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THE EFFICIENCY OF THE WATER PURIFICATION PROCESS FROM BACTERIA DEPENDING ON THE GAS NATURE

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Abstract

A study of the influence of different gas nature on the efficiency of water purification from rod-shaped bacteria of the Bacillus cereus type is presented. The action of oxygen, carbon dioxide and inert argon and helium were used. The investigated water was model microbial water obtained on the basis of deaerated distilled water with the introduction of a pure culture of bacteria in the amount of $7 \cdot 10^4$ CFU/cm^3. The total duration of the process was 2 hours at a reaction medium with temperature of 288 ± 1 K. The change in the number of microorganisms from the duration of gas bubbling is shown, depending on its nature. The degrees of destroyed microorganisms were calculated after each sampling of water (D_d), which was taken after each 30 min of the process. Studying the influence of different modes of water treatment, the largest number of destroyed bacterial cells was studied in an atmosphere of carbon dioxide (D_d = 91.0 %), and the smallest - in an atmosphere of oxygen (D_d = 34.73%). A two-stage process of number of microorganisms change was detected in the oxygen atmosphere: an increase in the first stage during 1800 s and a subsequent decrease in the second stage. After CO_2 bubbling with a rate of 0.2 cm^3/s through an aqueous medium with a volume of 75 cm^3, the microbial count decreased by two orders of magnitude, which is apparently due to an increase in the acidity of the test medium. Having found a high efficiency of CO_2 on the process of bacterial cells destruction, this gas should be used in water treatment processes, as well as in combination with other reagents or physical methods of water treatment to enhance the destructive effect on micro-objects.

Key words: Bacillus cereus, gas, water purification, bacteria destruction.

1. Problem statement. Common sources of pollution of open water bodies are insufficient wastewater treatment by industrial and communal enterprises, households, large livestock complexes, washing away by thawed and rainwater of pollutants from fields and urban areas. Wastewater generated during technological processes, especially after treatment, is discharged into rivers or reservoirs. Wastewater is mainly polluted by the products of life, household detergents, dyes, industrial waste discharged into the sewer. Wastewater contains a large amount of microorganisms (MO), infected with helminth eggs and therefore before being discharged into reservoirs, they are subject to mechanical and biochemical treatment, disinfection [1, 2]. Thus, a large amount of pollutants enter open water bodies, changing their chemical and microbiological composition [3–6]. That is why scientists are still searching for new methods of water purification, or improving existing methods.

Many effective and efficient factors of influence and water treatment not only by reagent, but also by non-reagent methods of water purification from MO are offered. Significant positive feedback was noted for the effect of ultraviolet radiation [2, 7], osmosis [7, 8], ozonation [9], ultrasound [10–14], etc. However, in the water, in addition to foreign pollutants, there are dissolved gases, which have a significant impact on the development and vital functions of various aquatic microflora. The presence of gaseous substances in natural waters is also due to the products of its activity [15]. Therefore, in water treatment technology, it is important to study the impact of gases on the growth and development of different MO, as its impact on micro-objects is currently poorly understood.

2. Analysis of the recent researches and publications.

In [12] found a decrease in the amount of yeast Saccharomyces cerevisiae by 55 % after 30 seconds of operation with a piezoelectric generator with a frequency of 800 kHz and an intensity of 7 W/cm^2) with an initial concentration of $3 \cdot 10^5$ cells/cm^3, after 2 min – 77 %, after 10 minutes – 90 %. During the supply of hydrogen in similar experimental conditions, an increase in the number of *Sacch servisiae* was observed. The effect of oxygen on bacterial cells at different concentrations in the range of $10^2…10^4$ CFU/cm^3 was studied in [16], in [17] the effect of oxygen concentration on the degree of cell death was studied. The effect of increased oxygen concentration on the growth and metabolism of eukaryotes and prokaryotes is presented in [18].

The presented work proposes the treatment of water with a high content of MO by the action of gases of different nature – oxygen, carbon dioxide, argon and helium, which will draw conclusions about the activity of bacteria in the atmosphere of specific gases.

3. Statement of the problem and its solution.

The task of the presented research is the following:

- to investigate the effect of gases on the process of water purification from aerobic microorganisms with the same initial content in the aquatic medium;

- to determine the gas nature in the atmosphere in which the highest degree of destroyed microbial cells per unit volume of water is investigated.

3.1. Materials and methods.

Monocultures of rod-shaped bacteria of the genus Bacillus cereus were used to study the process of MO destruction in the water. Pure cultures of these MO, subject to sterility, were added to distilled water, which was previously disaerated by boiling. That is, a model environment was created for experiments with an initial microbial load of $7 \cdot 10^4$ CFU/cm^3 in order to get as close as possible to the real concentration of water pollution from open reservoirs. The volume of the test medium was 75 cm^3, which was poured into a glass reactor and bubbled with the investigated gases for two hours. Thus, different modes of water treatment were used, namely: bubbling water with oxygen, carbon dioxide, argon and helium. The temperature of the reaction medium was 298 ± 1 K.

To determine the amount of MO per unit volume of water every 30 minutes with a sterile pipette was taken 1 cm^3 of the investigated water and inoculated on a solid nutrient medium – meat and peptone agar (MPA). Each water sample was seeded in at least three parallel Petri dishes by the deep method as follows. In a sterile slightly open Petri dish was introduced with a sterile pipette seed dose (1 cm^3) and immediately poured 15…20 cm^3 of molten and cooled to 45…48 °C nutrient medium. The lid was closed and with light rotating movements of the dish thoroughly mixed the nutrient medium with the seed to evenly cover the bottom. MPA was added to one Petri dish without a water sample to verify the sterility of the medium. The plates were left in a horizontal position to solidify the agar, with MO cells fixed at a certain point in the medium. Then the dishes were turned upside down and transferred MO cells multiply, their mass increases so that colonies are formed, visible to the naked eye.

The count of cells before and after treatment was expressed in colony-forming units, which grew on nutrient medium on Petri dishes. The method of counting colonies is described in [19].

3.2. Results and Discussion.

The studied MO were cells of aerobic bacteria of the genus *Bacillus cereus*, which is associated with the detection of this type of MO in a dominant amount, both in different natural waters and in wastewater among bacteria [20]. The percentage of its predominance was more than 60 %, compared to other forms of rod-shaped and spherical bacteria (*Bacterium*, *Pseudomonas*, *Micrococcus*, *Sarcina*, etc.).

Detailed characteristics of the studied microorganisms are given in [19], and morphological features of the studied microobjects – in [21].

Graphical dependences of the percentage of destroyed cells after each sampling of water are presented in the figure and the D_d values of the duration of bubbling gases, taking into account the mode of water treatment is in the table 1. The destruction degree of MO after treatment of microbial water with gas is calculated as the ratio of the number of microorganisms (NM) to its initial number, expressed as a percentage.

The highest efficiency of water disinfection was shown by carbon dioxide (D_d = 91.0 %), and not only in the final result, but also during the whole process (see Table 1). This is due to the acidification of the medium by almost two units due to the dissolution of carbon dioxide in the water. The initial pH of the water was 6.1, while the pH_{end} = 4.3. The change in pH of microbial water during carbon dioxide action is presented in the Table 2. Thus, the increase in the acidity of the aqueous medium due to water-soluble carbon dioxide with a total consumption of 1.4 dm^3 after a two-hour process led to the active destruction of cells.

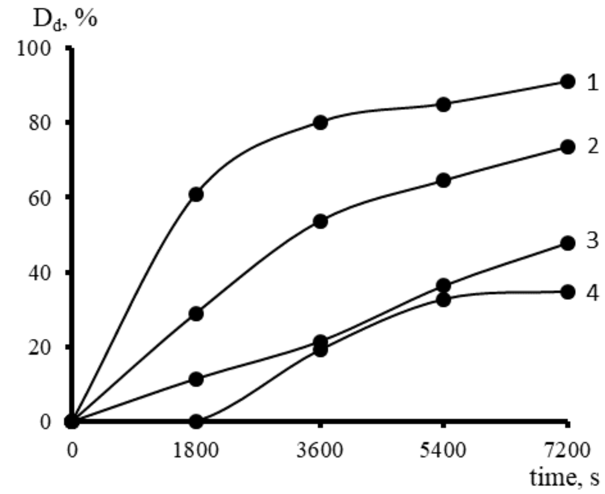

Figure 1 – Dependence of the destruction degree of bacterial cells on the process duration.
Experimental data are represented by points.
Experimental data: $NM_0 = 7 \cdot 10^4$ CFU/cm^3, CO_2 action (1), Ar action (2), He action (3), O_2 action (4). Process conditions: T = 288 ± 1 K.

Table 1 – The destruction degree of the duration of microbial water treatment and the treatment mode

Processing time, s	D_d depending on the mode of water treatment, %	
	O_2 action	CO_2 action
1800	–	60.86
3600	19.06	80.14
5400	32.63	85.0
7200	34.73	91.0
	Ar action	He action
1800	29.0	11.43
3600	53.71	21.43
5400	64.57	36.29
7200	73.57	47.71

Table 2 – Changing pH values of the reaction medium during bubbling of carbon dioxide

Processing time, s	pH values
0	6.1
1800	5.1
3600	4.8
5400	4.4
7200	4.3

Argon, compared with helium, purified water by 73.57 %, because the efficiency of helium after t = 7200 s is 47.71 %. In general, the effect of argon is almost twice the efficiency of helium during the entire duration of bubbling, which is explained by the physicochemical properties of the gas.

The least efficiency in water purification has been studied in an oxygen atmosphere, the process of which can be divided into two stages. In the I stage, there was a slight accumulation of MO cells, apparently due to oxygen consumption by aerobic cells of the studied bacteria. In the II stage, we were observed a slow and gradual decrease of the cells amount, which is explained by the saturation of the aquatic medium with oxygen and subsequent destruction of the cells. The presented results on the action of oxygen are consistent with the results of our previous work [16], which also revealed a two-stage process of NM change during oxygen bubbling through microbial water at different NM_0 and found that the duration of bacillary cell accumulation decreases with increasing microbial load in the water.

According to the results of research, it is possible to compare the efficiency of MO destruction in the aquatic medium under the action of different gases and, thus, to determine the gaseous atmosphere in which this process is most effective and in which gas the bacteria are destroyed most slowly.

Taking into account the effectiveness of the effects of the studied gases, we can display the relative series of the effective destruction of *Bacillus cereus* due to their action:

$$CO_2 > Ar > He > O_2.$$

Thus, the performed experiments allowed to describe and substantiate the processes of water purification from specific MO and to establish the effective nature of gas for its destruction.

4. Conclusion.

The influence of the nature of different gases on the process of aerobic MO destruction in the water with the same microbial load is shown. We used a quantitative method of calculating the initial and final NM by sowing samples of the investigated water on a nutrient medium in Petri dishes before and after experiments. It is established that the process of water purification from MO depends on the nature of the bubbled gas. The destruction degree of the studied microorganisms is calculated depending on the mode of water treatment and the process duration. The gas, the supply of which allowed to achieve the largest number of destroyed cells after 7200 s, was experimentally determined. The action curves of the gases on the process of water purification from bacteria were decreasing throughout the duration of the experiment, except for the oxygen action. It is investigated that the influence of oxygen on the process of MO destruction consists of two stages. They are an insignificant accumulation of the cells (stage I) and its subsequent destruction in the II stage. The percentage of cell accumulation during 3600 s of oxygen supply to the aqueous medium was 9.43 %, which is due to the consumption of bubbling oxygen by bacteria. This led to the lowest efficiency of the process for the action of oxygen in the end. The highest efficiency of water disinfection is achieved during the action of carbon dioxide by reducing the pH of the reaction medium. A relative series of effective action of the investigated gases on the microbial water purification is constructed.

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Коваль І. З.

ЕФЕКТИВНІСТЬ ПРОЦЕСУ ВОДООЧИЩЕННЯ ВІД БАКТЕРІЙ В ЗАЛЕЖНОСТІ ВІД ПРИРОДИ ГАЗУ

Представлено дослідження впливу різної природи газу на ефективність очищення води від паличкоподібних бактерій роду Bacillus cereus. Використовували дію кисню, вуглекислого газу та інертних аргону і гелію. Досліджуваною водою була модельна мікробна вода, отримана на основі дезаерованої дистильованої води з внесенням до неї чистої культури бактерій в кількості 7 $\cdot 10^4$ КУО/см^3. Загальна тривалість процесу становила 2 години при температурі реакційного середовища 288±1 К. Показано зміну числа мікроорганізмів (ЧМ) від тривалості барботування газу, в залежності від його природи. Розраховані ступені зруйнованих мікроорганізмів (МО) після кожного відбору проби води (D_d), яку відбирали після кожних 30 хв процесу. Вивчаючи вплив різних режимів обробки води, найбільшу кількість зруйнованих бактеріальних клітин досліджено в атмосфері вуглекислого газу (D_d = 91,0%), а найменшу – в атмосфері кисню (D_d = 34,73%). В атмосфері кисню виявлено двостадійний процес зміни ЧМ: збільшення на першій стадії тривалістю 1800 с та з подальшим зменшенням на другій. Після барботування CO_2 зі швидкістю 0,2 см^3/с через водне середовище об'ємом 75 см^3 мікробне число зменшилось на два порядки, що, очевидно, обумовлено збільшенням кислотності досліджуваного середовища. Виявивши високу ефективність дії CO_2 на процес руйнування бактеріальних клітин, цей газ доцільно застосовувати в процесах водоочищення, а також в поєднанні з іншими реагентами або фізичними методами обробки води з метою підсилення руйнівної дії на мікрооб'єкти.

Ключові слова: Bacillus cereus, газ, очищення води, руйнування бактерій.

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