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Research Article

The effect of media biofilter and aquatic plant (*Ceratophyllum demersum*) on water quality of recirculating aquaculture systems, growth and survival rates of ornamental fishes

Asadi Sharif E.[1*]; Imanpure Namin J.[1]; Ramezanpoure Z.[2]

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Abstract:
This study was conducted in order to compare the hornwort aquatic plant (*Ceratophyllum demersum*) with an artificial filter for purification in a recirculating aquaculture system (RAS). At first, the fluctuation of nitrogen components of the water reuse system equipped with artificial filtration was evaluated. Experiments were performed during three months. The hornwort aquatic plant was collected from Siadarvishan River and brought to the breeding and rearing system (JANAT Agriculture high school-Rasht). Subsequently, the plants were kept in river water for one month for adaptation. Initially, the plant wet biomass was weighted. For this purpose, 600 fishe specimens from the Cichlidae family (Oscar, Anjel, Burli and Zebra) provided from a local market were distributed between 12 aquarium tanks (160-liter capacity) in triplicate groups. Nitrogen component of water, such as ammonia (NH_3), nitrite (NO_2^-) and nitrate (No_3) were measured. Growth parameters and survival rate of fishe specimens at the end of the trial were evaluated. The results of this experiment indicated that nitrogen component when using hornwort aquatic plant as a biofilter not show better performance than the artificial filter treatment. The growth parameters performances when using the aquatic plant as biofilter in comparison with artificial filter declined.

Keywords: Recirculating aquaculture system, Cichlid, *Ceratophyllum demersum*, Biofilter

1-Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Iran, P.O.BOX: 1144.
2-Department of Ecology, Interntional Stugeon Research Institute, Agricultural Research, Education and Extension Organization, Rasht, Iran
*Corresponding author's Emial: ehsanasadisharif@gmail.com

Introduction

By introduction of a recirculating aquaculture system (RAS) in late 1970's the volume of fish production increased substantially worldwide (Rosenthal, 1981; Martins *et al.*, 2010). Recirculating aquaculture system involves biological and mechanical filtration, water pumps, oxygenation and aeration system, water supply system, and other units that maintain optimal water quality for fish (Hutchinson *et al.* 2004). Removal of nitrogen compounds is a very critical and important action to be taken in order to provide and maintain desirable water quality in RAS. Ammonia is the basic form of nitrogen emitted by aquatic animals with toxic effects on crustaceans and fish (Armstrong *et al.*, 1978). Nitrite is an intermediate product of nitrification with a strong poisonous effect. Nitrate is the final product of nitrification. Different studies confirmed that nitrate is the least toxic nitrogen compound in comparison to ammonia and nitrite (Noga, 2000; Timmons *et al.*, 2002).

Ceratophylum demersum (Hornwort or coontail) is a common submerged inland waters aquatic plant of Iran with rapid growth and is well-adjusted to aquarium conditions. *C. demersum* develops modified leaves once it is growing near the pond bottom. Considering this potential of *C. demersum*, the present study was conducted to compare purification capability of the plant, combination of plant and artificial media, and media to attenuate nitrogen components in water reuse system.

Materials and methods

Schematic diagram of the experimental water reuse system is illustrated in Figure 1. As shown in Figure 1 the system included several compartments, as follows: aquarium tanks, biofilter, microfilter, heating system, UV disinfection chamber, and bulbs.

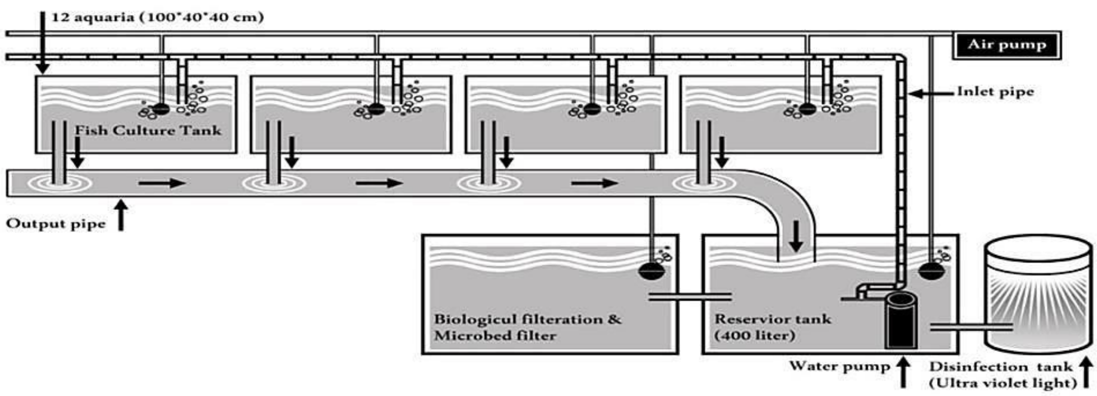

Figure 1: Schematic diagram of experimental water reuse system setup.

The experiments were carried out in 12 aquaria (dimension 100x40x40 cm, see Figure 1) equipped with an aeration system. Some 600 juvenile fish belonging to 4 species from Cichlidae family, including *Astronotus ocellatus* (oscar), *Pterophyllum scalare* (angel fish), *Amatitlania nigrofasciata* (zebra) and *Copadichromis borleyi* (borlieyi) were distributed in the aquariums. Two separate and identical aquaria were used as biofilter units. The volume of water in the reservoir tank of the system was 400 liters that was connected to a recirculation system pump. Experimental units were connected to the input pipe to let filtered water in and output pipes to drain water and waste materials from the units. The whole volume of water in the recirculation system was approximately 2500 liters. Before the experiment, the system was allowed to operate for one month in order to develop a bacterial biofilm. By the end of this period and knowing that the bacterial biofilm was already formed, the next phase began. At this phase, water was directed from biofilter units into the reservoir tank and subsequently into the experimental units (aquaria). Water temperature was adjusted and maintained at 27±1ºC which is almost optimum for the reared species of fish.

Submerged biological filter

The biological filter unit was composed of extremely light polystyrene bead, 1- 3 mm in diameter and submerged in water. Dimensions of the biological filter unit (tank) was100×40×40 cm.

Cultivation of nitrifying bacteria on submerged biological filter

Prior to the experiment, the polystyrene bead was placed at the aquaria (designated to be used as submerged biofilter) and subjected to constant water flow and aeration using Vortex Gas Pump-HG-370B for a period of one month. The process activated nitrifying bacteria in the unit. Once all parts of the recirculation systems were ready to operate, the experiment commenced. By the end of one month, water samples were collected from the inlet and outlet of the biofilter unit and analyzed. Simultaneously performance of submerged biofilter was evaluated by comparing nitrogen contents (ammonia, nitrite and nitrate) of inlet and outlet water.

Ceratophyllum demersum

The hornwort plant was collected from Siah-Darvishan River (Guilan-Iran) and transported to the ornamental RAS. Before any treatment or experiment, the plant was disinfected by diluted hydrochloric acid (3%).

Experimental conditions

Four species of cichlid family were stocked at a density of 50 fish/m^3 (November 2014). Two hundred fish specimens of each species were used for the experiments. The initial mean weight of angel fish was (14.5±1 gr), oscar (24.5±1 gr), borlieyi (17.50±1 gr) and zebra (17.5±1 gr). Fish from each species were distributed randomly in three tanks. The aquaria were supplied with constant aeration. During this time, photoperiod was adjusted from 7:00 Am to 7:00 Pm. Water temperature was automatically maintained at 27±1°C. Fish were fed %5 of their body weight with a diet containing 35% protein three times a day (7:00, 14:00 and 19:00) for the experimental period.

Purifying capacities of *C. demersum*, media and a combination of plant and media were examined in a recirculating system designed for aquaculture. Water samples from biofilter unit were tested for ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) regularly, every 10 days of the experimental period, by Spectrophotometer (DR5000) using phenate, sulfanilamide and Brucine method (USEPA, 1971). Survival rate and growth parameters of fish were examined at the end of the experiment. One- way analysis of variance was used on the experimental parameters including growth parameters and survival rate and Duncan's multiple range test was applied to verify the significance of differences amongst treatments at $P<0.05$ (values are presented as mean±standard error).

Results

Reduction of nitrogen content

The results of the experiments are illustrated in Figures 2, 3, and 4. Using artificial media as filter media reduced the amount of ammonia gradually to the final concentration of 0.02 mg/liter. Nitrite and nitrate concentration also showed a descending trend (Figs. 3 and 4). On the other hand, using hornwort plant alone as biofilter resulted in a tremendous increase in ammonia, nitrite, and nitrate concentrations. A combination of aquatic plant and media showed the best performance where concentrations of ammonia, nitrite, and nitrate dropped to 0.01, 0.02, and 40 mg/liter, respectively. The ammonia concentration in the onset of the treatments was adjusted to 0.15±0.03 mg/liter. As the experiment continued, levels of ammonia progressively raised to 0.8 mg/liter. Nitrite levels increased from an initial 0.03 mg/liter at the begging of the experiment to 1.21 mg/liter at the end of the experiment. Nitrate concentration also increased and reached to 115 mg/liter.

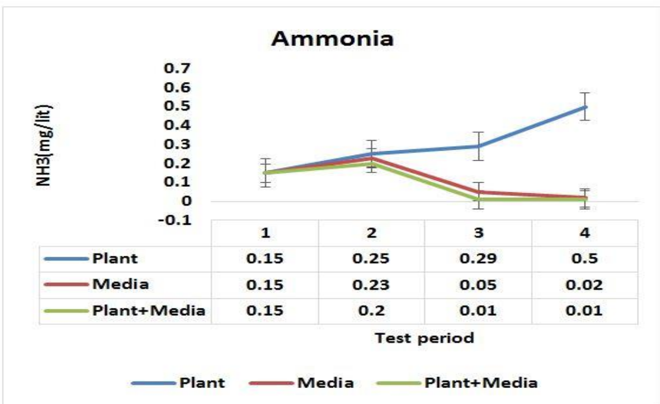

Figure 2: Ammonia concentration in different treatments during the experiment.

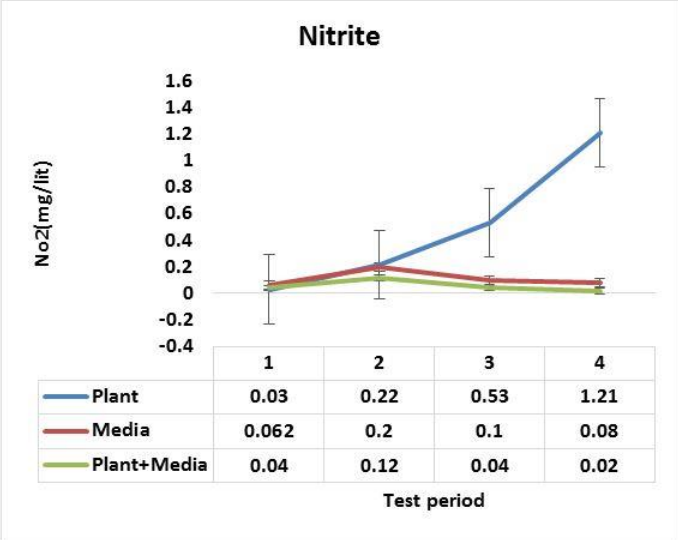

Figure 3: Nitrite concentration in different treatments during the experiment.

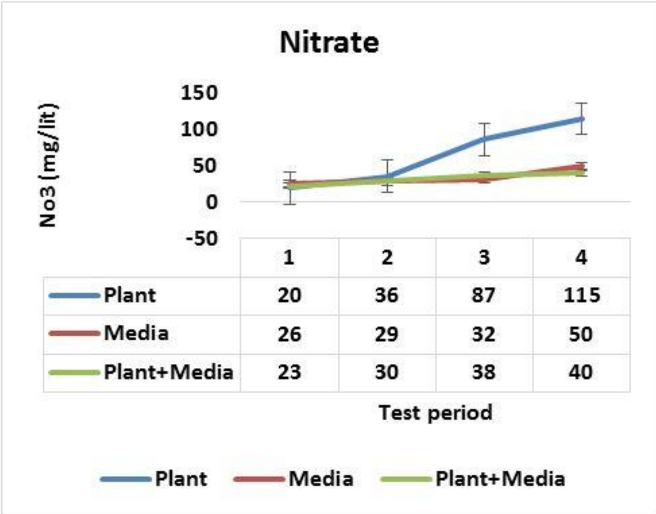

Figure 4: Nitrate concentration in different treatments during the experiment.

The initial concentration of ammonia in artificial media treatment was 0.15±0.03. At the end of the twentieth day of the experiment, the ammonia content reached a peak of about 0.23 mg/liter and dropped to 0.02 mg/liter by the end of the experimental period. The concentration of nitrite during the first 10 days of the experiment was 0.062 mg/liter and gradually increased to 0.2 mg/liter in day 20. The content of nitrite sharply declined and dropped to 0.08 mg/liter at the end of the trial, even in accordance with Timmons *et al.* (2006) this low concentration of nitrite is toxic for fish. In the combination of *C. demersum* and media used as biofilter, concentration of ammonia was 0.15±0.03 mg/liter at the onset of the experiment. It increased slightly to 0.2mg/liter but then decreased to 0.02 mg/liter by the end of experiment. The nitrite concentration was 0.04mg/liter at the beginning of the trial. Nitrite also showed an early increase and reached 0.12 mg/liter but afterward, it started decreasing and dropped to the final level of 0.02 mg/liter. Nitrate levels increased in comparison to initial concentration and reached 40 mg/liter by the end of the experiment.

Growth of Ceratophyllum demersum

Wet biomass of *C. demersum* specimens used in the two treatments (plant alone and plant+submerged biofilter) of the experiment is shown in Table 1. The results demonstrated that the biomass of *C. demersum* in plant alone treatment declined from 53 to 50.5 g/liter during 30 days, while when it was used in combined with artificial media, its biomass increased from 53.5 to 59.30 g/liter.

The effect of different biofilters on growth parameters and survival rate of fish are presented in Table 2. As shown in Table 2, the highest body weight (BW), specific growth rate (SGR), and survival rate were obtained in angel fish and oscar using artificial media as biological filter. However, zebra and borlieyi fishes showed the highest survival rate in the treatment using a combination of *C. demersum* andartificial media as biofilter (p<0.05).

Table 1: Biomass of the aquatic plant, *Ceratophylum demersum*, during the experiment.

Treatment	Initial biomass of Plant (mg/liter)	Final biomass of Plant (mg/liter)
Ceratophylum demersum	53	50.50
Media+ *C. demersum*	53.5	59.30

Table 2: Performance of growth parameters of fishes at the end of the experiment.

Fish survival (%)	Treatment	Initial body	Final body weight (gr)	Relative growth weight (gr)	Rate
Angel fish	M	14.27±0.23	22.19±0.16[a]	55.50±0.16[a]	6.3
	C	13.98±0.28	19.13±0.20[a]	36.83±0.17[b]	25
	M+C	14.60±0.15	26.63±0.22[b]	82.38±0.48[c]	100
Oscar	M	24.28±0.33	30.21±0.73[a]	24.42±0.68[a]	65
	C	23.90±0.30	28.30±0.80	18.3±0.80[a]	30
	M+C	24.30±0.39	41.31±0.68[b]	70.88±0.66	100
Borlieyi	M	17.23±0.20	31.98±0.23[a]	58.44±0.52[a]	100
	C	17.63±0.23	24.53±0.33[b]	39.13±0.42[b]	84
	M+C	17.50±0.21	72.30±0.18[c]	82.74±0.23[c]	100
Zebra	M	17.28±0.20	25.63±0.15[a]	48.75±0.51[a]	100
	C	17.70±0.14	23.63±0.48[a]	33.50±0.48[b]	86
	M+C	17.56±0.33	31.23±0.24[b]	77.84±0.33[c]	100

M= Media, C= *Ceratophyllum demersum*

Maximum body weight for anjel fish, oscar, zebra, and Borlieyi also achieved in the treatment combining plant and artificial media (26.63±0.22, 41.31±0.68, 31.23±0.24 and 31.98±0.23 gr) and minimum body weights were observed in the treatment using plant as biofilter (19.13±20, 28.3±0.80, 23.63±0.29 and 24.53±0.33 gr) respectively (p<0.05).

Discussion

The results suggested that a certain percentage of ammonia was converted to nitrite and then modified to nitrate. Nonetheless, *C. demersum* did not have the potential to absorb this amount of nitrate. Although nitrate is readily taken up by plants, however, in this experiment when *C. demersum* was used as biofilter the plants stopped growing completely.

Based on results, a combination of aquatic plant and media showed the best performance. Typical start-up curve for a new biological filter is designed by Timmons *et al.* (2002). Concentration of ammonia increased sharply in the early stages of the experiment. By incresasing the mobility of nitrifying bacteria, the nitrite content raised. Then the nitrate levels increased while ammonia and nitrite concentrations started to decline. It seems using *C. demersum* as a biofilter in the present study disordered the mechanism of nitrification.

As expected, the *C. demersum* could not absorb a high concentration of nitrate when using it as a biofilter. According to Ayyasamy *et al.* (2009), when plants are subjected to high levels of nitrate, their capability to absorb nitrate declines sharply. When using media as a biofilter, the absorption of ammonia increased to 0.02 mg/L at the end of the period. Tseng and Wu, (2004) evaluated the removal cycle of ammonia by artificial media in an eel recirculating system and obtained results similar to our results.

Little information is available on the use of a combination of artificial media and *C. demersum* as a biofilter. Fotedar (2016) was used duckweed (*Lemna minor*) as a biofilter medium in the RAS system. Their results showed that increasing the density above 18.75 kg in Baramundi fish led to decreased growth parameters, increased feed conversion ratio, and altered blood plasma levels, Therefore, the poor performance of the duckweed as a biofilter at high fish densities is consistent with the results of the present study.

According to the results, plant growth decreased when used as a biofilter independently, while plant growth increased when used with media as a biofilter simultaneously. In the present study, growth parameters and survival rate of fish significantly decreased in the *C. demersum* treatment. It seems that even the slightest increase in nitrogen compounds in recirculating water exerts severe stress on fish. Van Weerd and Komen (1998) reported similar results in their study where an increase in nitrogen compounds resulted in growth retardation and mortality due to stress.

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References

Armstrong, D.A., Chippendale, D., Knight, A.W. and Colt, J.E., 1978. Interaction of ioinized and un-ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. *Biology Bulletin*, 154(**1**), 15–31. https://doi.org/10.2307/1540771.

Ayyasamy, P.M., Rajakumar, S., Sathishkumar, M., Swaminathan, K., Shanthi, K., Lakshmanaperumalsamy, P. and Lee, S., 2009. Nitrate removal from synthetic medium and groundwater with aquatic macrophytes. *Desalination*, 242(**1–3**), 286–296. https://doi.org/10.1016/j.desal.2008.05.008.

Fotedar, R., 2016. Water quality, growth and stress responses of juvenile barramundi (Lates calcarifer Bloch), reared at four different densities in integrated recirculating aquaculture systems. *Aquaculture*, 458, 113-120. https://doi.org/10.1016/j.aquaculture.2016.03.001

Hutchinson, W., Jeffrey, M., O'Sullivan, D., Casemen, D. and Clark, S., 2004. *Recirculating aquaculture systems: minimum standards for design, construction and management.* South Australia Research and Development Institute,78 P.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., d'Orbcastel, E.R. and Verreth, J.A.J., 2010. New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. *Aquaculture Engineering*, 43(**3**), 83–93. https://doi.org/10.1016/j.aquaeng.2010.09.002.

Noga, E.J., 2000. *Fish disease, diagnosis and treatment*, 1st edition. Willey-Blackwell Publishing, Hoboken, New Jersey, USA, 367 P.

Rosenthal, H., 1981. Recirculation systems in western Europe. In: Aquaculture in heated effluents and recirculating systems.. Tiews, K., editor. Heenemann Publishing, Portsmouth, New Hampshire, USA, Volum 2, 305–316.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. and Vinci, B.J., 2002. *Recirculating aquaculture systems*, 2nd Edition. Cayuga Aqua Ventures, LLC, New York, USA, 769 P.

Timmons, M.B., Holder, J.L. and Ebeling, J.M., 2006. Application of microbead biological filters. *Aquacultural Engineering*, 34(**3**), 332–343. https://doi.org/10.1016/j.aquaeng.2005.07.003.

Tseng, K.F. and Wu, K.L. 2004. The ammonia removal cycle for a submerged biofilter used in a recirculating eel culture system. *Aquacultural Engineering*, 31(**1–2**), 17–30. https://doi.org/10.1016/j.aquaeng.2003.12.002.

USEPA, 1971. *Nitrogen, nitrate (colorimetric, brucine) by* *spectrophotometer*. United States Environmental Protection Agency, Washington, D.C., USA, Method 352.1, 5 P.

Van Weerd J.H. and Komen J., 1998. The effects of chronic stress on growth in fish: a critical appraisal. *Comparative Biochemistry and Physiology - Part A – Molecular and Integrative Physiology*, 120, 107-112. https://doi.org/10.1016/s1095-6433(98)10017.x.