Participation of Infusoria (Ciliophora, Ciliata) in Municipal Wastewater Treatment

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Abstract. The paper discusses the indicative role of infusoria as biological wastewater treatment participants. The study identified 57 infusoria species in the wastewater treatment plant in Khabarovsk, most of which belonged to the Oligohymenophorea class (45.6 %). The ciliate community core is represented by the following eurybiontic forms: Paramecium Caudatum, Litonotus Lamella, Coleps Hirtus, Aspidisca Cicada, Vorticella Convallaria, V. Microstoma. However, in general, the ciliate community under consideration turned out to be very specific relating to aerotanks in other world regions (according to the Chekanovsky-Sørensen index). It has been found that infusoria promptly respond to changes occurring in the treatment plant operating mode at the cellular and population-specific levels, which is expressed in the formation of specific complexes of species, as well as morpho-ecological changes in the protozoa cells.

1. Introduction
The growth of population, the continued expansion of the development and extraction of natural resources, and the increasing volume and diversity of industrial production lead to pervasive biosphere pollution. Herewith, water is the main resource of today’s humanity, spent to meet household, industrial, and agricultural needs. Wastewater treatment is among society’s most important environmental problems. It has been established that along with hazardous toxic compounds, untreated wastewaters may contain helminth eggs and pathogenic microorganisms.

Infusoria (Ciliophora, Ciliata) are among the most numerous hydrobionts inhabiting activated sludge, which is a biomass mix of microorganisms and pollutants entering the aerotank with wastewater [1]. Herewith, the role of infusoria in water purification is very significant. Ciliates are involved in clarifying water, reducing the number of pathogens, mineralization, and transformation of organic matter and energy, as well as serve as indicators of the treatment plant operation [2-4].

2. Research material and techniques
Wastewater treatment facilities located in Khabarovsk near the Berezovka Settlement were commissioned in 1983. Water is purified according to the classical scheme: mechanical and complete biological treatment and disinfection with liquid chlorine. The total approximate municipal wastewater volume is 300 thousand m³/day, of which 220.2 thousand m³/day is fully treated and the rest is discharged untreated [2].
The material studied was the hydrobiological samples taken from the aerotanks of treatment facilities within 2009-2012 and 2018-2020. In total, more than 300 samples taken using 500 ml and smaller glass wide-mouth samplers were analyzed for the identification of infusoria. In periods of poor faunal diversity, the samples were preliminarily centrifuged (1,500 rpm for 30-60 s) or settled.

Ciliophores have been studied in vivo and in vitro using the “slide on slide” technology [5, 6]. To do this, the sludge mix was evenly distributed on one slide. Another slide was placed with its narrow side on the first one (at an angle of about 45°), close to the sample edge. Then, the activated sludge was gently shifted to the opposite edge with the second slide, and the first one was tilted slightly to separate the water with protists from the sludge flakes. The motor activity of cells was decreased using immobilizing Da-Fano’s fluid and calcium–formol solution (according to Baker). Infusoria were colored intravitally using the following dyes: neutral red, methylene blue, eosin, etc. Protargol (0.3 %) was used to identify infraciliations.

The ciliate species were identified using guides and numerous descriptions given in multiple papers and monographs [7-11].

3. Results and discussion
During the faunistic revision of infusoria in the aerotanks of the Khabarovsky treatment facilities, 57 species have been identified, belonging to 2 subtypes and 8 classes (with 3 species identified to the genus only). The core of the ciliate fauna is represented by the Oligohymenophorea class numbering 26 species, accounting for 45.6 % of the total infusoria diversity in the treatment plant biocenosis. The largest number of species was found in the Epistylis and Vorticella genera (7). An important feature of the aerotank ciliocenosis is a large number of species belonging to the Peritrichia subclass (21 ones). Peritrichs are developed massively on the tank walls and activated sludge flakes, which, according to some authors [2, 10], is associated with the specific environmental conditions in aerotanks. These are constant mixing of the sludge mix (due to aeration), the optimal temperature for the infusoria life (+19 °C…+25 °C), organic substances dissolved in wastewater, the floculent structure of activated sludge, creating the prerequisites for successful attachment of periphyton forms, etc.

It should be noted that when comparing the faunal list with data on aerotanks in other settlements [12-15], the similarity of species (according to the Chekanovsky-Sørensen index) is rather low: Svobodny (Russia) – 31 %, Hovsan and Sahil (Azerbaijan) – 24.7 %, Borisoglebsk (Russia) – 21 %, and Wels (Austria) – 35.6 %. However, the core of the ciliate community in Khabarovsky wastewater treatment facilities turns out to be the same with most aerotanks in various world regions. These species include Paramecium Caudatum, Litonotus Lamella, Coleps Hirtus, Aspidisca Cicada, Vorticella Convallaria, V. Microstoma, etc.

Using literature data [2, 3, 7, 11] and our observations, we have identified complexes of ciliate species, which are indicative in determining the treatment plant operating mode and/or certain toxic substances in the environment. Thus, 8 ciliate species were markers of low and medium load on activated sludge, i.e., normal operation indicators. This category included Euplotes Patella, Acineria Uncinata, Amphileptus Pleurosigma, Coleps Hirtus, Colpidium Campylum, Epistylis Bimarginata, Vorticella Convallaria, and Zoohamnium Procerus. Normal activated sludge, typical of municipal wastewater, forms large well-settling flakes. The number of bacteria in such a biocenosis is small.

4 species indicated the sludge overload and low concentration of dissolved oxygen in water: Metopus Es, Paramecium Caudatum, Aspidisca Cicada, and Litonotus Lamella. Even a singular occurrence of these representatives indicates an unfavorable oxygen regime in the aerotank. Long-term aeration failure leads to stagnant zones and, consequently, the transition to anaerobic decay of pollutants. Some species of the Peritrichia subclass (Oparecularia Curvicaulis, O. Phryganae, Vorticella Infusionum, V. Microstoma) are also H₂S markers. Hydrogen sulfide in wastewater may be associated with the activity of sulfate-reducing microorganisms, as well as the use of sulfates in agriculture [16].
Thus, it becomes obvious that the treatment plant operating mode and the chemical composition of wastewater are the most important factors affecting the species composition of the ciliate community. Ciliate may quickly respond to changes in the aerotanks, which allows taking measures to improve the wastewater treatment even before chemical indicators show noticeable deterioration of the water quality. When the treatment plant operating mode is violated, the biocenosis transforms not only at the species level but also the cellular one, which is expressed in morpho-ecological changes in infusoria (Table 1).

**Table 1.** Morpho-Ecological Changes in Some Ciliate Species in Different Treatment Plant Operating Modes [2].

| Morpho-Ecological Sign | Cause of Occurrence | In a Favorable Aerotank Operating Mode | In an Unfavorable Aerotank Operating Mode |
|------------------------|----------------------|----------------------------------------|-----------------------------------------|
| Peristome retraction (closure) in *Epistylis* | insufficient aeration; effluent toxicity | ![Image](image1.png) | ![Image](image2.png) |
| Gas bubble forming in *Carchesium* | aeration mode violation | ![Image](image3.png) | ![Image](image4.png) |
Thus, in the aerotank biocenosis, the ciliate community is a complex biological system functioning at a high anthropogenic load. This is due to constantly changing the chemical composition, concentration of toxicants, and the volume of wastewater treated.

4. Conclusion
The faunistic diversity of infusoria in the Khabarovsk wastewater treatment plant is represented by 57 species, most of which (45.6%) belong to the Oligohymenophorea class. The core of structure-forming species (*Paramecium Caudatum, Litonotus Lamella, Coleps Hirtus, Aspidisca Cicada, Vorticella Convallaria, V. Microstoma*, etc.) is typical of many natural and anthropogenically transformed water bodies, which is largely due to their eurybionticity. However, in general, the ciliate community studied is highly specific. The faunal community diversity index is within 21-35.6%.

It has been found that infusoria may promptly signal changes occurring in aerotanks (violation of the aeration mode, high effluent toxicity, etc.) at the population-specific and cellular levels. At the first population-specific level, these processes are expressed in the formation of complexes of species indicating the starvation of activated sludge, violations of thermal and pH regimes, the formation of
stagnant zones, etc. At the cellular level, they are expressed in morpho-ecological changes in cells (closure of the peristome, retraction/reduction of actinophores, and the formation of gas bubbles).

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