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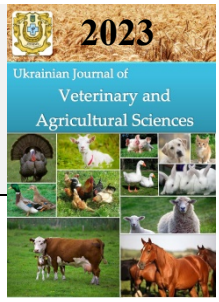

Study of the microbiological composition of sludge during the cultivation of *Chironomus* larvae

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Abstract

Silt (nutrition medium) is the habitat of many microorganisms that adapt well to water and soil. By extended aeration, it partially comes out of the sewage. The nature of organic impurities determines the composition of such a substrate, so its leading indicators may vary. Such a nutrient medium is one of the favorable conditions for the growth and development of *Chironomus* larvae and various microflora. The article presents the results of studies of bacteriological crops of the substrate for *Chironomus* larvae to establish the microbiological composition of the nutrient medium under various pasteurization modes. It was found that in native sludge, the growth of a colony of bacteria of the family *Pseudomonas spesialis* is 80 %, *Bacillus subtilis* is 15 %, and *Micrococcus luteus* is 5 % of the total number of colony-forming organisms. It was also found that different modes of pasteurization lead to a decrease in the number of microorganisms in the nutrient medium. When pasteurized at 65 °C (long-term), the number of microorganisms decreased by 16 times. When pasteurized at 75 °C (short-term), the number of colony-forming organisms decreased by 22 times, and at 95 °C (instant), it decreased by 358 times compared with the native form of the nutrient medium.

Keywords: sludge; bacteria; pasteurization modes; colony growth.

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1. Introduction

Silt is a habitat for many groups of microorganisms. Their composition and many parameters can differ from each other and be of different origins, namely, river, swamp, pond, and lake (Osadchyi, 2013; Bordiuh, 2013; Kummu et al., 2016).

Sludge located in pond water bodies may contain a significant amount of various substances that can be harmful and dangerous. It is also precious and contains many trace river and lake silt elements. Lake silt is also an organic fertilizer containing organic matter and lime. River silt contains a large number of valuable and nutritious properties. Such sludge is formed with the help of various microorganisms that help in water purification from various impurities and residues of animal organisms. More often, silt is found in stagnant water bodies or where there is a weak water flow. In its structure, it has the appearance of a jelly-like mass. Bog silt is also rich in composition and nutritional value for an organic matter of animal and plant origin (Kunst et al., 1997; Protasov, 2017; Abdelli et al., 2019; Merzlov et al., 2019; Pysarenko et al., 2022).

In the silt itself and the reservoir, there are different numbers of bacteria, which have their morphological characteristics, and show three primary forms: coca, rods, and spirilla. This species composition mainly refers to the morphological groups of bacteria of the genera *Bacillus, Actinomyces, Corynebacterium, Micrococcus, Desulfotomaculum, Arthrobacter, Sarcina, Bacterium, Pseudomonas,* and other representatives (Hilgren et al., 2009; Kryvytska et al., 2010; Kumar et al., 2012; Adler et al., 2012; Desloover et al., 2014; Smirnov et al., 2022).

The reservoirs' water column and bottom sediments are the largest habitat for such bacteria. Also, a significant number of bacteria can develop in such phases as phases of water and air and water and solid substrates.

A reservoir is the best environment for the life of bacteria; the motivation for the development of bacteria is the

quantitative and qualitative composition of organic substances, the presence of biogenic elements, and several regimes (gas, temperature, hydrological, salt, etc.) (Fedonenko et al., 2014; Fialko et al., 2018).

A large number of microorganisms is one of the factors, no less significant, which ensures a dynamic balance in the biosphere. Microorganisms are essential to any ecosystem, especially in aquatic environments; bacteria occupy one of these essential roles. Because due to their active participation, complex organic substances are changed into simple compounds directly suitable for better assimilation by hydrobionts of different levels (Furzikova et al., 2006; Gorshkova et al., 2018; Khilchevskyi & Karamushka, 2021).

The vast and accelerated spread of various microorganisms due to the speed of reproduction, small size, resistance, and adaptation to various unfavorable and detrimental factors to life, as well as their diversity.

Due to their high adaptability potential, microorganisms can coexist in various conditions and environments. Waters of different origins are the natural environment for the development and existence of various microorganisms. In water bodies (salt, fresh), different taxonomic groups of bacteria, algae, and protozoa enter with different organic residues, dust, and soil. The contamination and microflora of water depend on the composition of the primary medium and the microorganisms' origin (Fotina et al., 2019).

Also, the bacterial composition of the environment is formed and depends on environmental conditions, except for some groups of bacteria that can develop and coexist regardless of environmental conditions (Klymenko et al., 2014).

A different number of microorganisms is located not only in the thickness of the medium but also in the surface silt layer while forming a thin bacterial film. The zone of this film contains many bacteria of different origins, namely, iron and sulfur bacteria, which act as transforming substances in the water body (Ye et al., 2014; Kassich & Nechiporenko, 2020).

Among the bacteria, some are found in the aquatic environment and soils of different origins, air, terrestrial plants, and animals of different shapes and origins (Collins et al., 2016).

The aim of the study. These studies aim to establish the microbiological composition of the nutrient medium under various pasteurization regimes and identify bacterial colonies that can affect the quantitative and qualitative composition of the microflora of the substrate for *Chironomus* larvae.

2. Materials and methods

The study was conducted in the microbiological research methods laboratory of the Department of Microbiology and Virology of the Belotserkovsky National Agrarian University.

Nutrient medium from the river Ros is the material of research. Samples of river silt were taken from a sampling depth of 0.9–1 m and a thickness of 9–10 cm.

All research samples of the substrate were weighed into test tubes of 10 g, pre-sterilized. The total number of test tubes is 12, of which: the first three have native sludge; in the next three, the nutrient medium was subjected to long-term pasteurization (65 °C); and in three test tubes, there was sludge, which was pasteurized during short-term pasteurization (75 °C). The final stage of the pasteurization of the nutrient medium was sludge, which was acted upon at a temperature of 95 °C, flash pasteurization.

The study was carried out in a microbiological box sterilized by ultraviolet light. According to the serial dilution method (L. Pasteur), serial dilutions of the material were prepared in a sterile liquid nutrient medium (10^{-3}…….10^{-7}) (Fig. 1). Then, using a 3-fold and 7-fold dilution, our native and pasteurized substrate was inoculated on sterile meat-peptone agar (MPA), into the surface and thickness.

Fig. 1. Preparation of serial dilutions before inoculation

The material for inoculation into the medium was in a liquid state; it was taken with a sterile graduated pipette, in the amount of 1 ml of a diluted microbial suspension was poured into a sterile Petri dish and poured into the medium melted and cooled to 45–50 °C by MPA. In a circular motion and shaking the Petri dish, the material was mixed to be evenly distributed in the medium. After the complete thickening of the last cup, our samples were placed in a thermostat at a temperature of 37 °C, which is optimal for the growth of various microorganisms in the sludge (Fig. 2).

Fig. 2. Inoculation on the surface of the MPA medium

Microbiological cultures were performed to obtain and identify individual bacterial colonies. The description of our crops and the study of cultural properties were carried out on the third day of research.

3. Results and discussion

The microbiological characteristics showed that the nutrient media contained colonies of various microbes. A col-

ony is an accumulation of bacteria visible to the naked eye on the surface or in the thickness of a nutrient medium.

To determine the type of microorganisms grown on the surface of the agar, each Petri dish was carefully examined, and isolated colonies were studied. According to the general indicators of cultural properties, attention was paid to color, size, shape, consistency, the surface of the colonies, the nature of the edges, gloss, transparency, and other features.

After sowing, we saw the growth of such colonies as *Pseudomonas spesialis, Bacillus subtilis,* and *Micrococcus luteus* at various serial dilutions (Fig. 3).

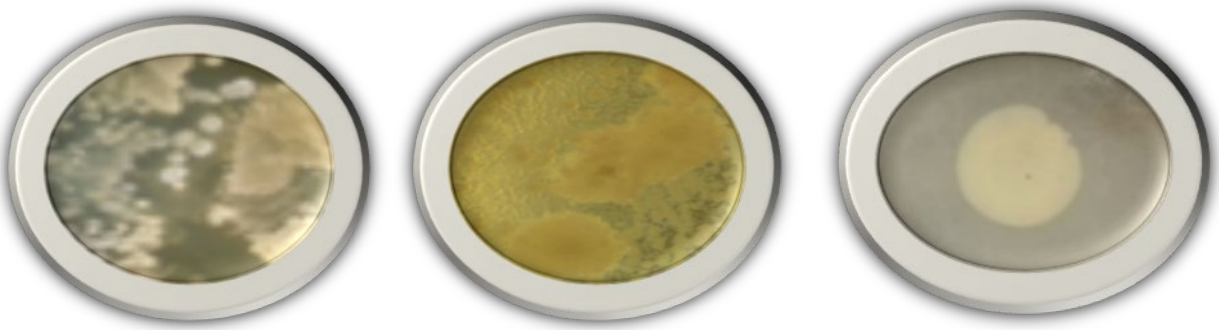

Fig. 3. Growth of the colony

Pseudomonas spesialis, identified in a nutrient medium, can metabolize various nutrients; they belong to rod-shaped bacteria and are the least picky about growth factors. Aerobes are about 0.5–1.0×1.5–5.0 microns in size. According to some indicators of cultural properties, the colonies of *Pseudomonas spesialis* were of different shapes (large, convex, shiny, flat, irregularly shaped, punctate, slimy, dwarf, and folded). In terms of color, they were yellowish, gray, and yellowish-gray and had an oily consistency.

Bacillus subtilis is a rod-shaped bacterium that produces endospores that do not exceed the size of the cell itself. According to its properties, the chemoorganoheterotroph breaks down starch and glycogen and ammonifies proteins. Reaches the size of 3–5×0.6 microns, each cell is mobile and has several peritrichous. Colonies can grow on different media: synthetic nutrients, vegetables, MPA, and MPB. The peculiarities of such bacteria are that they are colorless, velvety, pink, finely wrinkled, and dry. Also, according to the description of the edges of the colonies, one can note a characteristic feature for the species of these bacteria (coral and waviness).

Micrococcus luteus is a saprophytic bacterium, non-motile, Gram-positive, and widely distributed in various environments. It is unpretentious to different nutrient media. Therefore, it is well cultivated. It has a spherical regular shape, convex, smooth, shiny, and opaque, not exceeding 0.5–1.5 microns in size. The color of such bacteria can be different depending on the color of the pigment: bright yellow, golden, lemon yellow, fawn, and white. In the studying smears it was single or in the form of irregular clusters that did not form spores.

At the stage of further studies, a pure culture was obtained to have a good view of the form; preparation smears were prepared, which were stained using the Gram method. After performing preparation smears, microscopy was performed using an immersion system.

With the help of calculations of the results obtained, it was seen that the native sludge contained the most significant number of microorganisms (Table 1).

Table 1
Total bacterial colony counts in native and pasteurized sludge

	Temperature regime of pasteurized sludge	Bacterial colony count
1	native silt	$4{,}3\times10^7$ CFU/1g
2	lasted 65 °C	$2{,}6\times10^6$ CFU/1g
3	short-term 75 °C	$1{,}9\times10^6$ CFU/1g
4	instant 95°C	$1{,}2\times10^5$ CFU/1g

The data obtained indicate that, according to the number of counts of bacterial colonies in native and pasteurized sludge, the most significant number was in non-pasteurized sludge (native) 4.3×10^7 CFU/1g.

In crops where the nutrient medium was pasteurized at a temperature of 65 °C (long-term) and 75 °C (short-term), the growth of colony-forming organisms had a negligible amount of bacteria.

In crops of pasteurized sludge during instant pasteurization (95 °C), there was the least amount of bacteria, which was 1.2×10^5 CFU/1g.

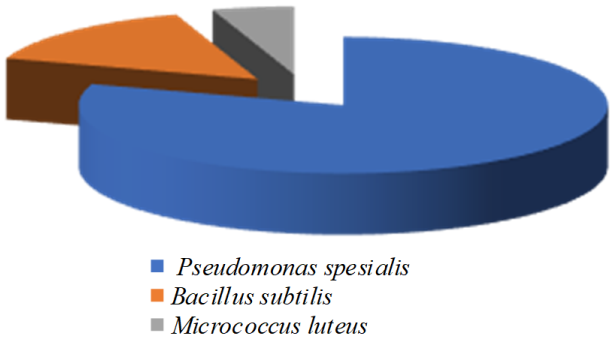

Fig. 4. Colonies of bacteria in native sludge, %

Also, according to the results of calculations (Fig. 4), it was found that native sludge has the most colonies of bacteria from the entire colonies: *Pseudomonas spesialis* 80 %, *Bacillus subtilis* 15 %, *Micrococcus luteus* 5 %.

4. Conclusions

1. It was revealed that in native sludge colonies of bacteria of the family *Pseudomonas spesialis*, 80 %, *Bacillus subtilis*, 15 %, and *Micrococcus luteus*, 5 %. Bacteriological inoculations on the MPA medium on the surface of the medium and in the thickness of the medium were introduced per 1 ml, showing that microorganisms affecting the growth and development of *Chironomus* larvae live in unpasteurized sludge.

2. The number of colony-forming organisms under various pasteurization regimes has decreased. During pasteurization at 65 °C (long-term) by 16 times, during pasteurization at 75 °C (short-term) by 22 times, during pasteurization at 95 °C (instant), the number of colony-forming organisms from the total number of colonies decreased by 358 times.

Conflict of interest

The authors declare that there is no conflict of interest.

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