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SYNERGISTIC EFFECT OF ULTRASOUND CAVITATION AND GAS IN THE WATER DISINFECTION

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Abstract. The paper considers water purification processes from *Bacillus* bacteria type under the conditions of gases bubbling only (argon, helium, oxygen, and carbon dioxide), cavitation and combined action of gas and cavitation. The synergistic effect was found under conditions of simultaneous action of gas and cavitation $(k_{d(gas/US)} > k_{d(gas)} + k_{d(US)}$ almost double) and it was shown that $k_{d(gas/US)} > k_{d(gas)}$ by almost an order of magnitude. Relative series of effective destruction of microbial cells was established: Ar/US > O₂/US > He/US > CO₂/US. Destruction degree of the cells reaches 70 % at the shortterm Ar/US exposure (~8 min), which is 7 times more active than cavitation action and 13.5 times more than bubbling of Ar alone.

Keywords: synergistic effect, gas, cavitation, microorganisms, oxygen, carbon dioxide, argon, helium.

1. Introduction

Gas bubbles serve as cavitation embryos in the reaction medium under the action of ultrasound (US) [1-4], which in turn accelerates the destruction of microorganisms (MO) present in water. Dissolved gas, on the one hand, provides embryos excess for the formation of bubbles in the cavitation zones, and on the other hand increases the pressure in the bubbles and reduces the rate of their splash [1]. The latter process is affected by the rate of the gas bubble dissolution in the liquid. During caverns collapse (their size is approximately 1 mm) the allocated energy causes the MO destruction. Pathogenic microflora is completely destroyed near the points of caverns collapse and the active radicals are formed. Caverns occur in the volume of the emitter ultrasound chamber with a frequency of several dozens of kilohertz, mainly on heterogeneities, which can be spores of fungi, bacteria, acting as a kind of target. In addition, under the influence of ultrasound radiation numerous microscopic air bubbles

appear (the process of bulk degassing) in the volume of the investigated water.

The pressure inside the cavern at the initial moment of its formation is very small compared to the pressure in the liquid itself, so the liquid and the dissolved gases are directed to the center of the cavity. Under the influence of ambient fluid pressure, the diameter of the bubble sharply decreases, and it bursts. Instant collapse of bubbles creates a local pressure increase of up to tens of thousands of atmospheres. A pressure peak is formed in the cavity, if bubble size is minimal, and a spherical shock wave is formed and propagates in the liquid in the direction from the center [3, 5-6].

Acoustic cavitation occurs when the passage of high-intensity sound waves and amplitudes of sound pressure is in excess of the threshold value. When acoustic oscillations propagate, sound pressure changes the sign with the frequency of the field, and therefore acoustic cavitation is characterized by small caverns.

Cavitation can cause fluid degassing and separation of the liquids [7], initiate free radical reactions [8-9], accelerate chemical reactions by facilitating the mixing of reagents, accelerate synthesis of alcohols in the cavitation field [10], increase the rate of emulsification [11], improve diffusion processes, help to form highly concentrated emulsions or homogeneous dispersions of particles and promote the extraction of substances [12-14].

One important feature of cavitation is a local concentration of relatively low average acoustic field energy in very small volumes, resulting in high energy densities [5]. Studies of reactions under the influence of ultrasound have shown that the effectiveness of chemical reactions is directly related to cavitation and its effects occurring in the reaction medium. The determining factor is the redox processes that are realized under the action of a beam of cavitation cavities. Important role in ultrasound chemical reactions is played by various gases bubbled through the reaction medium [4].

In a saturated air suspension of diploid yeast *Saccharomyces vini* ($NM_0 = 3 \cdot 10^5$ cells/cm³) with 30-seconds exposure of a piezocavator generator (800 kHz, 7 W/cm²) about 55 % of cells are dying, after 2 min – about 77 %, and after 10 min – 90 % [15]. Hydrogen

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bubbling through the aquatic environment contributes to the survival of Sacch. vini, compared to argon, oxygen, and air [15]. The efficiency of *Gilmaniella fungi* death by ultrasound is 85 % [16].

of Inactivation Escherichia coli and Staphylococcus aureus in the presence of ultrasound was investigated in [17]: 3.02±0.52 log and 0.18±0.14 log, respectively. The inactivation of Microcystis aeruginosa was evidenced by the results of cell cytometry (reduction in size, internal granularity, integrity, and activity of algae cells) due to mechanical and chemical effects caused by ultrasound [18, 19]. Systematic analysis of algae cell morphology with an algae removal efficiency of 80-90 % is presented in [19]. The influence of the ultrasound cavitation intensity on the structure of microorganisms during wastewater disinfection was investigated in [20]. The reduction of algae by 80 % in the volume of 100 l of water is achieved at the ultrasound frequency of 36 kHz and the power of 650 W for 10 min and by 50-90 % at 36-175 kHz (power of 650 W) in the volume of 4 m³ and processing time of 60 min. The use of ultrasound technology to prevent the formation of biofilms under real conditions arising under industrial conditions is described in [21] and recommendations are given for the construction of ultrasound cavitators [20], which provide a high level of ultrasound vibrations in the volume of a liquid.

The simultaneous action of gas and cavitation ensures a uniform dispersion of gas in the volume of water which is an intensifying factor in the process of its disinfection [2]. MO destruction under US action is explained by cavitation processes, that is, the formation, growth, and collapse in the fluid of pulsating cavitation bubbles filled with gas, steam or a mixture thereof. At the points of their splashing, the microflora of water is completely destroyed, since the collapse of the caverns gives off the energy that causes the destruction of the microbes. Therefore, it is important to investigate the effect of the gas nature on the water system in the processes of water cavitation.

2. Experimental

Cavitation processes in the aquatic medium were generated by US action (UZDN-2T) with the frequency of 22 kHz, the power of 35 W. Gas bubbling, namely oxygen, carbon dioxide, argon, and helium into the water is an additional factor for intensifying the cavitation process. The temperature of 298±1 K and pressure of 0.1 MPa were experiments condition. Duration of purification process was 2 h.

For sowing of the studied waters samples the nutrient media (meat and peptone agar), sterile Petri

dishes, pipettes, and test tubes were prepared in advance. Before sowing the samples were thoroughly mixed, depending on the expected contamination, 10-fold dilutions based on sterile water were prepared, *i.e.*, ten consecutive dilutions (1:10, 1: 100, 1: 1000, *etc.*). A separate pipette was used for each dilution and inoculated sequentially from the highest dilution to the lowest one. The multiplicity of dilutions depends on the initial amount of MO per unit of water volume.

Sowing of water samples was carried out by the deep method. For deep sowing (in the depth of the medium) the measured amounts of solid medium, harvested in test tubes of 10–15 cm³ are usually used. To do this, meat-peptone agar was poured into test tubes at 1/2 of its height and sterilized at 0.1 MPa. The suspension of microorganisms was introduced directly into a sterile Petri dish at the bottom, slightly opening the lid of the cup, and then filled it with molten and cooled agar. The culture medium was thoroughly mixed in a circular motion of the cup without lifting it from the table surface. After that, the cup was left on the table until the agar solidified. Next, Petri dishes were placed in a thermostat for their cultivation and subsequent counting.

To quantify the MO, colonies grown on Petri dishes were counted based on the fact that each colony developed from a single cell. The number of colonies was expressed in colony forming units (CFU).

The essence of the method of determining the number of microorganisms (NM) in 1 cm³ is to determine the total amount of MO capable of growing on meatpeptone agar (MPA) in Petri dishes at the temperature of 308 ± 0.5 K for 48 ± 2 h in 1 cm³ of water, followed by counting colonies grown on nutrient medium.

The number of living cells was calculated after the treatment by counting of colonies grown on a nutrient medium.

Experimental points presented below were obtained from arithmetic averages of three to four parallel seeding of water samples.

The morphological features of the studied MO were determined. MO Morphological features include the shape, size of cells, their relative location, coloration by Gram, ability to form spores, movement of MO, *etc.*

For visual observation of the number of cells, microscopy of fixed cell preparations was performed. The dye was an aqueous solution of safranin. To do this, the "crushed drop" was prepared, carefully distributing a drop of liquid on the surface of the slide in a thin layer. The smear was then dried and fixed by heating for 2–3 s over a burner flame. The dye solution was poured on it with a pipette. The duration of staining for aqueous magenta was 1–2 min. After staining the aliquot, the dye was washed off, followed by drying in air or with a strip of filter paper.

A drop of immersion oil was applied on the aliquot and microscopic investigations were carried out at $1500 \times$ magnification.

Effective rate constants of cells destruction (k_d) were calculated according to the kinetic reaction equation of the first order. The values of k_d were calculated after combined action of cavitation and gas influence.

3. Results and Discussion

The tested microorganisms were bacteria of the *Bacillus* type. The choice of microbial culture is related to the dominance of these rod-shaped sporogenic bacteria among prokaryotes found by us in natural waters and sewage at the brewery and pharmaceutical plant [22, 23]. Therefore, in the studies of cavitation water disinfection the influence of bubbled gas on the viability of these microbes are presented.

Morphological features of *Bacillus cereus* bacteria are presented in Table 1. *Bacillus cereus* bacteria are bacilli, rod-shaped bacteria, sporogenic (forming endospores) with the diameter of spores smaller than cell diameter belong to the *Bacillaceae* family. Physiological features of microorganisms and characteristics of colony growth on a nutrient medium in Petri dishes are presented in [22].

Experimental data on the process of bacterial death in distilled deaerated water during gas bubbling, cavitation and simultaneous action of gas and cavitation are presented in Fig. 1.

During the bubbling of He, O₂ and Ar (Fig. 1, curves 1-3) through the water system, the destruction rate of bacillary cells (D_d) is 47.7; 48.3 and 73.6%, respectively, while at CO₂ bubbling it is 90.0 %. Effective rate constants of cells destruction (k_d) for these gases are presented in Table 2. The dependence of NM on the duration of carbon dioxide bubbling only is represented in Fig. 2. The high rate of water decontamination is apparently due to the increase in medium acidity ($pH_0 = 6.1$; $pH_{end} = 3.8\pm0.1$, Table 3). Under the conditions of simultaneous CO2/US action, the pH value was not significantly decreased ($pH_0 = 6.1$; $pH_{end} = 5.0\pm0.2$), which can be explained by the penetration of CO_2 into the cavitation bubbles and its subsequent degassing from water. However, the value of k_d under the action of CO2/US is almost twice greater than in the case of water saturation by CO₂ alone (Table 4): $k_d(CO_2/US) =$ = $(8.68\pm0.01)\cdot10^4$ s⁻¹, whereas $k_d(CO_2) = (3.56\pm0.51)\cdot10^4$ s⁻¹. This testifies to the intensification of the process under gas/US conditions.

Taking into consideration that the concentration of carbon dioxide absorbed by water from the air is significantly lower than its concentration in water, saturated with CO_2 , it can be predicted that the concentration of CO_2 in microbial cells increases with its bubbling through water and may cause their destruction in a short period of time. More thorough studies of the effect of pH system on the viability of *Bacillus* under CO_2 bubbling are presented in [24], and the change in pH depending on the duration of storage of aquatic systems containing bacillary cells after the previous gas/US action is described by us in [25].

Table 1



Morphological features of Bacillus cereus bacteria

Fig. 1. Dependencies of NM/NM₀ (*Bacillus* bacteria) on the process duration under different conditions: O₂ (1), He (2), Ar (3); US (4); CO₂/US (5), O₂/US (6), Ar/US (7). Initial data: NM₀ = 10^4 - 10^5 CFU/cm³

Fig. 2. Dependence of NM (*Bacillus* bacteria) on the duration of only carbon dioxide bubbling. Initial data: $NM_0 = 7 \cdot 10^4 \text{ CFU/cm}^3$

The low rate of MO degradation under oxygen bubbling only ($D_d = 48.3 \%$) may be explained by the increase in dissolved oxygen in water that was used by the microbes as a power source, which led to a slight accumulation of NM (~9±1 %) during 1800 s of the oxygen bubbling. That is why in the case of only oxygen bubbling through the microbial water system, the NM_{max} obtained from the short-term exposure ($t_{02} = 1800$ s) was taken as the initial data (NM₀).

Curve 4 (Fig. 1) describes the US effect alone on the bacterial viability without gas bubbling. However, while contacting with air, water is capable of being saturated with gases dissolved in water. However, cavitation causes partial degassing of the liquid, which continues at an atmospheric pressure for $\sim 2-10$ min [3], depending on the initial concentration of dissolved gas in water, and ~ 60 % of dissolved gas is released during the first minute of degassing. Therefore, it can be assumed that under these conditions in the process of bacterial inactivation the action of US alone in the absence of dissolved gases in water was investigated. In addition, model water, based on distilled deaerated water, was used for research.

In the case of cavitation action, the efficiency of water disinfection is by 25–50 % higher than that of the gases alone (He, O₂ and Ar) (Table 2) with a shorter process duration (Table 5). The combination of these two factors of influence, namely gas and US in the same reaction medium (Fig. 1, *curves* 5-7) made it possible to achieve the efficiency of destruction of bacillary cells > 99 % at NM₀= 7·10⁴ CFU/cm³. This confirms the significant influence of the gas on water disinfection under cavitation conditions and indicates the acceleration of the process under condition of simultaneous use of gas and cavitation. The value of k_d with the combined action of gas and cavitation almost twice exceeds the sum of k_d values with the action of each factor alone (Table 2):

$$k_{d(\text{gas/US})} > k_{d(\text{gas})} + k_{d(\text{US})}$$
.

Table 2

Process conditions	NM_{\odot} CFU/cm ³	Rd	$k_{d} \cdot 10^{4} \text{ s}^{-1}$
US	8·10 ² ÷2.8·10 ⁴	0.993	4.33±0.01
Ar	7.104	0.992	1.91±0.01
Не	7.104	0.945	0.82±0.06
O_2	7.104	0.997	1.21±0.04*
CO ₂	7.104	0.885	3.56±0.51
CO ₂ /US	2.8.10	0.993	7.35±0.01
O ₂ /US	7.10 ⁵	0.993	9.76±0.01
Ar/US	7.10 ⁵	0.997	10.1±0.01

The effectiveness of water disinfection from Bacillus bacteria

Notes: * for the initial data, the NM_{max} was obtained as a result of short-term exposure

Table 3

The pH values before and after water disinfection process from *Bacillus* bacteria

Process conditions	pH ₀	pH _{end}		
US		5.9-0.3		
Ar				
He		5.9±0.1		
O_2	61			
CO_2	0.1	3.8±0.1		
CO ₂ /US		5.0±0.2		
O_2/US		5 8+0 1		
Ar/US		5.6±0.1		

Enective rate constants of <i>Ducuuus</i> Dacteria destruction					
Process conditions	NM ₀ , CFU/cm ³	R_d	$k_{d} \cdot 10^{4}, s^{-1}$		
CO ₂ /US	$(2.2-2.532)\cdot 10^3$	0.977	6.99±0.02		
	$2.8 \cdot 10^4$	0.993	7.35±0.01		
	8.5·10 ⁴	0.998	8.68±0.01		

0.885

Effective rate constants of Bacillus bacteria destruction

Thus, we observe a synergistic effect under conditions of simultaneous action of gas and cavitation. The detected synergistic gas/cavitation effect makes it possible to achieve high efficiency of water disinfection for shorter process time (Table 5), which allows to reduce energy costs of the process as a whole. In addition, gas bubbling in the cavitation field through a freshly prepared microbial system prevents the aggregation of microbial cells and the formation of stagnant zones in the process of water disinfection; promotes flow turbulence and efficient suspension mixing; contributes to the generation of additional germs of cavitation.

 7.10^{4}

 $\overline{CO_2}$

A similar process of reducing the duration of wastewater treatment, but with the simultaneous use of ultrasound and sorbent (coal, clay) was investigated by Malyarenko *et al.* [26], who reduces the amount of chemical oxygen consumption; the action of UV/US is described in [27]; Ag nanoparticles/US in [28]; a heterogeneous TiO₂/US catalyst in [29]. As we can see from the literature, combined methods of water disinfection with the US action led to the decrease in process duration.

The study of kinetic regularities of the MO destruction in the water system proved that these processes are described by the kinetic reaction equation of the first order (Fig. 3), under the influence of the gases



 3.56 ± 0.51

Taking into consideration the influence of He, O_2 , CO_2 and Ar gases (Table 2), it is possible to show the relative series of effective *Bacillus* cells destruction under the influence of gases alone:

$CO_2 > Ar > O_2 > He$

which (except for the CO_2 as justified above) reproduces its sequence under cavitation conditions when exposed to bacteria of the same type:

$Ar/US > O_2/US > He/US > CO_2/US$

However, under the cavitation conditions, the destruction of bacteria occurs much more efficiently and with lesser process duration (Table 5) than in the atmosphere of the gases alone. The k_d values of gas/US action are almost an order of magnitude greater than the action of gases alone:

$$k_{d(gas/US)} > k_{d(gas)}$$

proving the practicality of the combined action of gas and cavitation in the process of water disinfection. However, the greatest efficiency of MO destruction in water was investigated with simultaneous action of argon and cavitation. Thus, the process of water disinfection under Ar/US conditions is more efficient than under action of O_2/US , He/US, CO_2/US .



0 1800 3600 5400 7200 9000 0 t, s -0.5 -1 -1.5 -2 -2,5 -3 -3.5 -4 In NM/NM₀

Fig. 3. Semi-logarithmic dependences of NM/NM₀ (*Bacillus* bacteria) on the process duration under conditions of action: He (1), O₂ (2), Ar (3); US (4); CO₂/US (5), O₂/US (6) and Ar/US (7). Initial data: NM₀ = 10^4 – 10^5 CFU/cm³

Fig. 4. Semi-logarithmic dependence of NM/NM₀ (*Bacillus* bacteria) on the process duration under conditions of ultrasound. Initial data: $NM_0=8\cdot10^2-2.8\cdot10^4$ CFU/cm³

Table 4

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Table 5

Conditions	nditions NM ₀ , CFU/cm ³ The duration of water disinfection from <i>Bacillus</i> bacteria to achieve these percent of process efficiency, min					ercent of	$D_d, \%$ $t_{\rm US} =$ = 120			
		30 %	50 %	60 %	70 %	80 %	90 %	98 %	99 %	min
US	$8 \cdot 10^2 - 2.8 \cdot 10^4$	-	~27	38	58	69	90	-	-	95.6
CO ₂ /US	$2.8 \cdot 10^4$	-	-	~13	27	41	55	86	105	99.3
Не	7.10^{4}	80	-	-	_	-	_	_	_	47.7
O ₂	7.10^{4}	82	~125	-	_	-	_	_	_	48.3*
O ₂ /US	7.10^{5}	-	-	-	-	~18	42	65	84	99.8
Ar	7.10^{4}	32	56	78	108	_	_	—	_	73.6
Ar/US	7.10^{5}	_	_	-	~ 8	~27	46	68	83	99.9

Dependence of *Bacillus* destruction effectiveness on the process duration

Notes: * for the initial data, the NM_{max} was obtained as result of short-term exposure

The destruction of Bacillus bacteria under Ar/US action (99% efficiency) was achieved after 83 min (Table 5), while at the same time in the presence of ultrasound this value is only 86%, with the action of Ar alone – about 62 %, and with O_2 and He bubbling – only 30 %. During short-term process (~8 min) under Ar/US conditions the 70 % efficiency of MO destruction was obtained, whereas at the action of US alone such water disinfection efficiency was achieved only after 58 min (the activity is 7 times higher at Ar/US-action), and at Ar bubbling alone - after 108 min (the activity is more than 13.5 times higher at Ar/US-action). That is, cavitation accelerates the rate of water disinfection twice compared to bubbling the gas alone, and the simultaneous action of gas/cavitation is almost an order of magnitude greater than cavitation action alone. These data prove the intensification of MO destruction process when gas is applied under cavitation conditions and the need for gas bubbling for water treatment systems.

An explanation of the 70 % efficiency (instead of expected 98–99 %) achieved as a result of short-term

exposure (up to 10 min) under the action of Ar/US (Table 5) is based on the detected processes of disaggregation of microbial cells clusters in the initial water samples. The MO aggregates were broken up into isolated cells during subsequent exposure, leading to a slight increase in NM, which impacted the process efficiency during Ar/UStreatment after this short time. However, deaggregation processes are accompanied by the process of their destruction. The mechanical damage of individual cells after a short-term sonication confirms this fact. Water containing microbial aggregates in the initial water samples requires the longer process time to achieve the desired degree of water disinfection. These results were confirmed by the optical microscopy images of MO before and after gas/cavitation action (Fig. 5). Aggregates of microbial cells in the initial water samples were not detected during simultaneous action of CO2/US and O₂/US. The disaggregation processes after short-term exposure of cavitation in the presence of gas were studied by us and published in [30].



Fig. 5. Optical microscopy images of B.*cereus* before (a) and after (b, c) Ar/US-treatment for 5 min (b) and 80 min (c). Magnification of 1500×

However, even taking into account the existing disaggregation processes that were found in the initial water samples in a small amount under the action of Ar/US for 10 min, the cell death efficiency of 99% was obtained after the exposure for 83 minutes under these conditions. This result is best compared to the effect of oxygen and carbon dioxide under cavitation conditions at the same processing time (Table 5).

High performance of destroyed cells was established by simultaneous action of gas and cavitation, compared with the action of each agent separately. This indicates the intense destruction of the microbes in their combined action, as well as the feasibility of using gases in the processes of cavitation water disinfection. The presented experimental results demonstrate the synergism of the action of cavitation and gas, which determines the role and practical importance of the gas presence in the cavitation zone. This comprehensive approach ensures high efficiency of the water disinfection process and opens the prospect of practical application of the cavitation action in the atmosphere of gases of different nature.

4. Conclusions

The intensifying effect of gases in the processes of cavitation disinfection of water was confirmed, which proved the effectiveness of gas bubbling in the processes of destruction of aquatic microflora under cavitation conditions. A synergistic gas/cavitation effect was established: $k_{d(gas/US)} > k_{d(gas)} + k_{d(US)}$. It was shown that the value of the effective rate constant of bacteria destruction in water under the action of gas/cavitation is greater by almost an order of magnitude compared to the action of the gases alone: $k_{d(gas/US)} > k_{d(gas)}$. This indicates the feasibility of practical application of the joint action of gas and cavitation in the processes of water disinfection.

The destruction of *Bacillus* bacteria under cavitation conditions was found to be dependent on the nature of the bubbled gas. The highest efficiency of cavitation water disinfection was found for argon, compared to helium, oxygen, and carbon dioxide. 70% efficiency of MO destruction was achieved at the short-term cavitation exposure (8 min) in the argon atmosphere, compared to $D_d = 25\%$ under the action of cavitation alone and $D_d = 5.5\%$ under the action of argon during the same time. Argon bubbling under cavitation conditions accelerated the process by 13.5 times, compared to the argon bubbling alone.

The relative series of effective MO destruction under conditions of the gases alone and combined action of gas and cavitation were established. The series of effective destruction of bacillary cells under the action of the gases alone ($CO_2 > Ar > O_2 > He$) reproduces its sequence under cavitation conditions (Ar/US > O_2/US > He/US > CO₂/US), except for the reasonable action of CO₂, due to the increase of acidity of the reaction medium.

It was investigated that regardless of the initial number of MO cells per unit of water volume and the nature of the bubbled gas, the processes of MO destruction with simultaneous application of gas and cavitation are described by the kinetic reaction equation of the first order.

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СИНЕРГІЧНИЙ ЕФЕКТ УЛЬТРАЗВУКОВОЇ КАВІТАЦІЇ ТА ГАЗУ ПРИ ЗНЕЗАРАЖЕННІ ВОДИ

Анотація. Досліджено процеси очищення води від бактерій роду Васіllus в умовах барботування самих газів (аргону, гелію, кисню та вуглекислого газу), кавітації та спільної дії газ/кавітація. Виявлено синергічний ефект за умов одночасної дії газу та кавітації ($k_{d(cas)Y3} > k_{d(cas)} + k_{d(Y3)}$ майже вдвічі) та показано, що $k_{d(cas)Y3}$ більше за $k_{d(cas)}$ майже на порядок. Встановлено відносний ряд ефективного руйнування мікробних клітин: Ar/US > $O_2/US > He/US > CO_2/US$. При короткотривалій експозиції Ar/Y3 (~ 8 хв) досягнуто ступеня руйнування клітин (D_d) 70%, що активніше від дії самої кавітації в 7 раз та в 13.5 раз від барботування самого Ar.

Ключові слова: синергічний ефект, газ, кавітація, мікроорганізми, оксиген, вуглекислий газ, аргон, гелій.

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