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MICROBIAL DISAGGREGATION WITH AND WITHOUT GAS BUBBLING UNDER CAVITATION CONDITIONS

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Abstract

Short-term cavitational treatment (22 kHz, 1.65 W/cm^3, 35 W) of the water showed an increase in the amounts of the cells of Bacillus cereus bacteria type that was explained by the process of cell disaggregation usage microscopical investigations. The duration of disaggregation process was 3-5 minutes with gas bubbling during sonication but 30 minutes without gas bubbling during sonication. It was shown an acceleration of disaggregation process under simultaneous action of gas and cavitation by 10 times (synergistic effect).

Keywords: *short-term treatment, cavitation, gas, bacteria, cyanobacteria, disaggregation*

Introduction

Common methods of water disinfection among many chemical methods are chlorination, ozonation, hydrogen peroxide treatment and others, but modern technologies allow disinfection of water without using chemicals. More and more importance is attracted to physical factors of influence on water system – magnetic, electric, ultraviolet, ultrasound (US). Among them the practical importance is acquired by sonochemical methods of water treatment. Practical application of cavitational influence on the microorganisms cells leads to an expansion of the opportunities and to improvement of water purification technology. But many experimental studies are devoted to removal of microorganisms from water under US action (Zhang et al. 2006; Wu et al. 2012). Information about water treatment during short-term exposure of contaminated water is limited. Hence, the aim of current research is investigate short-term duration of cavitational treatment of some microorganisms (spore-forming bacillary cells) in the water medium.

Previously, we investigated the processes of water disinfection from different types of microorganisms: *Diplococcus*, *Bacillus*, *Sarcina*, *Pseudomonas* and *Saccharomyces* yeasts (Koval et al. 2011; Koval et al. 2012; Koval & Starchevskyy 2012; Koval & Falyk 2016; Koval 2016), natural water and sewages disinfection (Koval et al. 2012). It was found that the values of effective constants of microorganisms destruction rate were determined by the nature of bubbled gas (O_2, CO_2, Ar, He) and were independent on the initial amounts of cells in the water system (Koval et al. 2012; Koval et al. 2014).

It was achieved the hıghest efficiency of sewage disinfection (97.9%) after Ar/US-action with exposure of 2 hours and an efficiency of 99.8% of model water disinfection from bacillary cells. The next step of our investigation is to study the short-term exposure on the cells that could be valuable for explanation of mechanical cells destruction after cavitational impact on individual cells.

Materials and Methods

The water objects of the research were model water medium prepared from natural water with *Bacillus cereus* (N_01) and model medium from air-free distilled water which were added pure microorganism monocultures of *Bacillus cereus* bacteria type (N_02).

The experiments were carried out under T = 298±1 K, P = 0.1 MPa, US frequency of 22 kHz, intensity of 1.65 W/cm^3, power of 35 W and the process time (t) was from 3 to 60 minutes.

The change of number of microorganism (NM) was investigated with and without gas bubbling under cavitation condition. The source of cavitation was UZDN-2T generator. Ultrasonic vibrations were transmitted by the magnetostriction radiator which was immersed into the model medium with the volume of 75 cm^3. As additional gases for the research were used argon, helium, oxygen and carbon dioxide. Gases were bubbled into the water during the whole process with the rate of ~ 1 cm^3/s.

Morphological and cultural features of microorganisms were investigated. Fixed cells preparations with safranin as a coloring agent were used to study the morphological features of the cells. The nature of colony growth on a nutrient medium in Petri dishes was study for cultural features. Meat and pepton agar were used as a nutrient medium for bacteria growth on Petri dishes at 37°C for 48 hours.

Results and Discussion

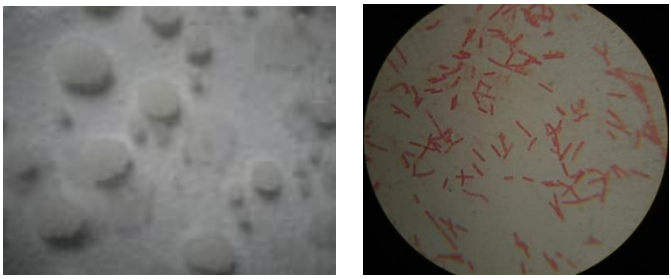
Bacillus cereus bacteria type as spor-forming cells were taken for investigation and as more resistant cells compared with vegetative cells. The initial NM (NM_0) of the model water N_01 was $8•10^2$ CFU/cm^3(NM_{01}) and $2•10^3$ CFU/cm^3(NM_{02}) and $8•10^2$ CFU/cm^3 (NM_0) of the model water N_02.

Cultural and morphological forms of *Bacillus* are illustrated on the Fig.1. On the base of general view of cellcolonies (Fig. 1-a) could estimate and describe colony characteristics, which are presented in Table 1. Optical microscopy images of the cells (Fig. 1-b) it is fixed cells preparation that allows to study morphological features. So, *Bacillus* are bacillary bacteria cells, belong to *Bacillaceae* family, they are spor-forming (form spore inside the cells, i.e. endospore within three days), spore diameter is smaller than cells diameter.

Here are presented dependences of NM changes of sonication time without gas bubbling (Figure 2) and with gas bubbling (Figure 3). It was shown that short-term exposure leads to the increasing of NM in both cases. But process duration was 30 minutes (Figure 2), unlike 3 minutes under simultaneous action of gas and cavitation (Figure 3). The results of cells increasing are represented in Table 2. The values of effective rate constants were calculated from kinetic equation of first order. Pitt and Ross have also reported about the increase of bacterial cell growth under cavitation but microorganisms were attached to a polyethylene surface (Pitt & Ross 2003).

Increasing of NM has been explained by the microscopical studies fixed cells preparations model water N_02 (Figure 4 a-c). It was found microorganisms which were connected into aggregates of different forms in an initial water samples before sonication (Figure 4-a). Cavitation broke the aggregates into smaller aggregates and into individual (separated) cells (Figure 4-b, 4-c). That is why, disaggregation processes took place during short-term exposure. The duration of disaggregation process was 30 minutes for model water N_01 (Figure 2) but 3-5 minutes for model water N_02 (Figure 3). Gas bubbling under cavitation conditions caused acceleration

of disaggregation process by 10 times. So, synergistic effects were observed during short-term exposure under the simultaneous action of gas and cavitation. But these effects considerably depended on the nature of gas bubbling. The highest efficiency was obtained for argon.



(a) (b)

Figure 1. Microorganisms of *B.cereus*: (a) character of colonies growth on a nutrient medium in Petri dish; (b) optical microscopy images of microorganism cells (image magnification is 1500).

Thereby, during short-term treatment suffer damage aggregates that are cause of destroying connections between cells in an aggregate. But bacteria retained morphological features and fully viably after disaggregation and cell walls were not damaged. But, despite this, the cells lost reproductive ability and ability to the physiological generation of new cells even after short-term process, because cells on the stage of cell division weren't found during microscopically investigations.

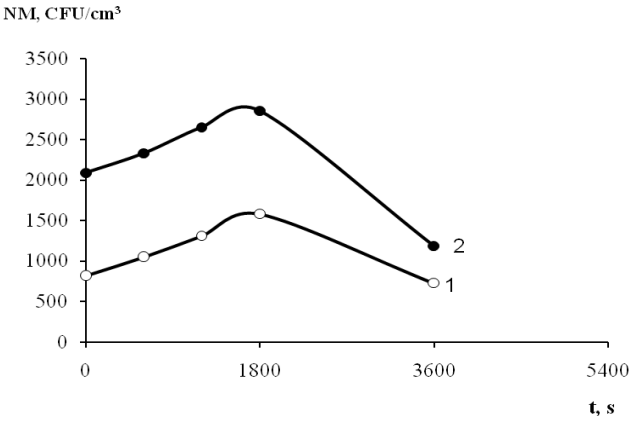


Figure 2. The change of NM values under US action in the model water $N_0 1$. Conditions: $N_0 1 = 8 \cdot 10^2$ CFU/cm^3 (curve 1); $N_0 2 = 2 \cdot 10^3$ CFU/cm^3 (curve 2).

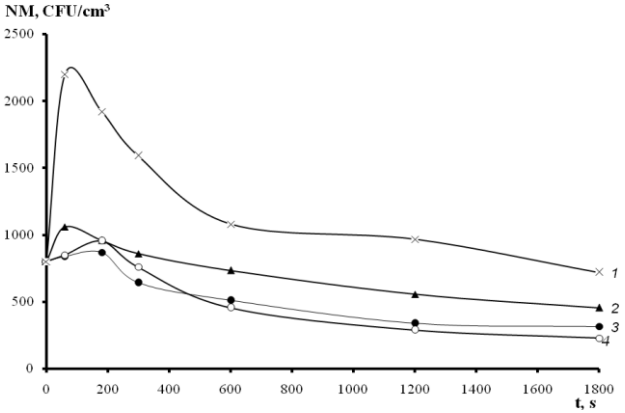
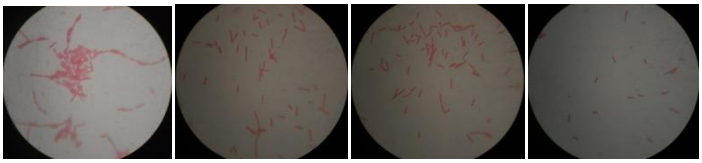


Figure 3. The change of NM values under the gas/US action in the model water $N_0 2$: Ar/US (4), O_2/US (3), He/US (2), CO_2/US (1). Conditions: $NM_0 = 8 \cdot 10^2$ CFU/cm^3.



(a) (b) (c) (d)

Figure 4. Optical microscopy images of *B. cereus* before (a) and after (b-d) cavitation. Magnification of images is 1500.

Decreasing of NM was observed after full disaggregation process, because individual cells were more susceptible to the destruction. The NM reduction was connected with mechanical destruction processes of separated cells. Continued cavitation impact reduced an acutance of cell membranes external contours of cells that survived after treatment (Figure 4-d).

Hence, in the case of presence of disaggregation process under cavitation conditions the process of water disinfection requires a greater length of treatment time (more than 60 minutes for natural water without gas bubbling). Because NM after t_{US} = 60 minutes is equal $8 \cdot 10^2$ CFU/cm^3 and approximately equal to NM_0 (Figure 2, curve 1) and $1.1 \cdot 10^2$ CFU/cm^3 (Figure 2, curve 2). So, after this treatment time we can speak about disinfection process that demonstrates further cells reduction only. In general, disaggregation increases duration of the disinfection process that depends on the NM_0 in the water sample before treatment.

Conclusions

Results of experimental researches are presented below:

- Short-term cavitation leads to the cells disaggregation that discrubes the NM increasing.
- Gas bubbling under cavitation conditions accelerates disaggregation process by 10 times, unlike cavitation conditions without usage of gas (synergistic effect).

- Sonication enhances to break up microbial aggregates into single cells, which are more susceptible to the destruction under cavitational conditions.
- Sonicated *B. cereus* bacteria cells and *O. brevis* cyanobacteria, have lost their ability to the physiological generation of the cells.

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