Biocenosis of cold-water and warm-water biofilter in recirculating aquaculture system

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Abstract. This article presents data on the species composition of periphyton organisms in biofilms of cold-water biological filtration systems intended for the cultivation of rainbow trout (Oncorhynchus mykiss) and warm-water species for the cultivation of (Clarias gariepinus) in a recirculating aquaculture system. Data on the occurrence of certain groups of periphyton organisms are presented, and their role in the process of water purification is indicated. A significant difference in the species composition of biofilter biofilm organisms is shown, depending on: temperature regime and planting density of aquaculture objects. The species composition of aquasysets is similar to that in urban wastewater treatment systems.

1. Introduction
The cultivation of aquaculture objects in recirculating aquaculture systems is widespread in the Russian Federation due to the possibility of full control over the parameters of the aquatic environment. This allows you to get fish products throughout the year, reduce the likelihood of diseases and grow fish species that have high demands on water temperature and hydrochemical conditions. The most common fish species cultivated in recirculating aquaculture systems are rainbow trout, sturgeon hybrids and Clarium catfish.

Trout is grown in recirculating aquaculture systems in order to produce offspring and much less frequently in a full cycle, since cage cultivation is economically much more appropriate. Sturgeon and Clarium catfish are cultivated to obtain marketable products [1].

A biofilter is an essential component of any installation of recirculating aquaculture systems. Regardless of the type of structures used, it performs the following functions: purification of water from the final nitrogenous metabolites of cultivation objects, utilization and removal of excess organic matter from the system, and partially maintaining optimal hydrochemical parameters, since the microbial community and other biofilm organisms change pH and chemical parameters aquatic environment [2].

The biocenosis of peripheral biofilter organisms is a complex dynamic community of aquatic organisms and organomineral components that serve as food and substrate for the development of aquatic organisms. In water purification from biogenic compounds involved: bacteria, algae, fungi, actinomycetes, euglena [2]. Algae, which are found in activated sludge and biofilm of treatment facilities, deserve special attention, but their participation in the treatment is questionable. It is believed that algae entering the biofilter do not find favorable living conditions there, so their occurrence there should be rather small [3].
The species composition of the biofilter is not constant and will dynamically change depending on the biological load on the biofilter, water temperature and the relationships within the phytocenosis. Due to active aeration [4], the basis of biofilm and activated sludge particles are saprophytic aerobic bacteria, whose biomass accounts for more than 90%.

In addition to the bacterial community, various types of fungi and actinomycetes, which decompose organic compounds poorly absorbed by microorganisms, are necessarily present in the biofilm. The initial destruction of organic matter is carried out by a variety of protozoa, nematodes rotifers, etc.

There are several ways organisms enter the biofilter biocenosis. Bacterial spores fall into the biofilter: from atmospheric air, a water supply source, with dust particles, artificial substrate can also be populated with special groups of microorganisms. Protozoa and rotifers, also widely represented in nature, settle on the developing bacterial film. The second source of entry of periphyton organisms into the filtration systems is drift with fish seed material, while pathogenic microorganisms and protozoan parasites can enter the filter. In this case, aeration tanks can become distributors of pathogenic organisms and lead to massive disease of aquaculture objects.

After a while, the species composition of the biofilter biocenosis stabilizes, although the number of certain species may be subject to significant changes, depending on the biomass of the fish grown, temperature, feeding regime and hydrochemical parameters of the environment [5].

Based on the foregoing, it is possible to determine the purpose of the study: to determine the species composition of saprophytic, as well as pathogenic for fish, aeration tank organisms of closed water supply systems in warm and cold waters.

2. Materials and methods

The study of the species composition of periphyton was carried out on the basis of the Russian-Scandinavian center for research and innovation in the field of aquaculture of K. G. Razumovsky Moscow State University of Technologies and Management (the First Cossack University). The paper presents data from two installations of recirculating aquaculture systems (fig. 1) for warm-water 26 °C and cold-water 18 °C aquaculture facilities.

The objects of experimental studies were a biofilm of a floating loading of biofilters and suspended silt particles.

Samples were taken from the inner surface of the biological substrate, where the mechanical effect on the biofilm organisms is minimal.

20 samples were taken from each biofilter and twenty samples of silt particles in the sump chamber after the biofiltration process. A particle of film and silt was placed on glass slides and microscopically using a standard technique. For added contrast, part of the samples was stained with methylene blue, which made it possible to better diagnose microorganisms, fungi and actinomycetes.

Sample surveys to assess the presence of microflora potentially dangerous for cultivation objects were carried out according to the generally accepted method. Microbiological material was inoculated on Endo-Agar and Soyabean Digest Casein Agar media at a temperature of 37 °C for 160 hours. A barrier of 37 °C is necessary for the separation of epidemiological hazardous species. After incubation,
tests were carried out to determine the species composition, in accordance with the established methodology.

3. Results and discussion

From the taken bacterial samples, 24 crops were made. According to the results of tests and microscopy, it was possible to reliably determine 9 genera of bacteria: *Salmonellae, Aeromonas, Pseudomonas, Escherhia, Staphylococcus, Vibrio, Acinetobacter, Enterobacteria, Streptobacillus* (Table 1). It was possible to determine the species affiliation in 5 cultures, among them all are potentially pathogenic for fish.

The genus *Aeromonas* was represented by one species *A. hydrophilia*. It has high virulence, is very dangerous for fish, subject to a high planting density and low quality of the aquatic environment, it causes a mass disease with aeromonosis [6].

Despite the fact that these species do not significantly affect the biofiltration processes, their role in the biofilm microbiocenosis is quite high.

Among the actinomycetes, the past is reliably defined: *Streptothrix actinomyces, Beggiatoa sp., Beggiatoa alba, Sphaerotilus sp., S. natans, Thiothrix tenuis* (Fig. 2), *Cladohrix dichotoma, Microthrix parvicella*. The temperature difference influenced the frequency of occurrence of these bacteria, in warm water (26 °C) their number was always higher. *Thiothrix tenuis* prevailed in cold water. Actinimicents are the most common representatives of activated sludge and biofilms, therefore they are assigned one of the main roles in the oxidation processes of organic and inorganic compounds.

The species *Cladohrix dichotoma*, also found in all samples, is widespread in the activated sludge of treatment facilities [3, 7]. It, as well as the species *Streptothrix actinomyces*, are distinguished by their ability to withstand low oxygen content and the presence of toxins in water [4]. For this reason, these species can be distinguished from the rest, as making a significant contribution to the destruction of organic compounds.

Serobacteria - *Beggiatoa alba* and *Thiothrix nivea, T. Tenuis*, were found as a constant component of the cold-water recirculating aquaculture system and were practically absent in the warm-water filtration system. The genus *Thiothrix* is capable of oxidizing sulfides, thiosulfates, and hydrogen sulfide. It is noted [8] that the species *T. tenuis* is capable of absorbing heavy metals, in the honesty of cadmium. All three of the above types are capable of synthesizing vitamins, as secondary or final metabolites [6].

| Microbiological culture number | Gram stain (+/-) | Catalase test (+/-) | Oxidase test (+/-) | Genus          |
|-------------------------------|-----------------|--------------------|--------------------|----------------|
| 1                             | -               | -                  | +                  | Acinetobacter  |
| 2                             | +               | +                  | +                  | Enterobacteria |
| 3                             | +               | +                  | -                  | Staphylococcus |
| 4                             | -               | +                  | +                  | Pseudomonas    |
| 5                             | -               | +                  | -                  | Escherhia     |
| 6                             | -               | +                  | +                  | Vibrio         |
| 7                             | -               | +                  | +                  | Aeromonas      |
| 8                             | -               | -                  | +                  | Acinetobacter  |
| 9                             | -               | +                  | +                  | Pseudomonas    |
| 10                            | +               | +                  | -                  | Staphylococcus |
| 11                            | -               | +                  | -                  | Escherhia     |
The composition of protozoa varied significantly in warm-water and cold-water systems. In all samples, shell amoeba species were found: *Arcella vulgaris*, *Centropyxis aculeata*, *C. Discoides* (Fig. 3 (B, C)). In warm waters, their occurrence was quite high, while in cold waters there were no more than 5 cells per field. This scatter may be due to the fact that nitrifying organisms predominate in the biocenosis of cold biofilms, which displace shell amoebas. In warm water, where dispersed flakes prevail and a high BOD index, a favorable environment is created for the development of amoebas [4]. The species *Arcella vulgaris*, as noted by several authors [9, 5], can be used to determine the quality of biological treatment, since its development primarily depends on the composition of wastewater.

**Figure 3.** Testate amoebae and infusoria biofilm biofilm: (A) Vorticella sp.; (B) Centropyxis aculeata; (C) C. discoides.
Among ciliate infusoria, two species were precisely identified: *Vorticella convallaria, V. Campanula*. Other groups of organisms that could not be differentiated were assigned to the genera *Carchesium, Zoothamnium, Vorticella* (Fig. 3 (A)). In warm water, prevalence of ciliates was noted, especially the genus *Vorticella*. In cold water, he did not meet at all. This degree of development indicates a well-developed nitrification in the biofilter [4].

*Opercularia coarctata, O. glomerate* were found in small quantities in both cold and warm water. The populations of these ciliates are associated with flocs of activated sludge.

In addition, 6 species of predatory ciliates found in single specimens of cold and warm waters were discovered: *Aspidisca costata, Litonotus lamella, Stylonychia pustulata, Oxytricha pellionella, Colpidium colpoda*. In the biofilm ecosystem, predatory ciliates take the place of consumers, eating rotifers and other ciliates. In biofilters with good nitrification, they are always present [10], while not achieving mass development, they are very sensitive to oxygen content. In case of violation of the technological process of cleaning, the number of predatory ciliates increases.

![Figure 4](image_url)  
**Figure 4.** Rotifers and nematodes biofilms biofilters: (A) Philodina acuticornis; (B) Nematoda sp.

| Species                  | Warm water | Cold water |
|--------------------------|------------|------------|
| **Opportunistic bacteria** |            |            |
| Pseudomonas ssp.         | +          | +          |
| P. fluorescens           | +          | +          |
| P. Aeruginosa            | +          | +          |
| Escherchia coli          | +          | +          |
| Staphylococcus sp.       | +          | +          |
| S. Aureus                | +          | -          |
| Aeromonas hydrophilia    | +          | +          |
| Salmonellae sp.          | +          | +          |
| Vibrio ssp.              | +          | -          |
| Acinetobacter ssp.       | -          | +          |
| Enterobacteria sp.       | +          | +          |
| Streptobacillus sp.      | -          | +          |
| **Actinomycetales**      |            |            |
| Streptothrix actinomycetes | +        | +          |
| Beggiatoa sp.            | +          | +          |

**Table 2.** Occurrence of species in biofilters with cold and warm water.
Rotifers, represented by three species: Callidina vorax, Philodina roseola, P. Acuticornis (Fig. 4 (A)), were found at waters of both temperatures, however, in warm water their number initially exceeded cold. This may be due to the weak development of the bacterial community in cold water. The presence of a greater number of rotifers is an indicator of good aeration of activated sludge and biofilm [4].

The organisms of phytoperiphyton were practically absent, although the light regime suggested the presence of diatoms. So in urban wastewater treatment plants, diatoms, euglena and green algae in the warm season make up the basis of the diet of predatory organisms [9]. Apparently, there is high competition in the biocenosis of aeration tanks of the recirculating aquaculture system, which, combined with a lack of light, leads to a weak development of phyto-planktonic and periphytonic communities.

As can be seen from the table (table 2), most organisms are present in both temperature conditions. The presence of organisms that cannot exist in cold water can be connected, past low temperature, with a weak biological load on the biofilter, since the system contains producers of rainbow trout with a planting density of 12-15 kg / m$^3$. 

| Organism Type          | Condition | Temperature |
|------------------------|-----------|-------------|
| Testate amoebae        |           |             |
| Arcella vulgari        | +         | +           |
| Centropyx aculeata     | +         | -           |
| C. discoides           | +         | +           |
| Ciliate                |           |             |
| Vorticella ssp.        | +         | -           |
| Vorticella convallaria | +         | -           |
| V. campanula           | +         | -           |
| Carchesium             | +         | +           |
| Zoothamnium            | +         | +           |
| Opercularia coarctata  | +         | +           |
| O. glomerate           | +         | +           |
| Predatory ciliates     |           |             |
| Aspidisca costata      | +         | +           |
| Litonotus lamella      | +         | +           |
| Styloynchia pustulata  | +         | +           |
| Oxytricha pellionella  | +         | -           |
| Colpidium sp.          | +         | +           |
| Colpidium colpoda      | +         | +           |
| Rotifer                |           |             |
| Callidina vorax        | +         | +           |
| Philodina roseola      | +         | +           |
| P. acuticornis         | +         | +           |
4. Conclusion
According to the results of a study and comparison of the species diversity of biofilms and activated sludge of biofilters recirculating aquaculture system in warm and cold waters, it was found that the composition of organisms in wastewater treatment plants of closed water supply systems is not much different from that in wastewater treatment biofilters. In total, 38 species of organisms were found, 8 of which lived only in warm waters, and 4 only in cold waters. At the same time, the active development of such groups of organisms in warm water as shell amoeba, ciliary infusoria and actinomycetes, as well as the presence of a small number of predatory ciliates, indicates an actively ongoing process of nitrification. In cold waters, this process does not proceed so actively due to the low nutrient load on the biofilter.

Microbiological studies on the presence of pathogenic bacteria biofilters in the biofilm showed that out of 12 differentiated organisms, only 5 are conditionally pathogenic: *Pseudomonas fluorescens*, *P. aeroginosa*, *Staphylococcus aureus*, *Aeromonas sp.*

Thus, the species diversity of the periphyton organisms of fish-breeding systems of the recirculating aquaculture system is formed under the influence of many factors and contains a number of species similar to those in biological wastewater treatment systems.

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