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SIMULTANEOUS ACTION OF CAVITATION AND INERT GASES ON THE WATER PURIFICATION FROM BACTERIAL CELLS

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Abstract

The simultaneous effect of cavitation and inert gases (argon and helium) on the process of microorganisms (MO) destruction has been studied. Sporogenic rod-shaped bacteria of the *Bacillus cereus* type were used for the study. The initial microbial load was 10^4 cells per 1 cm^3 of test water. The volume of water for the study was 75 cm^3. The source of the cavitation phenomenon was an ultrasonic generator with a frequency of 22 kHz. The gas was bubbled through the water system at a rate of 0.2 cm^3/s throughout the duration of the process (2 h) with a total flow rate of 0.7 dm^3/h. After every 30 min of simultaneous action of gas/cavitation, water samples were taken to determine the dynamics of the number of microorganisms (NM) from the time of water treatment. The change in the NM for each gas is given depending on the duration of the process and the initial number of cells per unit volume of water. To compare the effectiveness of the studied gases, the values of the effective rate constant of bacterial destruction (k_d) were calculated according to the kinetic equation of the first-order reaction. According to the calculated k_d values, the higher efficiency of bacterial destruction during argon bubbling under cavitation conditions was noted, in comparison with helium in similar experimental conditions. It is shown that k_d(Ar/US) > k_d(He/US). The efficiency of the process of water purification from pollutants of biological origin under cavitation conditions depends on the nature of the bubbled gas.

Key words: water, bacteria, purification, cavitation, argon, helium.

1. Problem statement. Nowadays, in Ukraine there are difficulties with the provision of natural resources due to qualitative and quantitative depletion of natural reservoirs, which is associated with pollution and irrational use of water. Cities of Ukraine suffer, first of all, from the discharge of wastewater (WW) into the waters of open reservoirs. The Dnieper River annually receives 370 million m^3 of polluted sewage or 14% of the total volume in the country [1], the Pripyat River basin - 6 million m^3 [2]. Among the western regions of Ukraine, Lviv and Ternopil regions reported the highest pollution [3]. Pollution of open reservoirs of Lviv region is caused mainly by flowering of reservoirs, rotting of vegetation and constant pollution by solid household waste.

Sanitary and biological studies [4] of the WW quality of the cities of Lubny, Kremenchuk, Svitlovodsk and Kropyvnytskyi indicate a low degree of purification after discharge of water from treatment plants into the Dnieper River basin. Thus, in some cases, helminth eggs (1-4 specimens/dm^3) and a high index of lactose-positive Escherichia coli were detected in WW samples in the cities of Lubny and Kremenchuk [4]. Several million bacteria are found per unit volume of wastewater and river water [5].

According to [6], after research of almost 18 thousand of approximately 27 thousand private wells in the Lviv region, deviations in bacteriological parameters were found in 15.7% of samples. Some indicators exceed the established norms for drinking water, in particular the coli index. In the well waters of some settlements of Lviv region (Dolishne village, Dovhe village and Morshyn town) the average annual coli-index is 3.3-8.2 times higher than the norm [6]. According to [7], 33% of tap water in Vinnytsia does not meet sanitary and microbiological standards. This is a far from complete list of direct pollution of open water bodies with industrial waste, but it is quite sufficient to understand the catastrophic state of water resources that pollute water not only with chemical but also with biological pollutants.

Although there are many ways to disinfect water, such as chemical and physical methods, many countries still use chlorine to disinfect drinking water. However, in the literature there are numerous reports of reactivation of microorganisms (MO) in chlorinated drinking water, the emergence of chlorine-resistant organisms that remain viable [8, 9] and worsen the sanitary and hygienic performance of water. It was studied [8] that even a chlorine concentration of 10 mg/dm^3 does not provide complete removal of microscopic fungi from water. Therefore, to obtain a guaranteed bactericidal effect, excessive doses of chlorine are used, but this, in turn, impairs the organoleptic characteristics of water, causes its denaturation.

The question of the need to abandon chlorination of water due to the formation of toxic, mutagenic and carcinogenic organochlorine compounds [10-12] is increasingly raised, which causes re-contamination of water. Such tap water is not spoken of as drinking water, but as man-made [12].

A promising method of water disinfection for today is the use of cavitation. Bubbling gas during cavitation leads to intensification of water treatment. Therefore, the paper proposes to consider the process of bubbling gases through microbial water in cavitation conditions and to investigate the viability of MO depending on the duration of water treatment. In addition, the effect of inert gases has been little studied in cavitation water treatment processes. The presented experimental studies can provide specific recommendations and conclusions on the choice of the nature of bubbling gas to remove bacteria from the aquatic environment not only as a direct reagent, but also in combination, for example, with physical methods of water treatment.

2. Analysis of the recent researches and publications.

In [13], inactivation of *E. coli* and *Staphylococcus aureus* in the presence of ultrasound was investigated: 3.02 ± 0.52 log and 0.18 ± 0.14 log, respectively. Inactivation of *Microcystis aeruginosa* as a result of mechanical and chemical effects caused by ultrasound [14, 15] was evidenced by the results of cell cytometry (reduction in size, internal granularity, integrity and activity of algae cells [14], systematic analysis of algae cell morphology with removal efficiency of 80-90 % [15] and the effect of ultrasonic cavitation intensity on the structure of microorganisms during WW disinfection [15]. Reduction of algae by 80% in 100 l of water was achieved at an ultrasonic frequency of 36 kHz and 650 W for 10 minutes and 50-90 % at 36-175 kHz (power 650 W) in a volume of 4 m^3 with a processing time of 60 minutes. The use of ultrasonic technology to prevent the formation of biofilm in realistic conditions arising in industrial conditions is described in [16] and provides recommendations for the construction of ultrasonic cavitators [17], which provide a high level of intensity of ultrasonic vibrations in the liquid volume.

In [18], the greater efficiency of argon compared to helium on the process of yeast destruction of *Saccharomyces cerevisiae* under cavitation conditions was investigated and it was shown that k_d (Ar/US) > k_d (He/US). This paper graphically presents the dynamics of the yeast number under the influence of gas/cavitation. The degree of water disinfection of more than 99% was detected after the duration of the process 1 hour in the conditions Ar/US.

However, experimental publications on the destruction of *Bacillus* bacteria under cavitation by bubbling inert argon and helium have not been found. Therefore, in the presented work it is proposed to consider the process of water purification from pollutants of biological origin, which consists in the MO destruction of a particular genus under the simultaneous action of cavitation and bubbling inert gas.

3. Statement of the problem and its solution.

The task of the presented research is the following:

- to study the simultaneous effect of cavitation and inert gases (argon and helium) on the process of water purification from rod-shaped *Bacillus cereus* bacteria type;

- to study the influence of the nature of bubbling gas on the process of bacteria destruction in water under cavitation conditions.

3.1. Materials and methods.

Bacillus cereus bacteria are served as the predominant microflora of different natural waters [19] with the initial microbial load of 10^4 cells per 1 cm^3 of the studied water. Pure cultures of these MO were introduced in sterility to sterile water. Sterile water is prepared on the basis of tap water by sterilizing it in an autoclave. Thus, separate model environments with different initial numbers of microorganisms (NM) were created in order to get as close as possible in quantitative content to the real level of microbiological contamination of natural and industrial WW. The number of bacterial cells per unit volume of water was within $10^4 \div 10^5$ cells per cm^3.

Cultivation of the studied MO was carried out by the deep method. MO grew in a thermostat at a constant temperature (T = 30 °C) for 48 h, which is typical for bacteria. NM calculation in 1 cm^3 of test water is to determine the total number of MOs that have the ability to grow on meat-peptone agar on Petri dishes. Cultivation of MO was carried out in an electric dry air thermostat TC-80M-3.

Model waters were bubbled with inert gases (argon, helium) for two hours (t = 7200 c). The gas was bubbled through the model water throughout the process at a rate of 0.2 cm^3/s. Its total consumption for two hours of the process was 1.4 dm^3.

The volume of microbial water tested was 75 cm^3, which was poured into a sterile glass reactor with built-in fittings for thermocouple, gas bubbling and water sampling. The reactor was cooled with running water, which provided a constant temperature of microbial water (T = 288 ± 1 K) throughout the experiment. Water samples were taken every 30 minutes to determine the microbial count per unit volume.

The calculated number of cells before and after treatment is expressed in colony-forming units (CFU). The experimental points of the diagram in the following experimental materials were obtained from the arithmetic mean of three parallel sowings of microbial water samples.

The calculated total microbial count in open water and various industrial wastewaters served to establish the initial number of microorganisms per unit volume of test water.

3.2. Results and Discussion

CFU was calculated in 1 cm^3 of natural water and WW of Lviv region to establish microbial contamination in model waters. Thus, in waters from open reservoirs the NM was within 10^4 CFU/cm^3, and in the case of production WW as follows: NM = $1.1 \cdot 10^5$ CFU/cm^3 was found in WW of brewery «Kumpel» and NM = $3.86 \cdot 10^4$ CFU/cm^3 was found in WW of pharmaceutical plant "Galichpharm". Therefore, for experiments, sterile water was introduced pure culture of sporogenic cells of *B. cereus* bacteria types with the calculation of microbial

contamination of water in the range of $10^4 \div 10^5$ CFU/cm^3.

Studies of the change in the number of bacteria from the duration of the bubbling of inert gases (argon and helium) are presented in tables 1 and 2, which allow us to assess and provide a comparative characterization of cell viability under experimental conditions. Although the bubbling of the studied gases through the microbial system leads to NM reduction throughout the experiment, but differ in the efficiency of the process.

According to the kinetic reaction equation of the first order, the values of the effective constants of the rate of bacteria destruction are calculated (Table 3), which can be used to determine the effective gas nature for the process of water disinfection from the studied MO.

Experimental waters with different NM_0 were used in the experiments, but according to our previous studies, the value of the effective constant of the viability rate of MO does not depend on the initial number of cells in the water system [20], which indicates the feasibility of comparing the results of experiments with different NM_0.

A decrease in the number of bacterial cells when bubbling argon and helium is observed throughout the process, regardless of the initial number of bacteria per unit volume of the water system. However, the processes for argon and helium are different in the efficiency of cell destruction. The value of the effective rate constant of bacterial destruction for argon is greater (table 3), which indicates a greater amount of destroyed cells under conditions of argon bubbling.

You can also compare the efficiency of destruction of *B. cereus* bacteria depending on the gas nature and, thus, to determine the gaseous atmosphere in which this process is more active. We see that the decrease in the number of bacterial cells occurs in the atmosphere of both studied gases, but with different activity. According to the results of comparing the values of the effective constant of the bacterial destruction rate for argon and helium, argon is more effective in the process of water purification:

$$k_d(\text{Ar}) > k_d(\text{He})$$

We see that the efficiency of bacterial cell destruction depends on the nature of the bubbled gas through the microbial system.

Thus, studies allow us to describe the processes of MO destruction cells in the presence of inert gases (Ar, He) and indicate the active purification of water from *B. cereus* bacteria under conditions of argon bubbling.

4. Conclusion

The viability of sporogenic bacteria in the bubbling of inert gases (argon and helium) through the water system undet cavitation conditions was studied and a comparison of the action of each of the studied gases on the process of bacterial cells destruction in water was investigated.

Table 1 – The change of the number of *B. cereus* bacteria type during Ar/cavitation condition with different initial NM (NM_0). Initial data: $NM_{01} = 7 \cdot 10^4$ CFU/cm^3; $NM_{02} = 1.157 \cdot 10^5$ CFU/cm^3.

Processing time, s	NM_{01}, CFU/cm^3	NM_{02}, CFU/cm^3
0	70000	115700
1800	49700	61600
3600	32400	43500
5400	24800	23200
7200	18500	19900

Table 2 – The change of the number of *B. cereus* bacteria type during He/cavitation condition with different initial NM (NM_0). Initial data: $NM_{01} = 3.4 \cdot 10^4$ CFU/cm^3; $NM_{02} = 4.8 \cdot 10^4$ CFU/cm^3.

Processing time, s	NM_{01}, CFU/cm^3	NM_{02}, CFU/cm^3
0	34000	48000
1800	26700	36600
3600	24300	27500
5400	22100	22500
7200	19800	13000

Table 3 – Effective rate constants of bacterial cells destruction (k_d)

Gas nature	R_d	k_d, s^{-1}
He	0.937	$(8.16\pm0.07)\cdot10^{-5}$
Ar	0.893	$(2.3\pm0.1)\cdot10^{-4}$

The number of microorganisms per unit volume of investigated water was determined by the total number of cells grown on nutrient medium on Petri dishes and expressed in colony-forming units. The dynamics of the bacteria number for gas/cavitation conditions from the time of sampling of the studied water is presented.

Comparison of the values of the effective rate constants of bacterial destruction for argon and helium showed that the number of cells decreased more actively under conditions of argon bubbling. Active destruction of microorganisms in the conditions of argon supply, in comparison with helium, irrespective of initial quantity of bacteria in 1 cm^3 of the investigated water is noted. The effect of argon on water containing bacteria is described by a larger value of the effective rate constant of cell destruction: $k_d(\text{Ar}) > k_d(\text{He})$. It is shown that the efficiency of water purification from microorganisms depends on the nature of the bubbled gas.

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Коваль І. З.

ОДНОЧАСНА ДІЯ КАВІТАЦІЇ ТА ІНЕРТНИХ ГАЗІВ НА ОЧИЩЕННЯ ВОДИ ВІД БАКТЕРІАЛЬНИХ КЛІТИН

Вивчено одночасну дію кавітації та інертних газів (аргону та гелію) на процес руйнування мікроорганізмів (МО). Для дослідження були використані спорогенні паличкополібні бактерії роду *Bacillus cereus*. Вихідне мікробне навантаження становило 10^4 клітин на 1 $см^3$ досліджуваної води. Об'єм води для дослідження становив 75 $см^3$. Джерелом кавітаційного явища був ультразвуковий генератор з частотою 22 кГц. Газ барботували через водну систему зі швидкістю 0,2 $см^3$/с впродовж всієї тривалості процесу (2 год) з загальною його витратою 0,7 $дм^3$/год. Після кожних 30 хв одночасної дії газ/кавітація відбирали проби води для визначення динаміки числа мікроорганізмів (ЧМ) від часу обробки води. Наведено зміну ЧМ для кожного газу в залежності від тривалості процесу та вихідної кількості клітин в одиниці об'єму води. Для порівняння результативності дії досліджуваних газів були розраховані величини ефективної константи швидкості відмирання бактерій (k_d) за кінетичним рівнянням реакції першого порядку. За розрахованими величинами k_d відзначено більшу ефективність руйнування бактерій під час барботування аргону в кавітаційних умовах, порівняно з гелієм в аналогічних умовах експерименту. Показано, що $k_d(Ar/US) > k_d(He/US)$. Ефективність процесу очищення води від забрудників біологічного походження в кавітаційних умовах залежить від природи барботованого газу.

Ключові слова: вода, бактерії, очищення, кавітація, аргон, гелій.

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