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# CHANGE OF BACTERIAL AMOUNT DURING SONICATION

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**Abstracts.** The dynamics of the change of microbial amounts in water medium in sonication time was determined. Gases bubbling of different nature into the reaction medium for water disinfection from bacteria under cavitation conditions is proposed in the article. The rate constants of destruction of *Bacillus cereus* bacteria type in the gas atmosphere are expected. It was determined that the processes microorganisms' destruction at bubbling of all investigated gases are described by the kinetic equations of the first order.

**Key words:** ultrasound, microorganisms, water disinfection.

### Introduction

Water ultrasound (US) treatment is one of the most effective decontamination methods resulting in the microorganisms (MO) inactivation [1, 2]. Water disinfection under the US action is explained by the cavitation process during sonication, i.e. formation, growth and collapse of gas bubbles in a liquid [3]. US effective disinfection action on various types of microorganisms is confirmed by numerous positive results but the information about kinetic reaction order of the disinfection process of natural waters and sewage was not found in the literature. A high decontamination degree has been achieved in our previous studies under acoustic cavitation effect in the model microbial suspensions [4, 5]. US can inactivate a wide range of bacterial pathogens, including E. coli [6] and Salmonella in a variety of liquids, effective against Microcystis aeruginosa. Microbial inactivation by ultrasound action is caused by the physical disruption of cells, lysis, and damage to DNA and the formation of free radicals. However, the mechanism of cells destruction is not fully understood. But at the same time, the researchers have noticed, that the US is highly effective nonchemical ecological method of water purification from organic and microbial components. On the contrary, in [7-9] it

has been noted that the complete destruction of pathogens is achieved when the ultrasound treatment is used only after long scoring or by increasing the power of ultrasound generators. It is clear that the need for a long disinfecting processing doubted the feasibility of using ultrasound for industrial conditions. Obviously, it is due to the result of an inadequate study of complex phenomena, which is accompanying with the cavitation effect, together with the ignorance of basic laws of the studied process that characterize the interaction of the components of water available to it specific organisms, the impact of additional gas supplied by the action of ultrasound, etc. This is because the effectiveness of bactericidal action as ultrasonic and hydrodynamic cavitation depends on several factors: the parameters of the physical factor (a capacity [10–12], an intensity [10], the oscillation frequency, an exposure [13]) and so on; some of the physical features of the environment that is exposed to cavitation (temperature, viscosity); the morphological features of the research facilities (sizes and shapes of bacterial cells, the presence of the capsule, the chemical composition of the membranes, an age, a culture [14–15] etc.); the nature of bubbling natural environment through the treated gas under the cavitation effect [4] and others.

### The main aim of the work

The purpose of this study has been to investigate the destruction of *Bacillus* bacteria type in the deaerated distilled water using ultrasound in the presence of different dissolved gases: oxygen, carbon dioxide, argon and helium.

# Materials and methods

Ultrasound oscillation frequency of 22 kHz lowfrequency generator UZDN-2T (power of 90 W) transmitted via magnetostriction emitter immersed in water volume study of the initial value of the number of known microorganisms (MO). The experiments have been carried out under T=298 K, P= $1 \cdot 10^5$  Pa. Investigated object was model medium with some amount of *Bacillus* is  $8 \cdot 10^2$  cells/cm<sup>3</sup>).

The thermostat for MO growth is shown in Fig. 1.

Gases were used as additional gases for the researches, which were bubbled into the microbial dispersion at the rate of ~  $1 \text{ cm}^3$ /s. The duration of the process was 2 h. The volume of the investigated dispersion (75 cm<sup>3</sup>) in the glass reactor was cooled by water during the whole process.



**Fig. 1.** The thermostat for microorganisms growth. Conditions: incubation in a thermostat at 37 °C during 48 h

#### **Results and discussion**

Water purification depends on the sonication time. For this reason, the effect of water sonicating was measured for the predefined periods of time. Water samples were sonicated during the periods of 30; 60; 90 and 120 min.

The effect of ultrasound on removal of *Bacillus* in argon (Ar), helium (He), oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ ) medium after 60 minutes exposure was 79.13; 65; 81.5 and 28.13 %, respectively, and after 120 minutes – 95.5; 93.38; 90.5 and 76.5 %. Study shows that increasing the exposure of sonication the number of bacteria in water samples significantly reduces; the power of bacterial cells destruction increases greatly (Fig. 2).

Moreover, the rate constants of microbial inactivation (*k*) have been determined. In argon saturated water *k* amounts to  $4.346 \cdot 10^{-4} \text{ s}^{-1}$ , for the helium saturated one it is  $3.566 \cdot 10^{-4} \text{ s}^{-1}$ , for the oxygen –  $3.211 \cdot 10^{-4} \text{ s}^{-1}$  and for the carbon dioxide –  $1.666 \cdot 10^{-4} \text{ s}^{-1}$  (table 1).

Table 1

Rate constants of cells disappearance. Conditions:  $\tau = 2$  hours, T=298 K, P=1·10<sup>5</sup> Pa and US frequency = 22 kHz

Investigated gases	k·10 <sup>4</sup> , s <sup>-1</sup>
carbon dioxide	1.666
oxygen	3.211
helium	3.566
argon	4.346

According to the results, the application of acoustic cavitation enhances the process of water purification in the presence of gases. Inactivation of *Bacillus* bacteria type is described by a reaction equation of the first-order. It has been found that the highest efficiency was obtained under conditions of the simultaneous action of ultrasound and argon (disinfection degree – 95.5 %, cells inactivation rate constant –  $4.346 \cdot 10^{-4} \text{ s}^{-1}$ ).



Fig. 2. Concentration of microorganisms as a function of sonication time in the presence of different gases: 1 - CO<sub>2</sub>, 2 - O<sub>2</sub>, 3 - He, 4 - Ar

Conditions:  $C_0 = 8 \cdot 10^2$  cells/cm<sup>3</sup>, T=298 K, P=1·10<sup>5</sup> Pa and US frequency = 22 kHz.

According to the results, the application of acoustic cavitation enhances the process of water purification in the presence of gases. Inactivation of *Bacillus* bacteria type is described by a equation of the first-order. It has been found that the highest efficiency was obtained under conditions of the simultaneous action of ultrasound and argon (disinfection degree – 95.5 %, cells inactivation rate constant –  $4.346 \cdot 10^{-4} \, \text{s}^{-1}$ ).

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