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Assessing Nutrient Removal Efficiency in an Integrated Recirculating Aquaculture System and its Applicability at Different Conditions in Fish Culture

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1. BACKGROUND AND OBJECTIVES

1.1. Background Information

Wastewater of intensive aquaculture contains considerable amounts of nutrients, is well documented as a serious problem to environmental deterioration. Recirculating aquaculture systems (RSAs) have been developed in response to the increasingly strict environmental regulations and limited access to land and water. The RAS offers many benefits in terms of reducing water requirements, recycling nutrients, improving waste management and better disease management. However, RAS is typically used for commercially important species with high stocking densities and low water exchange rates in order to cover the high investment costs. Due to increasing concerns about the problems of setup and operation costs, diseases, animal welfare, and the accumulations of the nitrate and phosphorus concentrations in the RASs, the research and developments in the RASs tend to focus on: (1) technical improvements within the recirculation loop and (2) recycling of nutrients through integrated farming.

Accordingly, integrated recirculating aquaculture systems (IRASs) have received a lot of attention as promising practices. These systems are considered as alternative solutions for the efficient utilization of available resources, recycling nutrients, managing water quality, and reducing environmental problems of aquaculture. These systems can also be operated at a lower cost in terms of water use, space and management. The IRASs refer to integrated systems where additional separated units are integrated into a RAS. These separated units have the ability to convert nutrients into harvestable products and higher nutrient retention can be achieved by the primary and secondary products while having positive impacts on water quality.

The IRAS is now accepted as an alternative solution to the conventional practice of farming, as the system is known to decrease the expenses involved in operations and reduce the environmental problems of aquaculture. However, optimizing growth conditions for both plants and fish is the biggest challenge to profitability because the amount of nutrients from different integrated modules differs and depends on the nutritional values of the feed, which in turn depends on the specific demands of the cultured species. Nutrients in the system such as nitrogen and phosphorus can have direct effects on water quality, plant growth, and nutrient removal capacity, and then indirectly affect fish growth. It is necessary to maintain the balance of nutrient production and uptake in order to ensure effective nutrient removal. However, several factors such as fish species, fish size, fish density, temperature, plant species, harvesting rate of plants and the microbial community can have a pronounced effect on the growth of cultured species and nutrient removal rates of the IRASs. These factors should be at an optimum to maximize the nutrient removal efficiency of these systems and growth conditions of cultured species. However, little information is available on the optimum of these factors in the IRASs. The integrated technology that combines the elements of the RAS and plant production is still under development, and there is a strong need to optimize the recycling rates of nutrients to achieve efficient nutrient removal in the overall system. Therefore, it is of prime necessity to understand the functioning of the nutrient cycles in the IRAS and optimise the recycling rates of nitrogen and phosphorus concerning the following criteria: (1) cultivated plant species, (2) size of fish, (3) harvesting biomass of plants and (4) magnetic water treatment technique.

1.2. Aim and Objectives of The Research

The research aimed to assess the nutrient cycling efficiency in the IRASs and evaluate the nutrient removal capacities at different modules of the IRAS for rearing common carp (*Cyprinus carpio*) considering the effects of different types of plant based biofilters (*Lemna minor*, *Hygroryza aristata* and *Phyllanthus fluitans*), size of fish, harvesting biomass of plants and magnetic water treatment technique.

The above aim is achieved by meeting the following objectives:

1- Evaluate and compare the growth and nutrient removal efficiencies of three plant-based biofilters (*Lemna minor, Hygroryza aristata* and *Phyllanthus fluitans)* with bacterial biofilms of a moving-bed filter in the IRASs culturing common carp.
2- Investigate the effect of different plant species (*L. minor, H. aristata* and *P. fluitans)* as biofilters in the IRASs on the growth and survival rates of the common carp.
3- Assess the effect of the initial body size of stocked common carp on water quality and the removal capacities of dissolved inorganic nitrogen in the IRASs.
4- Investigate the effect of harvesting different biomasses of watercress (*Nasturtium officinale*) on water quality and nutrient removal capacity in an IRAS for rearing common carp.
5- Examine the growth performance of both watercress and the common carp in an IRAS (Aquaponics) under different plant harvesting regimes.
6- Identify the potential of using magnetic water treatment to improve water quality and the growth performance of plant-based biofilter *(L. minor)* in the IRAS.
7- Investigate the impacts of magnetized water on the feeding efficiency and growth performance of common carp in the IRAS.

2. MATERIAL AND METHODS

2.1. Collection of Fish and Plants

Common carp (*Cyprinus carpio* L.) were collected from a local farm and transported to Georgikon Aquatic Research Laboratory (GARL), Keszthely, Hungary. Fish were acclimated to the laboratory conditions before the commencement of the experiment. In the third experiment, the fish were taken from a stocking tank at the Aquaculture Laboratory of Debrecen University.

Lemna minor, *Hygroryza aristata* and *Phyllanthus fluitans* plants were obtained from Interaqua-Flora Ltd., Hungary, cleaned and acclimated to the laboratory conditions before starting the experiment. The *Nasturtium officinale* plant was taken from a growing hydroponic bed in an operating aquaponic system at the Aquaculture Laboratory of Debrecen University.

2.2. Experimental Biological Filters

Different plant based biofilters were designed and established in this research. The biomasses of the plant based biofilters were estimated based on their ammonium uptake rates, which were estimated during a preliminary experiment. A bacterial biofilm filter was also designed as one of the experimental treatments. Plastic media with a specific surface area of 400 m^2 m^{-3} were used to place in the bacterial biofilm filter tanks. In order to determine the appropriate design of the bacterial biofilm filter, the calculation of the size of the filter was based on the calculations published by Timmons et al. (2002).

2.3. Design of Experimental Systems and Rearing Conditions

A series of experiments were conducted under laboratory conditions to investigate the nutrient cycling efficiency in the IRASs. The first trial was designed as four treatments with three replicates in a random arrangement. The biofilter tank of each of the four treatments was stocked with one type of filtration (bacterial biofilm filter; *L. minor* plant based biofilter; *P. fluitans* plant based biofilter; and *H. aristata* plant based biofilter). The second trial was performed whereas two sizes of fish (small and large fish) had two types of biological filters (bacterial biofilm filter and plant based biofilter). Three replicated tanks were randomly designed for each of the four treatments. In the third experiment, the effect of harvesting different biomasses of above-ground plants was evaluated by a random design with three replicates. There were four treatments: harvesting 0%, 25%, 33%, and 50% of plants biweekly from the surface area of each hydroponic bed. The fourth trial was designed as two treatments with three replicates in a random arrangement. One treatment was supplied with a magnetic field device, while the control treatment was set up without the device. The Electromagnetic field was generated in a coil by currents using a commercial magnetic field generator with a frequency of 25 kilohertz and an intensity of 0.8 millitesla.

In all the experiments, the experimental units have the same concept of structure and each unit consisted of three tanks: a fish tank, a waste collection tank, and a plant based biofilter unit. The fish and waste collection tanks were set on the floor, while the plant based biofilter unit was installed above the fish tank. Water from the waste collection tank was pumped to the plant based biofilter unit by a submerged pump, circulated to the fish tank and, then returned to the waste collection tank by gravity (Figure 1). The flow rate of water was set at 3 L min^{-1}. The natural light was used in the first and third experiments, while an artificial light (illumination) was provided 12 hours a day in the second and fourth experiments. The fish tanks were supplied with two air stones to provide dissolved oxygen for the fish, and a polyethylene mesh was put above the fish tank to prevent the fish from jumping outside. All fish were fed by hands twice a day at 09:00 and 16:00 hours with a commercial diet. The uneaten feed was collected one hour after feeding, while faeces were removed daily before the feeding commenced through a filter net with a mesh size of 100 µm and the remaining water returned into the waste-collection tank of the same system. Approximately 30% of the system water was siphoned out weekly (every ten days in the third experiment) and replaced with new water.

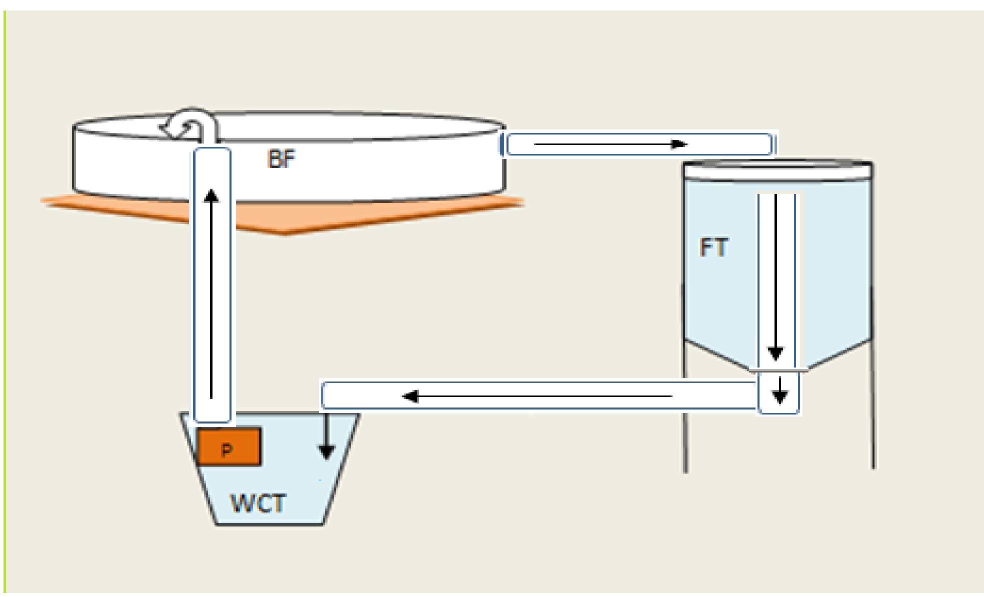

Figure 1. Diagram of the experimental recirculating units (the arrows show the direction of water flow), (FT): Fish tank, (WCT): Waste collection tank, (BF): Biological filter, (P): Pump

2.4. Sample Collection of Water and Analysis

Dissolved oxygen (DO), temperature and pH of water in the fish tanks were measured once a day before feeding commenced, using a dissolved oxygen meter, and pH meter. Triplicate water samples were collected from the fish tanks, as were the influent and effluent waters of each plant based biofilter unit to determine the nutrient removal rates in each system. Water samples were analysed under laboratory conditions and measured according to the European standard methods and the recommendations of the International Organization for Standardization. TAN, Ammonia nitrogen (NH_3-N), Ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N), total nitrogen (TN), orthophosphate (PO_4-P) and total phosphorus (TP) were measured using a spectrophotometer. The levels of NH_3-N, NO_2-N, and NO_3-N were determined using the Nessler method (Method 8038), diazotisation method (Method 8507), and cadmium reduction method (Method 8039), respectively. The TP and PO_4-P were determined by the phosphor-molybdenum blue method.

The nutrient removal rates were used to determine the nutrient removal cycle in each system. The nutrient removal capacities of the plant based biofilters were calculated after the water quality parameters were measured in all the systems.

2.5. Fish and Plant Growth Parameters

In all the experiments, the growth rates of both fish and plants were measured in all the systems. The growth and survival rates of the fish were recorded at the end of the experiment for each tank. The fish biomass, specific growth rates (SGR), fish weight gain, feed conversion ratio (FCR), and survival rates were calculated. All the plants were also harvested at the end of the experiment, and the weight of the plants was recorded. The final biomass, biomass gain, and specific growth rates of plants (SGRP) were calculated.

2.6. Statistical Analysis

All statistical analyses were performed using SPSS version 22.0 for windows. All the data obtained were tested for normality of distribution and homogeneity of variance. One-way analysis of variance (ANOVA) was conducted to test the differences between the parameters amongst treatments. Significant ANOVAs were followed by Duncan’s multiple range tests to recognize specific differences amongst treatments. A $P < 0.05$ was considered significant for all analyses. In the fourth experiment, the independent *t-test* was conducted to determine any significant differences between treatment means.

3. RESULTS

3.1. First Experiment: Effects of different plant species (*Lemna minor*, *Hygroryza aristata* and *Phyllanthus fluitans*) as plant based biofilters in integrated recirculating aquaculture systems.

The experiment aimed to evaluate and compare the nutrient removal efficiencies of three aquatic plant species (*Lemna minor*, *Hygrorysa aristata* and *Phyllanthus fluitans*) as plant based biofilters with the bacterial biofilms in the moving-bed filter.

The results showed that the mean NH_4-N removal rates of the bacterial biofilm filters were significantly higher than the values obtained with *L. minor* and *P. fluitans* biofilters. However, the NH_4-N removal rates in the *H. aristata* biofilters were significantly higher than those in the *P. fluitans* biofilters and comparable with those in the *L. minor* and bacterial biofilm filters. The bacterial biofilm filter had the highest NO_2-N removal rates and lowest NO_3-N removal rates, which significantly differed from those of other biofilters. The mean removal rates of NO_2-N and NO_3-N in the *H. aristata* biofilters were significantly higher than those in the *P. fluitans* biofilters and comparable with those in the *L. minor* biofilters. However, no significant differences in the mean removal rates of PO_4-P and TP were found between any of the treatments.

In this trial, the SGRs of fish reared in systems stocked with plant based biofilters (*P. fluitans*) were significantly lowest than in the other three treatments. However, the SGR of fish did not differ significantly between fish in systems stocked with *L. minor, H. aristata,* and bacterial biofilm filters. The highest SGRs were achieved for fish reared in systems using bacterial biofilm filter, followed by systems with *H. aristata*. The SGRP of plants in systems stocked with *H. aristata* was significantly higher than in the other treatments.

3.2. Second Experiment: Effect of the initial size of stocked fish and bio-filtration types in integrated recirculating aquaculture systems

The experiment aimed to investigate the effects of the initial size of stocked fish and biofiltration types on nitrogen removal efficiency and fish growth in an IRAS.

There were no significant differences in the mean removal rates of TAN, NO_2-N and NO_3-N in systems stocked by bacterial biofilm filters with small fish and those systems stocked by large fish with the same filters. Similarly, systems with plant based biofilters (*H. rotundifolia*) responded in the same way to remove TAN, NO_2-N and NO_3-N when they stocked with any sizes of fish. However, the bacterial biofilm filters with both sizes of fish had significantly higher removal rates of TAN and NO_2-N than the *H. rotundifolia*; while the *H. rotundifolia* filters showed significantly higher removal rates of NO_3-N than the bacterial biofilm filters. The mean TAN removal rate of all filters stocked with plant based biofilters (*H. rotundifolia*) decreased as the trial progressed; while the NO_2-N removal rates increased over time. All filters stocked with bacterial biofilm showed a decreasing trend in the NO_3-N removal rates as the trial progressed.

The small fish with both biofilters had significantly higher means of SGR than large fish. However, there were no significant differences in the SGRs of small fish reared in systems stocked with plant based biofilters (*H. rotundifolia*) and small fish in systems using bacterial biofilm filter. Similarly, large fish responded in the same way for growth when they stocked with any type of biofilters.

3.3. **Third Experiment**: Effects of harvesting different biomasses of plants in an integrated recirculating aquaponic system

The trial aimed to investigate the effects of harvesting different biomasses of watercress plants (*Nasturtium officinale*) on nutrient removal efficiency and growth performance of both plants and fish in an integrated recirculating aquaponic system.

In this trial, there were no significant differences in the mean removal rates of NH_3-N and NO_2-N amongst any of the treatments. The mean NH_3-N and NO_2-N removal rates increased in all the treatments over time. The highest removal rates for NO_3-N, PO_4-P, and TP were calculated in the unharvested system (0%), which significantly differed from those in the 33% and 50% systems. However, the mean NO_3-N, PO_4-P, and TP removal rates in the 25% harvested systems were comparable with those in the unharvested and 33% harvested systems. The mean NO_3-N removal rates in the unharvested systems increased over time, while the means for the other three treatments slightly decreased at the later stage of the culture period. The mean PO_4-P and TP removal rates increased in all treatments over time.

The results also showed that the SGRs of fish did not differ significantly amongst any of the treatments. However, the specific growth rates of the plants (SGRP) in the unharvested systems were significantly higher than the values for the 33% and 50% harvested systems. The SGRP of the 25% system was similar to the values for the unharvested (0%) and 33% harvested systems.

3.4. **Fourth Experiment:** Effects of using magnetic water treatment in an integrated recirculating aquaculture system

The experiment aimed to identify the potential of using magnetized water to improve water quality and growth performance of both fish and plants in an IRAS.

The results showed that the mean concentrations of temperature, DO, pH, NH_4-N, NO_2-N, and NO_3-N did not differ significantly between the electromagnetic field system and the control system. The results also indicated that the SGRP of plants in the electromagnetic field systems was significantly higher than in the control systems. The magnetized water also had significant effects on the SGR of common carp compared to the control system. The lower mean of FCR was recorded with the fish reared in the electromagnetic field system, which was significantly lower than those at the control system.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

Several factors such as fish species, fish size, fish density, temperature, plant species, and harvesting rate of plants can affect the nutrient removal rates and growth of cultured species in IRASs. Therefore, the research aimed to evaluate the nutrient removal capacities at different modules of the IRAS for rearing common carp (*Cyprinus carpio*), considering the effects of different plant species (*Lemna minor, Hygroryza aristata, and Phyllanthus fluitans*), size of fish, harvesting biomass of plants, and magnetic water treatment technique.

The research results proved that the use of plant based biofilters (*L. minor, H. aristata* and *P. fluitans*) in the IRASs was effective in maintaining water quality, removing nutrients, adding harvestable products and providing good conditions for common carp growth and survival. The nutrient uptake capacities of the tested plant based biofilters were different and strongly influenced by the growth rate of plants, which is affected by the environmental conditions. The research reveals that the *H. aristata* was the strongest plant in removing nutrients among the tested plant species, followed by *L. minor*. However, the bacterial biofilm in the moving-bed filter was the superior filter to reduce high concentrations of NH_4-N and NO_2-N.

The research findings also showed that the increase in the initial body size of stocked fish did not affect the removal efficiencies of TAN, NO_2-N, and NO_3-N; and both the bacterial biofilm and plant based biofilters (*H. rotundifolia*) were independent of the fish size. However, the bacterial biofilm filter showed higher removal rates of TAN and NO_2-N; while, the plant based biofilter (*H. rotundifolia*) had higher NO_3-N removal rates. The results also showed that the increase in the initial body size of fish significantly decreased the TAN excretion into the fish tank and the specific growth rate of fish.

The research results also revealed that increasing the biweekly harvested biomass of watercress plants (*Nasturtium officinale*) in the IRASs decreased the growth of the plants, while it did not affect the growth of the common carp. The 0% and 25% harvested systems were recorded to have the highest plant biomass production. Watercress plants were efficient in removing nutrients generated by fish in aquaponic systems. However, increasing the harvested biomass of watercress plants decreased the NO_3-N and PO_4-P removal efficiencies, while it did not affect the NH_3-N and NO_2-N removal efficiencies. The 0% and 25% harvested systems were recorded to have the highest NO_3-N and PO_4-P removal efficiencies.

The research results showed that the use of magnetized water in the IRASs increased the specific growth rate of common carp and decreased the feed conversion ratio. The growth of plants (*L. minor*) that used as a biofilter medium in the IRASs also improved after exposure to the magnetized water. However, the magnetized water had no significant effects on the concentrations of NH_4-N, NO_2-N, and NO_3-N in the IRASs.

4.2. Recommendations

The research recommends that the *H. aristata* is a more suitable plant to be used in removing nutrients, adding harvestable products and reducing the overall cost of the production systems, followed by *L. minor*, while the bacterial biofilm filter from a technical point of view is the strongest biofilter to reduce high concentrations of NH_4-N and NO_2-N. Regardless of the suitability of the bacterial biofilm and plant based biofilters, several factors must be considered when choosing appropriate biofilters, such as space, cost and benefit analyses, system location, climatic conditions and discharge regulations.

the small size of fish (initial body size of 33-46 g) should be stocked into the IRASs at the beginning of the rearing season to achieve better performance in fish. Moreover, the biweekly harvesting of less than 25% of the growing area of watercress plants is recommended for improving nutrient removal efficiency and sustaining the growth of both fish and plants in aquaponic systems. The research also suggests that the use of the magnetic water treatment technique in the IRAS can improve the growth of fish and plant, and this can increase the profitability, and makes these systems more cost-effective to be used.

5. NEW SCIENTIFIC RESULTS

Based on the results obtained from the four experiments conducted in the present research, the following points highlight the new scientific results and confirm the objectives of the research.

1. The use of plant based biofilter technique can be beneficial in decreasing nutrient overload, adding harvestable products and providing good conditions for common carp growth and survival. Among the tested plant species in the present research, *Hygroryza aristata* is a more suitable plant for removing nutrients, followed by *Lemna minor*. While from a technical point of view, the bacterial biofilm filter is the strongest biofilter to reduce high concentrations of NH_4-N and NO_2-N.

2. The size of fish did not affect the removal efficiencies of TAN, NO_2-N, and NO_3-N, and both the bacterial biofilm and plant based biofilters (*H. rotundifolia*) were independent of the fish size. The research shows that small size fish (initial size of 33-46 g) has better performance in the IRAS. This size of fish should be stocked into the IRASs at the beginning of the rearing season to achieve better performance in fish.

3. The biweekly harvesting of less than 25% of the growing area of watercress plants is recommended for improving nutrient removal efficiency and sustaining the growth of both fish and plants in the IRASs (Aquaponics).

4. Regarding the potential of using magnetized treated water in the IRASs, the research results show that the use of magnetized water in the IRASs can improve the growth performance of both fish and plants. However, the magnetized water had no significant effects on the concentrations of NH_4-N, NO_2-N, and NO_3-N in the IRASs.

6. SCIENTIFIC PUBLICATIONS OF THE AUTHOR

6.1. Articles in Peer-reviewed Journals

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6.2. Full Articles in Conferences

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6.3. Abstract in Conferences

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