The Usage of Aquatic Floating Macrophytes (\textit{Lemna And Wolffia}) as Biofilter in Recirculation Aquaculture System (RAS)

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Abstract

Conventional water treatment in recirculation aquaculture systems (RAS) is a limited technology to answer the challenges of so called “sustainable aquaculture”. This is why new and innovative technologies need to be invented and introduced in RAS. The aim of the conducted study was to determine the possible advantages of using two macrophytic plants – \textit{Lemna minor} and \textit{Wolffia arrhiza} and their quality as biological filter in a RAS for the cultivation of fingerlings from common carp. The temperature, dissolved oxygen, pH and conductivity were measured daily with a portable combined meter and with a probe appropriate for the parameters in the newly constructed control and experimental RAS (with floating macrophytes as a biological filter). Ammonium, nitrite, nitrate, total nitrogen and phosphorus were measured spectrophotometrically. At the end of the trial the fish were weighed and individual weight gain, specific growth rate and FCR (feed conversion ratio) were calculated. The utilization of two macrophytes (\textit{Lemna} and \textit{Wolffia}) in their quality as a biofilter in RAS increased dissolved oxygen in the water, significantly decreased the quantity of total dissolved solids, ammonia, nitrite, orthophosphate as well as total phosphorus in water, and significantly increased the growth of the cultivated carp’s fingerlings.

Keywords: Lemna, Wolffia, biofilter, RAS, water quality, carp’s fingerlings.

Introduction

One of the main problems in recirculation aquaculture systems (RAS) which are still object of investigation from aquaculture scientists is the removal of nitrogenous (Burrows, 1964; Leitritz and Lewis, 1976; Crab \textit{et al.}, 2007) and phosphorous metabolite compounds. The nitrogenous waste products are being created and excreted from fish through gill diffusion, gill cation exchange, as well as urine and feces excretion; in addition to this some nitrogenous wastes are accumulated from the organic debris of dead and dying organisms, uneaten feed, and from nitrogen gas in the atmosphere (Timmons \textit{et al.}, 2002). Phosphorous waste compounds, on the other hand are supplied together with feed, particularly compound feeds.

Piedrahita (2003), Gutierrez-Wing and Malone (2006) reported that approximately 75\% of nitrogenous and phosphorous compounds in food for hydrobionts remain unused and are accumulated in water like waste products.

Biological filtration is the method used for the destruction of organic and inorganic waste compounds in RAS (Boyd, 1990).

In the past few decades there has been considerable interest towards using floating macrophytes for the removal of nitrogenous and phosphorous compounds in the water (Steward, 1970). Macrophyte plants act as biological filters which accumulate nutritional compounds and inorganic waste products. Duckweed is a floating aquatic macrophyte which consists of four genera: \textit{Lemna}, \textit{Spirodela}, \textit{Wolffia} and \textit{Wolffiella}. Duckweed in wastewater treatment was found to be very effective in the removal of nutrients, soluble salts, organic matter, heavy metals and in the eliminating suspended solids, algal abundance and total and fecal coliform densities (El-Kheir \textit{et al.}, 2007). Duckweed has a high mineral absorption capacity and can tolerate high organic loading as well as high concentrations of micronutrients (Hasan and Chakrabarti, 2009).

Fresh duckweed is well suited to intensive fish farming systems with relatively rapid water exchange rates for waste removal (Gaigher \textit{et al.}, 1984), and duckweed is converted efficiently to live weight by certain fish, which include carp and tilapia (van Dyke and Sutton, 1977; Hepher and Pruginin, 1979).
Robinette et al., 1980; Hassan and Edwards, 1992). The research conducted with duckweed as a biological filter in the field of aquaculture is connected with the filtering of effluent water from fish farms (Sipaúba-Tavares et al., 2002) or improving water quality in fish ponds (Ferdoushi et al., 2008). The research pertaining to questions connected to the usage of duckweed as a biofilter in RAS are limited (Jo et al., 2002).

The aim of the conducted study was to determine the possible advantages of using two macrophytic plants – *Lemna minor* and *Wolffia arrhiza* and their quality as biological filter in RAS for the cultivating of fingerlings from common carp.

**Materials and Methods**

The experiment was conducted in two newly built recirculation aquaculture systems situated in the experimental aquaculture base at the Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

**The Recirculation Aquaculture Systems (RAS)**

For the purpose of our study two independent recirculation aquaculture systems were constructed and built. Each of them consisted of four tanks (50 litres each) and a module where the process of filtering the water was realized. The cleaning block of the experimental RAS consisted of a mechanical and biological filter (Figure 1). The biological filter consisted of two macrophytic plants – *Lemna minor* and *Wolffia arrhiza* freely floating on the surface of the water. The control RAS filtering block only consisted of a mechanical filter (Figure 1).

**Experimental Fish**

From a fish farm situated on the Jrebchevo dam, fingerlings from common carp were selected and transported to the experimental aquaculture base of the Faculty of Agriculture. The average fish weight at the start of the trial was:

- Experimental group fish – 8.13 g;
- Control group fish – 8.18 g;

Between the average weight of the carp from the two experimental variants a significant difference could not be found (P≥0.05). The stocking density was at 333 pcs/m³. The feeding level which we used in our trial was 2% of the biomass of the fish. The fish were fed manually three times per day. The content of granulated feed which we used in our experiment can be seen in Table 1.

At the end of the trial the fish were weighed and individual weight gain, specific growth rate and FCR (feed conversion ratio) were calculated.

The specific growth rate was calculated using the following formula:

$$SGR = \left( \frac{Bf \ln - \ln Bi}{T} \right) \times 100$$

Where:
- \(Bf\) – final biomass;
- \(Bi\) – initial biomass;
- \(T\) – time interval (days).

The food conversion ratio was calculated using the following formula:

$$FCR = \frac{F}{(Bf-Bi)}$$

Where:
- \(F\) – amount of food administered; 
- \((Bf-Bi)\) – growth gain; 
- \(Bf, Bi\) – final and initial biomass;

![Figure 1](image-url)
Hydrochemical Parameters

The temperature, dissolved oxygen, pH and conductivity were measured daily with a portable combined meter and with a probe appropriate for the parameters.

Samples for other analysis were taken once every ten days by the order showed below:
- B1 – 10th day after start of the trial
- B2 – 20th day after start of the trial
- B3 – 30th day after start of the trial
- B4 – 40th day after start of the trial

They were measured with a spectrophotometer DR 2800 (Hach Lange). The methods and range of tests which were used during the experiment are shown in Table 2.

The macrophytes were determined by Flora Reipublicae Popularis Bulgaricae, vol II (Jordanov et al., 1963). Data analysis were conducted by using ANOVA (MS Office, 2010).

Results

During the experimental period, the values of water temperature were similar in both RAS’s (Figure 2) and fluctuated between 19.7°C and 22.9°C (Table 3). Average values of temperature for the control RAS were 21.82°C and 21.97°C, but without statistically proven differences (Table 3).

The values of dissolved oxygen during the experimental period were higher in the experimental RAS in comparison to these of the controlled RAS and fluctuation in the values in the first system were much supple than these showed in the second system (Figure 3). Average values of dissolved oxygen were higher with 5.14% in experimental RAS compared with the measured values in the control RAS (P ≤ 0.01) (Table 3).

During our trial the measured pH was weakly alkaline in both recirculation systems too (Figure 4) and without statistically proven differences between control and experimental RAS (Table 3).

The values of conductivity in the controlled RAS were higher compared to those showed in the

<table>
<thead>
<tr>
<th>Table 1. Content of used feed in trial</th>
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<td>Content</td>
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<tr>
<td>Crude Protein</td>
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<tr>
<td>Crude Fat</td>
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<tr>
<td>Ash</td>
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<td>Crude fibre</td>
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<tr>
<td>Total phosphorus</td>
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<tr>
<td>Ca</td>
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<tr>
<td>Na</td>
</tr>
<tr>
<td>Vitamine</td>
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<tr>
<td>E300</td>
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<tr>
<td>E307</td>
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<tr>
<td>Antioxidants</td>
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<td>E324</td>
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<td>E321</td>
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<td>Cu</td>
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<td>Zn</td>
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<td>Fe</td>
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<th>Table 2. Methods and range of tests used for monitoring the water quality parameters during experiment</th>
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<tr>
<td>Quality parameters</td>
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<tr>
<td>Ammonium-nitrogen</td>
</tr>
<tr>
<td>Nitrite – nitrogen</td>
</tr>
<tr>
<td>Nitrate - nitrogen</td>
</tr>
<tr>
<td>Total nitrogen</td>
</tr>
<tr>
<td>Phosphorus (ortho + total)</td>
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Figure 2. Temperature in control and experimental RAS during experiment.
The average value of conductivity in experimental RAS were lower by 10.37% contrasted to those of the control variant (Table 3).

The minimal measured value of ammonia was 0.14 mg L\(^{-1}\) in the experimental RAS and the maximum value - 0.216 mg L\(^{-1}\) in the control system (Figure 6). The concentration of ammonium in experimental RAS were lower in every taken samples during the trial compared with the results which were received for control RAS (Figure 6).

The same tendency was found, even much more better expressed, for nitrite (Figure 7), the higher values were measured in control RAS and the maximum value - 0.216 mg L\(^{-1}\) in the control system (Figure 6). The concentration of ammonium in experimental RAS were lower in every taken samples during the trial compared with the results which were received for control RAS (Figure 6).

The same tendency was found, even much more better expressed, for nitrite (Figure 7), the higher values were measured in control RAS, compared with these which we received from our measurement in the experimental RAS and differences were statistically proven at a high level (P≤0.01) (Table 3). The minimal measured value of nitrite during our experiment were 0.053 mg L\(^{-1}\) and it was measured in the experimental RAS. The maximum value was 0.144 mg L\(^{-1}\) and it was measured in the control recirculation system (Figure 7).

During the experiment all measured quantities of nitrates were higher in the experimental RAS than those which we found for the control recirculation system (Figure 8), but differences between the control and the experimental variants were not statistically proven (Table 3). The maximum measured value for this parameter was 20.3 mg L\(^{-1}\) and the minimal quantity of nitrate was found to be 11.1 mg L\(^{-1}\) in the control system. Total nitrogen was higher in the control RAS compared to recirculation system using macrophytes as a biofilter every day when we took samples (Figure 9).

With respect to phosphorus compound we found a higher quantity of orthophosphate and total phosphorus in the control RAS compared to the experimental RAS in all conducted measurements (Figure 10 and 11).

### Discussion

Two RAS’s were not disposed with heating elements, which is the reason, the temperature inside
that they are dependent from air temperature. Nevertheless, the temperature during the experiment was optimal for the growth of experimental carp, because according to Huet (1970) a range of 20-28°C is the optimum temperature for the growth of common carp under laboratory conditions.

Our received results with respect to oxygen concentration are confirmed from the research conducted by Ferdoushi et al. (2008), which investigated the impact of macrophytic plants (Lemna)
as biofilters in fish ponds. They found out, that the quantity of dissolved oxygen was higher in the pond treated with *Lemna*. According Ondok *et al.* (1984) macrophytes oxygenate the water very effectively. Sengupta *et al.* (2010) explore the impact of duckweed growth on water quality in sub-tropical ponds and found out that pH values were mostly alkaline in the ponds studied and varied between 6.9 and 9.1. pH values of experimental ponds with macrophytes were found to be slightly alkaline and mean values of pH were 7.61±0.39 (Ferdoushi *et al.*, 2008).
The electrical conductivity (or specific conductance) of water depends on the concentration and charge of the dissolved ions. Because of this relationship, conductivity often is used as an indicator of the total dissolved solids (TDS) in the water. The slightly lower values of conductivity in the experimental RAS compared to those which we received in the control recirculation system are by our opinion the result from the decrease of total dissolved solids (TDS) in the water from the biofilter consisting of two macrophytes. Azeez and Sabbar (2012) stated that duckweed used in phytoremediation of the pollutants in wastewater from oil refinery reduced 48.9% from TDS in the water.

Ammonia toxicity is thought to occur from osmoregulatory imbalance causing renal failure and gill epithelial damage resulting in suffocation, decreased excretion of endogenous ammonia and general neurological and cytological failure (Meade, 1985). The total ammonia in the water is the sum of the un-ionized ammonia and the ionized form (ammonium). The main factor which determines the

![Figure 10. Dynamics evolution of orthophosphate](image1)

![Figure 11. Dynamics evolution of total phosphorus.](image2)

<table>
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<th>Table 4. Technological indicators of common carp’s fingerlings growth</th>
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<td><strong>Experimental variant</strong></td>
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<tr>
<td>Fish (recalculated pcs/m³)</td>
</tr>
<tr>
<td>Initial fish weight (g/fish)</td>
</tr>
<tr>
<td>Final fish weight (g/fish)</td>
</tr>
<tr>
<td>Individual weight gain (g)</td>
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<tr>
<td>SGR (Specific growth rate) (%/day)</td>
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<tr>
<td>FCR Feed conversion ratio (g/g)</td>
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<tr>
<td>Feeding level (% biomass)</td>
</tr>
<tr>
<td>Survival (%)</td>
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<tr>
<td>Days of growth (days)</td>
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The growth of bacteria from genus Nitrobacter, which in their turn are involved in the nitrification process. This possibly leads to an accumulation of significant quantity of nitrate in the water, that could be another possible reason of the higher concentration of nitrate in the experimental recirculation system, compared with the nitrate concentration in the control RAS.

Contrary to ammonia and nitrite, nitrate is relatively non-toxic to aquatic organisms. However, the research with octopus (Hyrayama, 1966), trout (Berka et al., 1981), shrimp (Muir et al., 1991) and eel (Kamstra et al., 1998) showed that a high concentration of nitrate can affect the growth of commercially grown hydrobionts.

The quantity of nitrate in our trial was higher in both RAS’s than recommended for the cultivation of common carp - 2 mg L\(^{-1}\) (Zajkow, 2006). The general reason for that was the lack of a denitrification process in both systems. Average values of total phosphorus and orthophosphate were respectively by 20% and 25.8% lower in experimental RAS compared with values of phosphorus compound measured in control RAS (P<0.05) (Table 3).

Our results are in confirmation to those received from Boyd and Queiroz (1997) who stated that aquatic plants in biofilter systems are able to remove 97% of the phosphorus compound in the water. The lower purification effect of a biofilter consisting of the two above cited macrophytic plants, concerning phosphorus and some of the other waste products voided in water from fish, could be results from the lower temperature during the experiment, which reduced the assimilation of soluble compounds from the plants. For example Landolt, (1986) stated that the optimal temperature for Lemna minor is 26°C.

Ferdoushi et al. (2008) who conducted an experiment with macrophytes as biofilter in a fish pond throughout the study period found out that phosphate phosphorus (PO\(_4-\)P) were 3.5 mg L\(^{-1}\) higher in the treatment without macrophytes. Landolt and Kandeler (1987) reported that Lemna sp. requires high phosphorus concentrations to grow in water.

The better water quality in the experimental RAS compared with those in the control recirculation system are logically expressed in better growth rate and better food assimilation in carps fingerlings from recirculation system which used floating macrophytes like biological filter. Timmons et al. (2002) stated that deterioration of the water in RAS caused negative effects on fish growth, increased fish stress and caused health problems in the fish.

**Conclusion**

The utilization of two macrophytes (Lemna and Wolffia) in their quality as a biofilter in RAS increased dissolved oxygen in water and decreased significantly the quantity of total dissolved solids, ammonia, nitrite, orthophosphate and total phosphorus in water.
Better water quality in a RAS using macrophytes (Lemna and Wolffia) as biofilter result in better growth and better assimilation of food in carp’s fingerlings compared to growth parameters and FCR of fingerlings from the control RAS whose cleaning section consists just from a mechanical filter.

References


Ferdoushi, Z., Haque, F., Khan, S. and Haque, M. 2008. The Effects of two Aquatic Floating Macrophytes (Lemna and Azolla) as Biofilters of Nitrogen and Phosphate in Fish Ponds. Turkish Journal of Fisheries and Aquatic Sciences, 8: 283-288.


