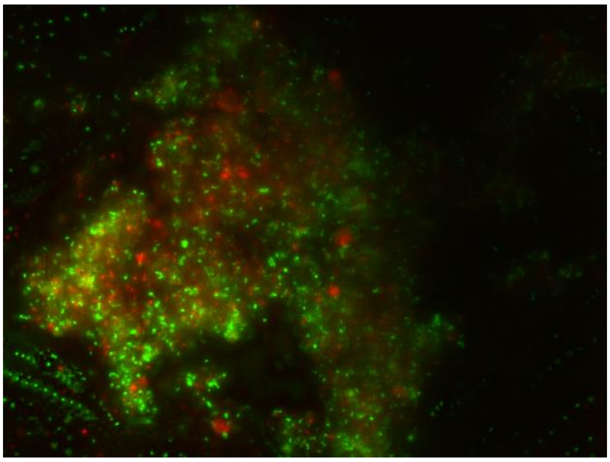

Fig. 8. The evaluation of live/dead bacteria after one minute of ultrasound.

4 Conclusion

Visualization is an essential and helpful tool in the study of fluid flow. Here we used the PIV method to calculate the flow velocity, which is shown using a vector field. Furthermore, the interaction of flow with the water surface was determined.

Ultrasonic cavitation was used to disintegrate foam-forming bacteria and determine the system's most suitable and effective input parameters. The viability of the foam-forming bacteria (Microthrix, Gordonia, Skermania) depending on the properties of the setting ultrasound parameters were studied.

During our research, it was shown that the ultrasonic effect followed by cavitation disintegrates bacteria. The cavitation effect plays an essential role in the disintegration of bacteria because we were used very low power of acoustic intensity; the result was totally evident in Fig. 6 and Fig. 8.

The effect on the viability of the fibrous bacteria was most pronounced at a distance of 2 cm. Fiber viability was not wholly suppressed, but we were observed bacterial deformation and total loss of viability.

Acknowledgements.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic and the European Union - European Structural and Investment Funds in the frames of Operational Programme Research, Development and Education - project Hybrid Materials for Hierarchical Structures (HyHi, Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000843), and this work was partly supported by the Student Grant Competition of the Technical University of Liberec under the project No. SGS-2020-3059.

References

1. D. Jasikova, *Proceedings of SPIE*, **10151,** (2016)
2. D. Jasikova, *EPJ Web of Conferences*, **143,** (2017)
3. D. Jasikova, *EPJ Web of Conferences*, **180**, (2018)
4. P. Schovanec, W. Garen, *ACC Jrnl* **25**, 58 (2019)
5. P. Schovanec, D. Jasikova, M. Kotek, V. Kopecky, *Proceedings of conference EFM 2019*
6. P. Schovanec, D. Jasikova, *MATEC Web of Conf.*, **328**, 05003 (2020)
7. Ch. E. Brennen, *Cavitation and bubble dynamics*, (Cambridge University Press, 2015)
8. P. K. Kennedy, *Prog.QuantElectr*. **21**, 3, (1997)
9. Vogel, A., *App. Physics B: Lasers and Optics* **68**, 271-280 (1999)
10. W. Lauterborn, *Archives of Acoustics* **33**, (2008)
11. K. Havlicek, L. Svobodova, *Materials & Design*, **194**, (2020)
12. J. Nemecek, M. Nechanicka, et al. *Chemosphere*, **237** (2019)
13. K. Havlicek, M. Nechanicka, *VTEI*, **62**, (2020)
14. J. B. Winterburn, P. J. Martin, *Asia-Pacific Jrnl of Chem. Eng*, **4**, 184-190 (2009)