UDC 66.084, 541,182; 628.1

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EFFECTIVENESS OF GAS BUBBLING DURING SHORT-TERM CAVITATIONAL ACTION ON THE WATER DISINFECTION FROM *BACILLUS CEREUS* BACTERIA TYPE

Gases bubbling of different nature into the reaction medium for water disinfection from bacteria under cavitation conditions is proposed in the article. It was shown that gas bubbling within the first three minutes of the process leads to acceleration the process of aggregates breaking in the aqueous medium under acoustic conditions, due to the formation of additional cavitational embryos compared with the process without gas bubbling. It was found that in the initial moment of reaction the cavitation energy was spent for breaking of aggregates into a single bacterial cells but cell disruption is slower process unlike disaggregation.

Key words: wastewater, microorganisms, cavitation, water purification, micro-organism's number.

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ЕФЕКТИВНІСТЬ ПОДАЧІ ГАЗУ ПРИ КОРОТКОЧАСНІЙ ДІЇ КАВІТАЦІЇ НА ЗНЕЗАРАЖЕННЯ БАКТЕРІЙ *BACILLUS CEREUS* У ВОДІ

Запропоновано подачу газів різної природи в реакційне середовище для знезараження води від бактерій в умовах кавітації. Доведено, що подача газу впродовж перших трьох хвилин процесу пришвидшує розбиття скупчень агрегатів у водному середовищі в акустичних умовах, що пов'язано з утворенням додаткових зародків кавітації порівняно із протіканням процесу за відсутності подачі газу. Встановлено, що у початковий момент реакції енергія кавітації витрачалася на розбивання скупчень бактеріальних агрегатів в поодинокі клітини, а відмирання відбувається повільніше, ніж дезагрегація.

Ключові слова: стічна вода, мікроорганізми, кавітація, очищення води, мікробне число.

Formulation of the problem. In the prevailing majority of the main cause of pollution natural water pools are getting water and untreated insufficiently treated wastewater different origin. Various branches of petroleum, chemical and food industries cause significant damage to natural reservoir; outdated and imperfect technology forms a substantial amount of harmful waste and wastewater to the environment. Mostly businesses these industries consume large amounts of water, which in some cases used directly for manufacturing processes, while others - for the support and maintenance of technology, ie for treatment products and semi-finished products, washing packing container and equipment etc. Thus, the amount of wastewater (WW), contaminants concentration dependent primarily on the capacity of companies and introduced technologies.

The analysis of recent research and publications. In the recent decades, a general trend of the world practice is the growing of the impact of technological component on the degree of WW pollution and on the related to it water quality of natural water bodies. This effect leads to the need of rapid development of new energy-saving water-treatment technologies that ensure not only successful, but economically justified efficient neutralization of complex pollution of natural water sources.

Among them the advanced water treatment technologies, the cavitation methods are still important on processing liquid. Thus, a number of works is dedicated to the investigation of the ultrasound (US) effect in water by the indicating of its high efficiency in the viruses, protozoa [4–6] etc. 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 [16] and others.

The main aim of the work. This work is dedicating to the feasibility of various gas bubbling in the reaction medium under the short action of cavitation process.

The discussed results. We have conducted complex of microbiology research of natural water from the lake in the Lviv region (Sudova Vyshnya city). In this water the *Diplococcus, Pseudomonas fluorescens, Bacillus cereus, Sarcina lutea* bacterias were detected. The bacteria *Bacillus cereus* was available in a huge number that is why it was chosen as the data to create model environment. The basis was to identify not only microscopy preparations like a "crushed drop", with the fixed preparations of cells to study their morphological criteria, including Gram staining, but the study of culture and physiological reaction (oxygen) MO properties identified in the examined water.

The determination of the total number of MO in the water for the purpose of bacteriological study of universal culture medium used – meat peptone agar.

Cultivation was carried investigated by the MO depth method. MO was grown in an incubator at a constant temperature (37 °C) with the duration of 48 hours.

To investigate the impact of natural gas to the cavitation effectiveness of water treatment experiments performed under argon, helium, oxygen, carbon dioxide in the ultrasound field at atmospheric pressure, temperature of 298 K, US frequency – 22 kHz. Processing time 1–120 min. Ultrasound oscillation frequency of 22 kHz low-frequency generator UZDN-2T (power 90 W) transmitted via magnetostriction emitter immersed in water volume study of the initial value of the number of known microorganisms (NM). Outputs NM machining water samples were in the range NM_0 = 820–2090 CFU/cm^3.

The further research aimed at studying the simultaneous effect of gas and US perturbed by the rod-shaped bacteria of *Bacillus cereus* family *Bacillaceae* an insignificant microbial contamination of water ($NM_0 = 8*10^2$ CFU/cm^3) (Fig. 1). In the short-term action of the acoustic field under argon, oxygen, helium and carbon dioxide gas ($t_{gas/US} = 60{\div}180$ s) there was a sharp increase NM (phase I). Availability of simultaneous action of carbon dioxide and ultrasound led growth in order NM ($2.4*10^3$ CFU/cm^3) in the first three minutes of the experiment. The further steps of gas/ultrasound processes in all cases leads to a sharp reduction of NM throughout the whole treatment process.

For detailed studying of this phenomenon the microscopy of water samples before and after the joint gas/ultrasonic action was made. As the result, in the control water samples a large number of microbial units was revealed, while after t = 180 s system was dominated mostly by the separated cells. We can therefore say that the accumulation of microbial cells on the stage of the process and due process of disaggregation. Obviously, increasing the value of NM directly proportional to the number of cells in units identified in the initial samples of water, and may affect the process of disaggregation nature of the bubbling gas. The growth of NM in the presence of carbon dioxide by 1600 CFU/cm^3, in contrast to the growth in the presence of argon (only 100 CFU/cm^3) may have an impact on the final value of the NM.

Thus, when cutting in the ultrasound field water in which existing microbial aggregates is disaggregation dominant process in the presence bubbling gases, which in turn, is characterized by a determining influence on the overall efficiency of water disinfection. Therefore, the studying of the process of disaggregation would make the process without bubbling gases only in acoustic terms.

To study the process of disaggregation, the real natural water from lake was selected in the Lviv region (Sudova Vyshnya city). Samples of water were taken during the summer period (June and July), as the water temperature is the highest and the water level is the lowest, when the oscillations of NM are maximal.

The number of MO ranged 820÷2090 CFU/cm^3, depending on many physical and chemical factors: temperature regime of a reservoir, which influences on the growth of MO, the concentration of dissolved oxygen in water, the value of pH of water and redox potential, and also temperature of the air, quantity of the atmospheric precipitations, hydrodynamic regime of the reservoir, etc.

Investigating a joint action of gas/US on the example of bacteria *Bacillus cereus* at NM_0=800 CFU/cm^3, and the influence of acoustic cavitation on the natural water from the lake in the Lviv region (Sudova Vyshnya city) with an increased content of bacteria Bacillus cereus at NM_{01}= 820 CFU/cm^3 and NM_{02}=2090 CFU/cm^3, it was found a pattern of growth of NM at the first stage of acoustic cavitation process. The difference was only at the time of sound treatment, at which the maximal values of NM were observed.

The abrupt increase of NM was observed during a short-term action of acoustic field on the bacteria *Bacillus cereus* in atmosphere of carbon dioxide during the first 60÷180 s of experiment. At the joint action of US and argon or helium, although there was observed an increase in NM at the beginning of the experiment, but with much lower maxima than at the joint action of carbon dioxide and US (Fig. 1). The exception was the action of oxygen, where the increase of NM was not observed.

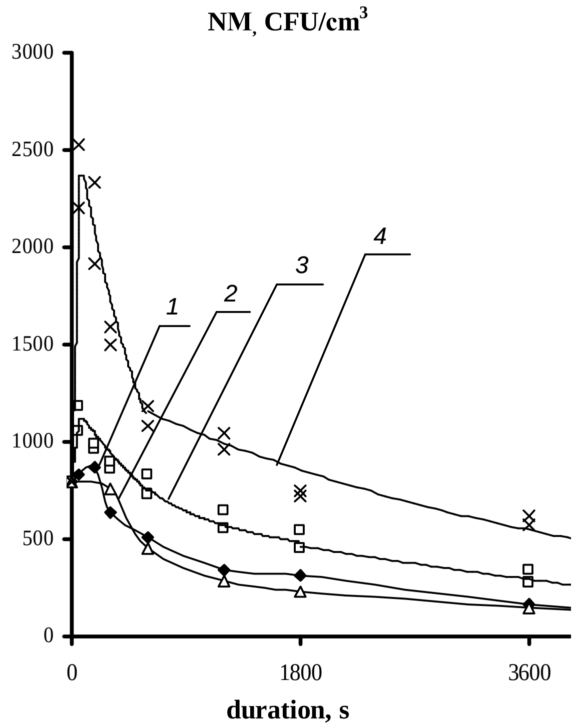

Fig. 1. NM dependence of B.cereus bacteria during process duration: Ar/US (1), O_2/US (2), He/US (3) and CO_2/US (4). Initial data: $NM_0 = 8{\cdot}10^2$ CFU/cm^3. Conditions: T = 298±1 K

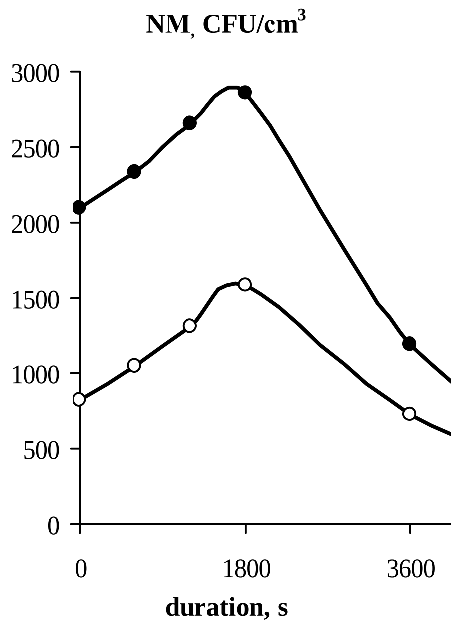

Fig. 2. NM change of natural water during US treatment. Initial data: NM_{01} = 820 CFU/cm^3 (1) and NM_{02} = 2090 CFU/cm^3 (2). Conditions: T = 298±1 K

When lake water is treated by a sound, NM reaches its maximal value after 1800 s of experiment in the absence of gas bubbling (Fig. 2). By microscopic examination of the water, it was discovered the aggregates of MO of different types, while the number of single cells was negligible. Energy of acoustic cavitation at this stage was spent mostly on the breaking of the aggregates to the single cells, and the dying occurs more slowly than disaggregation. A similar picture was observed during the action of acoustic cavitation on the clusters of detected bacteria aggregates *Bacillus cereus* under atmosphere of argon, oxygen, helium and carbon dioxide. The decrease of the time spent on the breaking of aggregates in the water environment, is likely due to saturation of one or another gas, because in their absence only dissolved oxygen is available in aqueous solutions.

Aqueous solutions are not only favorable environment to create cavitation, but also the environment, which enables electronic breakdown of cavitation cavern. This is about a set of complex phenomena based on the formation of molecules with excess energy and the transfer of this energy to other molecules or atoms as a result of mutual collisions or radiation. From a chemical point of view, the process of excitation of water molecules, their rate of dissociation is mainly governed by the presence in cavitation cavern of various gases, which are in the excited state. Chemically active gases dissolved in the solution treated by a sound, influence in different ways on the ultrasonic chemical reactions. Firstly, the atoms of oxygen and hydrogen are involved in the reactions of radical transformations; secondly, chemically active gases penetrating into the cavitation cavern, participate in the energy transfer of electronic excitation by water molecules, and also, possibly, in the processes of recharging [10, 11].

Some authors investigated the synthesis of hydrogen peroxide occurs easier in the water saturated with a gas mixture of oxygen and inert gas (argon or helium). The influence of the inert gases, according to Park and Taylor, is provided by an amplification of the ionization processes, particularly by the processes of dissociation of oxygen molecules. During the studies of the chemical reaction rates in the water treated by a sound in the absence of oxygen, it was found the influence of the inert gases on the reaction rate. It was proved by results on the aqueous solution of methylene chloride (CH_2Cl_2) treated by a sound to form new compound with an elementary formula ($C_{10}H_7O_3Cl_2$). Unfortunately, the structural formula of the formed compound was not determined. However, it was investigated that synthesis of that substance in the presence of US waves runs slowly in the nitrogen environment, a little faster – in the presence of air, and the fastest – in argon environment, contrary to helium, where it runs very slowly. Taking into account that the new synthesized compound contains oxygen, then its source in the presence of argon should be a product of decomposition of water molecules (˚OH-radicals), because in the previous solution saturation with argon, the oxygen was forced out from the investigating sample, that was checked by polarographic method. Based on these results, another authors concluded that the argon, bubbled into the water environment treated by a sound, promotes more effective decomposition of water molecules in the cavitation cavern, thus amplifying the processes of oxidation. Methylene chloride undergoes chemical transformations directly in the cavitation caverns.

Similar studies were conducted with a serum albumin, where in the presence of argon under US waves formaldehyde production was in 10 times larger than in the presence of air. Also, the amplification of coagulation effects of US waves on protein solutions was observed.

From these results it can be concluded that the amplification effect of argon on oxidation processes occurring in the US field depends on the structure and characteristics of the reacting substances. In the presence of argon, the rate of ˚OH-radical formation in the treated water increases.

Based on the above mentioned literature data on the oxidation of aqueous solutions of organic substances in cavitation conditions, the obtained experimental data on disinfection of aqueous solutions from bacteria *Bacillus cereus* (Fig. 1–2) demonstrate the effectivity of simultaneous bubbling of gases and the action of US waves on the studied water environment. The presence of a gas decreases the accumulation period of the number of MO comparing with the water treated by a sound without gases in 10 times. Thus, for the water sample 1 NM_{max} = 1500 CFU/cm^3 for 1800 s without gas bubbling; and for the sample of water with a pure culture of bacteria *Bacillus cereus* with the same initial value NM, it increased accordingly: NM_{max}= 1100 CFU/cm^3 for helium, NM_{max} = 850 CFU/cm^3 for argon, and NM_{max}= 2400 CFU/cm^3 for carbon dioxide within 60÷180 s of the experiment. As it was found that the increase of the value NM is proportional to the number of cells in the aggregates, found in the initial water samples. Thus, the process of disaggregation is also affected by the nature of bubbling gas. In the atmosphere of argon, it was achieved the highest value of the sonochemical effective death rate constant of MO $k_d = 8{,}92*10^{-4}$ s^{-1}, comparing with $k_d = 7{,}47*10^{-4}$ s^{-1} for oxygen, in the presence of which the increase of NM was never observed. In the atmosphere of carbon dioxide, where the highest value of NM_{max} was observed, the process of water disinfection from bacteria *Bacillus cereus* proceeds the most slowly, at the value of $k_d = 6{,}99*10^{-4}$ s^{-1}. Calculation of the sonochemical effective death rate constants of MO once again confirms the expediency of the gas usage (in particular, argon) to disinfect the water from bacteria *Bacillus cereus*, compared with the use of only US. Without bubbling of gases under the action of US waves on water samples 1 and 2, only dissolved oxygen is available in the reaction medium, the amount of which is insufficient for the formation of additional centers of cavitation nucleations.

Conclusions. It was proved that the supply of gas in the first three minutes of the process promotes acceleration of breaking of bacterial aggregates in 10 times in the water environment at acoustic conditions, comparing with the process run in the absence of gas supply, where a similar process occurs after 30 minutes, due to a formation of additional cavitation nucleations during gas supply.

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