

Cavitation action on the cocci bacteria

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IRYNA KOVAL¹, AMIR HAJIALI²

¹Lviv Polytechnic National University, Department of Physical, Analytical and General Chemistry, 12 Bandera, 79013, Lviv, Ukraine

²Postdoctoral Fellow in Geoecology at Yerevan State University, 1 Alex Manoogean, 0025, Yerevan, Armenia
Corresponding author (e-mail): irynazk@gmail.com, a.hajiali.env@gmail.com

Abstract

Features of growth of microorganisms on a nutrient medium and their microscopic researches were studied. Cavitation treatment (22 kHz, 91 W, 1.65 W/cm³) of water with the simultaneous action of bubbled inert gases (argon and helium) on the viability of microbial cells (*Diplococcus* and *Sarcina*) are presented. The highest water disinfection was obtained for water samples with *Sarcina lutea* cells for both used gases under cavitation conditions. Both investigated types of cocci bacteria were destroyed faster under Ar/US-action after comparison of the effectiveness of the gas nature action on the water disinfection.

Keywords: destruction, gas, cavitation, cocci bacteria

INTRODUCTION

Natural and wastewaters include not only biological compounds but also organic and toxic compounds [1]. Studies in recent years have shown high efficiency of cavitation during microorganisms' removal [2-4]. However, the effectiveness depends on the microorganisms' type. All microorganisms are different in features, properties, etc. that may have an ambiguous effect on the process of their destruction. Therefore, the purpose of the present research was to investigate cavitation influence in the gas atmosphere on the viability of cocci bacteria in the water medium.

MATERIALS AND METHODS

Water samples based on deaerated distilled water with added *Diplococcus lanceolatus* and *Sarcina lutea* were used for investigations. The studied pure cultures of microorganisms were grown in test tubes in the laboratory at 30°C for 96 h on agar medium followed by storage at 4°C.

Carried out microscopy of fixed cell preparations. Microscopy of fixed cell preparations includes several successive steps. At the first stage, a drop of test water is applied to the degreased slide with a bacterial loop. Then this drop is evenly rubbed with a loop on an area of 1-2 cm². The smear should be thin, uniform in thickness, oval. The second stage is the process of drying this smear. The smear is

Investigated microorganisms of *Diplococcus lanceolatus* and *Sarcina lutea* types from the Coccaceae family have different sizes and numbers of connections. Differences in morphological, physiological, and cultural characteristics are presented in detail in [5], which describes the response to oxygen during the intensity of growth after injected in a melted substrate (physiological characteristics), characteristics of colony growth on a nutrient medium in Petri dishes (cultural characteristics).

best dried at room temperature in the air for 15-20 minutes.

The fixed drug is placed on a parallel glass bridge over the cuvette and the drug was filled with magenta for 1-3 minutes. After staining, the drug is washed with water until the water becomes colorless, the drug is dried in the air, applied to the smear a drop of immersion, and microscopy.

Simultaneous action of gas and cavitation, namely Ar/US and He/US on the individual bacterial cells were used. Effective rate constants of cell destruction (k_d) were calculated after the combined action of cavitation and gas influence. Cells were grown on the nutrient medium – meat and peptone agar (MPA) on Petri dishes.

The source of cavitation was US waves from generator UZDN-2T with an oscillation frequency of 22 kHz, power of 91 W, and intensity of 1.65 W/cm³. US oscillation was transmitted by the magnetostrictive emitter immersed into the volume of investigated water (V = 75 cm³). Experimental conditions were T = 298±1K, P = 0.1MPa, process duration (t) – 2 h.

$$X = a \cdot 10^n \quad (1)$$

where *a* is the number of colonies grown in the

To quickly calculate the total number of colonies (X), determine their number in 1 cm²

$$S = \pi \cdot r^2 \quad (2)$$

where *r* is the radius of the dish, then:

$$S = m \cdot \pi \cdot r^2 \quad (3)$$

The experimental data presented below of the experimental part of the work were obtained

For the quantitative determination of the MO, the colonies grown on Petri dishes were counted based on the fact that each colony evolved from a single cell.

The results obtained were converted to the initial water sample, taking into account the dilution according to the formula:

dish, and *n* is dilution.

(*m*), multiplying this number by the area of the Petri dish:

from arithmetic averages of three to four parallel seeding of water samples.

RESULTS AND DISCUSSION

The test microorganisms were bacteria of the *Diplococcus* sp. and *Sarcina lutea* types from the Coccaceae family. They are related to spherical microorganisms; they differ by size and by numbers of connected cells. *Diplococcus* sp. cells are connected in pairs, whilst *Sarcina* cells are in blocks. This shape for *Sarcina* occurs as a result of cell division in three mutually perpendicular planes.

Argon and helium were used as an additional

source of bubbles in an aqueous medium and were bubbled during the whole process of cavitation action on the cell in the water medium. Saturation of the treated aqueous medium by gases of different natures created additional cavitation embryos in the reactive zone.

Some morphological features of *Diplococcus* sp. and *Sarcina* sp. types are presented in Table 1.

Table 1. Morphological features of investigated microorganisms

Investigated microorganisms	Morphological features				
	Coloration by Gram	Width, micrometers	Length, micrometers	Ability to the form spores	Ability to the movement
<i>Diplococcus lanceolatus</i>	Gram +	0.6	-	-	-
<i>Sarcina lutea</i>	Gram +	2.2	-	-	-

Superficial, deep, and bottom colonies were observed during the growth of the studied MOs in Petri dishes. Colonies corresponded in size to point and small colonies. The nature of colony growth on MPA in a Petri dish and fixed preparations of the studied microorganisms are shown in Figures 1 and 2.

Over time, the color (pigmentation) of the colonies of the microorganisms increased, so when studying their cultural properties, the change in pigmentation of the colonies of the studied cultures during growth in Petri dishes

on a solid nutrient medium was investigated and presented (Fig. 1, 2a, and 2b).

The choice of microbial culture is related to the dominance of these bacteria types among prokaryotes found by us in natural waters [4]. Therefore, in the processes of cavitation water disinfection studies of the influence of bubbled gas on the viability of these microorganisms are presented.

According to the intensity of growth as physiological features in cocci bacteria after being injected in a solid substrate in a test tube

the superficial thin layer was present, the superficial layer is all-round, dense, smooth and after being injected in a melted substrate in a test tube evenly distributed superficial thin layer was investigated for *Diplococcus* sp. and

Sarcina sp. bacteria, the superficial layer is the same as in the case of a solid medium, explicit growth of colonies distributed on the tube wall over the medium.

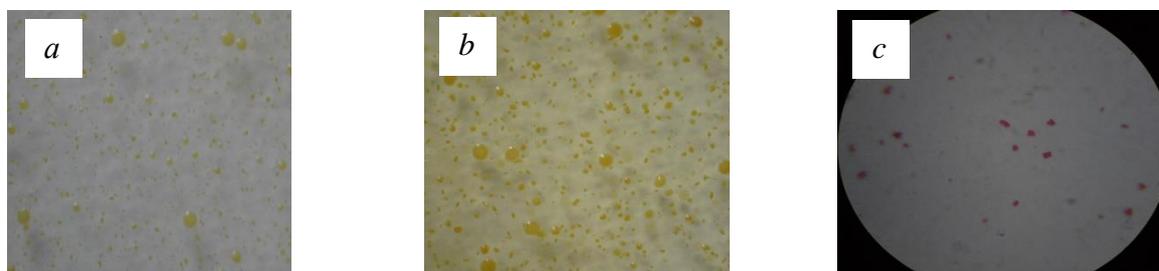


Fig. 1. *Sarcina* bacteria type: a) and b) – the character of the colonies growth on the MPA in a Petri dishes: a) - lemon pigment colonies (1 day); b) - yellow pigment of colonies (4-5 days); c) - cells of the 3-day culture of fixed cells during microscopy (x1500).

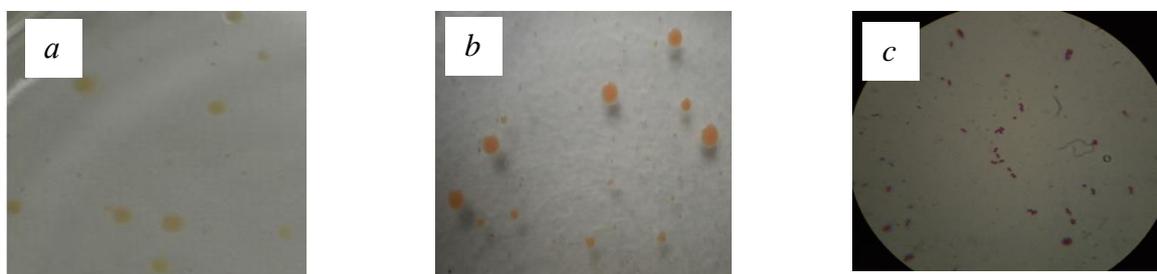


Fig. 2. *Diplococcus* sp.: a) and b) – the character of the colonies grown on the MPA in a Petri dishes: a) - orange pigment of the colonies (1 day); b) - orange pigment of colonies (4-5 days); c) - cells of a 1-day culture of fixed cells during microscopy (x1500).

According to the characteristics of colony growth on a nutrient medium in Petri dishes as cultural features for both types of studied microorganisms were investigated the next characteristics: shape and profile were compact, round, convex profile; the surface was smooth; shine and transparency was brilliant, moisture and opaque; structure and consistency were homogeneous with soft texture; edges was smooth and flat.

According to calculated effective rate constants for microorganisms destruction, both *Diplococcus* sp. ($k_d = 3.25 \pm 0.06 \cdot 10^{-4} \text{ s}^{-1}$) and *Sarcina* sp. ($k_d = 5.95 \pm 0.07 \cdot 10^{-4} \text{ s}^{-1}$) are destroyed most readily in the presence of argon

rather than in helium - $k_d = 1.72 \pm 0.03 \cdot 10^{-4} \text{ s}^{-1}$ and $k_d = 3.30 \pm 0.09 \cdot 10^{-4} \text{ s}^{-1}$, respectively. The value of k_d is less by 0.52 and 0.55 times under He/US for *Diplococcus* sp. and *Sarcina* sp., respectively. Comparing the efficiency of the process for monocultures, more active destruction of *Sarcina* sp. (both in argon and in helium) is observed, which may be related to its significantly larger cell size compared to that of *Diplococcus* sp. (~3.5 times).

Thus, the destruction of *Diplococcus* sp. and *Sarcina* sp. under cavitation conditions has been shown to occur most effectively in argon. Destruction degree is approximately twice higher under Ar/US condition than for He/US.

CONCLUSIONS

The effect of gas and cavitation on the process of water purification from spherical bacteria was studied. The results of morphological features of bacteria during growth on a nutrient medium and results of microscopic researches of the fixed cells are presented. The

effectiveness of this action depending on the microorganisms' type, as well as the nature of the bubbled gas have been compared. The values of the effective rate constant of bacterial destruction for argon and helium under cavitation condition are compared. The greater

efficiency of the destruction process of cocci bacteria at the simultaneous action of argon and cavitation regardless of their types has been experimentally shown. It has been found that

the efficiency of cell amount reduction per unit volume of the water system depends on the nature of the bubbled gas.

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