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Research Article

Nutrient bioremediation efficiency of bacterial biofilms and plant based biofilters in a recirculating common carp (*Cyprinus carpio* L.) culture system

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Abstract

The study aimed to evaluate and compare the nutrient cycling efficiencies of bacterial biofilms of a moving-bed filter and three aquatic plants (*Lemna minor*, *Hygroryza aristata* and *Phyllanthus fluitans*) as biological filters in recirculating aquaculture systems (RASs). The nutrient cycling capacities were tested in 12 independent RASs used for culturing common carp (*Cyprinus carpio* L.) in greenhouse conditions. The efficiencies were determined by comparing fish survival and growth performance, water quality parameters and removal rates of NH_4-N, NO_2-N, NO_3-N, PO_4^3-P and TP among biofilters. All biofilter types were efficient in maintaining water quality parameters, removing nutrients and providing an acceptable environment for fish growth and survival. However, the bacterial biofilm filter had the highest removal efficiencies of NH_4-N and NO_2-N than those of the other filters; while *H. aristata* and *L. minor* filters had higher removal efficiencies of NO_3-N and PO_4^3-P than those of *P. fluitans* and bacterial biofilm filters. The bacterial biofilm filter had a higher ability to deal with higher concentrations of NH_4-N and NO_2-N; whereas plant based filters were more effective in maintaining NO_3-N concentrations. Nutrient uptake capacities of selected plants differ and are strongly influenced by the growth rate of plants. The present study suggests that plant based filters in this filtration technique could be beneficial in removing nutrient overload in RASs, adding harvestable product and reducing the overall cost of RASs; whereas bacterial biofilm filter is a superior filter in maintaining ammonium and nitrite concentrations in RASs.

Keywords: Ammonia removal, Aquaculture, Macrophytes, Nitrogenous waste, Water quality

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Introduction

Production systems in the aquaculture sector differ in their capacity to increase production and reduce the negative environmental impacts while achieving food security. Recirculating aquaculture systems (RASs) have been developed in response to increasingly strict environmental regulations and limited access to land and water (Martins *et al.*, 2010). However, RASs are usually characterized by high stocking densities for marketed and higher-price species with large quantities of feed and low water exchange rates to cover the high investment cost (Timmons *et al.*, 2002). Approximately, 20–50% nitrogen and 15–65% phosphorus supplied through the feed is converted into the fish biomass when harvest, while large amounts of nutrients in the form of uneaten feed and excretory products are discharged into the water, leading to deterioration in water quality (Schneider *et al.*, 2005). The accumulation of nutrients can be a major concern in RASs if not properly managed. Aquaculturists often rely on bacterial biofilm filters to control nutrient concentrations in RASs. However, in biological filters, natural colonization of nitrifying bacteria works well for initiating a biological filter but can take about 4–8 weeks to establish a healthy and effective population of bacteria (Timmons *et al.*, 2002). The population of nitrifying bacteria can be affected by many factors that cause inhibition of the nitrification process, such as temperature (Malone and Pfeiffer, 2006), pH, ammonia concentration (Groeneweg *et al.*, 1994), salinity (Tseng and Wu, 2004), dissolved oxygen (Hao and Huang, 1996) and organic loading (Ling and Chen, 2005). Nitrifying bacteria also gradually acidify the system (van Rijn, 1996).

In order to deal with the problems associated with bacterial biofilm filters in RASs and nutrients overloading in the environment; integrated recirculating aquaculture systems (IRASs) are getting popularity. IRASs refer to integrated systems where additional separated units are integrated into a RAS (Chien and Tsai, 1985). These separated units that have the ability to convert nutrients could be aquatic plants (Redding *et al.*, 1997; Jo *et al.*, 2002). The utilization of aquatic plants as a biofilter in RASs can have positive impacts on water quality (Jo *et al.*, 2002) and add significant income; for example as food for humans, animal feed, fibre and ornamental plants (Wersal and Madsen, 2012). The plants in this study were chosen because of their potential to recover nutrients into the useful products as well as they have rapid growth and simplicity of harvest. *Lemna minor* and *Phyllanthus fluitans* use as animal feed and ornamental plants. *Hygroryza aristata* also uses as cattle feed and as a diuretic, emollient, galactagogue, strangury, diarrhea, fatigue and general debility (Malik *et al.*, 2014).

Although numerous studies have been proven the usefulness of bacterial biofilm filtration (van Rijn, 1996; Timmons *et al.*, 2002; Ling and Chen,

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2005; Malone and Pfeiffer, 2006; Guerdat *et al.*, 2010) and aquatic plants (Redding *et al.*, 1997; Jo *et al.*, 2002; Ardiansyah and Fotedar, 2016; Nakphet *et al.*, 2017) to remove nutrients in RASs, none of them have evaluated and directly compared the efficacy of such systems under greenhouse laboratory conditions and the comparison between these systems remains unclear. Therefore, the aim of the present study was to evaluate and compare the nutrients removal efficacy of bacterial biofilm in a moving-bed filter with three aquatic plant species (*L. minor, H. aristata* and *P. fluitans*) as biological filters in RASs culturing *Cyprinus carpio* L. under greenhouse conditions.

Materials and methods

Collection of fish and plants

Common carp (*Cyprinus carpio* L.) with an average weight of 45.81±0.06 g were collected from a local farm and transported to Georgikon Aquatic Research Laboratory (GARL), Keszthely, Hungary. The fish were held in 12 plastic tanks for one week to adapt them to the laboratory conditions. The fish were fed with a commercially formulated feed, Nutra MP T (50% protein, 20% fat, 1% fibre, 8.5% ash, 0.5 Na, 1.8% Ca and 1.4% P) (Skretting a Nutreco Co., Mozzecane, Italy) until the start of the experiment.

Lemna minor, *Hygroryza aristata* and *Phyllanthus fluitans* were obtained from Interaqua-Flora Ltd., Hungary, cleaned and put in 9 plastic tanks for two weeks as an acclimatization period.

Biological filters

A moving-bed biological filter was designed and established as one of the experimental treatments. Media with established biofilms were obtained from an operating recirculating system in GARL. Plastic media in the form of "bio-balls" with a specific surface area of 400 m^2 m^{-3} were used to place in moving-bed filter tanks. In order to determine the appropriate design of biofilters, the calculation of the size of the moving-bed filter was based on the calculations published by Timmons *et al.* (2002). Hence, 0.003 m^3 of plastic media was placed in each moving-bed filter tank.

L. minor, H. aristata and *P. fluitans* were also used as biofilters in this study. The biomass of the plants to be used for the trial was estimated by their ammonium uptake rates, which was estimated during a preliminary experiment. In that experiment, three beakers (1 L) were filled with water and ammonium nitrate (NH_4NO_3) to give an initial ammonium nitrogen concentration (NH_4-N) of 0.32 mg L^{-1}. Approximately 20.7 g (wet weight) of each plant was added to each beaker and the ammonium uptake rate was calculated based on the reduction in NH_4-N after one hour and 24 hours. The NH_4-N uptake rates of the plants were approximately 5.80 mg NH_4-N kg^{-1} h^{-1}, 3.14 mg NH_4-N kg^{-1} h^{-1} and 1.45 mg NH_4-N kg^{-1} h^{-1} for *L. minor*, *P. fluitans* and *H. aristata*, respectively. Therefore, biomasses of 162 g of *L. minor*, 299 g of *P. fluitans* and 647 g of *H. aristata* were stocked into the

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biofilter tanks which had the same surface area of 0.41 m^2.

Design of recirculating systems

The trial comprised 12 independent experimental units; each unit consisted of three tanks: a fish tank, a waste-collection tank and a biological filter tank. The fish and waste-collection tanks were put on the floor, while the biological filters were installed on a wooden stand to elevate it higher than the fish tanks. Water from the bottom of the fish tank was drained through a PVC pipe to the waste-collection tank, and water from the waste-collection tank was pumped through a plastic tube to the biological filter tank by a submerged pump. Water from the biological filter tank was circulated back to the fish tank by gravity (Fig. 1).

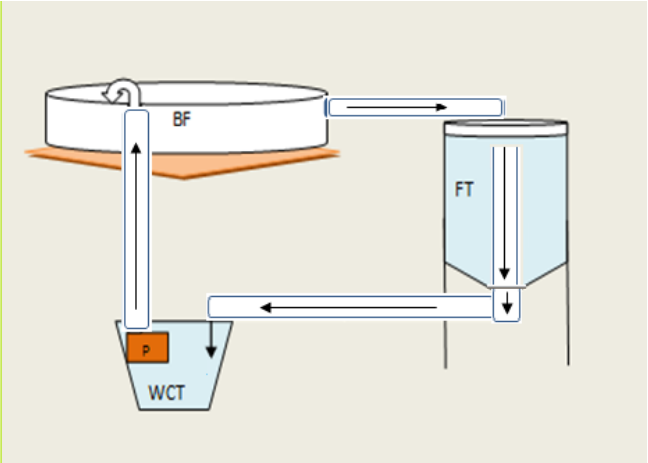

Figure 1: Diagram of the experimental recirculating unit (the arrows show the direction of water flow), (FT): Fish tank, (WCT): Waste collection tank, (BF): Biological filter, (P): Pump.

The water flow rate was set at 3 L min^{-1}. The water volumes in the fish and waste-collection tanks were kept at 55 and 36 litres, respectively, while the water volume in each biofilter was kept at 60 litres. The tank of the moving-bed filter was provided with two air stones and covered with a black plastic cover to prevent algae from growing. The fish tank was also aerated with two air stones to supply dissolved oxygen for fish. A polyethylene mesh (1.0–1.5 cm in diameter) was put above the fish tank to prevent fish from jumping outside.

Experimental setup and rearing conditions

The trial was conducted for five weeks in an insulated greenhouse, in which the environmental conditions were recorded but not completely controlled, and the only natural light was used throughout the study. The 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 filtration, *L. minor* filtration, *P. fluitans* filtration and *H. aristata* filtration). The fish were initially stocked at 7.5 kg m^{-3}, and the

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total biomass was approximately 412 g $tank^{-1}$. All fish were fed by hands twice a day at 09:00 and 16:00 hours with a commercial diet (pellet size 2 mm). The feeding rate of fish was 2.5% of body weight per day and the amount of feed after two weeks was adjusted according to the actual weight of the fish until the end of the trial. 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. A weekly amount of 40 litres of water was siphoned out of the waste collection tanks and replaced with new water, amounting to 27% of the water system. Also, new water was added to compensate for the water lost by evaporation which was 2–3% of the water system per week.

Sample collection and analysis

Survival and growth rates of the fish were recorded after the first two weeks and at the end of the experiment for each tank. Dissolved oxygen (DO), temperature and pH of water in the fish tanks were measured once a day before feeding commenced. Temperature and DO were measured by using the OxyGuard Handy Polaris meter (OxyGuard International A/S, Denmark), while pH was measured by using the Lovibond Senso Direct pH 110 meter (Tintometer Group, Germany). Ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), total nitrogen (TN), orthophosphate (PO_4^3-P) and total phosphorus (TP) were measured weekly in the fish tanks and in the influent and effluent waters of each biofilter to obtain the removal rates. Water samples were analysed under laboratory conditions, using the MSZ EN ISO 11732:2005 method to determine NH_4-N levels and the MSZ EN ISO 13395:1999 method for NO_2-N and NO_3-N, while TN was determined by using the MSZ EN ISO 11905-1:2000 method. PO_4^3-P and TP levels were determined using the MSZ EN ISO 15681-1:2005 C Annex and the MSZ EN 1189:1998 methods, respectively. Water quality parameters were measured according to the European standard methods and the recommendations of the International Organization for Standardization.

Data calculation and statistics

Fish biomass, specific growth rate (SGR) and survival rate were calculated using the following formulas:

Fish biomass (g) = Sum of individual fish weights (g);

$$SGR\ (\%\ day) = 100 \times (\ln W_t - \ln W_0)/t \text{ and survival rate } (\%) = 100 \times (n_t / n_0),$$

Where W_t and W_0 are the weight of fish at the testing time and at the start of the trial respectively and (t) is the number of rearing days. The n_t is the number of fish at the testing time and n_0 is the number of fish at the start of the trial.

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The feed conversion ratio (FCR) was calculated as follows:

FCR = WF (g) / WG (g),

where WF is the weight of feed given to the fish (g) and WG is the weight gain (g).

Nutrient removal rates were used to determine the nutrient removal cycle of biofilters. A nutrient removal rate is defined as the percentage of a particular nutrient that is reduced after passing through the biofilter (Tseng and Wu, 2004). The nutrient removal rate (NR %) was calculated using the following equation:

$NR\ (\%) = [(N_I - N_E)/ N_I] \times 100$,

Where N_I and N_E are the amounts of a particular nutrient in influent and effluent waters of biofilter, respectively. The removal rates of NH_4-N, NO_2-N, NO_3-N, PO_4^3-P and TP were calculated using the same equation.

The plants were harvested from the biofilter tanks at the end of the experiment and the weight of the plants was recorded. The specific growth rates of plants (SGR) were calculated as follows:

$SGR\ of\ plant = 100 \times (\ln B_f - \ln B_i)/t$;

Where B_f and B_i are the final biomass of the plant and the initial stocked biomass respectively, while (t) is the number of rearing days. All statistical analyses were performed using the SPSS version 22.0 for Windows package. All of 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 in parameters amongst treatments. Significant ANOVAs were followed by Duncan's multiple range tests to recognize specific differences amongst treatments. The 5% level of probability was considered to be the significance level.

Results

Water quality parameters in fish tanks

There were no significant differences (p>0.05) in the overall means of DO, pH and temperature amongst treatments . Dissolved oxygen in all treatments showed a slight decline from 6.99 to 6.5 mg L^{-1}, whereas the mean temperature increased as the trial progressed; from 21°C to 30°C. The mean pH in all treatments fluctuated between 7.0 and 7.9. The overall means of NH_4-N and NH_3 in fish tanks of systems using bacterial biofilm filtration and *H. aristata* were significantly lower (p<0.05) than those stocked with *L. minor* and *P. fluitans* (Table 1).

The lowest mean was recorded in fish tanks of systems using bacterial biofilm throughout the study (Fig. 2 A and D). However, no significant differences (p>0.05) were found between systems using bacterial biofilm filtration and those stocked with *H. aristata*. The mean NO_2-N in fish tanks of systems stocked with *P. fluitans* was significantly higher (p<0.05) than that of the other three treatments. The mean NO_2-N in fish tanks of systems using bacterial biofilm filtration remained below 0.44±0.11 mg L^{-1} over the entire period of the trial (Fig. 2 B). The mean NO_3-N and TN in fish tanks of systems using bacterial biofilm filtration were significantly higher (p<0.05) than in the other three treatments (Table 1). The mean NO_3-N and TN concentrations in all treatments increased

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as the trial progressed (Fig. 2 C and F). No treatments significantly affected (p>0.05) the PO_4^3-P and TP means (Table 1). In all treatments, the PO_4^3-P concentrations increased gradually while the TP concentrations fluctuated over time (Fig. 2 E and G).

Table 1: Overall mean water quality parameters of tanks used for culturing *Cyprinus carpio* in recirculating aquaculture systems using different biological filters.

Parameter	*Lemna minor*	*Hygroryza aristata*	*Phyllanthus fluitans*	Bacterial biofilm
NH_4-N (mg L^{-1})	1.50±0.33[a]	0.72±0.16 [b]	1.71±0.12 [a]	0.32±0.01 [b]
NH_3 (mg L^{-1})	0.0091±0.002[a]	0.0031±0.001[b]	0.0093±0.002[a]	0.0019±0.0004[b]
NO_2-N (mg L^{-1})	1.16±0.37[b]	1.07±0.19[b]	2.51±0.33[a]	0.39±0.03[b]
NO_3-N (mg L^{-1})	8.68±1.43[b]	8.96±1.76[b]	8.53±0.88[b]	20.11±2.34[a]
TN (mg L^{-1})	16.01±1.46[b]	18.14±2.19[b]	18.08±1.64[b]	28.55±3.07[a]
PO_4-P (mg L^{-1})	0.059±0.004[a]	0.062±0.003[a]	0.057±0.003[a]	0.059±0.004[a]
TP (mg L^{-1})	0.219±0.023[a]	0.243±0.027[a]	0.186±0.021[a]	0.172±0.019[a]
DO (mg L^{-1})	6.71±0.04[a]	6.72±0.05[a]	6.69±0.04[a]	6.64±0.03[a]
pH	7.43±0.07[a]	7.41±0.06[a]	7.44±0.05[a]	7.52±0.07[a]
Temperature (°C)	26.08±0.93[a]	26.37±0.96[a]	26.34±0.97[a]	26.67±0.99[a]

Values (means±SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; p<0.05); data are the means of three replicates.

Removal rates of biological filters

The mean removal rates of NH_4-N by bacterial biofilm filters were significantly higher (p<0.05) than the values obtained with *L. minor* and *P. fluitans* filters. However, no significant differences (p>0.05) were found between systems using bacterial biofilm filtration and those stocked with *H. aristata*. The bacterial biofilm filter had the highest removal rate of NO_2-N and lowest removal rate of NO_3-N, which significantly differed (p<0.05) from those of the other filters. However, no significant differences (p>0.05) in the mean removal rates of PO_4^3-P and TP were found between any of the treatments (Table 2).

The mean PO_4^3-P removal rates by *H. aristata* and *P. fluitans* increased in the early stage of the trial and decreased afterwards, while the mean removal rates of bacterial biofilm and *L. minor* filters increased over time (Fig. 3 D). The mean removal rate of TP increased gradually in all filters (Fig. 3 E).

Growth and survival rates

After five weeks, the growth rate of common carp increased in all treatments. The mean biomass gain, SGR and weight gain of fish reared in systems stocked with *P. fluitans* were significantly lowest (p<0.05) than the other three treatments. However, the mean biomass gain, SGR and fish weight gain did not differ significantly (p>0.05) between fish reared in systems stocked with *L. minor*, *H. aristata* and bacterial biofilm filtrations. The highest mean biomass gain, SGR and fish weight gain were achieved for fish reared in systems using bacterial biofilm filtration. The mean FCR of fish reared in systems stocked with *P. fluitans* was significantly highest (p<0.05) than the other three treatments. In all treatments, the survival rate of

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fish was the same (p>0.05), and 100% survival rate was achieved in all systems. The mean SGR of plants in systems stocked with *H. aristata* was significantly highest (p<0.05) than the other treatments (Table 3).

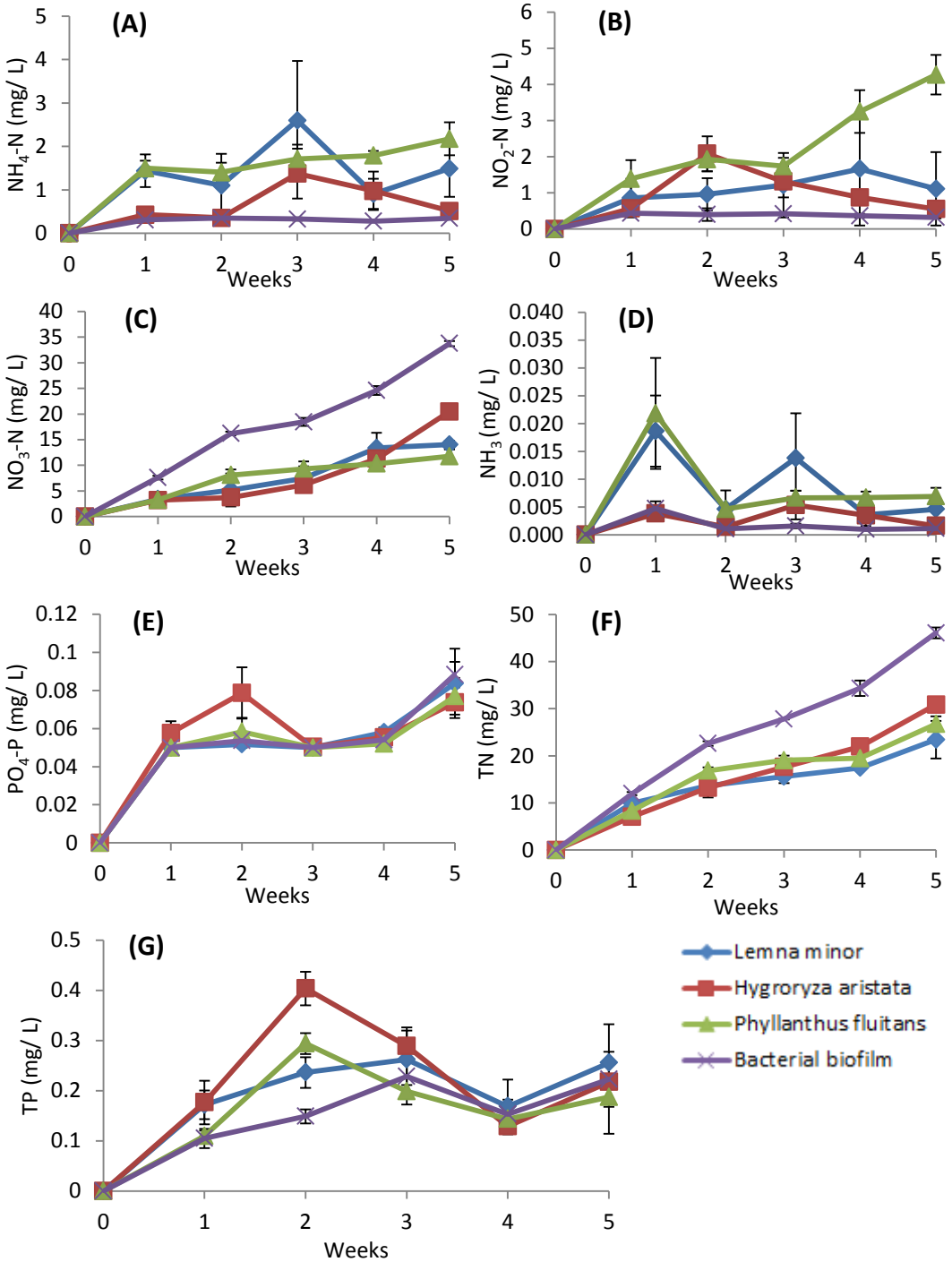

Figure 2: Weekly mean concentrations of (A) ammonium nitrogen, (B) nitrite nitrogen, (C) nitrate nitrogen, (D) unionized ammonia, (E) orthophosphate, (F) total nitrogen, and (G) total phosphorus in tanks of *Cyprinus carpio* reared in recirculating aquaculture systems during 5-week trial (error bars indicate the standard error).

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Table 2: Overall mean removal rates of four biological filters used in recirculating *Cyprinus carpio* systems during 5 weeks trial.

Parameter	*Lemna minor*	*Hygroryza aristata*	*Phyllanthus fluitans*	Bacterial biofilm
NH_4-N (%)	10.96±2.80^{bc}	17.96±3.20^{ab}	6.67±0.78^{c}	25.00±2.51^{a}
NO_2-N (%)	9.25±1.70^{bc}	11.30±3.51^{b}	2.14±0.62^{c}	25.95±4.29^{a}
NO_3-N (%)	7.53±1.22^{ab}	8.15±0.90^{a}	5.07±1.02^{b}	2.27±0.31^{c}
PO_4-P (%)	4.05±1.16^{a}	4.97±0.83^{a}	2.51±0.69^{a}	2.94±0.91^{a}
TP (%)	19.04±4.64^{a}	11.50±3.42^{a}	17.73±3.62^{a}	14.49±4.36^{a}

Values (means±SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; p<0.05); data are the means of three replicates.

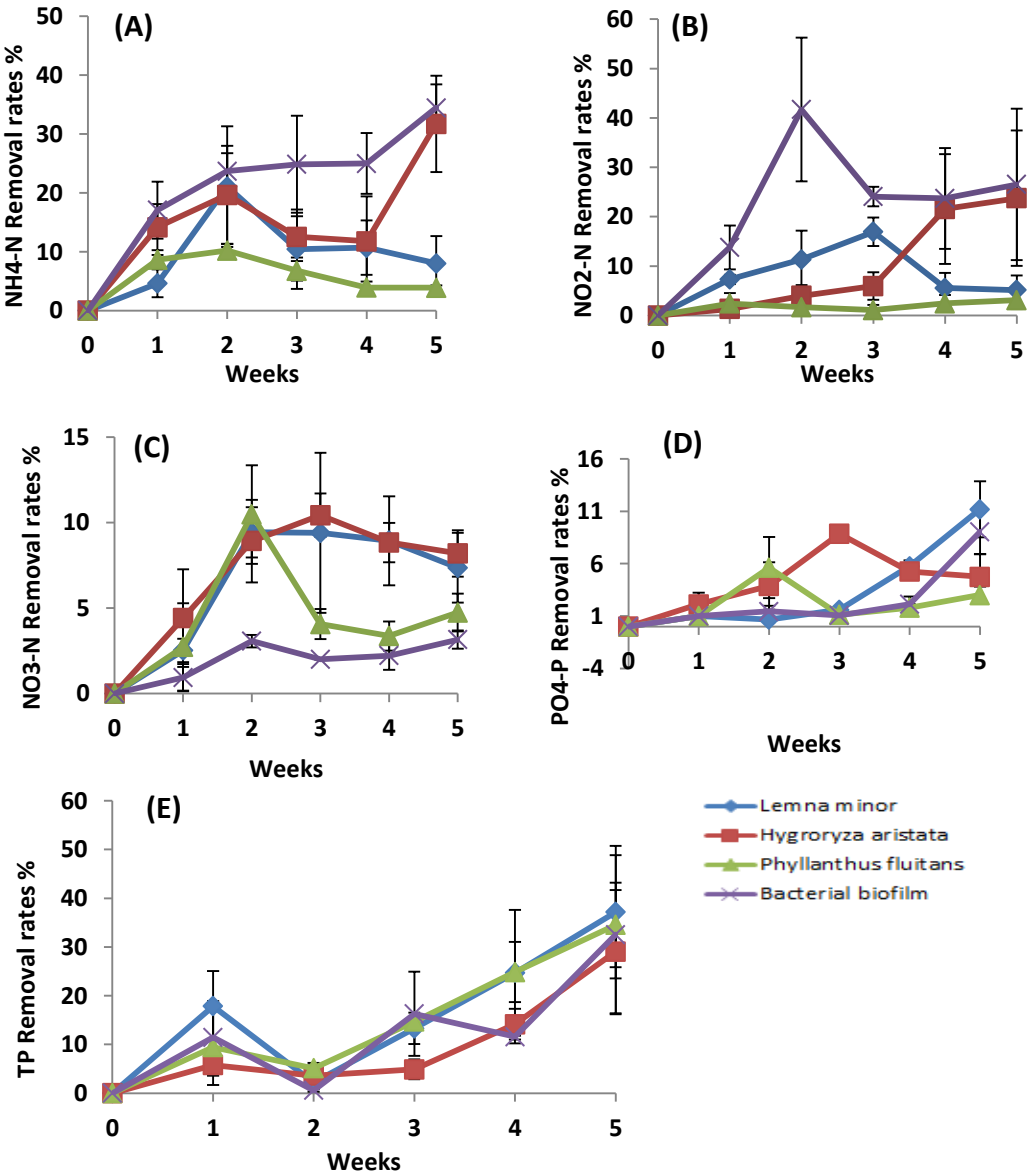

Figure 3: Weekly mean removal rates by four biological filters for (A) ammonium nitrogen, (B) nitrite nitrogen, (C) nitrate nitrogen, (D) orthophosphate, and (E) total phosphorus in recirculating *Cyprinus carpio* systems during 5-week trial (error bars indicate the standard error).

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Table 3: Effects of bio-filtration types on growth and survival rates of *Cyprinus carpio* reared in recirculating aquaculture systems.

	Lemna minor	*Hygroryza aristata*	*Phyllanthus fluitans*	Bacterial biofilm
Stocking fish				
Fish biomass (g tank^{-1})	412.667±1.28^{a}	412.133±0.81^{a}	411.533±1.34^{a}	412.833±1.62^{a}
Stocking density (kg m^{-3})	7.503±0.023^{a}	7.493±0.014^{a}	7.482±0.024^{a}	7.506±0.029^{a}
Number of fish	9	9	9	9
Mean fish weight (g fish^{-1})	45.85±0.14^{a}	45.79±0.09^{a}	45.73±0.15^{a}	45.87±0.18^{a}
Harvesting fish				
Final fish biomass (g tank^{-1})	731.23±4.05^{a}	740.37±7.59^{a}	690.37±10.97^{b}	753.80±11.54^{a}
Final stocking density(kg m^{-3})	13.295±0.07^{a}	13.461±0.14^{a}	12.552±0.20^{b}	13.705±0.21^{a}
Number of surviving fish	9	9	9	9
Final fish weight (g fish^{-1})	81.25±0.45^{a}	82.26±0.84^{a}	76.71±1.29^{b}	83.76±1.28^{a}
Fish weight gain (g fish^{-1})	35.40±0.52^{a}	36.47±0.92^{a}	30.98±1.10^{b}	37.89±1.32^{a}
Biomass gain (g tank^{-1})	318.57±4.72^{a}	328.23±8.30^{a}	278.83±9.90^{b}	340.96±11.90^{a}
SGR (% d^{-1})	1.61±0.23^{a}	1.66±0.33^{a}	1.46±0.33^{b}	1.70±0.57^{a}
Daily growth rate (g fish^{-1})	1.00±0.00^{a}	1.07±0.032^{a}	0.87±0.033^{b}	1.07±0.031^{a}
Feed consumption (g fish^{-1} d^{-1})	1.308±0.017^{a}	1.314±0.009^{a}	1.335±0.009^{a}	1.328±0.012^{a}
Feed conversion ratio	1.29±0.012^{b}	1.26±0.043^{b}	1.49±0.084^{a}	1.23±0.032^{b}
Survival (%)	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}
Stocking plant biomass (g biofilter tank^{-1})	162±0.00^{c}	647±0.00^{a}	299±0.00^{b}	0.0±0.00^{d}
Final plant biomass (g biofilter tank^{-1})	894.80±10.07^{b}	5429.90±117.32^{a}	466.76±15.52^{c}	0.0±0.00^{d}
Plant biomass gain (g biofilter tank^{-1})	732.8±10.07^{b}	4782.90±117.32^{a}	167.76±15.52^{c}	0.0±0.00b^{d}
Specific growth rate of plant (SGR % d^{-1})	4.86±0.033^{b}	6.10±0.57^{a}	1.26±0.08^{c}	0.0±0.00^{d}

Values (means±SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; p<0.05); data are the means of three replicates

Discussion

In RASs, water quality parameters should be maintained within recommended limits for optimum fish growth and survival. The results of the present study indicated that all types of biofilters provided acceptable water quality parameters and good conditions for common carp growth and survival in RASs. The temperature, pH and DO concentrations in the fish tanks of all systems remained within the tolerance range for common carp growth and survival (Horváth *et al.*, 2002). The

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maximum concentrations of NH_3, NO_2-N, and NO_3-N in all systems were lower than the lethal levels reported by various authors for common carp (Solbé *et al.*, 1985; Tarazona *et al.*, 1987; Iqbal *et al.*, 2004; Biswas *et al.*, 2006; Kroupova *et al.*, 2010; Kim *et al.*, 2013) (Table 4).

Table 4: Comparative description of the maximum values of some water quality parameters with the lethal levels of common carp.

The maximum values / system (mg L^{-1})		Concentration levels (mg L^{-1})			Reference
		No Effect	Lowest Effect	Lethal level	
NH_3	0.042 (*P. fluitans*)			1.3	(Tarazona *et al.*, 1987)
		0.0286	0.034	0.043	(Biswas *et al.*, 2006)
NO_2-N	5.29 (*P. fluitans*)			16	(Solbé *et al.*, 1985)
		7	28	88	(Kroupova *et al.*, 2010)
NO_3-N	34.7 (*bacterial biofilm*)			865	(Iqbal *et al.*, 2004)
Phosphate		No toxicity reported			(Kim *et al.*, 2013)

Although the water quality parameters were maintained at the levels recommended for fish in all treatments, the concentrations of NH_4-N, NO_2-N, and NO_3-N in the fish tanks were different among the treatments (Table 1). These variations between treatments in the ability to maintain the concentrations of water quality could be attributed to differences in mechanisms for the uptake of nutrients, specific environmental requirements and biomass (Redding *et al.*, 1997; Cahill *et al.*, 2010). Redding *et al.* (1997) concluded that purification in culture systems could be processed by different mechanisms such as nitrification, denitrification, microbial assimilation and sedimentation as well as plant uptake and the growth form of plants can play an important role in the design criterion. Our results also indicated that the bacterial biofilm filter was a powerful filter to maintain NH_4-N concentrations within the lowest level and this was due to the highest removal rate of NH_4-N which tended to increase as the trial progressed. The increasing trend was probably due to the increase in the number of nitrifying bacteria in response to the rise in ambient ammonia concentrations as a consequence of increasing fish biomass and the amount of feed given to fish. Previous studies have also shown the removal efficiency of biofilm filter improved with increasing ambient ammonia concentrations (Zhu and Chen, 1999; Brazil, 2006). In contrast, the higher concentrations of NH_4-N in the *P. fluitans* and *L. minor* systems were possibly due to the lower removal rate of NH_4-N which tended to decrease over time. The decreasing trend could be attributed to differences in the growth performance of plants, since different plant species have different growth performance in the current study. Vymazal (2007) reported that the potential rate of nutrients uptake by

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plants is influenced by their growth rate and the concentration of nutrients in the plant tissue. Another explanation for decreasing trend of NH_4-N removal rate in plant based systems may be related to the biological factors, such as the age of the plant, its nutritional history and interplant variability (Ahn *et al.*, 1998). The highest removal rate of NO_2-N by bacterial biofilm filters corresponded to the NH_4-N removal rate and the nitrification process. However, the NO_2-N removal rate remained relatively constant in the later stages of the trial; suggesting that the nitrite-oxidizing bacteria were probably limited in responding to the increase in nitrite concentrations in the systems (Guerdat *et al.*, 2010), since the ammonia-oxidizing bacteria have a higher kinetic reaction rate than the nitrite-oxidizing bacteria in the nitrification process (Timmons *et al.*, 2002). In contrast, the lower NO_2-N and higher NO_3-N removal rates by plant based systems could be attributed to the fact that ammonium and nitrate are directly taken up by plants as a nitrogen source (Fang *et al.*, 2007), and it is most likely that nitrifying bacteria were responsible for the NO_2-N removal rate in the plant based systems (Wei *et al.*, 2011). The reduction in the nitrate concentrations has been reported previously for *Lemna* sp. by Ferdoushi *et al.* (2008), who found that the introduction of *Lemna* sp. in a fish pond efficiently removed nitrate and improved water quality. Tan *et al.* (2014) also found that *Hygroryza aristata* removed the excessive amount of nitrogen under the real operational conditions in a canal, while Han *et al.* (2013) reported that *Hygroryza aristata* removed the concentration of nitrate from the pond water. In the current study, different trends in NO_3-N removal rate between selected plants could be related to differences in the nutrient utilization capacity and growth performance of plants, since different plant species have different nutrient utilization capacity and growth characteristics. Hu *et al.* (2015) investigated the effect of plant species on nitrogen recovery and concluded that plant species had a significant influence on nitrogen transformations, and the higher plant biomass translates to higher plant uptake rate resulting in a higher nitrate removal efficiency. Different growth performance between selected plants species in the current study may be attributed to the continuous water flow and/or specific environmental requirements for each plant (Redding *et al.*, 1997; Van der Steen *et al.*, 1998). Crowding of plants and the limited surface area also may slow down the growth of plants (Driever *et al.*, 2005), since no harvesting regime was applied in the current study to give the plants more space for extending and increased their growth.

In the present study, the artificial feed was the only source of phosphorus and a large part of it was removed by removing uneaten food and fish faeces; which resulted in a large portion of soluble phosphorus and suspended particles in the water column. The removal of phosphate in culture systems

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can be processed through the plant uptake and the mechanism of sedimentation (Redding *et al.*, 1997). In fact, the biofilter tank bottom had little sediments which can play an important role in removing phosphate. Midlen and Redding (1998) reported that over half of the phosphorus inputs are bound in the soils of the pond bottom in a relative insoluble form. Although the differences in the removal rate of PO_4^3-P between treatments were not significant; the plant systems exhibited different trends; which could be related to differences in the concentration of nutrients in plant tissue (Vymazal, 2007).

Previous studies indicated that the growth and survival of fish are influenced by water quality parameters in the culture system (Colt, 2006; Ridha and Cruz, 2001; Timmons *et al.*, 2002; Ardiansyah and Fotedar, 2016). Different growth performance of fish between treatments in the current study was probably due to differences in water quality during the study period. The elevated but acceptable levels of NH_4-N, NH_3 and NO_2-N may be one of the reasons for the lowest growth performance and highest FCR of fish reared in the system stocked with *P. fluitans*. The SGRs of common carp in the present study ranging from 1.46 to 1.70% d^{-1} were higher than the 1.03-1.06% d^{-1} reported by Karakatsouli *et al.* (2010) for 52 g mirror common carp and the 0.84% d^{-1} obtained by Ridha and Cruz (2001) for 62 g Nile tilapia (*Oreochromis niloticus* L.). However, the SGRs of fish were lower than those (1.6-2.14% d^{-1}) achieved by Ahmed *et al.* (2013) for common carp reared in RAS on different diets. The lower SGR may be related to the lower feeding rate and feeding frequency used in this study (2.5% body weight twice a day) compared to those used by Ahmed *et al.* (2013) (3% body weight, four times a day). In the present study, no fish mortality was found in any of the treatments. Fish were probably unaffected because a lower feeding rate was used in this study. Huisman (1976) suggested 3% of the body weight per day as a suitable amount of feed for 42.1 g common carp. In addition, the stocking density of fish was probably below the carrying capacity of these systems and did not reach the threshold at which survival rates would be affected.

The economic assessment is required to identify the benefits of the systems designed in this study. From an economic point of view, our results indicated that it would be more cost-effective to use the plant based biofilters in RAS. Data in Table 5 presents the profitability of applying 1 m^2 of different plant based biofilters in RASs. It is clear that the profitability of plant based systems was higher compared to the bacterial biofilm system. However; the profit depends on the growth rate of plants, which may be influenced by environmental conditions, surface area and nutrients concentrations. The production and nutrient uptake capacity of the plant

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based systems could be increased by providing specific environmental requirements for the plants, increasing surface area and/or applying an appropriate harvesting regime.

Table 5: Economic analysis of applying different biofilters in recirculating *Cyprinus carpio* systems for 5 weeks trial (1 m^2 area of biofilters and 1 m^3 water volume of fish tank).

Parameter	*Lemna minor*	*Hygroryza aristata*	*Phyllanthus fluitans*	Bacterial biofilm
Fish biomass gain (g $tank^{-1}$)	318.57	328.23	278.83	340.65
Fish biomass gain (kg m^{-3})	5.792	5.968	5.070	6.194
Sale of fish (Euro) (Farm gate price=1.91 Euro kg^{-1})	11.06	11.40	9.68	11.83
Plant biomass gain (g biofilter $tank^{-1}$)	732.8	4782.9	167.8	0
Plant biomass gain (g/ m^2)	1787.32	11665.61	409.19	0.00
Plant sales of 1 m^2 (Euro)	44.68 40g = 1Euro	115.96 100.6g = 1 Euro	6.08 67.3 g = 1 Euro	0.00
Profitability of the system (Euro) (Sales of fish +plants)	55.75	127.36	15.76	11.83

The price of plants was according to the supplier company (Interaqua-Flora Ltd., Hungary).
The price of fish was according to the Hungarian Research Institute of Agricultural Economics.

The results presented in this paper demonstrate that the use of plant based biofilters under the conditions used are effective in maintaining water quality, removing nutrients and providing good conditions for fish growth and survival in RASs. However, bacterial biofilm in the moving-bed filter was the strongest filter to deal with the higher concentrations of NH_4-N and NO_2-N; and had generally the highest removal rates of NH_4-N and NO_2-N; whereas plant based filters had higher NO_3-N removal rate. The nutrients uptake capacities of plant based systems were different and are strongly influenced by the growth rate of plants, which is affected by environmental conditions, surface area and nutrient concentration. The use of plant based biofilters in this filtration technique can be beneficial in decreasing the high investment and operation costs associated with RASs; while from a technical point of view, the bacterial biofilm filter is the strongest biofilter to be used. Regardless of the suitability of bacterial biofilm and plant based filters in RASs, several factors must be considered when choosing appropriate biological filters, such as space, cost and benefit analyses, system location, climatic conditions and discharge regulations.

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