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Ultrasonic Intensification of the Natural Water and Sewage Disinfection

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The effectiveness of ultrasound action on the disinfection processes of natural water and sewage, as well as the kinetic regularities of these processes have been shown. Disinfection processes of the investigated waters under ultrasonic cavitation and gas bubbling correspond to the kinetic regularities that are described by the reaction equations of the first order regardless of the different origins of polluted waters, different initial number of microorganisms and their different types. In the given work the natural water disinfection is the combination of the processes containing deaggregation and cells destruction. The highest efficiency of the sewage disinfection has been determined under the simultaneous action of argon and ultrasound.

1. Introduction

Manufacturing application of ultrasonic devices is substantially conditioned by the prospects of their use in various industries: chemical, food, medicine (Alupului and Lavric, 2008), technological fields, particularly, in water purification. Water ultrasound (US) treatment is one of the most effective decontamination methods resulting in the microorganisms inactivation (Dehghani, 2005; Stamper et al., 2008). Water disinfection under the US action is explained by the cavitation process during sonication, i.e. formation, growth and collapse of gas bubbles in a liquid (Ashokkumar et al., 2007). US effective disinfection action on various types of microorganisms is confirmed by numerous positive results but the information about kinetic reaction order of the disinfection process of natural waters and sewage was not found in the literature.
A high decontamination degree has been achieved in our previous studies under acoustic cavitation effect in the model microbial suspensions (Koval et al., 2010; Starchevskyy et al., 2008). Therefore, the aim of further research is to study the kinetic regularities of microorganisms inactivation in the natural water and the industrial sewage in the presence of US and combined action of gas/US. Such regularities will allow to determine the most effective conditions for these processes.

2. Materials and Methods

The investigated water objects were the natural water with the different initial number of microorganisms (NM_0): NM_{01} = 820 cells/cm^3; NM_{02} = 2090 cells/cm^3 and industry

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sewage N_o1 (from brewing production with the $NM_0 = 1.1 \cdot 10^5$ cells/cm^3) and N_o2 (from pharmaceutical enterprise with the $NM_0 = 3.86 \cdot 10^4$ cells/cm^3).

The experiments were carried out under T=298±1K, P=0.1MPa, the process time (t) was 2 h. As additional gases for the research oxygen, argon and carbon dioxide were used. Gases bubbled into the water during the whole process with the rate of ~ 1 cm^3/s. The gas bubbling and US took place simultaneously in the same vessel.

The source of ultrasonic waves was UZDN-2T generator with the frequency of 22 kHz, power of 35W and intensity of 1.65W/cm^3. Ultrasonic vibrations were transmitted by the magnetostriction radiator, immersed into the investigated water (V_{H2O}=75 cm^3).

Samples of sonicated water (1cm^3) were poured out in Petri dishes on a nutrient medium (meat water (1dm^3), pepton (10g), agar (15g)). Petri dishes were placed in TS-80M-3 thermostat at the temperature of 37^{0}C for 48 h. Water samples taken before US action served as the control samples. The concentration of microbial cells was determined by the number of colony forming units, assuming that each colony developed from a single cell.

Based on the range of microbiological tests the NM dynamics depending on the aqueous medium conditions and water sampling date was established. For this purpose the samples of natural water were periodically taken during June and July.

Microorganisms identification was carried out by microscopic investigation using “crushed drop” preparations and fixed cells preparations with safranin as a coloring agent to study the morphological features of the cells. “Crushed drop” preparations means the direct research of the water samples using the microscope. Physiological and cultural features of colonies were also investigated.

3. Results and Discussion

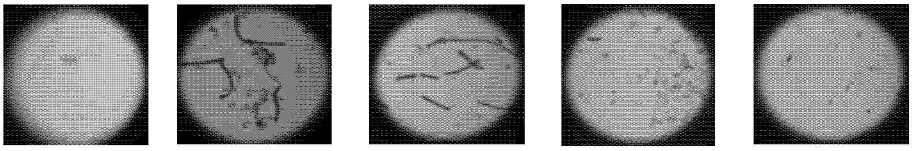
3.1 Natural Water Disinfection

Before the study of the US action on the natural water disinfection the type of microbes were determined using microscopic investigations. It is of great importance for understanding of this process. Among microbial cells bacteria of *Bacillus* types were identified in dominant amounts.

After a series of parallel microscopic studies of water samples we found that the microorganisms are closed in an overall slime and form aggregates (Fig. 1*a*) using “crushed drop” preparations, while the number of individual cells was low. During the examination of the fixed preparations we observed the presence of cells tightly adjacent to each other and covered with a thin colloidal case; thus long filamentary shapes are formed (Fig. 1 *b,c*). The number of cells in them is varied widely from 5 to 40 cells. The colony sizes are from 2-3 to 40-50 microns.

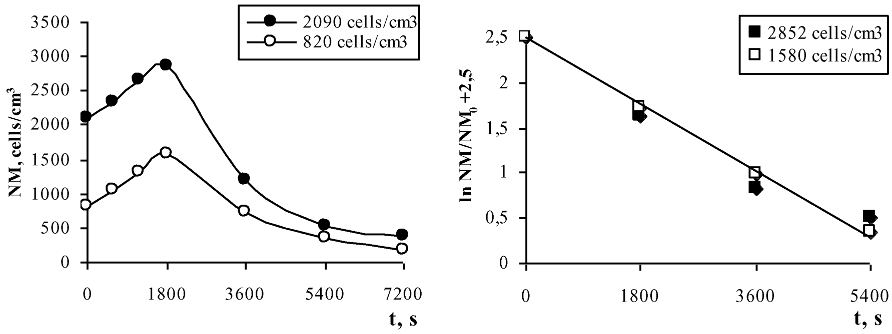
The values of NM in the natural water varied from 820 to 2090 cells/cm^3 during June and July because the water temperature increased from 13±2^{0}C to 21±1^{0}C. Thus, the investigation of US disinfection was performed with minimum (NM_{01}=820 cells/cm^3) and maximum (NM_{02}=2090 cells/cm^3) initial number of microorganisms (Fig. 2*a*). Previous studies showed that the NM_0 did not change during 2 h before the US action in control experiments, what is enough for the experiment.

Under the natural water US treatment not only expected decrease of NM (the second stage) was observed but also the increase of their number at the first stage during of 1800s. Therefore, each of these processes was considered separately.



a) b) c) d) e)

Figure 1: Scanning microscopy images of microorganism cells before (a-c) and after (d,e) US exposure: d) t_{US}=1800s; e) t_{US}=7200s. Increase of images is 1200.



a) b)

Figure 2: Effect of US action on the NM in the natural water during all process (a) and at the stage of microorganisms destruction (b). Conditions: T=298±1K, P=0.1MPa, US frequency of 22 kHz, power of 35 W.

Table 1: Efficiency of US action of the natural water disinfection

NM_0, cells/cm^3	N_a at the appropriate t_{US}, %			R^2_a	$k_a \cdot 10^4$, s^{-1}	N_d at the appropriate t_{US},%			R^2_d	$k_d \cdot 10^4$, s^{-1}
	600 s	1200 s	1800 s			3600s	5400 s	7200 s		
820	28.05	59.76	92.68	0.995	3.75±0.01	53.92	78.16	88.29	0.978	4.06±0.03
2090	11.48	26.79	36.46	0.989	1.81±0.02	58.35	81.17	86.50		

According to Fig. 2*a*, the number of cells during t_{US}=1800s essentially increased. The results are represented in Table 1. Pitt and Ross (2003) have also reported about the increase of bacterial cell growth under US action but microorganisms were attached to a polyethylene surface.

Microscopic investigations of sonicated water after 1800s explain the reasons of cells accumulation at the first stage of the process. The NM considerably increases due to the breaking of microbial aggregates into smaller clusters, which break up in their turn into a great number of individual cells (Fig. 1*d*). However, deaggregation processes are accompanied by the process of their destruction. The mechanical damage of individual cells after a short-term sonication (t_{US}=60÷180s) confirms this fact. Obviously, initial individual cells of the natural water undergo instantaneous destruction after US generator is switched on. This phenomenon is additionally confirmed by us during US treatment of model microbial suspension infected by bacteria of *Bacillus* type. The suspension without microorganism aggregates has cells separated one from another. In the model water bacteria of *Bacillus* type were used as dominant microbial species in the investigated waters.

The number of cells accumulation at the first stage of the process considerably exceeds the number of killed cells, so in Fig. 2*a* we see only an increase of the NM during t_{US}=1800s. Thus, we can assume that the NM increases in the natural water due to the gradual deaggregation processes under the US action.

The maximum NM was observed at 1580 cells/cm^3 (NM_{max1}) in the water with NM_{01} and at 2852 cells/cm^3 (NM_{max2}) in the water with NM_{02} (Fig. 2*a*). The number of accumulating cells (N_a), expressed in percentage and effective rate constants of this process (k_a) during the first stage are shown in Table 1. At the NM_{01} the deaggregation process is more efficient, what is explained by the larger size of aggregates. The experimental points reflecting the process of NM increase under the US action are in agreement with the coordinates of the first order equation, what is mainly caused by the aggregates destruction processes with a gradual breaking into individual cells.

The NM_{max} were taken as initial data while consideration of the microorganism destruction stages. The number of destroyed cells (N_d), expressed in percentage and effective rate constants of this process (k_d) during the second stage (t_{US}=1800÷7200s) are shown in Table 1. The NM reduction is connected with the mechanical destruction processes of individual cells during the long US treatment. The long US action reduces the acutance of cell membranes external contours (Fig. 1*e*).

The NM/NM_{max} slightly varies during the cells destruction at various NM_{max}, therefore, the processes of the NM reduction can be described by one straight line in semilogarithmic coordinates, i.e. these processes are also described by the first order equation (Fig. 2*b*). The value of k_d is determined from the tangent of the straight line slope and equal to $(4.06\pm0.03)\cdot10^{-4}$ s^{-1} (Table 1). Moreover, $k_d>k_a$, what indicates a greater efficiency of cells inactivation compared with the deaggregation processes.

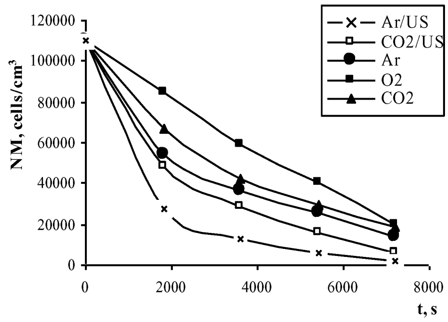
3.2 Sewage Disinfection

The bacteria of *Micrococcus, Sarcina* and *Bacillus* types were identified among bacterial cells and only *Saccharomyces* – among yeast in the sewage N_o1. Sewage N_o2 contained bacteria of *Sarcina* and *Bacillus* types in dominant amounts.

Dependences of the NM decrease in the sewage N_o1 during gases bubbling and simultaneous gas/US treatment are shown in Fig. 3*a*. Using the US influence on the sewage but changing the gas nature (O_2, CO_2 and Ar) under the same experimental conditions one can estimate the gas effect on the water disinfection process under ultrasonic cavitation. During the whole process the most effective decontamination agent among the used gases was argon under US action (97.86%) and without US action

(87.41%) in contrast to oxygen and carbon dioxide under similar conditions (Table 2). Accordingly, the effect of argon is described by the most effective rate constants of cells destruction (k_d) (Table 2).

As for the oxygen action under US cavitation and despite the different NM_0 of the sewage of both types, it should be noted that the NM/NM_0 ratio slightly varies (Table 3), what indicates similar regularities of the disinfection processes for the sewage N_o1 and N_o2 under the O_2/US treatment. The cells destruction in the sewage from various enterprises under the O_2/US action proceeds according to the equation of the first order reaction (Fig. 3*b*) and the process may be described by only one value of k_d (Table 2).



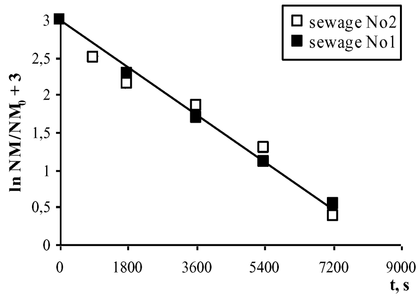


Figure 3: Effects of different gases and gas/US action on the NM in the sewage N_o1 (a) and effect of O_2/US action on the NM in the sewage N_o1 and N_o2 (b). Conditions: T=298±1K, P=0.1MPa, US frequency of 22 kHz, power of 35 W.

Table 2: Efficiency of different gases and gas/US action on the sewage disinfection

Process conditions	NM/NM_0	N_d at the t=7200s, %	R^2_d	$k_d \cdot 10^4$, s^{-1}
O_2	0.18	82.15	0.951	2.12±0.05
CO_2	0.17	83.31	0.997	2.51±0.01
Ar	0.13	87.41	0.982	2.89±0.02
O_2/US	0.08	91.59	*0.981	*3.51±0.02
CO_2/US	0.05	95.01	0.983	3.94±0.02
Ar/US	0.02	97.86	0.974	5.48±0.03

*Note: * – for the sewage N_o1 and N_o2.*

The microorganisms destruction considerably depends on the nature of gas bubbling. The row of gas nature effect in the US field on the cells destruction of sewage N_o1 is established by us: Ar/US > CO_2/US > O_2/US.

The NM/NM_0 ratio in a water volume unit under the US action in the presence of gases is the one order lower than that under the gases effect (Table 2), what confirms the advantage of gas using for the treatment of contaminated water under the US and indicates the intensification of water disinfection under the simultaneous gas/US action.

Table 3: Relative number of microorganisms in the sewage under O_2/US treatment

Investigated waters	NM/NM_0 at the appropriate t_{US}, %			
	1800 s	3600 s	5400 s	7200 s
Sewage N_o1	0.49	0.27	0.15	0.08
Sewage N_o2	0.42	0.32	0.18	0.07

Thus, the obtained results of the sewage disinfection indicate the prospective use of the combined action of US and gases of different nature for water purification. The sewage after the previously sonication may be wasted to the water bodies without any additional microbe contamination.

4. Conclusion

A high disinfection degree of the natural water under the US action and higher efficiency of microorganisms destruction in the sewage using gas/US treatment compared to the gases effect have been shown. Thus, the investigation results of the acoustic effect on microorganisms destruction in contaminated waters extend the possibilities of the US use during their disinfection and allows to determine the most effective conditions of this process with the aim of achieving a high degree of microorganisms inactivation.

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