Evaluating the UV radiation effectiveness in industrial aquaculture

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Abstract. The paper presents an analysis of the bacterial purification effectiveness by UV radiation of wastewater in modern industrial aquaculture recirculation systems. The effectiveness of water purification in the ultrasound system by a set of filters for mechanical treatment and ultraviolet radiation is experimentally established. UV lamp allows to process water with rays that effectively destroy the organic matter formed in it. Pathogenic and conditionally pathogenic genera, such as: Aeromonas, Pseudomonas, Staphylococcus, Vibrio, Enterobacteria were differentiated.

1. Introduction
Ultraviolet, as a means of water purification in conditions of closed fish rearing, is used almost everywhere [1]. The principle of action is that when passing through a UV filter, the water is treated with ultraviolet radiation and the microorganisms that are present in it, die or lose the ability to reproduce. It is customary to use it in conjunction with other methods of mechanical, biological and chemical water treatment [2]. Despite the fact that the effectiveness of UV for water disinfection is beyond doubt [3], it is worth noting that the aquatic microflora may vary in different farms, and directly depend on the specific conditions of the enterprise. To all this, it is worth adding that farms can be equipped with various treatment systems having different degrees of resource development, which can affect the efficiency of treatment, especially with regard to cultural, hatchery and decorative reservoirs [4]. In addition, the species composition of the microflora of water also depends on the type of cultivated fish, which is accompanied by characteristic types of microbes. It should also be amended that some fish species are demanding to the simulation of seasonal factors creation, which also entails changes in the microflora of water.

Based on the foregoing, it follows that for each individual business it is necessary to conduct its own research to analyse the effectiveness of the UV cleaning systems and identify possible problems.

The aim of the work is to display the current efficiency of water treatment by a set of measures, coupled with the ultraviolet water exposure installation in relation to the water microflora, as well as to check the compliance of the water environment with veterinary and sanitary standards. The research issue is the need to conduct research in this industry each time for a separate system of RAS (recirculating aquaculture system).

2. Study methodology
The study was conducted at the Russian-Norwegian Center for Innovation research and aquaculture at K.G. Razumovsky Moscow State University. The sampling of water was carried out in accordance with
the established methodology: GOST P 51592 2000 "General sampling requirements," GOST 31942-
2012 (ISO 19458:2006) "Sampling for microbiological analysis."

The following water sampling points have been identified:
1. Water from the recirculating aquaculture system (RAS) pool, which has not been cleaned;
2. Water after the initial mechanical cleaning on the basis of 50 microns filters;
3. Water after being cleaned with biofilters;
4. Water after undergoing secondary mechanical cleaning on the basis of 50 microns filters;
5. Water after passing the UV cleaning system based on the Jebao UV Clarifier installation with a
capacity of 18W with throughput capability of 20 l/min.

Two types of environments were selected for the study:
Endo-Agar (Agar Endo-GRM). This nutrient medium was chosen because of selective properties
(suppression of gram-negative microorganisms) and the expected results of crops from this type of
environment are aimed at determining water contamination by a group of enterobacterales
microorganisms that meets the epidemiological parameters of the study.
Soyabean Digest Casein Agar (Tripton Soybean Environment). This nutrient medium was chosen
because of the study of aquatic crops, which implies the selection of aquatic microorganisms (such as
Aeromonas, Pseudomonas, etc.), whose requirements meet this nutrient environment. The control of
microbiological environments was carried out in accordance with the conventional methodologies: ICC
4.2.2316-08 "Methods of control of bacteriological nutrient environments." Microbiological
environments with water sowing were divided in pairs and incubated at temperatures of 21 and 37
degrees Celsius for 160 hours to produce objective results.
The temperature barrier of 37 degrees Celsius has been determined for the preliminary differentiation
of epidemiological-hazardous species.

Based on the results of incubation within 18-24 hours, the most characteristic colonies were selected
for the subsequent incubation of clean culture and tests, tests were carried out in accordance with the
established methodology: GOST 24849-2014 "Water. Methods of sanitary and bacteriological analysis
for field conditions, MUK (methodological instructive regulations) 4.2.2316-08 "Methods of control of
bacteriological nutrient environments." The tests most relevant to the study's objectives were defined as
daily tests for catalase, oxidase, and Gramo coloration test. By this, microscopy was carried out on the
morphology of individual bacteria.

The definition of the total microbial number was made according to the standard method.

3. Results and discussion
Total microbial number

According to the results of incubation, the following results of TBC were obtained (figure 1; figure
2; figure 3; figure 4).
Based on the results obtained, it can be seen that water that did not undergo purification in ultraviolet
installations showed a high contamination. Rapid growth of colonies was noted on all types of media.
The maximum number of sprouted colonies was noted on Trypton-Soybean medium, since it
provides a wide range of cultivated microorganisms. So, in water samples taken directly from the RAS
pool, it was 120 TMN on average, and samples that underwent primary mechanical cleaning showed
about 115 TMN on average, which is the highest result.
TMN from the Endo-medium, compared with Tryptone-soybean, was lower, due to its selective
properties in relation to bacteria of the intestinal group. The highest result on it was 58 TMN, for water
samples from the RAS pool. The water that underwent primary mechanical treatment also had high
TMN indicators (about 55).
The total microbial number in the two types of media that underwent UV treatment (figure 1; figure
2), as expected, was the smallest - 10 TMN for Trypton Soya and 0 for Endo-medium.
Figure 1. TMN at 21 °C from Tryptone-Soya medium for 1-7 days.

Figure 2. TMN at 21 °C from Endo-medium for 1-7 days.

The second group of samples was cultured at a temperature of 37 °C to identify bacteria of the intestinal group, which are also pathogenic dangerous for humans groups of bacteria [6]. High temperature inhibited the growth of aquatic microflora (figures 5; figures 6), because of this, TMN decreased in relation to the previous series of experiments.

The highest indicator of TMN was recorded in samples taken from the RAS basin in Tryptone-soybean medium and amounted to 72 TMN on average (figure 3). Samples taken from the systems:
primary mechanical cleaning, secondary mechanical cleaning, and biofilter cleaning showed similar quantitative results of TMN, but significantly lower than similar ones at a temperature of 24 °C. This may be due to the inability of the aquatic microflora to such a temperature regime.

Indicators of TMN for Endo-agar at all sampling points were the lowest of all. The greatest growth was observed in the sample after the initial mechanical cleaning, which amounted to 22 TMN. Water treated with UV, as well as at a temperature of 24, showed extremely low values of TMN (0-1) (figure 3; figure 4).

![Figure 3](image1.png)

**Figure 3.** TMN at 37 °C from Tryptone-soybean medium for 1-7 days.

![Figure 4](image2.png)

**Figure 4.** TMN at 37 °C from Endo-medium for 1-7 days.
In general, at a temperature of 37 °C, there is a general inhibition of the growth activity of microorganisms. On the seventh day of cultivation at 37 °C, an increase in the number of colonies was noted due to the growth of spore-forming bacteria, but due to temperature inhibition, this indicator is much lower than in media at a temperature of °C 24.

Figure 5. Tryptone-soybean medium - the point of water intake from the RAS pool, incubation at 37 °C

Figure 6. Endo-medium – the point of water intake from the RAS basin, incubation at 37 °C.
Figure 7. Tryptone-soybean medium water sampling point after treatment with ultraviolet radiation, incubation at 37 ℃

Figure 8. Endo-medium water intake point after treatment with ultraviolet radiation, incubation at 37 ℃.

Species differentiation
A total of 39 bacterial samples were taken from 17 water samples.
In the course of the tests, some cultures (8) gave the same results; later, their belonging to the same genus was determined. Samples of crops belonging to the same genus will be presented once in one water sample, the rest will be skipped.
Tests showed the presence of the following bacteria in the water samples.
Table 1. The results of tests for generic assignment.

| Water sample number | Gram-test (+\(-) | Catalase test (+\(-) | Oxidase test (+\(-) | Specific genus       |
|---------------------|------------------|-----------------------|----------------------|---------------------|
| 1                   | +                | +                     | +                    | Enterobacteria      |
| 1                   | -                | +                     | +                    | Aeromonas *          |
| 1                   | -                | +                     | +                    | Pseudomonas *        |
| 1                   | -                | -                     | +                    | Acinetobacter       |
| 1                   | +                | +                     | -                    | Staphylococcus      |
| 1                   | -                | +                     | -                    | Escherhia           |
| 2                   | -                | +                     | +                    | Vibrio              |
| 2                   | -                | -                     | -                    | Streptobacillus     |
| 2                   | -                | +                     | +                    | Aeromonas *          |
| 2                   | -                | +                     | -                    | Escherhia           |
| 2                   | -                | +                     | +                    | Pseudomonas *        |
| 2                   | +                | +                     | -                    | Staphylococcus      |
| 3                   | -                | +                     | +                    | Aeromonas *          |
| 3                   | -                | -                     | +                    | Acinetobacter       |
| 3                   | +                | +                     | +                    | Enterobacteria      |
| 3                   | +                | +                     | -                    | Staphylococcus      |
| 3                   | -                | +                     | +                    | Pseudomonas *        |
| 3                   | -                | +                     | -                    | Escherhia           |
| 3                   | -                | +                     | +                    | Vibrio              |
| 4                   | -                | +                     | +                    | Aeromonas *          |
| 4                   | -                | -                     | +                    | Acinetobacter       |
| 4                   | -                | +                     | +                    | Pseudomonas *        |
| 4                   | +                | +                     | -                    | Staphylococcus      |
| 4                   | -                | +                     | -                    | Escherhia           |
| 5                   | -                | +                     | +                    | Aeromonas *          |
| 5                   | -                | +                     | -                    | Escherhia           |
| 5                   | -                | +                     | +                    | Pseudomonas *        |

* According to the test results, some cultures had identical test results, according to the totality of all known facts, it was determined that the same test result belongs to two genera: Pseudomonas and Aeromonas.
Further, on the basis of gender, information obtained by microscopy and known facts, 7 genera were discovered: Salmonellae, Aeromonas, Pseudomonas, Escherhia, Staphylococcus, Vibrio, Acinetobacter. Among them, the species affiliation was determined in 9 cultures.

The genus Salmonellae most likely belongs to S. Salmonella.

To the genus Aeromonas, it was possible to prove for certain that A. hydrophilia belongs, A. eucrenophilia may be present. These organisms were found in all samples except UV samples; there it was not possible to reliably determine the species affiliation. Both species are pathogenic for fish [1].

Pseudomonas is represented by two species of P. fluorescens and P. aeroginosa. P. fluorescens was present in all samples, but after undergoing purification by ultraviolet radiation, its number dropped significantly. The presence of P. aeruginosa was seen only in samples taken directly from the RAS pool. Both of these bacteria are conditionally pathogenic, that is, they cannot be independent pathogens, but they are not virulent enough for effective damage to healthy fish [7].

E. Coli belongs to the genus Escherhia, as well as two indefinite species Escherhia spp. E. Coli, being a conditionally pathogenic species, was found in all samples, but its maximum amount was noted at the first stages of RAS water purification. In the case of cage farming, water pollution by Escherichia coli is not critical, with a low fish density and high-quality purification of incoming water. In addition, E. Coli has a veterinary hazard factor.

In the genus Staphylococcus, only one species, S. Aureus, was identified. It is a conditional pathogen for fish, but for humans it is a sanitary and epidemiological danger [8;9]. These species were found only in samples from the early stages of mechanical water treatment. After treating the water with UV, it was not detected.

Vibrio probably belongs to V. anguillarium. This species is the causative agent of vibriosis in fish.

4. Conclusion

1. The water from the pool water recirculation systems that has not been cleaned with ultraviolet light has a high enough microbial contamination, among which there are pathogenic and conditionally pathogenic kinds, such as Salmonellae, Aeromonas, Pseudomonas, Staphylococcus. The maximum value of TMN for water taken directly from the RAS pool was 120.

2. The highest microflora growth took place with the incubation temperature at 210 C. The reason for this could be the recreation of the most similar temperature conditions with water taken from the system pool recirculation systems and filtration systems.

3. The least growth of bacteria was observed at incubation temperature of 370 C. because of the reason for the initial domination of the microflora of aquatic bacteria that do not possess the necessary degree of adaptability to the temperature conditions data. It should also be noted that the microflora that showed the highest growth at a given temperature, was pathogenic for humans, or was not exclusively aquatic, that reinforces the possibility of its sanitary-veterinary insecurity.

4. Stage purification of water by ultraviolet radiation has an extremely high efficiency (over 95%) relative to water, not held or held only mechanical clean up. The effectiveness of mechanical cleaning as antibacterial measures is insufficient, since they do not have antimicrobial properties affecting water, however, there was a slight decrease in microbial contamination of water and some genera ceased to occur as water passed through the mechanical filtration system. This may be due to the influence of microflora of mechanical cleaning systems and thus suppression of microflora of the RAS system.

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