

The use of biofilters with different types of fillers in recirculation aquatic system (RAS)

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Abstract. The aim of this study was to evaluate the effectiveness of using highly porous materials as fillers for biofilters in RAS, such as those with a much higher specific surface area than classical polymer floating bioload, and to determine the optimal proportion of biofilter with these fillers to the total volume of fish tanks. During the experiment, additional nitrogen in the form of ammonium chloride was added to the water of the RAS with different filler types. The effectiveness of biofiltration systems with different fillers was evaluated by the oxidation time of $\text{NH}_3/\text{NH}_4^+$ to NO_2 and the physiological condition of sterlet fry (*Acipenser ruthenus* L.), which was used as a test organism. It was proven that highly porous fillers oxidized ammonium compounds in RAS water 1-3 hours faster than polymer floating bioloading. The optimal proportions of the volumes of highly porous fillers to the total volume of fish tanks were determined to be 1:100 for foamed glass and 1:50 for porous ceramics. Under the experimental conditions, the survival of the test object corresponded to current technological standards. Observations of the physiological state and behavioral responses of young sterlets did not reveal any adverse effects of nitrogen compounds on fish.

Keywords: sterlet, RAS, ammonia-ammonium, biofilter filler, foamed glass, porous ceramics

Introduction

In recent years, there has been a steady trend towards the creation of recirculating aquatic systems (RAS) for hydrobiont cultivation. RAS, in which water is repeatedly reused owing to purification in the water treatment unit, is an alternative to traditional resource-intensive methods for producing marketable products. The use of such systems allows for the minimization of water consumption and efficient use of land (Schuster and Stelz 1998, Godoy-Olmos et al. 2016). The intensity of the technological process in RAS does not depend on the season because of the ability to form optimal parameters of the aquatic environment for cultivated hydrobionts (Guerdat et al. 2013). The productivity of RAS, due to the maximum intensification of the hydrobiont cultivation process, is much higher than that of open aquaculture systems (Hrinevych 2016).

The RAS water treatment unit provides optimal water quality for cultivated hydrobionts during their growth period. The task of the biological filter in the water treatment unit is to remove harmful

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nitrogenous compounds from water. The capacity of the biological filter is filled with various fillers, the surfaces of which serve as substrates for the settlement and growth of nitrifying bacteria (Hagopian and Riley 1998, Eding et al. 2006, Sharylo and Kovalenko 2022). The main parameters of the quality of the aquatic environment that affect the process of growing hydrobionts are the content of dissolved oxygen, carbon dioxide, nitrogen in $\text{NH}_3/\text{NH}_4^+$, NO_3^- , NO_2^- , suspended solids, water pH, alkalinity, and water hardness (Portz et al. 2006). All of the above indicators of aquatic environment quality inevitably deteriorate with each water recirculation cycle. Oxygen is rapidly consumed by fish and bacteria, and organics (both dissolved and suspended) accumulate in water and are sources of ammonium and ammonia. The presence of ammonium and ammonia, even at low concentrations, poses a threat to the health and life of most fish species (Hargreaves 1998). Increasing the concentration of ammonia to critical values leads to the inhibition of ammonia excretion through the gills, and its accumulation in the blood causes negative changes in the body of fish (Wilkie 1997, Eddy 2005).

The first stage of biological water purification is the conversion of ammonium to nitrite. The NH_4^+ ion is not toxic to fish because their bodies secrete free ammonia (NH_3) through the gills. The release of ammonia is usually directly proportional to the amount of food consumed, inversely proportional to the nutritive ratio, and dependent on the composition of the feed. Ammonia and ammonium ions are in the chemical equilibrium „ $\text{NH}_3 - \text{H}^+ = \text{NH}_4^+$ “, which in the alkaline environment is shifted to the left, and in acidic – to the right, with the binding of H^+ ions. In addition to pH, the process is affected by temperature. The concentration of free ammonia, from which the vital activity of most fish species begins to be suppressed, is $0.05 \text{ mg NH}_3 \text{ dm}^{-3}$. Standard methods of hydrochemical analysis of water allow for the measurement of only the total amount of ammonia-ammonium (Spotte 1979, Timmons and Ebeling 2006). Nitrates are believed to be non-toxic to fish. The fish can withstand nitrate concentrations in water up to $100 \text{ mg NO}_3^- \text{ dm}^{-3}$. In typical RAS, such

high nitrate concentrations are usually not observed (Braynballe 2010). It is also believed that nitrates do not penetrate the tissues, and fish raised in water with high concentrations of nitrates do not accumulate them in their muscles.

The presence of NO_2^- in water is the next negative factor after $\text{NH}_3/\text{NH}_4^+$ for fish farming because the oxidation of ammonium compounds to nitric acids makes them dangerous for fish. The allowable concentration of nitrites in water is up to $0.2 \text{ mg NO}_2^- \text{ dm}^{-3}$ (Chebanov and Halych 2011). If this norm is considered the maximum allowable value, it is impossible to start fish in RAS until the nitrification processes in the biofilter reach the required level, and the nitrite content in the water will steadily decrease to a safe concentration. Otherwise, it can lead to fish death. Information on the toxicity of nitrites without data on the concentration of chlorine ions in water is not very informative. Thus, with a chloride content of $32 \text{ mg Cl}^- \text{ dm}^{-3}$, the safe concentration of nitrites for salmon fish increases from 0.2 to $8.9 \text{ mg NO}_2^- \text{ dm}^{-3}$ (Proskurenko 2003). In this regard, the maximum allowable concentration (MPC) of nitrites in the water of RAS is considered to be $2 \text{ mg NO}_2^- \text{ dm}^{-3}$ (Timmons and Ebeling 2006).

Currently, all existing types of biofilters for RAS require a large amount of load, the surface of which serves as a substrate for bacterial settlement. The cost of the aquasystem and the level of costs for the construction and operation of RAS are directly proportional to the volume of the biofilter and the area of its placement. Accordingly, the use of fillers with a high specific surface area can reduce the cost of fish production (Timmons and Ebeling 2006). The entry of nitrogen compounds into water is directly related to the process of fish farming in fisheries and usually determines the necessary technical characteristics of the installed biofilters and other equipment (Eding et al. 2006).

The nature of the influence of various abiotic factors on the operation of biological filtration units in RAS has been described in many scientific papers. Thus, Nijhof (1995) and Kamstra et al. (1998) evaluated the influence of external factors on the rate of removal of nitrogen compounds by several parameters,

such as the type of filter substrate, concentration of total ammonium nitrogen (TAN), and level of dissolved oxygen. The dependence of the concentration of nitrogenous compounds in RAS water on the characteristics of the biofiltration system has also been described in studies by Greiner and Timmons (1998), Van Rijn and Rivera (1990) and Watten and Sibrell (2006). Thus, the efficiency of the biofilter is evaluated mainly by the specific surface area of the filler, regardless of the type of the latter, which was proved by studies of many authors (Nijhof 1995, Greiner and Timmons 1998, Kamstra et al. 1998, Lekang and Kleppe 2000, Timmons et al. 2006). The above studies are mainly devoted to the analysis of discrete values of TAN in RAS water. There are almost no publications on the peculiarities of the daily dynamics of TAN content in water during the cultivation of aquatic organisms and the neutralization of toxic nitrogenous substances by biofilters (Dosdat et al. 1996, García-Romero et al. 2014, Godoy-Olmos et al. 2016). This study aimed to evaluate the effectiveness of using highly porous JBL Micromec foamed glass and porous ceramics as fillers for RAS biofilters in the cultivation of aquatic organisms and to select the optimal proportions of biofilter volume with these fillers to the volume of fish tanks.

Materials and Methods

The study was conducted in the fish farming laboratory of the Department of Aquaculture, National University of Life and Environmental Sciences (NULES) of Ukraine in July 2020. For research, a complex of six autonomous aquarium units was designed and installed, which corresponded to the main characteristics of the RAS. Each RAS included a 100 dm^{-3} fish tank made of glass and a water filtration and aeration unit. Porous foam sponges connected to the water-supply pump were used as mechanical filters. The mechanical filter was cleaned manually and periodically (if required). During the course of the work, a daily planned replacement of water was performed.

The biofilter consisted of a plastic tank (dimensions $90 \times 14 \times 15 \text{ cm}$, working volume 10 dm^{-3}) located above the fish tank (aquarium), a water supply pipe (hose) from the pump to the overflow column, and a filler. MinJang NS F801 pumps with a capacity of $1200 \text{ dm}^3 \text{ h}^{-1}$ and a power consumption of 15 W h^{-1} were used to lift the water into the biofilter. A water supply hose was laid at the bottom of the biofilter tank and was perforated. This allowed water to be evenly distributed in the biofilter. The water was aerated using a compressor. The spillway was equipped with a retaining wall that maintained a constant water level in the biofilter, with a self-return to the aquarium.

Two experiments were performed: the first compared the effectiveness of RAS biofilters with different types of fillers, and the second determined the optimal ratio of the volumes of highly porous fillers and fish tanks. In the first experiment, the following substrates were used for bacterial film formation:

- 1) porous ceramics for biofilters with a useful area of $250 \text{ m}^2 \text{ dm}^{-3}$ in a volume of 5 dm^{-3} (experimental variant 1 with double repetition, RAS 1.1 and 1.2);
- 2) high-porosity foamed glass JBL Micromec with a usable area of $1600 \text{ m}^2 \text{ dm}^{-3}$ in a volume of 5 dm^{-3} (experimental variant 2 with double repetition, RAS 2.1 and 2.2). According to the manufacturer, 10% of the area of this type of filler is an aerobic zone available for nitrifying bacteria, and 90% is an anaerobic zone available for denitrifying bacteria.
- 3) floating load Aqua $16 \times 12 \text{ mm}$ with a usable area of $1000 \text{ m}^2 \text{ dm}^{-3}$ in a volume of 5 dm^{-3} (control variant, one RAS).

In the second experiment, the following substrates for bacterial colonization were used:

- 1) highly porous foamed glass JBL Micromec in a volume of 1 dm^{-3} in the proportion of the volume of the biofilter filler to the fish tank as 1 of 100 (experiment E1);
- 2) highly porous foamed glass JBL Micromec in a volume of 2 dm^{-3} in the proportion of the volume of the biofilter filler to the fish tank as 1 of 50 (experiment E2);
- 3) high-porosity foamed glass JBL Micromec in a volume of 3 dm^{-3} in the proportion of the volume of

the biofilter filler to the fish tank as 1 of 33 (experiment E3);

- 4) porous ceramics in a volume of 1 dm^{-3} in the proportion of the volume of the biofilter filler to the fish tank as 1 to 100 (experiment E4);
- 5) porous ceramics in a volume of 2 dm^{-3} in the proportion of the volume of the biofilter filler to the fish tank as 1 to 50 (experiment E5);
- 6) porous ceramics with a volume of 3 dm^{-3} in the proportion of the volume of the biofilter filler to the fish tank as 1 to 33 (experiment E6).

All experimental variants were performed in triplicates.

The efficiency of the biofilters was evaluated by the oxidation time of toxic ammonia to relatively safe nitrate, as well as by the oxygen content in the water and the pH at different stages of the experiment. Sterlet fry (*Acipenser ruthenus* L.) with an average body weight of 150 mg were selected as biological test items with a stocking density of 330 specimens m^{-2} for the first experiment and an average weight of 1.5 g for the second experiment, and a stocking density of 250 specimens m^{-2} for the latter. This study was conducted during the rearing stage of young sturgeons in RAS. This technological stage allows the use of small fish tanks (up to 1000 dm^{-3}) for aquaculture. At the same time, the young sterlet at this stage of ontogenesis has a high sensitivity to environmental conditions, which allows it to be used as a test object to assess the quality of water in RAS.

The experiments included observations of the aquatic environment and the condition of the fish (survival, reaction to feed, nature of respiration, swimming, etc.) and a comparative evaluation of the effectiveness of the nitrification process in biofilters with different filler types. At the beginning of the experiment, the RAS biofilters were launched using special preparations containing cultures of nitrifying bacteria and nutrients for their growth. The quality of the biofilters was checked by determining the amount of nitrogenous substances in water by measuring the content of ammonia-ammonium ($\text{NH}_3/\text{NH}_4^+$), nitrate (NO_3^-), and nitrite (NO_2^-) content. The dynamics of the concentration of nitrogen compounds in water were observed for several hours, from artificial raising to the maximum

allowable level by adding a solution of ammonium chloride to reduce the content of these compounds in water due to the operation of the biofilter. The concentration of nitrogen compounds in water was verified according to the generally accepted methods of hydrochemical research in fish farming, using ready-made test systems 2010-2021 TM Ptero (Pravdin 1966, Chakchir and Alekseyeva 2002, Isayenko et al. 2009). Fish survival was determined as a percentage based on the results of direct counting of fish at the beginning and end of the experiment. The observation of fish growth rate was not included in the experimental plan, given the short research period.

Results

Experiment 1

After establishing the biological equilibrium phase in the system and the output of the biological filter at operating power, the level of $\text{NH}_3/\text{NH}_4^+$ in the water was artificially increased to 1.5 mg dm^{-3} by adding ammonium chloride. The NH_4Cl solution was administered gradually over 2 h to simulate mass feeding of fish. The dynamics of the changes in these indicators during the experiment are shown in Table 1. As shown in the table, the oxidation times of ammonia-ammonium in different systems differed. The time difference ranged from 1 to 3 h for the different experimental variants tested. Thus, in RAS 2.1, a significant decrease in the concentration of $\text{NH}_3/\text{NH}_4^+$ was observed as early as 4 h after the start of the experiment. However, complete oxidation of $\text{NH}_3/\text{NH}_4^+$ occurred most rapidly in RAS 1.2 and lasted for 5 h. In RAS 1.1, the worst result among the experimental variants concerning the oxidation of $\text{NH}_3/\text{NH}_4^+$ was fixed at 7 h; however, in this system, the complete oxidation of $\text{NH}_3/\text{NH}_4^+$ from water occurred faster than that in the control variant (8 h).

In general, the experimental variants showed better results for the oxidation rate of $\text{NH}_3/\text{NH}_4^+$ than the control variant.

The highest nitrite levels in water were observed throughout the experiment in systems with porous

Table 1Dynamics of changes in $\text{NH}_3/\text{NH}_4^+$ nitrites, pH and O_2 in experiment 1

VE	Time							
	09:30	11:30	13:30	14:30	15:30	16:30	17:30	18:30
$\text{NH}_3/\text{NH}_4^+$, mg dm^{-3}								
1.1	0	1.5	1	0.5	0.25	0	0	0
1.2	0	1.5	0.5	0	0	0	0	0
2.1	0	1.5	0.25	0.25	0	0	0	0
2.2	0	1.5	0.5	0.25	0	0	0	0
Control	0	1.5	1.5	1	0.5	0.25	0	0
NO_2^- , mg dm^{-3}								
1.1	0.2	0.8	0.8	0.5	0.5	0.5	0.5	0.3
1.2	0.2	0.8	0.8	0.5	0.5	0.3	0.3	0.2
2.1	0.8	0.8	1.0	0.8	0.8	0.5	0.5	0.3
2.2	0.5	0.5	1.0	0.5	0.5	0.5	0.5	0.3
Control	0.1	0.3	0.8	0.8	0.5	0.5	0.5	0.3
pH								
1.1	7.9	8.1	8.0	8.0	8.0	8.1	8.1	8.1
1.2	7.6	7.8	7.8	7.8	7.8	7.9	7.9	7.9
2.1	7.8	7.9	7.9	7.9	7.9	7.9	7.9	7.9
2.2	7.8	7.9	7.8	7.9	7.8	7.9	7.9	7.9
Control	7.8	8.0	7.9	7.9	7.9	7.9	7.9	7.9
O_2 , mg dm^{-3}								
1.1	6.1	6.0	5.7	5.7	5.8	5.8	6.0	6.0
1.2	5.7	5.3	5.4	5.3	5.5	5.5	5.5	5.5
2.1	5.7	5.4	5.4	5.5	5.7	5.6	5.6	5.5
2.2	6.0	5.7	5.6	5.4	5.8	5.6	5.7	5.7
Control	6.1	5.7	5.7	5.5	5.8	5.7	5.7	5.8

glass. At the peak, the nitrite concentration reached 1 mg $\text{NO}_2^- \text{ dm}^{-3}$. This can be explained by the presence of anaerobic zones inside the filler granules, where denitrification processes occur, with the reduction of some nitrates to nitrites. The increase in the level of NO_2^- in the water of the control RAS occurred later than in the experimental RAS, indicating a less efficient operation of the classical polymer filler compared to foamed glass and highly porous ceramics.

The level of nitrates during the experiment in all RAS was within acceptable limits and averaged 30 mg dm^{-3} without significant fluctuations. The water pH was also in the optimal range for the operation of the biofiltration system (pH 7.6-8.1), with a slight increase in pH proportional to all systems after the introduction of NH_4Cl solution and a decrease in this indicator due to active biofiltration 2-4 hours after

the start of the experiment. The concentration of dissolved oxygen in water varied within acceptable limits for biofilters, from 5.3 to 6.1 mg dm^{-3} , and was acceptable for growing Sterlet. The water temperature during the experiment averaged 23.4°C, with slight fluctuations in the range of 22.9-24.1°C. No changes in the behavior or physiological conditions of the sterlet fry were observed during the experiment. No fish death was observed.

Experiment 2

After the establishment of biological equilibrium in all RAS, the concentration of $\text{NH}_3/\text{NH}_4^+$ was artificially increased to 1.5 mg dm^{-3} by adding an NH_4Cl solution. The experimental results are summarized in

Table 2Dynamics of changes in $\text{NH}_3/\text{NH}_4^+$, nitrites, pH and O_2 in experiment 2

VE	Time						
	09:00	11:00	13:00	14:00	15:00	16:00	17:00
Average values of $\text{NH}_3/\text{NH}_4^+$, mg dm^{-3}							
E 1	0	1.67 \pm 0.17	0.92 \pm 0.54	0.5 \pm 0.38	0.33 \pm 0.04	0.23 \pm 0.11	0.08 \pm 0.04
E 2	0	1.5	0.5	0.25	0.17 \pm 0.04	0.08 \pm 0.04	0
E 3	0	1.5	0.25	0.03 \pm 0.01	0	0	0
E 4	0	1.5	0.42 \pm 0.04	0.17 \pm 0.04	0.12 \pm 0.03	0.03 \pm 0.01	0
E 5	0	1.33 \pm 0.17	0.25	0.12 \pm 0.03	0.03 \pm 0.01	0.03 \pm 0.01	0
E 6	0	1.33 \pm 0.17	0.25	0.03 \pm 0.01	0	0	0
Average values of NO_2^- , mg dm^{-3}							
E 1	0.35 \pm 0.135	0.63 \pm 0.167	0.80	0.60 \pm 0.06	0.50	0.60 \pm 0.06	0.50
E 2	0.05	0.13 \pm 0.007	0.27 \pm 0.007	0.23 \pm 0.007	0.17 \pm 0.007	0.15 \pm 0.015	0.15 \pm 0.015
E 3	0.07 \pm 0.002	0.20	0.27 \pm 0.007	0.10	0.08 \pm 0.002	0.08 \pm 0.002	0.08 \pm 0.002
E 4	0.13 \pm 0.007	0.37 \pm 0.027	0.50	0.30	0.27 \pm 0.007	0.27 \pm 0.007	0.27 \pm 0.007
E 5	0.07 \pm 0.002	0.27 \pm 0.007	0.27 \pm 0.007	0.20	0.17 \pm 0.007	0.13 \pm 0.007	0.10
E 6	0.08 \pm 0.002	0.30	0.38 \pm 0.042	0.23 \pm 0.007	0.17 \pm 0.007	0.17 \pm 0.007	0.17 \pm 0.007
Average pH values							
E 1	7.79 \pm 0.093	7.91 \pm 0.029	8.00	8.02 \pm 0.001	7.99	7.99	7.99
E 2	7.77 \pm 0.121	7.84 \pm 0.015	7.92 \pm 0.001	7.95	7.93 \pm 0.001	7.93 \pm 0.001	7.94 \pm 0.003
E 3	7.71 \pm 0.086	7.68 \pm 0.256	7.81	7.82	7.81 \pm 0.002	7.84 \pm 0.002	7.84 \pm 0.003
E 4	7.81 \pm 0.085	7.71 \pm 0.259	7.95	7.98	7.97 \pm 0.001	7.95 \pm 0.005	7.95 \pm 0.004
E 5	7.81 \pm 0.055	7.65 \pm 0.411	7.95	7.95	7.94	7.94 \pm 0.002	7.95 \pm 0.004
E 6	7.83 \pm 0.055	7.63 \pm 0.427	7.90	7.90	7.89 \pm 0.001	7.92 \pm 0.004	7.92 \pm 0.005
Average values of dissolved oxygen in water, $\text{mg O}_2 \text{ dm}^{-3}$							
E 1	7.80 \pm 0.420	7.60 \pm 0.380	7.65 \pm 0.605	7.65 \pm 0.605	7.35 \pm 0.125	7.40 \pm 0.080	7.50 \pm 0.080
E 2	7.87 \pm 1.207	7.24 \pm 1.635	7.50 \pm 0.980	7.55 \pm 0.845	7.20 \pm 0.180	7.60 \pm 0.180	7.65 \pm 0.245
E 3	7.17 \pm 1.127	6.67 \pm 0.327	7.00 \pm 0.5	6.80 \pm 0.5	6.65 \pm 0.045	6.84 \pm 0.054	6.95 \pm 0.045
E 4	7.77 \pm 0.327	7.27 \pm 0.527	7.65 \pm 0.125	7.70 \pm 0.5	7.40 \pm 0.18	7.60 \pm 0.02	7.65 \pm 0.045
E 5	7.72 \pm 0.802	6.73 \pm 0.247	7.30 \pm 0.32	7.10 \pm 0.18	6.95 \pm 0.125	7.20 \pm 0.08	7.20 \pm 0.180
E 6	7.43 \pm 0.327	6.40 \pm 0.78	7.00 \pm 0.08	6.90 \pm 0.02	6.95 \pm 0.125	7.25 \pm 0.245	7.45 \pm 0.125

Table 2. As can be seen from the table, the fastest elimination of ammonia-ammonium from water was observed in variants E3 and E6, and a decrease in the concentration of $\text{NH}_3/\text{NH}_4^+$ to 0.1 mg dm^{-3} occurred, on average, within five hours. In the first and second repetitions of these variants, complete oxidation of $\text{NH}_3/\text{NH}_4^+$ occurred within 5 h in both cases. In the third repetition, this was observed after 6 h, although in 5 h, the concentration of $\text{NH}_3/\text{NH}_4^+$ decreased to 0.1 mg dm^{-3} . In variant E5, the reduction of $\text{NH}_3/\text{NH}_4^+$ content to 0.03 mg dm^{-3} occurred within

6 h. Variants E4 and E2 showed similar results, with a decrease in the $\text{NH}_3/\text{NH}_4^+$ content in water to the level of 0.1 mg dm^{-3} occurring within 7 h.

The lowest rate of nitrification processes was observed in the variants E1 – E8 h before the decrease of $\text{NH}_3/\text{NH}_4^+$ concentration below the level of 0.1 mg dm^{-3} , namely 0.08 mg dm^{-3} . It should be noted that during the second and third repetitions of the experiment, complete oxidation of $\text{NH}_3/\text{NH}_4^+$ occurred at 7 h of the test. Simultaneously, the highest NO_2^- content was observed in the system. Changes in

NO_2^- concentration in all experimental systems corresponded to the normal scheme of the nitrogen cycle in the RAS with a biofilter: at the beginning of the experiment, an increase in NO_2^- was observed, with a peak at the minimum concentration of $\text{NH}_3/\text{NH}_4^+$ (on average, at 5 h), with a subsequent decrease in the values of NO_2^- to the starting values. Throughout the experiment, the low concentration of NO_2^- in RAS water (from 0.05 to 0.3 mg dm^{-3}) was constant, which was due to the processes of nitrification in the biofilter during the cultivation of aquatic organisms. A significant increase in NO_2^- content was observed only after the artificial introduction of nitrogenous substances into the water.

The concentration of dissolved oxygen in the water corresponded to the normative values for young sterlet. Fluctuations in the oxygen content in water ranged from 5.7 to 8.5 $\text{mg O}_2 \text{ dm}^{-3}$, with an average value of 7.3 $\text{mg O}_2 \text{ dm}^{-3}$. In all systems, a slight decrease in the oxygen content in the water was observed at 2–4 h from the beginning of the experiment, which corresponded to the period of the highest oxygen consumption by the biofilter bacteria, with a gradual return of oxygen concentration to baseline values. The average water temperature during the experiment was 25.1°C, with fluctuations in the range of 24.6–25.7°C. The water pH was within the optimum range for both the sturgeon fish and the normal functioning of the biofilter. The pH values ranged from 6.2 to 8.2, with an average of 7.3. The influence of biological filtration processes on pH was not observed. The survival of sterlets in variants E4 and E5 was 99%, in variant E1 was 98%, and in variants E2, E3 and 6 was 100%. No changes in the behavior or physiological conditions of the fish were observed.

Discussion

The purpose of experiment 1 was to comparatively evaluate the efficiency of using highly porous fillers (porous ceramics and foamed glass) for biofilters of RAS and classical polymer fillers. It is believed that the rate of oxidation of nitrogenous substances in the

biofilter entering the water during the cultivation of aquatic organisms is directly proportional to the value of the useful specific surface area of the substrate. Therefore, a biofilter loaded with a filler with a larger specific surface area will oxidize $\text{NH}_3/\text{NH}_4^+$ from water faster than a biofilter with the same volume of filler with a smaller specific surface area. This was confirmed by the experimental results (Table 1). The oxidation of the additionally added ammonium nitrogen in the experimental variants occurred in 5 (RAS 1.2), 6 (RAS 2.1 and 2.2), and 7 (RAS 1.1) h, and in the control variant, it occurred in 8 h. The best result was obtained in the second repetition of the experimental variant with porous ceramic as a filler for the biofilter. However, in the first repetition of the same variant, the oxidation time of the additionally added ammonium nitrogen was slightly longer than the other variants and repetitions, probably due to the fact that the nitrifying bacteria did not cover the entire useful area of this filler. However, the purpose of this experiment, which was to provide a comparative description of highly porous and classical fillers, was achieved.

Biofilters with porous glass showed a higher oxidation rate of additionally added ammonium nitrogen after 2 h than the control. According to the researchers, the potential of the rate of purification of water from ammonium compounds per unit volume of this type of filler was not exhausted during their experiment. However, this assumption requires further investigation to be confirmed. As shown in Table 1, a slightly higher level of NO_2^- was observed in experimental variant 2 (RAS 2.1 and 2.2). This may be due to the presence of an anaerobic zone in the foam glass, which, according to the manufacturer of this material, acts as a denitrifier. Presumably, a certain part of NO_3^- formed during biological filtration was reduced to NO_2^- , which increased the concentration of nitrites. In general, the nitrite content in the water of the experimental RAS was significantly higher than the norm for fish farms (0.2 $\text{mg NO}_2^- \text{ dm}^{-3}$). However, recent data on the reduction of the toxic effects of nitrites on fish in the presence of chlorine ions in the water are the basis for considering the maximum allowable content of nitrite concentration

of $2 \text{ mg NO}_2 \cdot \text{dm}^{-3}$ (Timmons and Ebeling 2006, Chebanov and Halych 2011).

Thus, during the period of maximum load on the biofilter, there was a decrease in the concentration of O_2 , with a gradual increase in the 6th hour of the experiment, after reducing the concentration of $\text{NH}_3/\text{NH}_4^+$ to a minimum level. As the technological scheme of operation of the research systems included aeration of both fish tanks and biofilters separately, no significant differences in the oxygen content of the water were observed between the treatments. Thus, the decrease in oxygen concentration in different systems during the experiment was only $0.3\text{--}0.6 \text{ mg O}_2 \cdot \text{dm}^{-3}$.

The survival rate of the sterlets was 100% in all experimental variants. After an artificial increase in the concentration of nitrogen compounds in water, no negative reactions were observed in the young sterlets. Observations were made on the activity of behavior and the nature of swimming and breathing movements of the fish. No differences were observed in the normal physiological state of the fish after the experiment was completed. In general, the first experiment confirmed the assumption of a more efficient operation of highly porous fillers, porous ceramics, and foamed glass compared with the classic polymer floating bio-load of the same volume.

The analysis of the experimental materials revealed a direct dependence of the nitrogen oxidation rate on the volume of the highly porous filler in the biofilter. Thus, in systems with foamed glass in biofilters (options E1 – E3), the decrease in the content of $\text{NH}_3/\text{NH}_4^+$ in water to a level below 0.1 mg dm^{-3} occurred the fastest in the variant with 3 dm^{-3} of filler – on average in 5 h, in the system with 2 dm^{-3} – in 6 h, and in the system with 1 dm^{-3} – in 7 h. A similar trend was observed in the variants with porous ceramics in the biofilter (E4 – E6), where the decrease of $\text{NH}_3/\text{NH}_4^+$ to a safe level below 0.1 mg dm^{-3} most likely occurred in the variant with the largest amount of filler (3 dm^{-3}) after 5 h. In the system with 2 dm^{-3} of filler, this process took 7 h, and in the system with 1 dm^{-3} of porous ceramics, it took 8 h (see Table 2). The dynamics of ammonium nitrogen oxidation processes in all systems were similar, with differences in

the duration of these processes depending on the experimental variant (VE) used. In general, in the experimental systems, the hydrochemical parameters were better than those recorded in the control version of the first experiment, although the volume of the classical polymeric filler was larger and the biomass of the cultivated aquatic organisms was smaller. Thus, the complete oxidation of $\text{NH}_3/\text{NH}_4^+$ in the experimental variants of experiment 2 occurred in 5–7 hours, and in the system with the classic polymeric filler of the biofilter in experiment 1, it occurred in 8 h.

However, it should be noted that although the level of dissolved oxygen in the water was optimal for nitrification processes, its average values in the first repetition of the experiment were slightly lower than in subsequent repetitions, which could also affect the rate of oxidation of $\text{NH}_3/\text{NH}_4^+$. In general, the dynamics of changes in the concentration of dissolved oxygen in water were as expected: a slight decrease in the concentration of O_2 was observed during periods of maximum load on the biofilter, with a gradual increase until the end of the experiment.

The concentration of NO_2^- during the experiment was higher than that specified by the regulations for fish farms. However, the toxic effect of nitrites is directly proportional to the presence of chlorine ions in water, and the maximum allowable concentrations of this indicator can increase by hundreds of times (Chebanov and Halych 2011). In experimental system E1, higher NO_2^- values were observed compared to the other experimental variants at all stages of the experiment. As the lowest rates of $\text{NH}_3/\text{NH}_4^+$ oxidation were observed in this system, and the indicators of dissolved oxygen in water were among the best, it should be considered that 1 dm^{-3} of porous ceramics per 100 dm^{-3} of fish tank volume is insufficient for normal nitrification processes under conditions similar to those in this experiment. During the research period, fish deaths were observed in experimental systems E4 and E5 (one specimen each) and E1 (two specimens). No symptoms of deterioration in the physiological condition of the test objects, which are characteristic of nitrogen poisoning, were observed.

Based on the analysis of the experimental results, it was determined that the proportions of the volume of porous ceramics to the volume of fish tanks, as 1 of 20 and 1 to 33, are excessive. A proportion of 1 to 50 was optimal under conditions similar to the experimental biological load on the system. Thus, at the stage of preparation for the experiment, in the presence of 2 dm^{-3} of porous ceramics per 100 dm^{-3} of fish tank in the biofilter, when keeping sterlet fry with an average weight of 1.5 g at a standard planting density of 250 specimens m^{-2} , the system maintained zero ammonia-ammonium concentration. In the experiment, with an artificial increase in the concentration of $\text{NH}_3/\text{NH}_4^+$ to 1.5 mg dm^{-3} , the biofilter purified water from ammonium compounds for seven hours. The proportion of 1:100 showed a result similar to the control variant of experiment 1 (5 dm^{-3} of the classic polymer filler per 100 dm^{-3} of fish tank). The ratios of porous glass to fish tank volumes, such as 1:20, 1:33, and 1:50, were excessive for the experimental conditions. The variant with a proportion of 1:100 showed a result similar to that of the variant with 2 dm^{-3} of porous ceramics (proportion 1:50), which can be considered optimal.

Conclusion

According to the results of this study, the following has been established:

- the use of highly porous materials, foamed glass JBL Micromec and porous ceramics as fillers for the biofilter proved to be more effective than the use of classic floating polymer load.
- the optimal proportion of biofilter volume with highly porous fillers to fish tank volume under conditions similar to the experimental ones was 1:100 for porous glass and 1:50 for porous ceramics.

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