Investigation of the effects of cavitation on different media in a device with a discrete secondary

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Abstract. Preserving food and improving the quality of drinking water are of paramount importance to the public. Despite the very different objectives of food preservation and drinking water disinfection, these problems have in common the destruction of microorganisms. In this regard, the actual problem, which has been the focus of research in recent years, is the development of new technologies capable of non-thermal treatment of liquid substances and foodstuffs and not using chemical reagents. Hydrodynamic cavitation can be a promising technology for non-thermal treatment of liquid substances, including wastewater and food liquid products. This paper presents the results of our own study of the effect of cavitation on microorganisms under given conditions in a device with a discrete secondary part.

1 Introduction

So far, hydrodynamic cavitation has shown promising characteristics and industrialization potential into various industrial processes: water purification [1], sludge disintegration [2], emulsification [3], biomass pretreatment [4], food processing [5], petroleum and petroleum product treatment [6], petroleum desulfurization [7], cellulose slurry treatment, etc. In addition, the conditions created by hydrodynamic cavitation make it suitable for effective synergy with other intensification processes including ultrasound, chemicals, plasma, electrochemistry, etc.

Today, the search and development of non-thermal methods of antimicrobial action on liquid substances is actively developing. For many years, the main methods of antimicrobial action on liquid products have traditionally been either thermal methods for food processing or chemical methods, for example, for wastewater disinfection. Thermal methods, common in the food industry, have significant disadvantages in terms of deterioration of product quality due to vitamin destruction as well as flavor alteration [8]. Wastewater disinfection is performed due to its danger to human health due to the presence of various pathogenic microbes in it. Being the most important component and source of life on earth, water can also be hazardous to health when contaminated. The disadvantages of common methods associated with exposure to various chemical compounds, such as chlorine, are related to

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both incomplete disinfection, the formation of unsafe organochlorine compounds, and high cost, for example, when using bromine and iodine.

2 Main part

2.1 Investigation of the impact of cavitation on various media

Cavitation is a fairly actively researched technology for wastewater treatment and disinfection and can be an alternative to already implemented technologies. The slamming of cavitation bubbles, creating localized 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. [9] 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.

In [10], the effectiveness of hydrodynamic cavitation generator for sludge disintegration was investigated. According to the results presented in the work, the particle size reduction after treatment was 88%. Analyses of water samples confirmed the damage of Epistilys yeast cells.

Various radicals are formed in water when the liquid medium is affected by hydrodynamic cavitation [11]. The destruction of organic compounds in this case can occur in three places, including inside the bubble (gas phase), at the gas-liquid interface.

For disinfection of various sources of drinking water, both thermal methods and methods associated with water treatment with chlorine are common. A breakthrough in the development of new unique systems of water disinfection by non-thermal methods was the study of the effects of cavitation. At the same time, scientists mainly studied the antibacterial effect of acoustic cavitation [12].

The main factor, according to the members of the scientific team, reducing the intensity of the study of antimicrobial effects is the lack of a common understanding of cavitation in certain devices. In each separate research, each separate team often use their own modifications of hydrodynamic cavitation generators. For example, in the absolute majority of works either Venturi tubes or tubes with diaphragms are used. In [13], scientists noticed that depending on the nozzle geometry, the inactivation rate of microorganisms changes.

This is not surprising, since the number of orifices in the diaphragm, while keeping the flow velocity at the appropriate required level, provides a higher cavitation intensity over a larger volume of the working chamber. At the same time, the use of tubes with or without diaphragm but with a narrowing neck requires a high-pressure pump. In the pump at rotation of the blades may already be present some cavitation, which in the study of antimicrobial effects of cavitation can lead to significant errors. As a result, there is an extensive list of articles, where some connection between the number of cavitation and the intensity of antimicrobial action appears.

Thus, in [14] scientists chose the range of 0.13-0.18 as the optimal cavitation number. In [15], when studying the removal of insecticides (neonicotinoids) by cavitation, 0.067 was recommended as the optimal value of cavitation number. In [16], in a study of KI decomposition, the best results were achieved at a cavitation number of 0.4, and in a study of cavitation efficiency in wastewater treatment [17], other scientists gave a range from 0.1 to 1.

An equally important problem in studies of antimicrobial effects of cavitation is the approach of a number of scientists in the study. A number of peer-reviewed scientific

publications often present studies of the effects of cavitation on particular species of microorganisms (bacteria). Gram-positive bacteria in most studies were found to be more resistant to cavitation compared to Gram-negative bacteria. This is thought to be due to a tougher and stronger cell wall [18-20].

At the same time in some studies, even using the same bacterium lead to opposite results. In [21], almost no antimicrobial effect of cavitation was observed in cavitation treatment of liquid medium with Staphylococcus epidermidis. Low antimicrobial efficacy was also observed in cavitation treatment of seawater in [22].

In contrast to these results, most studies claim that cavitation provides inactivation of at least 70% of bacteria.

Additional complexity in cavitation binding and antimicrobial efficacy is introduced by the medium in which the microorganisms under study are kept. Microorganisms are incubated in a nutrient medium to stimulate their growth. However, a non-complex aqueous medium is desirable for cavitation because different media may affect the cavitation phenomenon [23]. Therefore, some authors used distilled water. Distilled water has a negative effect on bacteria because it acts as a hypotonic solution, which leads to cell swelling and possible cell rupture. Therefore, this may affect the results [24].

As a result, it is difficult to find a correlation between shape, cell size and cavitation efficiency in the scientific literature. It is this correlation that this study will focus on.

Cavitation is a unique physical phenomenon that provides the creation of a large energy density per unit volume of matter. The general picture of cavitation bubble formation is as follows. In the rarefaction phase, a gap is formed in the liquid in the form of a cavity, which is filled with saturated liquid vapor. In the compression phase, under the action of increased pressure and surface tension forces, the cavity collapses. Significant effect of cavitation processes is associated with high concentration of energy released in the process of collapse in the treated medium. At the moment of collapse, gas pressure and temperature reach significant values, and according to some data reach 100 MPa and 1000 0C, respectively [25]. This phenomenon is explained by the small volume of the substance at the moment when the bubble reaches its minimum radius preceding collapse. Based on the results of scientific studies [26-28], it can be stated that the radius of a cavitation bubble at the moment of collapse can reach, as a rule, 10-7-10-8 m, against the radius in the equilibrium state of 10-10-6 m. The change in the volume of the cavitation bubble, reaching thousandth values, leads to the achievement of high values of the stored energy.

The uniqueness of cavitation allows its application in various industrial fields, including food industry [29], wastewater treatment [30], biomedical applications [31], treatment of liquid hydrocarbons to change their rheological properties [32-34], etc. Hydrodynamic cavitation was used to prepare mustard oil in aqueous nanoemulsion with the smallest droplet size of 87 nm [35].

Hydrodynamic cavitation is mainly induced in the devices investigated by scientists by several types of devices, including:

1. venturi tube (the most commonly investigated);
2. a tube with diaphragms;
3. rotary oscillators;
4. devices with a discrete secondary.

Most often in scientific research in various conditions of application from oil products treatment to wastewater, either Venturi tube or diaphragm tube is used. A number of publications state that it is in the Venturi tube that the densest cavitation cloud is created in the area after contraction [36].

Venturi tubes have become widespread in various spheres of industry due to their constructive simplicity and efficiency. A kind of analog of Venturi tubes are diaphragm tubes. Unlike Venturi tubes and tubes with diaphragms, devices with a moving part, including

rotary hydrodynamic cavitation generators [37], as well as devices with a discrete secondary part [38] have lower power consumption and provide better scalability, and most importantly do not require a high-pressure pump.

In this study, a device with a discrete secondary part was chosen to evaluate the impact of cavitation (Fig. 1)

Fig. 1. Device with discrete secondary part

2.2 Materials and Methods

Microorganisms used.

The bacteria selected as study subjects were: gram-negative Escherichia coli, gram-positive Bacillus subtilis in the form of resting spores, and the micromycete Saccharomyces cerevisiae.

Preparation of media.

Distilled water, physiological solution, LB (Luria-Bertani), and chicken artificial intestinal medium (hereinafter referred to as ACI) developed by us [41, 43] were used as media in which microorganisms were placed.

E. coli was prepared for the experiment as follows. A day before the study, E. coli culture was inoculated into 100 ml of liquid LB medium, placed on a shaker and incubated at 37ºC. After 24 hours, the suspension was centrifuged (5 min, 3500 rpm), the supernatant was drained, and the resulting biomass was resuspended with physiological solution to OD600 1.0. The same manipulations were performed with S. cerevisiae.

To prepare a suspension of B. subtilis spores, the cell culture was sown by surface seeding on solid LB medium, incubated for 3 days at 37ºC, and then placed at 4ºC for 3 days. Thus, by the day of the experiment, the vast majority of cells in the culture were in the form of spores. Before the experiment, the culture was microscoped to confirm successful spore formation. The biofilm of B. subtilis biofilm containing spores was thoroughly mixed by repeatedly passing the suspension through the pipette tip to crush the biofilm. It was then shaken until a visually homogeneous suspension was formed. The remaining large biofilm

particles were then allowed to settle to the bottom of the flask for 5 min, and the remaining suspension was transferred to a sterile flask and centrifuged (5 min, 3500 rpm), the supernatant was drained, and the resulting biomass was resuspended with saline to an OD600 of 1.0.

All the obtained suspensions were poured together and mixed thoroughly. The resulting suspension containing all types of microbial cells was added to the prepared sterile media at the rate of 3 ml of suspension: 100 ml of medium.

The media were immediately placed in a refrigerator and transported to the experiment site on ice.

2.3 Sampling

Before the cavitation treatment, 1.5 ml of suspension was taken from each medium into sterile microtubes and considered as control. Then 1.5 ml each was taken after 1, 3 and 5 minutes from the start of treatment. All the samples were placed on cold and so were transferred to the laboratory within one hour.

Fig. 2. Sampling from the working area of a plant with a discrete secondary part

Investigation of the number of surviving microorganisms.

The study of the number of surviving microorganisms was carried out on the day of the experiment immediately after delivery of samples to the laboratory. A series of serial decimal dilutions were prepared from each sample and sown on solid nutrient media. To determine the number of E. coli was used Endo medium, S. cerevisiae - Sabouraud medium with the addition of antibiotic gentamicin, B. subtilis - on solid nutrient medium LB. In the latter case, bacillus colonies were determined morphologically. All experiments were carried out in triplicate.

3 Discussion

We chose three groups of microorganisms as objects of research. E. coli is a Gram-negative bacillus and is a classical object in microbiological studies. B. subtilis, also known as hay bacillus, belongs to the widespread p. Bacillus, some representatives of which can be pathogenic or contribute to spoilage of products (Ehling-Schulz et al., 2019). The main feature of Bacillus is the formation of a resting form called a spore. Physiologically, spores are almost inactive and are covered by a complex multilayered shell (Setlow et al., 2014; Driks, Eichenberger, 2017). Due to this, spores have the ability to remain viable for many years, tolerate desiccation, treatment with high temperatures (100ºC), pressure, etc. (Setlow

et al., 2014; Driks, Eichenberger, 2017). We hypothesized that spore bacteria, due to their dense envelope and heat tolerance, would be able to better tolerate the effects of cavitation and have higher survival rates.

S. cerevisiae are not bacteria. They are full-fledged eukaryotic cells with a much larger size, possessing a cell nucleus and all other structures of a eukaryotic cell. In this case, S.cerevisiae can be considered as a non-pathogenic cell model of micro-mycetes, which also include pathogenic Candida as well as mold fungi.

Thus, we will be able to evaluate the effects of the same cavitation conditions on bacterial and eukaryotic cells, which will help us to assess the difference of the effects depending on the cell structure.

The selection of media for bacterial treatment was based on the following criteria. Distilled water is the purest medium, devoid of most of the ions present in ordinary water. Cavitation action in such a medium will affect the microorganisms directly. On the other hand, distilled water can negatively affect cells, especially those with cell walls damaged by cavitation. Therefore, we also used physiological solution, which is isotonic with respect to microbial and yeast cells, but contains sodium and chlorine ions. The LB medium was chosen because it contains peptone and yeast extract, which are a mixture of peptides, proteins, lipids, and other large organic molecules that can also be damaged by all kinds of cavitation effects and thus take over some of the energy applied to the fluid. ICS is closest to the conditions under which cavitation action can be applied in different industrial fields (food, medical, sewage treatment, etc.): in addition to large organic molecules, protein conglomerates up to 0.5-1 mm in diameter and lipid micelles are present in the medium. These substances can absorb a significant amount of cavitation energy and weaken the effect of cavitation on microorganisms.

The results obtained in the unit with a discrete secondary are presented in Table 1.

Table 1. Number of microorganisms after different treatment times in the unit with discrete secondary part, CFU/mL

	Microorganism	Control	1 min	3 min	5 min
Distillate	*E. coli*	$6{,}9\pm0{,}4\cdot10^6$	$3{,}6\pm0{,}6\cdot10^6$	$7{,}1\pm0{,}4\cdot10^6$	$6{,}9\pm0{,}4\cdot10^6$
	B. subtilis	$3{,}1\pm0{,}6\cdot10^6$	$2{,}0\pm0{,}3\cdot10^6$	$3{,}2\pm0{,}5\cdot10^6$	$4{,}7\pm0{,}2\cdot10^6$
	S. cerevisiae	$9{,}8\pm0{,}4\cdot10^5$	$9{,}0\pm0{,}5\cdot10^6$	$5{,}5\pm0{,}3\cdot10^6$	$2{,}2\pm0{,}3\cdot10^6$*
Physiological solution	*E. coli*	$6{,}0\pm0{,}2\cdot10^6$	$6{,}1\pm0{,}3\cdot10^6$	$5{,}9\pm0{,}4\cdot10^6$	$6{,}5\pm0{,}5\cdot10^6$
	B. subtilis	$5{,}9\pm0{,}9\cdot10^6$	$7{,}7\pm0{,}2\cdot10^6$	$5{,}8\pm0{,}4\cdot10^6$	$6{,}0\pm0{,}4\cdot10^6$
	S. cerevisiae	$8{,}4\pm0{,}4\cdot10^5$	$8{,}1\pm0{,}4\cdot10^6$	$6{,}1\pm0{,}3\cdot10^6$	$2{,}4\pm0{,}2\cdot10^6$*
LB	*E. coli*	$6{,}0\pm0{,}3\cdot10^6$	$2{,}7\pm0{,}3\cdot10^6$	$3{,}4\pm0{,}4\cdot10^6$	$5{,}5\pm0{,}2\cdot10^6$
	B. subtilis	$4{,}1\pm0{,}4\cdot10^6$	$2{,}8\pm0{,}4\cdot10^6$	$2{,}8\pm0{,}3\cdot10^6$	$4{,}5\pm0{,}4\cdot10^6$
	S. cerevisiae	$8{,}9\pm0{,}3\cdot10^5$	$7{,}2\pm0{,}3\cdot10^6$	$7{,}3\pm0{,}2\cdot10^6$	$5{,}2\pm0{,}6\cdot10^6$
ICS	*E. coli*	$3{,}0\pm0{,}3\cdot10^6$	$3{,}9\pm0{,}3\cdot10^6$	$3{,}4\pm0{,}4\cdot10^6$	$3{,}2\pm0{,}2\cdot10^6$
	B. subtilis	$6{,}8\pm0{,}4\cdot10^6$	$5{,}7\pm0{,}4\cdot10^6$	$3{,}7\pm0{,}3\cdot10^6$	$4{,}0\pm0{,}4\cdot10^6$
	S. cerevisiae	$7{,}2\pm0{,}3\cdot10^5$	$9{,}3\pm0{,}3\cdot10^6$	$8{,}5\pm0{,}2\cdot10^6$	$8{,}2\pm0{,}6\cdot10^6$

*$p < 0.05$

4 Conclusions

It follows from the presented data that the effect was insufficient for bacteria, both in the form of spores and vegetative cells. Even after 5 minutes of treatment there were no significant differences in the number of cells from the control.

In distillate and physiological solution, by the fifth minute of treatment, there was a slight drop in the number of micro-mycetes - by 78% and 72%, respectively. At the same time in LB and ICS no such significant changes in the number of micromycetes were observed. Apparently, large molecules and particles contained in the medium can really reduce the cavitation efficiency. It should also be noted that in the case of LB, and, less pronouncedly,

of ICS, significant foaming of the medium was observed, which probably disturbed cavitation processes.

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