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INFLUENCE OF INERT GASES ON YEAST IN CAVITATION CONDITIONS

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Abstract

The process of water disinfection with high content of *Saccharomyces cerevisiae* yeast type under cavitation conditions (frequency of 22 kHz) at saturation of the studied water with inert gases of different nature (argon, helium) was investigated in this work. The use of additional gas increases the bactericidal effect and facilitates the cavitation formation. The results of morphological features of yeast are presented, as well as images of cells based on the results of microscopic studies at the appropriate magnification, characteristic of a particular type of microorganisms. The values of the effective rate constants of yeast destruction are calculated. It is shown that the efficiency of water purification from yeast under gas/US conditions depends not only on the duration of the process, but also on the nature of the bubbled gas. The effective gas nature in the process of water purification from microorganisms is noted. A decrease of the number of bacterial cells was observed in the atmosphere of both investigated gases, but with different activity. Argon under cavitation conditions showed greater efficiency in the process of yeast destruction: $k_d(\text{Ar/US}) > k_d(\text{He/US})$. The dynamics of the yeast number under the influence of gas/cavitation is graphically presented. The degree of water disinfection > 99% was detected after the duration of the process for 1 hour in an argon atmosphere. $NM = 100 \text{ CFU/cm}^3$ under Ar/US reached after $t=3600 \text{ s}$, and for He/US only after $t=5400 \text{ s}$. It was shown that the nature of gas under cavitation conditions affects the duration of the water purification process.

Key words: cavitation, argon, helium, water, yeasts, destruction.

1. Problem statement. There are chemical contaminants of different composition (pesticides, phenols, petroleum products, salts of heavy metals, etc.) together with impurities of natural origin in the natural and wastewater, which is due to the discharge into the water of insufficiently treated or untreated industrial and domestic wastewater [1]. The presence of microbiological pollution has a significant negative impact on the state of water resources, in addition to the available organic pollution in these waters [2]. These can be pathogenic microorganisms, bacteria, fungi and small algae. The fight against its mass reproduction in wastewater discharge systems, in the technological environments, in the water supply systems should be aimed at the emergence of new cost-effective water treatment technologies.

Such new technologies include cavitation purification of the water. At the stage of cavitation treatment, free radicals are formed in the water, which significantly increases its chemical activity and provides an opportunity for effective use of the cavitation to neutralize pathogenic microflora, pasteurization, etc. A successful combination of cavitation treatment of the water in the presence of specific gases can significantly improve disinfection degree and purification of natural water and wastewater. The presence of gases in the reaction medium not only reduces energy consumption for perturbation and stable maintenance of cavitation phenomena in the liquid media, but also provides an intensifying effect of cavitation.

The effect of inert gases on the viability of microorganisms (MO) has not been studied under

cavitation conditions as chemically inactive gases. However, the study of the action of inert gases causes considerable interest in scientific purposes due to its nature, as the additional bubbling of the gas into reaction medium leads to the acceleration of the cavitation effect on the MO [3].

2. Analysis of the recent researches and publications.

After reviewing the scientific material with the maximum approximation to the research topic, it was found that the cavitation influence on the MO of various kinds is represented in both domestic and foreign works. The results of such studies are presented in the scientific publications [4-9], which investigated the effect of ultrasonic cavitation on the algae, fungi, bacteria.

Cavitation effect on the *Saccharomyces cerevisiae* yeast type is presented in [10, 11]. The initial cell numbers (from 10^2 to 10^5 ml^{-1}) had an influence on the inactivation of the yeast cells by ultrasonic irradiation by horn-type sonicator with frequency of 27.5 kHz [10]. A sharp decrease of the number of yeast cells, followed by a decrease in its ability to ferment occurs under the optimum ultrasonic conditions such as frequency 28 kHz, power 140 W/l and ultrasonic time 1 hour [11].

As we see, these data indicate the destruction of yeast cells under ultrasound (US) action alone without the use of gases. However, the bubbling of hydrogen through a suspension of yeast of *Saccharomyces cerevisiae* with the simultaneous use of a piezoelectric generator with a frequency of 800 kHz and an intensity of 7 W/cm^2 revealed the survival of yeast cells [11].

The influence of different gases nature on the MO is presented in the works [12-14].

The influence of carbon dioxide alone on the viability of MO was studied by us in [12], where the influence of gas pressure in the microbubble as the most probable cause of cell death was studied. To study this process used carbon dioxide as the most soluble in the water among the subjects with a known dependence of the solubility of CO₂ in the aqueous medium at different pressures in the gas phase.

The influence of oxygen and carbon dioxide alone on the water purification from yeast under cavitation conditions is presented in [13]. The paper noted more active destruction of bacterial cells compared to yeast under gas/cavitation. The viability of sporogenic bacteria in the atmosphere of inert gases without the use of cavitation we studied in the work [14]. The active destruction of *Bacillus cereus* bacteria type in the argon atmosphere, compared with helium, regardless of the initial number of bacteria in 1 cm³ of the investigated water. However, it is interesting to study the effect of inert gases not only on bacterial cells, but also on yeast cells, which are differ significantly in structure from bacteria. In addition, experimental data that would confirm the effect of inert gases under cavitation conditions on such a species of yeast as *Saccharomyces cerevisiae* in the literature we have not found.

3. Statement of the problem and its solution.

In the experimental part of the work presented below, it is proposed to carry out the process of processing yeast cells under cavitation conditions, because the joint action of cavitation and gas intensifies the processes in the aquatic medium [3]. Therefore, the task of the study is as follows:

- to study the influence of cavitation and inert gases of different nature (argon and helium) on the viability of *Saccharomyces cerevisiae* yeasts.
- to determine the effective nature of the gas during the cavitation treatment of the water system.

3.1. Materials and methods.

The pure microorganism cultures investigated were grown in test tubes in laboratory conditions at 30°C for 96 hrs, on an agar nutrient medium with subsequent storage at 4°C. Model dispersions as a investigated water was prepared from sterile natural water to which were added pure monocultures of the yeast cells. Ten-fold dilutions were made from this investigated water, depending on the initial number of yeast cells per unit volume of the water. 1 cm³ of the investigated water from the last dilution by the pipette was placed into a Petri dish. Each water dilutions were seeded into three parallel Petri dishes. Next, Petri dishes were placed into a thermostat TC-80M-3 under the following conditions: T= 25-30°C with duration of 96-120 hours. The nutrient medium for yeast growth is agar wort.

“Crushed drop” preparations were prepared for microbiological researches. “Crushed drop” preparations with Lugol solution as a coloring agent were used to study the morphological features of the cells. “Crushed drop” preparation involved the evaluation of water samples with the aid of a microscope.

Preparations are prepared, as a rule, on slides, the thickness of which should not exceed 1.2-1.4 mm. When working with cover glasses, their thickness should not exceed 0.15-0.17 mm. Thicker glasses degrade image quality. Bacteriological loops have to be decontaminated over the flame. To prepare the drug "crushed drop" on the slide put a loop of a drop of the investigated water, and then "crushed" with a cover glass. The drop of cells suspension should be such that after its crushing the liquid does not protrude beyond the glass.

For greater visualization of cells during microscoping, the method of differential staining of intracellular structures and inclusions with Lugols solution was used.

Staining of glycogen in the yeast was carried out as follows: on a clean slide was applied a small drop of a suspension of the microorganism and to it was added the same drop of a solution of iodine in potassium iodide. The cover glass is placed on top, the excess liquid is removed with filter paper. Increase of images was 480 during microscoping.

The source of cavitation was an, namely UZDN-2T generator was used as an ultrasonic wave source with frequency of 22 kHz and power of 35W. Ultrasonic vibrations were transmitted by the magnetostriction radiator, which was immersed in the water being studied. The volume of investigated water was 75 cm³. Glass reactor was cooled throughout the process with tap water.

An experiments conditions were: T = 298±1 K, P = 0.1 MPa, with a process time (t) = 2 hrs. Inert gases such as argon and helium were bubbled at the rate of ~ 1 cm³/s into the water under investigation.

The nutrient medium for yeast is wort agar, that was heated in the glass test tubes in a water bath, then cooled medium up to T = 45°C was poured into Petri dishes, where there was already 1 cm³ of investigated water of a particular dilution.

The concentration of microbial cells was determined by the number of colony forming units (CFU), assuming that each colony developed from a single cell.

3.2. Results and Discussion

The test microorganisms were *Saccharomyces cerevisiae* yeast cells from the *Saccharomycetaceae* family as the least studied type of MO. They are related to oval microorganisms with morphological features presented in Table 1. Physiological and cultural features of yeast cells are described in our previous work [15].

Table 1. Morphological features of investigated microobjects

Investigated microobjects	Morphological features				
	Coloration by Gram	Width, micrometers	Length, micrometers	Ability to form spores/buds	Ability to move
<i>Saccharomyces cerevisiae</i>	Gram +	5.17	8.67	buds	–

Argon and helium were used as an additional source of bubbles in an aqueous medium. Saturation of the treated aqueous medium by gases of different nature created additional cavitation embryos in reactive zone.

Features of yeast growth on the nutrient medium and microscopic studies of cells of different ages are presented at the Figure 1. Young yeast cells were used for research, namely: 1-day yeast cultures, which is shown by microscopy of cells (Fig. 1b). This figure clearly shows the presence of glycogen in the yeast cells, which is stained with Lugols solution in brown. Glycogen incorporation should be well investigated in 1-2 daily cultures, as was done during microbiological studies, which confirms the age category of selected microobjects.

The nature of colony growth on wort agar in a Petri dish and "crushed drop" preparations of the studied microorganisms are shown at the Figure 1.

According to the results of the experiments presented at Figure 2, it is shown that the survival of yeast cells depends on the gas nature in which the sonication took place.

An active decrease of the NM was observed during $t_{\text{gas/US}} = 3600\text{s}$ on the constructed curves of yeast destruction in the coordinates of $\text{NM}=f(t)$, and then a slow decrease of NM, that was expressed by relatively straight lines of the curves.

The efficiency of cells destruction when bubbling inert gases during $t_{\text{gas/US}} = 5400 \div 7200\text{s}$ practically does not change. That is, the destruction curves passed into the plateau of resistance, on which the number of yeast cells in 1 cm^3 did not exceed 100. This is evidence that the microbiological parameters of the investigated water fully correspond to drinking water. $\text{NM} = 100 \text{ CFU/cm}^3$ under Ar/US was achieved after a treatment time of 1 hour. That is, bubbling argon reduces the duration of the process by 25% compared to helium.

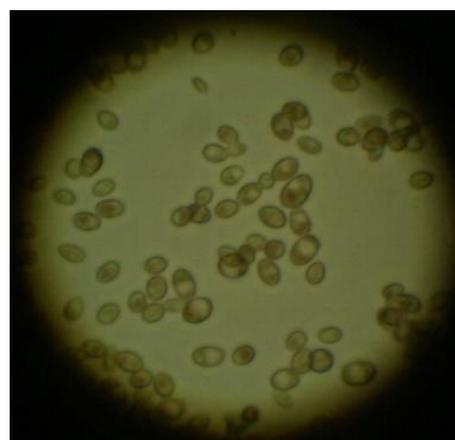
The calculated rate constant of microorganisms inactivation (k_d), calculated by the kinetic reaction equation of the first order, in an atmosphere of argon and helium is $(7.94 \pm 0.09) \cdot 10^{-4} \text{ s}^{-1}$ and $(5.73 \pm 0.08) \cdot 10^{-4} \text{ s}^{-1}$, respectively. The effect of argon on the water containing bacteria is described by a larger value of the effective rate constant of the cells destruction: $k_d(\text{Ar/US}) > k_d(\text{He/US})$.

Inert gases, not having reactivity, have an effect on chemical and biochemical processes. After all, when a yeast cell is destroyed under cavitation action, its mechanical damage occurs and their functional properties can change. In microbiological studies of voiced yeast cells, destruction of the cell membrane was observed, which was not observed in bacteria destroyed by heat [10].

During bubbling inert gases under cavitation conditions was possible to achieve almost complete water purification from yeast in a relatively short duration of the process. It should be noted that the unicellular yeast (eukaryotes) studied by us, in contrast to bacterial cells (prokaryotes), differ significantly in structure. Yeast cell with a fully formed nucleus and a well-developed vacuolar apparatus, in contrast to the bacterial, although characterized by a stronger shell, but much larger in size. These facts may be directly related to the effect of inactivation under gas and cavitation.



a



b



c

Figure 1 – Features of microorganisms growth on a nutrient medium and microscopic researches of yeast of different age (images enlargement is 480):

a – the nature of the colonies growth on agar wort in a Petri dish;

b – cells of culture with in vivo staining by microscopic examination (x480) stained with Lugols solution: 1-day cultures with stained glycogen in brown color;

c – cells of culture with in vivo staining by microscopic examination (x480), stained with Lugols solution: 10-day cultures in a light field

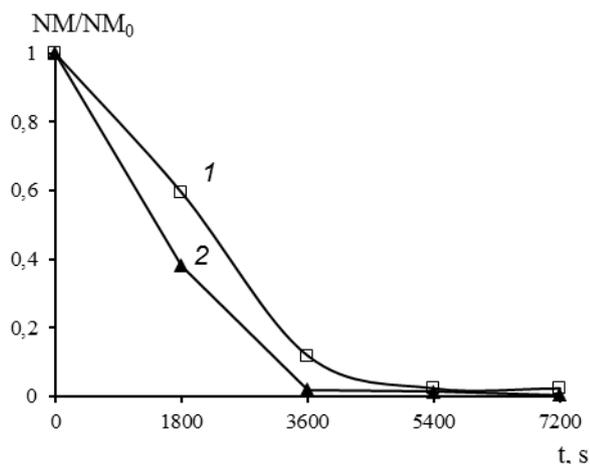


Figure. 2 – Dependences of the relative number of yeasts on the process time under the gas/US action: He/US (1) and Ar/US (2).

Conditions of the process:

$NM_0 = 4.2 \cdot 10^3$ CFU/cm³ - for He/US and

$NM_0 = 2.07 \cdot 10^4$ CFU/cm³ - for Ar/US;

$T = 298 \pm 1$ K; $P = 0.1$ MPa.

Hence, the effectiveness of the process of investigated water disinfection under cavitation depends not only on the process duration and the ability of yeast to be exposed to ultrasound action, as previously determined [10, 11], but also on the nature of the bubbled gas.

4. Conclusion

The yeast viability at the conditions of bubbling inert gases (argon and helium) through the water system was studied and the effect of each of the investigated gases on the cell destruction process in the water was compared. On the example of the destruction of *Saccharomyces cerevisiae* yeast type during bubbling of argon and helium under cavitation conditions, the effectiveness of its joint action in the water treatment processes is presented. Peculiarities of yeast growth on the nutrient medium and microscopic studies of their cells are given. The values of the effective rate constant of the yeast destruction for argon and helium are compared. Experimentally showed greater efficiency of the process of cell destruction under conditions of Ar/US. It is proved that the efficiency of reducing the NM in the unit volume of the water system depends on the nature of the bubbled gas under cavitation conditions.

REFERENCES

1. Andronov, V., Makarov Ye., Danchenko Yu. et al. (2020). Research of the regularities of forming and chemical composition of sewage water of a dairy processing company. Scientific and technical journal «Technogenic and Ecological Safety», 7(1), 13-21. (In Ukrainian) DOI: 10.5281/zenodo.3780011
2. Haseena, M., Malik, M.F., Javed, A. et al. (2017). Water pollution and human health. Environmental Risk Assessment and Remediation, 1(3), 16-19. DOI: 10.4066/2529-8046.100020
3. Naddeo, V., Cesaro, A., Mantzavinos, D. et al. (2014). Water and wastewater disinfection by ultrasound irradiation - a critical review. Global Nest Journal, 16(3), 561-577.
4. Kong, Y., Peng, Y., Zhang, Zh. (2019). Removal of *Microcystis aeruginosa* by ultrasound: Inactivation mechanism and release of algal organic matter. Ultrasonics Sonochem., 56, 447-457. <https://doi.org/10.1016/j.ultsonch.2019.04.017>
5. Park, J., Son, Y., Lee W. H. (2019). Variation of efficiencies and limits of ultrasonication for practical algal bloom control in fields. Ultrasonics Sonochem., 55, 8-17.
6. Dehghani, M.H., Mahvi, A. H., Jahed, G. R. et al. (2007). Investigation and evaluation of ultrasound reactor for reduction of fungi from sewage. J. Zhejiang Univ. Sci B., 7(8), 493-497.
7. Iorio, M. C., Bevilacqua, A., Corbo, M. R. et al. (2019). A case study on the use of ultrasound for the inhibition of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in almond milk. Ultrasonics Sonochem., 52, 477-483. DOI: 10.1016/j.ultsonch.2018.12.026
8. Li, Y., Shi, X., Zhang, Zh. et al. (2019). Enhanced coagulation by high-frequency ultrasound in *Microcystis aeruginosa*-laden water: Strategies and mechanisms. Ultrasonics Sonochem., 55, 232-242. DOI: 10.1016/j.ultsonch.2019.01.022
9. Carrillo-Lopez, L. M., Huerta-Jimenez, M., Garcia-Galicia, I. A. (2019). Bacterial control and structural and physicochemical modification of bovine *Longissimus dorsi* by ultrasound. Ultrasonics Sonochem., 58, 104-108. DOI: 10.1016/j.ultsonch.2019.05.025
10. Tsukamoto, I., Yim, B., Stavarache, C. E. et al. (2004). Inactivation of *Saccharomyces cerevisiae* by ultrasonic irradiation. Ultrasonics Sonochem., 11, 61-65. [https://doi.org/10.1016/S1350-4177\(03\)00135-4](https://doi.org/10.1016/S1350-4177(03)00135-4)
11. Dai Ch., Xiong F., He R., Zhang W., Ma H. (2017). Effects of low-intensity ultrasound on the growth, cell membrane permeability and ethanol tolerance of *Saccharomyces cerevisiae*. Ultrasonics Sonochem., 36, 191-197. DOI: 10.1016/j.ultsonch.2016.11.035
12. Koval I. Z., Kislenco V. N., Starchevskii V. L. et al. (2012). The effect of carbon dioxide on the viability of bacteria of *Bacillus* and *Diplococcus* genera. Journal of Water Chemistry and Technology, 349(2), 112-116. <https://doi.org/10.3103/S1063455X12020075>
13. Koval I. Z. (2020). Influence of oxygen and carbon dioxide on water purification from bacteria and yeast under cavitation conditions. Visnyk of V. N. Karazin Kharkiv National University series «Ecology», 22, 75-82. (In Ukrainian) DOI: <https://doi.org/10.26565/1992-4259-2020-22-07>
14. Koval I. Z. (2020). Viability of sporogenic bacteria in an inert gas atmosphere. Scientific Herald of Chernivtsy University. Biology (Biological Systems), 12(1), 8-13. (In Ukrainian)
15. Koval I., Starchevskyy V. (2020). Gas nature effect on the destruction of various microorganisms under cavitation action. Chemistry & Chemical Technology, 14(2), 264-271. <https://doi.org/10.23939/chcht14.02.264>

Коваль І. З.**ВПЛИВ ІНЕРТНИХ ГАЗІВ НА ДРІЖДЖІ В КАВІТАЦІЙНИХ УМОВАХ**

В даній роботі досліджено процес знезараження води з підвищеним вмістом дріжджів роду *Saccharomyces cerevisiae* в умовах кавітації (частота 22 кГц) при насиченні досліджуваної води інертними газами різної природи (аргон, гелій). Використання додаткового газу сприяє збільшенню бактерицидного ефекту та легшому утворенню кавітації. Представлені результати морфологічних ознак дріжджів, а також знімки клітин за результатами мікроскопічних досліджень при відповідному збільшенні, характерному для конкретного роду мікроорганізмів. Обчислені величини ефективних констант швидкості руйнування дріжджів. Показано, що ефективність очищення води від дріжджів в умовах газ/УЗ залежить не лише від тривалості процесу, але від природи барботованого газу. Відзначено ефективну природу газу в процесі очищення води від мікроорганізмів. Зменшення кількості бактеріальних клітин спостерігали в атмосфері обидвох досліджуваних газів, однак з різною активністю. Більшу ефективність на процес руйнування дріжджів проявив аргон в кавітаційних умовах: $k_d(\text{Ar/УЗ}) > k_d(\text{He/УЗ})$. Графічно представлено динаміку чисельності дріжджів під впливом газ/кавітація. Ступінь знезараження води $> 99\%$ виявлено вже після тривалості процесу 1 година в атмосфері аргону. ЧМ = 100 КВО/см³ в умовах Ar/УЗ досягнуто після $t=3600$ с, а для He/УЗ - лише після $t=5400$ с. Показано, що природа газу в кавітаційних умовах впливає на тривалість процесу очищення води.

Ключові слова: кавітація, аргон, гелій, вода, дріжджі, руйнування.

ЛІТЕРАТУРА

1. Andronov V. A., Makarov Ye., Danchenko Yu. et al. Doslidzhennya zakonimirostey formuvannya ta khimichnoho skladu stichnykh vod molokopererobnoho pidpryyemstva // Scientific and technical journal «Technogenic and Ecological Safety». 2020. Vol. 7. No 1. P. 13-21. (In Ukrainian) DOI: 10.5281/zenodo.3780011
2. Haseena M., Malik M. F., Javed A. et al. Water pollution and human health // Environmental Risk Assessment and Remediation. 2017. Vol. 1, Issue 3. P. 16-19. DOI: 10.4066/2529-8046.100020
3. Naddeo V., Cesaro A., Mantzavinos D. et al. Water and wastewater disinfection by ultrasound irradiation - a critical review // Global Nest Journal. 2014. Vol. 16, No 3. P. 561-577.
4. Kong, Y., Peng, Y., Zhang, Zh. Removal of *Microcystis aeruginosa* by ultrasound: Inactivation mechanism and release of algal organic matter // Ultrasonics Sonochem. 2019. Vol. 56. P. 447-457. <https://doi.org/10.1016/j.ultsonch.2019.04.017>
5. Park, J., Son, Y., Lee W. H. Variation of efficiencies and limits of ultrasonication for practical algal bloom control in fields // Ultrasonics Sonochem. 2019. Vol. 55. P. 8-17.
6. Dehghani M.H., Mahvi A.H., Jahed G.R. et al. Investigation and evaluation of ultrasound reactor for reduction of fungi from sewage // J. Zhejiang Univ. Sci B. 2007. Vol. 7, No 8. P. 493-497.
7. Iorio M. C., Bevilacqua A., Corbo M. R. et al. A case study on the use of ultrasound for the inhibition of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in almond milk // Ultrasonics Sonochem. 2019. Vol. 52. P. 477-483. DOI: 10.1016/j.ultsonch.2018.12.026
8. Li Y., Shi X., Zhang Zh. et al. Enhanced coagulation by high-frequency ultrasound in *Microcystis aeruginosa*-laden water: Strategies and mechanisms // Ultrasonics Sonochem. 2019. Vol. 55. P. 232-242. DOI: 10.1016/j.ultsonch.2019.01.022
9. Carrillo-Lopez L. M., Huerta-Jimenez M., Garcia-Galicia I. A. Bacterial control and structural and physicochemical modification of bovine *Longissimus dorsi* by ultrasound. Ultrasonics Sonochem. 2019. Vol. 58. P. 104-108. DOI: 10.1016/j.ultsonch.2019.05.025
10. Tsukamoto I., Yim, B., Stavarache, C. E. et al. Inactivation of *Saccharomyces cerevisiae* by ultrasonic irradiation. Ultrasonics Sonochemistry. 2004. Vol. 11. P. 61-65. [https://doi.org/10.1016/S1350-4177\(03\)00135-4](https://doi.org/10.1016/S1350-4177(03)00135-4)
11. Dai Ch., Xiong F., He R. et al. Effects of low-intensity ultrasound on the growth, cell membrane permeability and ethanol tolerance of *Saccharomyces cerevisiae* // Ultrasonics Sonochem. 2017. Vol. 36. P. 191-197. DOI: 10.1016/j.ultsonch.2016.11.035
12. Koval I. Z., Kislenco V. N., Starchevskii V. L. et al. The effect of carbon dioxide on the viability of bacteria of *Bacillus* and *Diplococcus* genera // Journal of Water Chemistry and Technology. 2012. Vol. 349, Issue 2. P. 112-116.
13. Koval I. Z. Vplyv kysnyu ta vuhlekysloho hazu na ochyshchennya vody vid bakteriy ta drizhdzhiv v kavitatsiynykh umovakh // Visnyk of V. N. Karazin Kharkiv National University series «Ecology». 2020. Vol. 22. P. 75-82. (In Ukrainian) DOI: <https://doi.org/10.26565/1992-4259-2020-22-07>
14. Koval I. Z. Zhytlyezdatnist sporohennykh bakteriy v atmosferi inertnykh haziv // Scientific Herald of Chernivtsy University. Biology (Biological Systems). 2020. Vol.12, Issue 1. P. 8-13. (In Ukrainian)
15. Koval I., Starchevskyy V. Gas nature effect on the destruction of various microorganisms under cavitation action // Chemistry & Chemical Technology. 2020. Vol. 14, No. 2. P. 264-271. <https://doi.org/10.23939/chcht14.02.264>