Current state of research on the effect of cavitation on microorganisms and viruses

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Abstract. Cavitation, both hydrodynamic and acoustic, is a promising technology for non-thermal treatment of liquid products in order to inactivate microorganisms and viruses. Aggressive conditions and high energy density per unit volume of the treated substance allows to sterilize liquid products, destroying also pathogenic microorganisms. This paper presents a mini-review of the current state of research in the field of studying the mechanisms of destruction of the cellular structure of microorganisms and viruses. Chemical and physical mechanisms of destruction of the cell structure of microorganisms and viruses are evaluated. Recommendations for further research are presented.

1 Introduction

The 20th century was marked by a boom in demand for a variety of consumer goods, ranging from food products to pharmaceuticals and other industries. The increased demand predetermined a number of problems and tasks to be solved. Industrial enterprises faced the tasks of improving the quality of products, reducing the cost of manufacturing products, increasing the environmental friendliness of production. Especially relevant for industry is the problem of finding a non-heat technology for processing liquid food products, allowing to preserve vitamins, flavour and overall quality of the product. The boom in industrial growth has also been marked by the emergence of a serious threat of pollution of the world's clean water reserves. The problem of wastewater treatment is not only the need to remove solid and chemical impurities, but also the removal of various viruses and microorganisms, including bacteria, which can cause serious infections, and microalgae, which cause algal blooms (release toxins into the aquatic environment). At the same time, the presence of many microorganisms in various liquid substances has negative consequences for humans.

All currently existing methods of pathogenic microorganisms as well as viruses elimination have some limitations, such as high cost of cleaning, duration of treatment, the need for a large infrastructure requiring high temperature, filtration and sedimentation, or directly the disinfection process itself, including the use of chemicals, leads to the formation of new toxic chemical compounds in the treated liquid product. An advanced and environmentally safe in these conditions can be the process of cavitation treatment.

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Cavitation is a rather actively researched technology for wastewater treatment and disinfection and can become an alternative to already implemented technologies. Cavitation has confirmed its efficiency in various fields, including oil products treatment [1,2], food industry [3], biomedical areas [4]. The slamming of cavitation bubbles, creating local pressure and temperature increases, can effectively destroy many chemical compounds. The main reactions occurring in the cavitation zone are thermal decomposition of chemical compounds, as well as oxidation by oxygen [5]. The great interest in this topic is supported by the increasing number of publications devoted to a number of aspects related to the formation of cavitation phenomena and their application to water and wastewater treatment, as well as hybrid processes based on external oxidizers that provide efficient formation of radical forms under cavitation conditions.

2 Main part

2.1 Physical and chemical effects of cavitation

At present, a large number of studies of microbiological sterilization of liquids using cavitation have been conducted. The mechanism of energetic effect of cavitation is the creation of high energy density per unit volume of substance. During cavitation, regardless of its type, including acoustic or hydrodynamic, small caverns are formed in the treated medium, which undergo successive stages of growth, compression and subsequent collapse. At the moment of collapse, the gas pressure and temperature reach significant values, according to some data reaching 100 MPa and 1000 °C, respectively [6]. This phenomenon is explained by the small volume of matter at the moment when the bubble reaches its minimum radius before collapse. Based on the results of scientific studies [7-19], it can be stated that the radius of a cavitation bubble at the moment of collapse can reach 10^{-7}-10^{-8} m as a rule, against the radius in the equilibrium state of $10 \cdot 10^{-6}$ m. The change in the volume of the cavitation bubble, reaching thousandths, leads to the achievement of high values of stored energy. For cavitation generation, the most common Venturi tubes (Fig. 1) are often used for hydrodynamic cavitation generation, or various acoustic emitters for acoustic cavitation. Less popular in microbial inactivation studies are vortex generators (Fig. 2) or vortex bed activators (Fig. 3). Extreme temperatures can disrupt the integrity of the outer layer of cells, making them susceptible to substances.

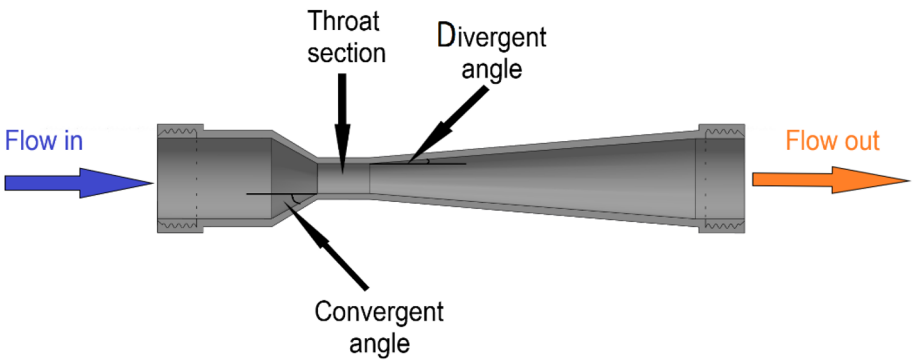

Fig. 1. Venturi ring tube configurations [10]

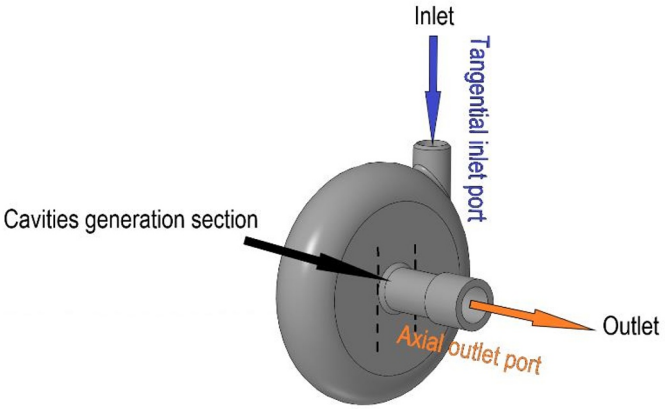

Fig. 2. Vortex apparatus configurations [11].

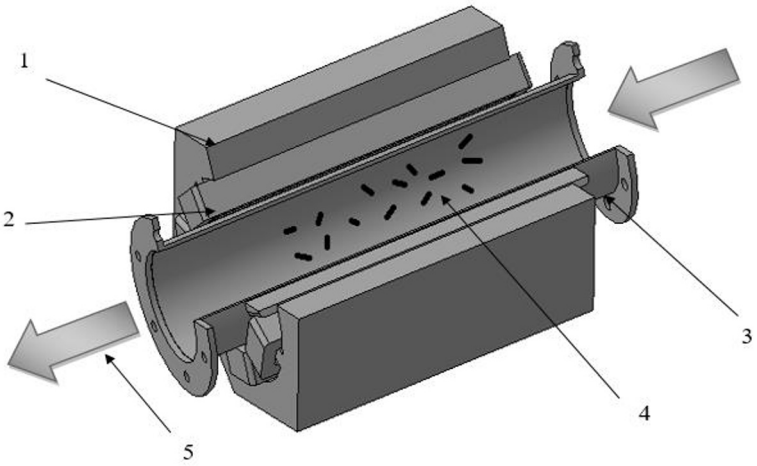

Fig. 3. Design of electromechanical converter with a secondary moving part: 1 - inductor; 2 - 3-phase distributed winding; 3 - tube of nonmagnetic material; 4 - set of ferromagnetic elements; 5 - flow of processed material [2].

When hydrodynamic cavitation is applied, extreme conditions occur in the raw material to be treated, resulting in shock waves, liquid microjets, flow turbulence and high shear forces. Microjets are formed by bubble collapse and possess high energy and are capable of impacting even metal surfaces. According to a study [12], the velocity of solid particles in a cavitation field can reach 100 μm/s.

In addition to the above-mentioned, acoustic cavitation produces a number of additional physical effects, including microflows. In ultrasonic cavitation through the action of acoustic oscillations in the treated liquid, streams are created that oscillate from a piezoelectric transducer. The created flow in different directions is accompanied by mass transfer effects [13-15]. Streamer development during cavitation bubble evolution is carried out under the influence of primary and secondary Bjerknes forces [16].

Chemical effects are inherent to both acoustic cavitation and hydrodynamic cavitation and are associated with the splitting of water molecules and the formation of free radicals (OH and H) [17]. At the moment of compression of the cavitation bubble, the pressure and temperature inside the bubble increase to critical values. Inside the heated bubble, water

vapor and oxygen dissociate and oxidizing agents such as OH radicals, O radicals, H_2, O_2 and O_3 are formed inside the bubble [18,19]. The formed radicals diffuse from the bubble into the treated medium where they react chemically with the contained substances [20-22], including the formation of H_2O_2, which is an effective antiseptic agent. In the presence of other dissolved gases in the treated raw materials, other radicals can be formed.

2.2 Mechanism of cavitation effect on microorganisms

The inactivation of microorganisms by cavitation, using ultrasound as an example, was first reported in 1928 [23], when the dimming of luminescent bacteria was recorded when exposed to high-frequency sound waves. The mechanism of microbial inactivation, according to several studies, varies not only with the type of microorganism but also with the associated treatment parameters such as temperature, ultrasonic frequency and acoustic power for acoustic cavitation and flow velocity for hydrodynamic cavitation. A comparative ultrasound treatment of two microorganisms (Escherichia coli and Klebsiella pneumonia) in physiological solution with phosphate buffer was reported in [24]. According to the results of experimental studies at ultrasound treatment inactivation of microorganisms and reduction of their number is observed, and the degree of effectiveness depends on the intensity and frequency of ultrasound.

In [25] and [26] the results of comparative studies of the efficiency of microorganisms inactivation by simultaneous exposure to ultrasound and pressure (manosonification (MS)) and the combined method of heating, ultrasound and pressure (manothermosonification (MTS)) are presented. The inactivation rate of vegetative cells in MS increased with increasing static pressure [27,28]. The inactivation rate of microorganisms remained constant with temperature fluctuations in the low temperature range. At high temperatures, the inactivation rate increased dramatically [27].

In order to elucidate the mechanism of cavitation effect, the authors of [29] conducted experimental studies of the effect of ultrasonic cavitation on algae (haetoceros gracilis, Chaetoceros calcitrans and Nannochloropsis sp), microcapsules and plankton. In this work, microcapsules (pseudo-algae) and plankton were used to evaluate the inactivation rate at different frequencies and to observe the mechanism. According to the results, coincident inactivation frequencies were found for algae and microcapsules, which confirmed mechanical resonance from nearby collapsing bubbles as one of the causes of algae damage. The plankton damage confirmed the presence of shear mechanism, which is the local effect of cavitation bubble collapse. Also in this work, an important conclusion was made, according to which for each species of microorganism it is necessary to select a different frequency of ultrasound.

The influence of ultrasound frequency was also confirmed in [30], where the authors evaluated the efficiency of ultrasound destruction of algae Chaetoceros gracilis, Chaetoceros calcitrans and Nannochloropsis sp. The researchers found that suitable frequencies of ultrasound oscillations are related to the mechanical properties of cells, and the physical effects of ultrasound exposure, rather than chemical effects, are responsible for cell destruction.

Hydrodynamic cavitation also has an effective effect on the process of inactivation of microorganisms. In general, hydrodynamic cavitation has a number of advantages over acoustic cavitation, the most important of which is the possibility of processing the target material in the flow mode and compliance with the scaling of the process to industrial scale, as well as lower cost of organization of the technological process.

Inactivation of microorganisms is caused by the combined chemical and physical effects on cell destruction. At the same time, according to [31] in acoustic cavitation, chemical processes play a more important role in the inactivation process, while in the case of

hydrodynamic cavitation, disinfection is primarily associated with mechanical destruction. Mainly, the reduction of the influence of chemical action in hydrodynamic cavitation is related to low frequencies and the occurrence of cavitation bubbles of large sizes.

Thus, previously shown numerous studies have confirmed the effectiveness of the effect of both acoustic and hydrodynamic cavitation on the inactivation of microorganisms. At the same time, despite the high intensity of scientific research, the understanding of the mechanisms of microbial inactivation at the fundamental level remains insufficient. A number of early studies presented conclusions that shear forces induced by bubble collapse cause deformation of nearby highly pliable biological structures, which can lead to both local pore formation in the bacterial cell membrane and complete destruction of the liposome [32].

Nevertheless, the world of pathogenic microorganisms is represented by a wide variety of waterborne bacteria, which vary in shape from simple spherical to bacillary and spiral, with sizes ranging from 0.5 to 5 μm [33], which will undoubtedly influence the degree of deformation from shear forces during the collapse of cavitation bubbles. At the same time, various bacteria possess a complex cell envelope with rigid membranes capable of resisting internal pressure, but also capable of adapting to a wide range of external influences and elastically deforming upon impact, subsequently returning to their original shape [34].

To assess the mechanical damage of bacterial cells by cavitation bubble collapse, the authors of the study [32] conducted a study of cavitation bubble interaction on a microscale. The authors' modeling confirmed the postulate that in the absence of thermal and chemical effects, the damage of bacterial cells can be explained solely by mechanical effects. At the same time, the temperature impact during cavitation bubble collapse is the least probable mechanism of cell structure destruction. The results of modeling [32] also show that microflows formed during cavitation bubble collapse are the main mechanism responsible for the destruction of more pliable cells and when the bubbles are the same size as the cells.

The results of [32] are also consistent with earlier work [35,36] where microflows were responsible for the creation of pores in the cellular structure. Shock waves have the greatest effect on cells that have more rigid characteristics or in cases where the vesicles are significantly larger than individual bacterial cells [32].

The authors [32] drew the main conclusions from their modeling studies that both the geometric parameters of bubble-bacterial interaction (distance, size ratio) and the mechanical properties of bacterial cells determine the predominant mechanism of cell damage, and there is no clear relationship between the different modes of bubble collapse and the mechanisms of cell damage.

2.3 Mechanism of cavitation effect on viruses

The COVID-19 pandemic has intensified scientific research in the field of studying advanced methods of water purification from potential pathogenic viruses. In [35], studies of the effect of hydrodynamic cavitation on the process of inactivation of bacteriophage phi6 (enveloped virus), used in studies as a surrogate for SARS-CoV-2, were conducted. Through experimental studies, it was observed that the inactivation of envelope viruses is always slower at 20°C and increases at 30°C. In [36], it was observed that it took only 48 hours to reduce the viruses to 5 logs at 30 °C medium temperature, whereas it took 6 days to achieve comparable virus reduction at 22 °C medium temperature. In [37], it was previously hypothesized using the example of MS-2 virus inactivation in water by hydrodynamic cavitation that the formation of OH radicals during hydrodynamic cavitation treatment due to the presence of high shear forces was responsible for virus inactivation. According to the authors' conclusions [37], the OH radical can damage the recognition receptors located on the bacteriophage surface. However, in subsequent studies these conclusions were not confirmed, so in the study [35], when viruses were exposed to hydrodynamic cavitation, OH

radicals did not significantly affect the inactivation rate. To confirm these results, radical scavengers were added to the medium during the treatment process, which did not affect the inactivation rate. These results were confirmed in a study [38] on inactivation of plant viruses by hydrodynamic cavitation.

It should also be noted that in a number of scientific studies, including [39], it was confirmed that the presence of OH radical has no effect on the intensity of virus inactivation.

Despite a number of experimental studies confirming the effectiveness of cavitation for virus inactivation, the mechanism of the inactivation process remains unknown. To date, the main mechanisms include damage to the capsid itself or recognition receptors on the capsid surface, formation of OH radicals under the influence of shock waves, and local temperature and pressure increases during the collapse of cavitation caverns.

The difficulty in determining the cavitation mechanism is hampered by the uncertainty of the process of creation and development of cavitation in different geometries of the system. For example, in an experimental study [40], in a comparative analysis of two types of units with a treated volume of 3 mL and 1 L, respectively, the virus inactivation efficiency was greater for the larger reactor, at comparable flow rates.

In addition to issues related to the volume of product treated, the intensity of virus inactivation is also related directly to the type of virus and medium. The authors of the study [41] evaluated the effect of ultrasound on three surrogates of foodborne viruses, including murine virus (MNV-1), feline calicivirus (FCV-F9), and bacteriophage MS2. The viruses were diluted in saline solution and orange juice. The results showed that the efficacy of ultrasound exposure depended on the virus type, initial virus titer, and virus suspension solution, with virus inactivation in orange juice being significantly lower than in saline.

3 Discussion

The results of experimental studies of most scientific publications prove the possibility of effective application of both acoustic cavitation and hydrodynamic cavitation for inactivation of microorganisms and viruses. Nevertheless, the study of the mechanisms of influence is difficult for two main reasons, including the difficulty of parametric study of the cavitation process itself, as well as the excellent dependence of types of microorganisms and viruses on chemical and mechanical influences.

The assessment of mechanical effects is primarily hampered by the difficulty of varying the parameters of cavitation intensity. If in the case of acoustic cavitation the mechanism can be studied by changing the frequency of exposure, thus regulating the cavitation intensity in the target area, in the case of hydrodynamic cavitation the intensity is regulated by changing the design of the cavitation generator and the conditions of the experimental study.

At the same time, the physical effects of cavitation themselves largely depend on the process of formation of cavitation bubbles, their sizes, collapse velocity, etc. It is the size of cavitation bubbles at different stages of its evolution (initial radius, maximum achieved radius and bubble radius directly at the moment of collapse) that are of the utmost importance. It is also worth noting that in laboratory studies to evaluate the effects of cavitation, the authors of studies often use standard parameters of both the medium and external effects. As a medium they often use either distilled water or isotonic solutions, occasionally in some studies they use microorganism nutrient medium, and standard expressions of dimensionless cavitation number are used for cavitation assessment. Nevertheless, the multifaceted nature of the parameters influencing the process of cavitation creation, including the concentration of dissolved salts, the presence of solid and gas impurities, the medium temperature, the rheological properties of the medium, etc., are not taken into account in the expressions of the dimensionless cavitation number, distorting the results of laboratory studies [42,43]. In [44], m-PSi nanoparticles were added to the aqueous suspension during the preparation

process. The acoustic cavitation index was 2-5 times higher compared to pure water, which significantly increased the reduction of bacterial viability. The m-Psi nanoparticles are able to trap small air bubbles on their surface, which increase the cavitation intensity when exposed to ultrasound. On the other hand, the use of different media, despite the possibility of a more detailed assessment of the effect of cavitation in media close to natural, requires scientists to adjust the cavitation intensity according to changes in the rheological properties of the liquid and the concentration of impurities, but the applied cavitation numbers practically do not allow taking into account the properties of the liquid. Thus, based on the above, the first problem arises, which consists in the method of evaluating the effect of cavitation on the intensity of disinfection and the degree of destruction of cellular structure using the calculation of cavitation number. Taking into account the selected parameters for each experiment, the environment in which there are microorganisms that significantly affect the resistance of microorganisms, we can talk about the inexpediency and unreliability of linking in this way the number of cavitation and the effectiveness of antimicrobial action.

Most scientific papers on the effects of acoustic cavitation often apply the standard and most common frequency of ultrasonic oscillations of 20 kHz. Nevertheless, the analysis of scientific literature allows us to assert that the frequency cannot remain standard for different microorganisms. For example, [45] stated that the inactivation of Nannochloropsis sp. was highest at 4300 kHz ultrasound frequency and lowest at 400 kHz, C. gracilis was highest at 3300 kHz and lowest at 400 kHz. On the other hand, scientists may often apply different cavitation intensities and use only one group of microorganisms as a test subject [46,47], whereas the literature repeatedly shows a difference in the effect of cavitation on microorganisms with different cell wall structure. This makes it difficult to compare different cavitation units with each other.

4 Conclusions

The presented mini-review analyses the effectiveness and extent of the study of the technology of inactivation of microorganisms and viruses by cavitation. Despite significant progress in the study of technology of inactivation of various types of microorganisms and viruses, the understanding of inactivation mechanisms at the fundamental level is lacking. Various hypotheses from the influence of oxidative processes by oxygen radicals to mechanical damage by shear forces or microflows during cavitation bubble collapse are presented. It is also worth noting that rotary hydrodynamic cavitation generators and vortex layer activators are neglected in fluid inactivation studies. Nevertheless, in studies related to liquid treatment in other industries, e.g. in the oil refining industry, such installations have shown more significant results in comparison to Venturi tubes or acoustic cavitation generators.

The contradictory efficiency of the effect of physical and chemical mechanisms on the destruction of cellular structure is related not only to the type of microorganism or virus, but also to the intensity of cavitation, which in turn depends on a large number of factors, including the rheological properties of the medium, the amount of dissolved salts, gas and solid impurities and their type, the temperature of the medium, etc. As it was said above, aggressiveness of cavitation, including the value of shear forces, as well as the radius of bubbles have a direct impact on the efficiency of inactivation of microorganisms and viruses. At the same time, the properties of the medium and parameters of the cavitation generator will directly influence both the intensity and aggressiveness of cavitation and the size of the created bubbles. The high degree of mutual influence of factors of the process of inactivation of microorganisms and viruses from the parameters of intensity and aggressiveness of cavitation puts first of all before researchers the necessity of revealing the mechanisms of influence of medium parameters on cavitation intensity and linking it with the mechanism of

influence on cell structure. Since the efficiency of cavitation in destruction of microorganisms is proved, for the greatest organisation of these experimental studies it is necessary to pass from direct assessment of cavitation influence on inactivation of certain microorganisms to the design of experimental studies implying assessment of separate chemical and physical mechanisms on the degree of cell destruction.

Such experimental studies will make it possible to find the relationship between bubble size, bubble life rate and bubble collapse with the mechanisms of destruction of the cell structure of certain microorganisms, allowing to design cavitation generators and adjust the technological process to create cavitation with the required intensity and aggressiveness.

Acknowledgements

The research was supported by the Russian Science Foundation grant No. 23-29-00972, https://rscf.ru/project/23-29-00972/

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