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RESEARCH ARTICLE

Application of alginate-immobilized microalgae beads as biosorbent for removal of total ammonia and phosphorus from water of African cichlid (*Labidochromis lividus*) recirculating aquaculture system

Mehrdad Sarkheil[1] · Maryam Ameri[2] · Omid Safari[1]

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Abstract

Immobilized microalgae are a promising approach to incorporate microalgae in recirculating aquaculture system (RAS) for water purification. In the present study, two types of biosorbents including sodium alginate–immobilized *Scenedesmus* spp. and *Chlorella* spp. beads (algal beads) and sodium alginate beads without microalgae (alginate beads) were prepared. In the first experiment (static test), the potential of two biosorbents to remove different concentrations of total ammonia nitrogen (TAN) and total phosphorus (TP) from water was investigated. In the second experiment, two prepared biosorbents were used as biofilter in a RAS for rearing African cichlid (*Labidochromis lividus*) for 30 days. The survival rate and growth indices of fingerling fish and removal efficiency of two biosorbents for TAN, NO_3^{-}–N, and TP were determined. The results of static test showed that the removal efficiency and uptake capacity of the two biosorbents for TAN and TP increased during 30 days of the experiment, and these values for the algal beads were higher than the alginate beads. The TAN removal efficiency of the two biosorbents increased with increasing TAN concentration from 0.5 to 5 mg L^{-1}. The application of algal beads in the RAS improved the survival rate, final weight, final length, weight gain, and daily growth index (DGI%) indices of fish compared to those cultured in the RAS containing the alginate beads and the control ($P<0.05$). The algal and alginate beads decreased the TAN concentration by 42.85% and 28.57% compared to the control after 30 days of cultivation period, respectively. The uptake of nitrate was not observed by the two biosorbents during cultivation period. The TP removal efficiency of algal beads reached 44.90% after 30 days. The findings of this study indicated that the sodium alginate–immobilized microalgae could be considered as a suitable biofilter to be incorporated into a RAS to improve water quality and consequently enhance the growth and health of fish.

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Highlights • Synthesis of two biosorbents of sodium alginate–immobilized *Scenedesmus* spp. and *Chlorella* spp. beads and sodium alginate beads for removal of total ammonia nitrogen (TAN) and total phosphorus (TP) from water.
• Increasing the removal efficiency and uptake capacity of the two biosorbents for TAN with increasing TAN concentration from 0.5 to 5 mg L^{-1} and time of experiment.
• TAN and TP removal efficiency and uptake capacity of the alginate-immobilized microalgae beads were higher than those of the alginate beads.
• Application of two biosorbents as biofilter in a recirculating aquaculture system (RAS) to culture African cichlid (*Labidochromis lividus*) fish for 30 days.
• Improvement of survival rate and growth performance of African cichlid (*Labidochromis lividus*) fingerlings cultured in the RAS with the alginate-immobilized microalgae beads.

✉ Mehrdad Sarkheil
sarkheil@um.ac.ir; mehrdadsarkheil@gmail.com

[1] Department of Fisheries, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad P.B. 91773-1363, Iran

[2] Industrial Microbial Biotechnology Department, Academic Center for Education, Culture and Research, Mashhad, Iran

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Introduction

The global demand for animal protein sources is increasing rapidly (Boer et al. 2006). Fish and seafood products meet part of demands for animal protein. Aquaculture has been expanded in recent decades to meet human needs for finfish and shellfish around the world (Sapkota et al. 2008; Little et al. 2016). The majority of the world's aquaculture systems use intensive cultivation methods coincident with the increased demands for aquaculture products (Sapkota et al. 2008). The application of these methods can increase the production of nutrients such as nitrogen and phosphorus compounds (Ferdoushi et al. 2008), which adversely affect water quality and ultimately the growth and health of aquatic animals. Discharge of nutrient-rich effluent from aquaculture farms into aquatic environments may also resulted in eutrophication and consequent algal bloom (Selvarani et al. 2015). Therefore, elimination or reduction of the concentration of these nutrients from wastewater is a vital factor for usage of intensive and super-intensive aquaculture systems such as recirculating aquaculture system (RAS).

Ammonia is the main form of nitrogen excreted by aquatic animals and can be found in water resources in two forms of molecular ammonia (NH_3) and ammonium ions (NH_4) (Hagopian and Riley 1998; Rezagama et al. 2017). The proportion of each form depends on pH and temperature, so that higher pH and temperature values lead to the formation of more molecular ammonia (Rezagama et al. 2017; Timmons and Ebling 2010). The molecular ammonia penetrates more than ammonium ions into biological membranes, resulting in more toxicity to aquatic animals (Chen et al. 2006; Rezagama et al. 2017). The removal of ammonia from water is a challenging issue in the RAS and predominantly occurs through nitrification process in biofilm systems. In the RAS, the management and maintenance of nitrifying bacteria function to oxidize ammonia/ammonium to nitrite and nitrate is one of the most challenging and time-consuming steps (Foore 2016; Pulkkinen et al. 2018). The potential use of aquatic plants (Nizam et al. 2020; Sarkheil and Safari 2020), macroalgae (Marinho-Soriano et al. 2009; Arumugam et al. 2018; Liu et al. 2019), microalgae (Ramli et al. 2017; Pacheco et al. 2020; Wirth et al. 2020), and biochar (Ocampo-Perez et al. 2019; Medellín-Castillo et al. 2021) as biosorbent and ion exchange sorbents (Garipov et al. 2020; Huang et al. 2020) for treatment of wastewater has been considered in recent years. Therefore, the use of alternative methods of nitrification process especially biosorbents can be useful for removal of various nutrients and pollutions such as nitrogen and phosphorus compounds from water, which ultimately improves the performance of the RAS.

Microalgal cells have been widely used as a low-cost and effective method to remove the excessive nutrients and other contaminants from wastewater (Martínez et al. 2000; Chevalier et al. 2000; Shawky et al. 2015; Ameri et al. 2020a, b; Nasoudari et al. 2021). Microalgae are capable to directly assimilate ammonium into amino acids, and they preferentially absorb ammonium ions with less energy consumption compared to nitrate (Dortch 1990; Perez-Garcia et al. 2011). However, one of the major limitations of using microalgae in wastewater treatment is the harvesting or separation of algal biomass from treated effluent (Mallick 2002). The application of microalgae in the RAS also increases risks of contamination and clogging of biofilter pores and pipes (Ramli et al. 2017). The immobilized or attached microalgae are a potentially alternative approach to prevent these problems (Endong et al. 2008; Ramli et al. 2017). The additional advantages of immobilized microalgae compared to their free-living counterparts include (1) more flexibility in the reactor design, (2) more uptake rate of nutrients due to increased surface area and cell density, and (3) more stability of cells and reduced leakage of cells into all parts of the aqueous phase (Brouers et al. 1989; Zhang et al. 2012; Ramli et al. 2017; Whitton et al. 2018). Green microalgae such as *Chlorella* sp., *Scenedesmus* sp., and *Botryococcus* sp. have been dominantly used in immobilization technology for wastewater purification (Kobbai et al. 2000; Zhang et al. 2012; Ramli et al. 2017; Ameri et al. 2019; Ameri et al. 2020b). Alginate as a high molecular weight biopolymer because of nontoxicity, permeability, transparency of formed matrix, and low cost is often used in algal immobilization technology (Smidsrød and Skja°k-Br k 1990; Arau´jo and Andrade Santana 1996). Alginates have also been applied as a hydrophilic, biocompatible, and inexpensive biosorbent (Klock et al. 1997) for metal removal from aqueous solutions (Chen and Wang 2001; Soni et al. 2012; Rahman and Wilfred 2018). It has been reported that alginate or modified alginate beads effectively remove the ammonium and soluble phosphorus ions from wastewaters (Zhang et al. 2012; Cruz et al. 2013; Ramli et al. 2017; Malicevic et al. 2020). Ramli et al. (2017) used the epiphyte *Stigeoclonium nanum* immobilized in alginate beads for removal of ammonium and nitrate in recirculating aquaculture systems. They found that algal growth and TAN removal by *S. nanum* immobilized in alginate beads were significantly higher than for *S. nanum* in free suspension culture. Immobilized *S. nanum* absorbed TAN more efficiently than NO_3-N and preferred ammonia to nitrate. They suggested that immobilized *S. nanum* is a suitable candidate for removal of total ammonia, which is produced daily by fish in aquaculture systems.

One of the most profitable sectors of the aquaculture industry is the reproduction and culture of ornamental fish (Jaleel et al. 2015). Increasing global demand for ornamental could be met through intensive and super-intensive methods used in the RAS. Therefore, the purpose of this study was to synthetize sodium alginate beads and immobilized microalgae in alginate beads as biosorbents and evaluate their efficiency in reducing total nitrogen of ammonia (TAN) and total phosphorus from water in static test. Afterward, the synthetized biosorbents were administrated as a biofilter in a RAS for rearing ornamental African cichlid (*Labidochromis lividus*) fish. The survival rate, growth indices of fingerling fish, and water quality including TAN, nitrate, and total phosphorus concentrations were also determined.

Materials and methods

Algal culture

Scenedesmus spp. and *Chlorella* spp. were obtained from the Research Institute of Industrial Biotechnology, Microbial Industrial Biotechnology Department, Mashhad, Iran. *Scenedesmus* spp. and *Chlorella* spp. were separately cultured in BBM (Bold Basal Medium, Sigma-Aldrich) and N8 culture (Sigma-Aldrich) media at 25 °C under 16/8 photoperiod and continuous aeration. After 14 days, the microalgae cells were collected at logarithmic growth phase by centrifugation at 8000 rpm for 10 min and rinsed three times with double distilled water. Afterward, the cells were diluted in 20 mL of double distilled water to mix with a 2% sodium alginate solution.

Preparation of biosorbents

Two types of biosorbents were prepared according to the method described by Ameri et al. (2019). Briefly, 2% sodium alginate (Sigma-Aldrich) was placed on a stirrer at 70 °C for 1 h to prepare the uniform suspension. The temperature of suspension was allowed to reach the room temperature, and then, *Scenedesmus* spp. and *Chlorella* spp. solutions were separately added to the 2% Na-alginate suspension at concentrations of 5 and 10 g L^{-1}, respectively. In the following, 50 mL of the mixture was poured dropwise into 100 mL of 2% calcium chloride (Sigma-Aldrich) solution for 20 min (rate of 2.5 mL/min). The synthetized algal beads of approximately 3-mm diameter were rinsed with 0.85% NaCl solution and then, kept in distilled water for further usage. The prepared beads without adding microalgae solutions were considered as blank alginate beads.

Static test

In this experiment, 18-glass aquariums with a capacity of 54 L were used and filled with 15 L of tap water. The physicochemical properties of used water were presented in Table 1. Two experimental concentrations of the TAN and TP including 0.5 and 5 mg L^{-1} were chosen according to literature review on their concentrations existing in wastewater of different aquaculture farms (Mustapha and Akinshola 2016; Coldebella et al. 2018). These concentrations were prepared by dissolving certain amounts of the analytical salts [NH_4Cl and K_2HPO_4] (Merck) into water of each glass aquarium. The efficiency of two types of biosorbents in removing TAN and TP from water was investigated based on the following treatments: (1) sodium alginate–immobilized microalgae (algal beads); (2) blank alginate beads (without algal cells); (3) control (without algal beads or alginate beads). All treatments were set up in triplicate. Each algal and alginate beads was inoculated at density of 25-g wet weight in each aquarium. The water temperature and photoperiod were adjusted to 25 °C and 16-h light/8-h dark cycle using an aquarium heater and a white fluorescent lamp (5000 lux), respectively. The continuous aeration for 24 h was provided by using a central air pump (Hailea ACO 318, India) and an air stone in each aquarium. The experimental period was 30 days and the water TAN and TP concentrations of each aquarium were measured every 10 days. The TAN concentration was measured based on the Ammonia-Nessler method using ultraviolet visible spectrophotometer (DR 5000TM model, HACH Co., USA) at the wavelength of 425 nm. The TP concentration also was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES, Spectro Arcos-76004555 plasma model, Germany). The TAN and TP removal efficiency of each biosorbent was calculated using Eq. 1.

$$R\ (\%) = [(C_A - C_B)/C_A] \times 100 \quad (1)$$

where R is the removal efficiency (%), C_A refers to the measured concentration in the control group (mg L^{-1}), and C_B is the measured concentration in the algal bead or alginate bead group (mg L^{-1}). Uptake capacity of each biosorbent was also calculated with Eq. 2 (Can-Terzi et al. 2021).

$$Uptake\ capacity = (C_i \times V) \times R\ (\%)/Bead\ weight \quad (2)$$

where C_i is the initial TAN or TP concentration (mg L^{-1}) and

Table 1 The physicochemical properties of tap water used in the static test

Parameters	Unit	Concentration
Total ammonia nitrogen (TAN)	mg L^{-1}	0.045±0.006
Nitrate (NO_3^--N)	mg L^{-1}	3.58±0.083
Total phosphorus	mg L^{-1}	0.041±0.005
pH	-	7.21±0.041
Dissolved oxygen	mg L^{-1}	6.56±0.13
Electrical conductivity (EC)	μS cm^{-1}	713.33±7.63

V and R (%) refer to the volume of the treated water and TAN or TP removal efficiency of each biosorbent. Bead weight (g) is the weight of algal beads or alginate beads in each aquarium.

Fish culture experiment

Fingerlings of African cichlid (*L. lividus*) fish were cultured in 12 water recirculating systems as experimental units. The components of each unit included a glass aquarium (45×30×35 cm) for fish keeping, a plastic container (50×30×20 cm) for placing physical and biological filters, a water pump (RS-4000 model, China) and pipes for water recirculation in the system, a controllable valve, an aquarium heater, and an air stone for aeration (Fig. 1). The physical filters of each experimental unit consisted of polyurethane foam aquarium sponge filter (20 × 10 × 5 cm; 6 pieces) and aquarium filter pad (32 × 12 × 2 cm; 5 pieces) to remove suspended solid particles including fish feces and uneaten fish food from water. The synthetized algal beads (100 g wet wt.) and alginate beads (100 g wet wt.) were also used separately as biofilter in each experimental unit to investigate their TAN and TP removal efficiency from water. The glass aquarium and plastic container were filled with 60 L of dechlorinated tap water in each unit. The water inside the aquarium was pumped into plastic container at a flow rate of 6 L min^{-1}. The water passed through the filters in sinusoidal motion and then returned to the aquarium. A valve was used to control the water height inside the plastic container at level of 15 cm. The algal beads were kept under 16/8 photoperiod by using a white fluorescent lamp placing top of the plastic container. The experimental units without biosorbent (algal or alginate beads) were considered as the control group.

A total of 270 fingerling fish were obtained from a local ornamental fish farm, transferred to the Aquatic Research Laboratory (Ferdowsi University of Mashhad, Iran) and were stocked into a 500-L tank to acclimatize to laboratory conditions. After launching the water recirculating systems and water aeration for 48 h, the 20 fish with average weight of 0.10 ±0.1 g and average length of 2.16±0.25 cm were randomly introduced to each of the aquarium. The fish were fed with commercial pellets (Salvia 9015F, BioMar®, France) that contained 42% crude protein, 28% crude lipid, 20.9% carbohydrates (NFE), and 6.1% ash (Sarkheil and Safari 2020). The fish were cultured for 30 days and fed three times daily at 8:00, 12:00, and 16:00 at rate of 4% of body weight. The uneaten food and fish feces left in the aquarium were siphoned daily, and evaporated water was also replaced with double distilled water. The TAN and TP concentrations on days 0, 10, 20, and 30 were measured according to the methods explained in the "Static test" section. The concentration of nitrate (NO_3^-) was also determined using UV/visible spectrophotometer (DR 5000TM model, HACH CO., USA) at the wavelength of 500 nm. The electrical conductivity (EC) and pH of water were also measured every 5 days using the portable multi-meter model AZ-8603. The fluctuations of water temperature and dissolved oxygen during 30 days of the experiment were 25.44–27.90 °C and 7.12–7.26 mg L^{-1}, respectively. At the end of the cultivation period, the weight and length of all the fish were measured after anesthetizing with 500 mg L^{-1} of clove powder. The growth indices and survival rate of the fish were determined using following equations:

$$\text{Weight gain (g)} = (W_{\text{f}} - W_{\text{i}})$$

$$\text{Specific growth rate } \left(\text{SGR; \%body weight day}^{-1}\right) = \left[(\text{LnW}_{\text{f}} - \text{LnW}_{\text{i}})/\text{Time}\right] \times 100$$

$$\text{Feed conversion ratio (FCR)} = \left(\text{Feed consumed}/W_{\text{gain}}\right)$$

$$\text{Condition factor (CF)} = \left[W_{\text{f}}\ (\text{g})/L_{\text{F}}^{3}\ (\text{cm})\right] \times 100$$

$$\text{Daily growth index (DGI)} = \left[(W_{\text{f}} - W_{\text{i}})/\text{Time}\right]$$

$$\text{Body weight increase\%(BWI)} = \left[(W_{\text{f}} - W_{\text{i}})/W_{\text{i}}\right] \times 100$$

$$\text{Survival rate (\%)} = (N_{\text{f}}/N_{\text{i}}) \times 100$$

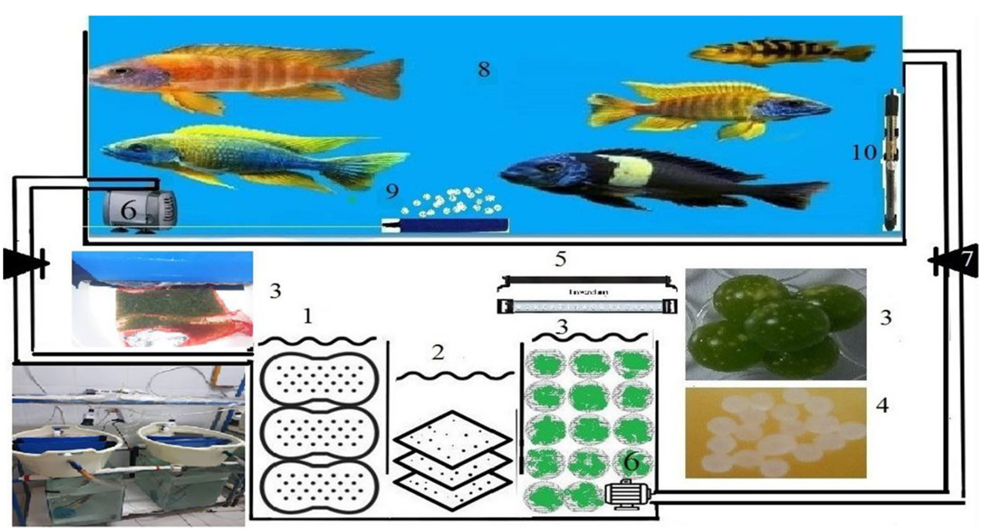

Fig. 1 Schematic figure of a recirculating aquaculture system for culture of African cichlid (*Labidochromis lividus*) fingerlings for 30 days. 1 polyurethane foam aquarium sponge filter; 2 aquarium filter pad; 3 immobilized algal beads; 4 alginate beads; 5 white fluorescent lamp; 6 water pump; 7 controllable valve; 8 glass aquarium; 9 air stone; 10 aquarium heater

where W_i, W_f, L_F, N_i, and N_f refer to initial weight, final weight, final length, initial number of fish, and final number of fish, respectively.

Statistical analysis

All data were shown as mean ± SD. The statistical analysis of data was performed using SPSS software (Version, 19). Normality assumption of data was investigated using Kolmogorov-Smirnov test. Significant differences between the means were evaluated using one-way analysis of variance (ANOVA) and two-way ANOVA (concentrations of TAN or TP × biosorbent type) followed by the Duncan multiple range test. One-way repeated measures analysis of variance (ANOVA with repeated measures) was applied to determine the significant differences between the means in each treatment. The P-value of <0.05 was employed to assay the significant differences.

Results

Static test

Fluctuations of TAN concentration in different groups during the 30 days of static test are presented in Fig. 2. The significant differences between means were evaluated using two-way ANOVA with main effects of TAN concentrations (0.5 and 5 mg L^{-1}) and types of biosorbents (control, alginate beads, and algal beads). On day 0 of the test, there was a significant main effect of TAN concentration (F=2775.88; P=0.00), but the main effect of biosorbent type (F=2.76; P=0.10) and TAN concentration × biosorbent type interaction effect (F=2.04; P=0.17) were not significant. The main effects of TAN concentration (F=4502.99; P=0.00) and biosorbent type (F=1255.48; P=0.00) and their interaction (F=853.90; P=0.00) were significant on the 10th day of the experiment. The TAN concentration in the alginate beads and algal beads groups decreased compared to the control in both 0.5 and 5 mg L^{-1} of TAN (P<0.05). The TAN concentration in the algal beads group was significantly lower than that in the alginate beads group in 5 mg L^{-1} of TAN (P<0.05). On the 20th day of the experiment, it was found that main effects of TAN concentration (F=822.85; P=0.00) and biosorbent type (F=629.72 P=0.00) and their interaction effect (F=468.80; P=0.00) were statistically significant. In 0.5 mg L^{-1} of TAN, the algal beads group showed lower TAN concentration than the control and the alginate beads groups (P<0.05), while there was no significant difference between the alginate beads group and the control (P>0.05). In 5 mg L^{-1} of TAN, the TAN concentration in both alginate and algal beads groups was significantly lower than that in the control (P<0.05). The algal beads group had lower TAN concentration in comparison to the alginate beads group (P<0.05). After 30 days of the experiment, the results of main effects of TAN concentration (F=4210.42; P=0.00) and type of biosorbent (F=6050.14; P=0.00) and their interaction effect (F=4374.51; P=0.00) were also significant. In 0.5 mg L^{-1} of TAN, the alginate and algal groups reduced TAN concentration compared to the control (P<0.05). The lowest TAN concentration was observed in the algal beads group (P<0.05). In 5 mg L^{-1} of TAN, the alginate and algal beads reduced the TAN concentration compared to the control, but there was no significant difference in the TAN concentration between these two biosorbent groups (P>0.05). In all groups, the TAN concentration reduced significantly during 30 days of the experiment (P<0.05).

The TAN removal efficiency and uptake capacity of the alginate and the algal beads at different time intervals of the static test are shown in Table 2. The TAN removal efficiency and uptake capacity of the two biosorbent types in both 0.5 and 5 mg L^{-1} of TAN increased during 30 days of the experiment. In both TAN concentrations, the removal efficiency and uptake capacity of the algal beads was approximately 1.30–1.92 times higher than those of the alginate beads at different times of the experiment except on the 30th day at 5 mg L^{-1} of TAN. Here, a 10-fold increase in the TAN concentration from 0.5 to 5 mg L^{-1} resulted in a 12.38-, 17.15-, and 14.60-fold increase in uptake capacity for the alginate beads and a 11-, 11.18-, and 10.37-fold increase for the algal bead on days 10, 20, and 30, respectively.

Figure 3 shows the variations of total phosphorus (TP) concentration treated with two biosorbent types during 30 days of static test. The significant differences between the main effects of TP concentrations (0.5 and 5 mg L^{-1}) and types of biosorbents (control, alginate beads, and algal beads) and their interaction were determined using two-way ANOVA. At the first day of the experiment, the main effect of TP concentration was significant (F=10336.97; P=0.00) but no significant differences was observed between the main effect of biosorbent type (F=1.38; P=0.28) and between TP concentration × biosorbent type interaction effect (F=0.47; P=0.63). On the 10th day of the experiment, the main effects of TP concentration (F=6170.13; P=0.00) and biosorbent type (F=16.35; P=0.00) were significant, but their interaction effect was not significant (F=3.4; P=0.067). In 0.5 mg L^{-1} of TP, the alginate and algal beads reduced the TP concentration compared to the control (P<0.05). In 5 mg L^{-1} of TP, the lowest TP concentration was recorded in the algal beads groups (P<0.05), while there was no significant difference between the alginate beads group and the control (P>0.05). On day 20 of the test, the main effects of TP concentration (F=549.69; P=0.00), biosorbent type (F=58.80; P=0.00), and their interaction effect were significant (F=25.89; P=0.00). In 0.5 mg L^{-1} of TP groups, the TP concentration in the algal and alginate beads groups decreased in comparison with that in the control (P<0.05). In 5 mg L^{-1} of TP, the alginate and algal

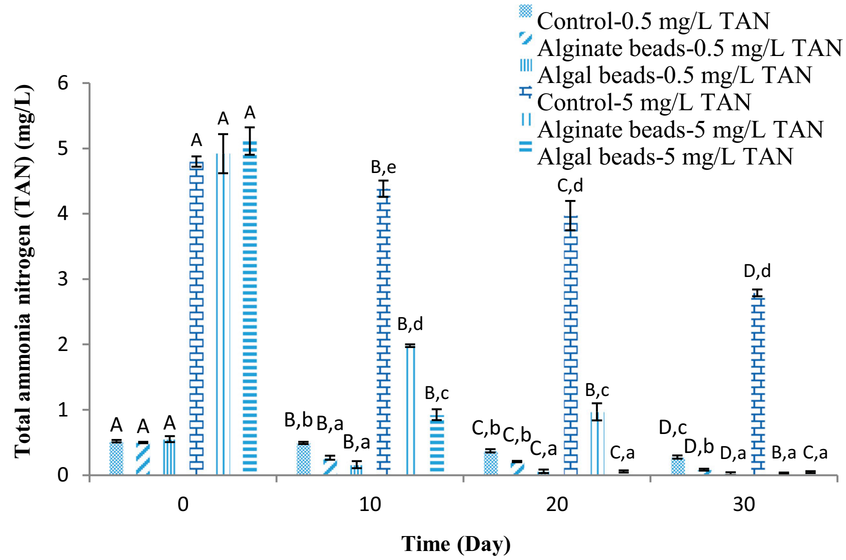

Fig. 2 Total ammonia nitrogen (TAN) concentration of water in different groups of static test at various time intervals. Bars with different lowercase letters in each time are significantly different (mean± SD, two-way ANOVA, $P<0.05$). Bars with different capital letters in each group are significantly different (mean± SD, ANOVA with repeated measures, $P<0.05$)

beads reduced the TP concentration compared to the control, and the lowest TP concentration was observed in the algal beads group ($P<0.05$). The statistical analysis showed that the main effects of TP concentration ($F=2397.58$; $P=0.00$), biosorbent type ($F=173.80$; $P=0.00$), and their interaction effect were significant ($F=93.60$; $P=0.00$) on the 30th day of the test. The use of algal beads decreased the TP concentration compared to the alginate and control groups in 0.5 mg L^{-1} of TP. The TP concentration in the alginate and algal beads groups was lower than that in the control, and the algal bead group showed the lowest TP concentration in 5 mg L^{-1} of TP ($P<0.05$). In the control-5 mg L^{-1} of TP and the alginate-0.5 mg L^{-1} of TP groups, the TP concentrations decreased significantly from day 0 to day 10 ($P<0.05$), but no significant differences was observed until day 30 of the experiment ($P>0.05$). The TP concentration in other experimental groups showed a significant reduction from day 0 to day 30 ($P<0.05$).

Table 3 shows the percentages of TP removal efficiency and uptake capacity of alginate and algal beads during different days of static test. The TP removal efficiency and uptake capacity of the alginate and algal beads increased during 30 days of the test in both 0.5 and 5 mg L^{-1} of TP groups. In both 0.5 and 5 mg L^{-1} of TP, the removal efficiency and uptake capacity of algal beads were 4.45–8.91 and 6–10.66 times higher than those of the alginate beads at different time intervals, respectively. The results showed that TP uptake capacity of alginate beads increased 3-, 5.66-, and 8-fold by increasing TP concentration from 0.5 to 5 mg L^{-1}, while a 4.91-, 5.2-, and 5.37-fold increase was recorded for the algal beads on days 10, 20, and 30, respectively.

Fish culture

Growth performance of fish

The results of culture of African cichlid (*L. lividus*) fingerlings in the RAS containing two types of biosorbents for 30 days showed that the growth performance of fish including final

Table 2 Total ammonia nitrogen (TAN) removal efficiency and uptake capacity of two biosorbents in different TAN concentrations and times of static test (mean ± SD; $n=3$)

Treatment	Total ammonia nitrogen (TAN)					
	Removal efficiency (%)			Uptake capacity (%)		
	Time (day)			Time (day)		
	10	20	30	10	20	30
Alginate beads-0.5 mg/L TAN	45.99±5.68	43.69±1.14	69.76±2.68	0.13±0.01	0.13±0.19	0.20±0.01
Algal beads-0.5 mg/L TAN	67.73±9.97	84.16±6.30	91.09±8.15	0.22±0.03	0.27±0.04	0.29±0.02
Alginate beads-5 mg/L TAN	54.81±0.83	75.65±1.90	98.94±0.30	1.61±0.07	2.23±0.19	2.92±0.17
Algal beads-5 mg/L TAN	79.02±1.06	98.58±0.31	98.32±0.51	2.42±0.09	3.02±0.12	3.01±0.12

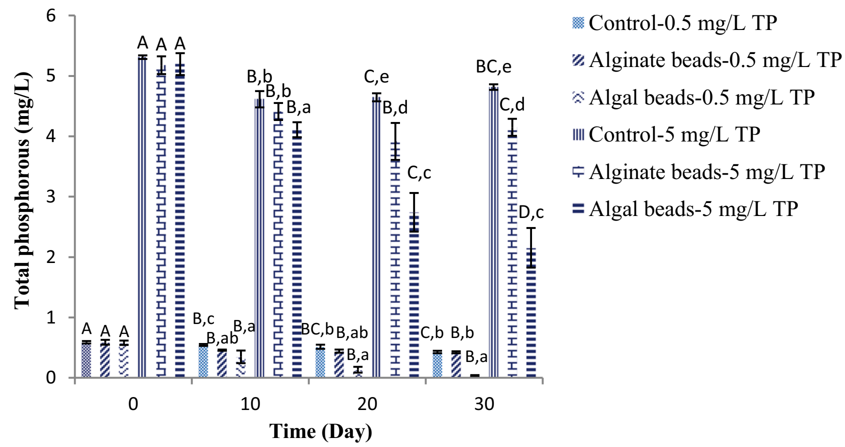

Fig. 3 Total phosphorus concentration of water in different groups of static test at various time intervals. Bars with different lowercase letters in each time are significantly different (mean± SD, two-way ANOVA, *P*<0.05). Bars with different capital letters in each group are significantly different (mean± SD, ANOVA with repeated measures, *P*<0.05)

weight, final length, weight gain, and DGI were significantly higher in the algal beads group than those in the alginate beads and control groups (*P*<0.05). The SGR%, BWI%, and FCR of fish increased significantly in the alginate and algal beads groups compared to the control group (*P*<0.05). The CF% value showed no significant difference between the experimental groups (*P*>0.05). The survival rate of fish increased significantly in the algal beads group in comparison to that in the control (*P*<0.05), but no significant differences was observed between the alginate beads group and other groups (*P*>0.05) (Table 4).

Water quality assay

The variations of water TAN, nitrate, and TP in the RAS with two types of biosorbents during cultivation period are presented in Fig. 4. The TAN concentration increased significantly from day 0 to day 20 (*P*<0.05), but this value did not increase until the 30th day of the experiment in the control group (*P*>0.05). In the alginate beads group, the TAN concentration increased significantly during the first 10 days (*P*<0.05), but no significant change was observed until day 30 (*P*>0.05). The TAN concentration did not change significantly during 30 days of the experiment in the algal beads group (*P*>0.05). The TAN concentration was not significantly different between the experimental groups on days 0 and 10 (*P*>0.05). The alginate and algal beads decreased the TAN concentration compared to the control on the 20th and 30th days of the experiment (*P*<0.05). The lowest TAN concentration was also observed in the algal beads group (*P*<0.05) (Fig. 4(a)).

The nitrate concentration increased significantly in the first 10 days but decreased during the next 10 days and then increased until day 30 in the control group (*P*<0.05). In both alginate and algal beads groups, the nitrate concentration increased significantly during 30 days of the experiment (*P*<0.05). The nitrate concentration showed no significant differences between the groups on different days of the experiment (Fig. 4(b)).

The TP concentration increased significantly during 30 days of fish culture in all three groups (*P*<0.05). No significant differences was observed between the TP concentrations of the three groups on days 0, 10, and 20 (*P*>0.05). The algal beads group had a lower TP concentration than the alginate beads group on day 30 (*P*<0.05), while no significant difference was observed between TP concentrations of the algal bead group and the control group (*P*>0.05) (Fig. 4(c)).

The pH of water varied between 7.18–7.29, 7.20–7.23, and 7.26–7.38 during 30 days of fish culture in the RAS without

Table 3 Total phosphorus (TP) removal efficiency and uptake capacity of two biosorbents in different TP concentrations and times of static test (mean ± SD; *n*=3)

Treatment	Total phosphorus (TP)					
	Removal efficiency (%)			Uptake capacity (%)		
	Time (day)			Time (day)		
	10	20	30	10	20	30
Alginate beads-0.5 mg/L TP	8.22±2.29	9.89±2.35	10.31±4.01	0.02±0.007	0.03±0.008	0.03±0.01
Algal beads-0.5 mg/L TP	36.61±18.16	73.85±10.09	91.95±5.48	0.12±0.06	0.25±0.04	0.32±0.02
Alginate beads-5 mg/L TP	2.22±1.36	5.68±1.88	8.10±5.48	0.06±0.04	0.17±0.05	0.24±0.16
Algal beads-5 mg/L TP	19.16±3.66	41. 72±7.35	55.30±6.60	0.59±0.12	1.30±0.25	1.72±0.24

Table 4 Growth performance, feed utilization parameters, and survival rate of African cichlid (*L. lividus*) fingerlings cultured in the RAS containing two biosorbent types for 30 days (mean ±SD; *n*=3)

Parameter	Biosorbent type		
	Control	Alginate beads	Algal beads
Initial weight (g)	0.11±0.005	0.10±0.008	0.11±0.000
Initial length (cm)	2.15±0.082	2.21±0.110	2.14±0.052
Final weight (g)	0.54±0.035[a]	0.56±0.014[a]	0.65±0.043[b]
Final length (cm)	3.43±0.022[a]	3.47±0.025[a]	3.58±0.093[b]
Weight gain (g)	0.43±0.030[a]	0.46±0.021[a]	0.54±0.042[b]
SGR (%BW day^{-1})	5.45±0.05[a]	5.71±0.35[ab]	5.95±0.19[b]
FCR	2.54±0.66[b]	1.90±0.23[ab]	1.31±0.33[a]
DGI (g)	0.014±0.001[a]	0.015±0.0007[a]	0.018±0.0014[b]
BWI%	414.00±8.86[a]	457.95±57.57[ab]	501.36±36.32[b]
CF (g/cm^3)	1.33±0.060	1.34±0.057	1.42±0.025
Survival rate (%)	60±3.57[a]	73.09±8.24[ab]	83.80±7.43[b]

The values with different letters in the same row are significantly different (ANOVA, *P*<0.05). *SGR*, specific growth rate; *FCR*, feed conversion ratio; *CF*, condition factor; *DGI*, daily growth index; *BWI*, body weight increase

biosorbent (control) and the RAS containing the alginate beads and algal beads, respectively. The variations of electrical conductivity (EC) of water in the control, alginate bead, and algal bead groups were also found to be 715–805.66, 705–791, and 720.66–785 µs cm^{-1}, respectively.

Discussion

Nowadays, a recirculating aquaculture system (RAS) is known as a sustainable aquaculture practice due to controlled waste discharge into the environment, optimization of water usage, increasing biosecurity, and reducing the use of disinfectants (Verdegem et al. 2006; Martins et al. 2010). Increasing the efficiency of biofilters to improve water quality and fish production is one of the most important factors in the success of RAS performance. Therefore, in the present study, the efficiency of two types of synthetized biosorbents for removal of TAN and TP from water in a static test and also as biofilter in a RAS to culture ornamental African cichlid (*L. lividus*) fish was evaluated.

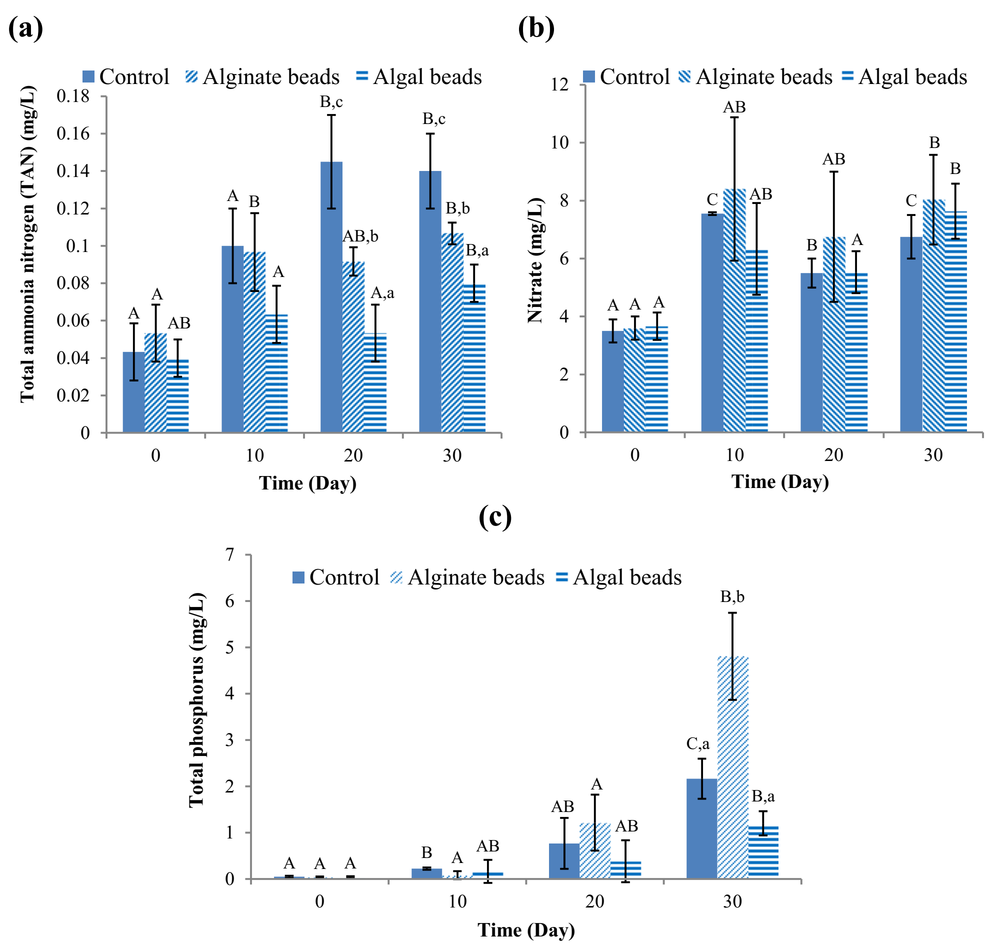

Fig. 4 Fluctuations in water total ammonia nitrogen (TAN) (a), nitrate (b), and total phosphorus (c) concentrations in the RAS containing two biosorbents for culture of African cichlid (*L. lividus*) fingerlings for 30 days. Bars with different lowercase letters in each time are significantly different (mean± SD, ANOVA, *P*<0.05). Bars with different capital letters in each group are significantly different (mean± SD, ANOVA with repeated measures, *P*<0.05)

Microalgae prefer to utilize inorganic nitrogen (N) sources in order of $NH_4^+ > NO_3^- > NO_2^- >$ urea (Perez-Garcia et al. 2011). The assimilated N is further transformed into protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) (Wheeler 1983). The results of static test revealed that both alginate and algal beads significantly reduced the TAN concentration compared to the control in both 0.5 and 5 mg L^{-1} of TAN. The TAN removal efficiency of two biosorbents ranged between 45.99–79.02% on day 10 and 69.67–98.32% on day 30 of the test in the different treatment groups. It is worth to mention here that TAN removal efficiency of both biosorbents increased during the 30-day bio-treatment period. Ruiz-Marin et al. (2010) found that removal efficiency of alginate-immobilized *Chlorella vulgaris* and *Scenedesmus obliquus* growing in batch culture of artificial wastewater for N-NH_4^+ after 48 h were 65.6% and 95.4%, respectively. Differences in nutrient removal efficiency and time required for immobilized cells to remove nutrients from solutions may be due to the number of beads, the density of cells per bead, and the number of active cells in each bead (Ruiz-Marin et al. 2010). In the current study, increased TAN removal efficiency of microalgal cells immobilized in alginate beads may be due to growth and multiplication of cells (Tam and Wang 2000), decreasing self-shading of light by gel, thus increasing the synthesis of pigments (Vichez and Vega 1994) and increasing anabolism and physiological activity of algal cells (Yan et al. 1995). The removal capacity of green microalga *Scenedesmus quadricauda* immobilized in sodium alginate beads reached up to 100% for ammonia and 61% for phosphate after 4 and 6 days, respectively (Shawky et al. 2015). In contrast, Ruiz-Marin et al. (2010) reported that immobilized microalgae of *S. obliquus* removed 97% of N-NH_4^+ from artificial wastewater during first 48 h, but N-NH_4^+ removal decreased to 80% and 30% after 74 h and 250 h, respectively. Algal beads remove nitrogen and phosphorus in different immobilization matrices (Al-Jabri et al. 2021). Nutrient removal occurs in two possible ways in algal beads: (1) their entrapment in alginate matrix (Mallick 2002) and (2) immobilization on the surface of matrix (Abe et al. 2008). The rate of nutrient removal by algae beads depends on their photosynthetic activity. *Chlorella* beads in alginate matrix showed 80 and 70% reduction in ammonium and phosphate, respectively, from wastewater (Travieso et al. 1992). Nutrient removal by algal beads also depends on its concentration. Garbayo et al. (2000) showed that algal beads uptake nutrients in concentration lower than 0.14 mM because of limitation of the nutrient diffusion through the immobilizing matrix. In spite of limited growth of immobilized cell in beads, they could be more active than free cell. So, immobilized cells are recommended for wastewater treatment due to their higher removal efficiency (Vasilieva et al. 2016).

The findings of the present study showed that the alginate beads without microalgae reduced the TAN concentration by 69.76% and 98.94% after 30 days in 0.5 and 5 mg L^{-1} of TAN, respectively. In contrast to our results, Shawky et al. (2015) reported nearly no ammonia removal by neat sodium alginate beads from water solution. They stated that the alginate beads just act as a carrier tool for microalgal cells to solve the common harvesting problem of cells from solutions. In agreement to our results, Ruiz-Marin et al. (2010) reported that alginate beads reduced N-NH_4^+ of artificial and urban wastewaters by approximately 15% after 48 h. They attributed the uptake of ammonium to ionic interactions between the ammonium ions and the carboxyl groups of the gel.

The results of the current study revealed that the TAN removal efficiency of algal beads in 0.5 mg L^{-1} of TAN was 21.74%, 40.47%, and 21.33% higher than that of the alginate beads, and these values were recorded as 24.21%, 22.93%, and 0% in 5 mg L^{-1} of TAN, on days 10, 20, and 30 of static test, respectively. These results indicated that the immobilization of *Scenedesmus* spp. and *Chlorella* spp. improved the TAN removal efficiency of alginate beads. Kube et al. (2020) found that immobilization of *C. vulgaris* in alginate beads did not compromise the rate of N and P uptake. Kaya et al. (1995, 1996) also found that *Scenedesmus* cells immobilized in alginate screens had higher nutrient uptake rate than alginate beads.

Initial TAN concentration was an important variable for TAN removal by both biosorptions as their TAN removal efficiency increased with elevation of TAN concentration from 0.5 to 5 mg L^{-1} due to an increase in their uptake capacity. Furthermore, the increase in TAN concentration could increase the contact between the biosorbent and TAN. Ruiz-Marin et al. (2010) indicated that N-NH_4^+ uptake rate of both microalgae of *S. obliquus* and *C. vulgaris* immobilized in alginate beads increased with increasing ammonium concentration in urban wastewater. In disagreement with our results, Shawky et al. (2015) reported that removal efficiency of ammonia reduced coincident with increasing ammonia concentration from 1 to 5 mg L^{-1}.

Microalgae utilize phosphorus (P) to synthetize nucleic acids (e.g., RNA and DNA), ATP, and phospholipids as the main membrane component (Dyhrman 2016). It has been found that luxury P uptake by microalgae would enhance P removal in wastewater treatment (Powell et al. 2011). Inorganic nutrients such as phosphate are freely available for immobilized algal cells as their free counterparts (Lau et al. 1997). Zhang et al. (2012) reported that immobilized *Chlorella* sp. in calcium alginate sheets had great efficiency in removing PO_4^{3-}-P from wastewater. Rai and Mallick (1992) found that immobilized *Chlorella* had a higher uptake rate for phosphate than their free-living counterparts. In the current study, despite the presence of phosphate ions in medium, both biosorbents did not lose their integrity, which could be due to the initial low phosphate concentration in water (<6 mg L^{-1}) (Tam and Wang 2000). Both sodium alginate–

immobilized *Scenedesmus* spp. and *Chlorella* spp. and sodium alginate beads without microalgae were capable to decrease total phosphorus (TP) concentration significantly compared to the control in different time intervals. The TP removal efficiency of algal beads reached up to 91.95% and 55.30% after 30 days of the experiment in 0.5 and 5 mg L^{-1}, respectively. Gonzalez et al. (1997) reported 55% phosphorus removal by *C. vulgaris* and *Scenedesmus dimorphus* from agro-industrial wastewater. In contrast to our findings, Shawky et al. (2015) found that sodium alginate beads had no phosphate removal from wastewater during 6 days (Shawky et al. 2015). The findings revealed that the TP removal efficiency of the algal beads was clearly higher than that of the alginate beads on all times of the experiment due to their higher uptake capacity. This result indicates the contribution of algal cells in removing TP from water. Tam and Wong (2000) also demonstrated that the PO_4^{-3}-P removal efficiency of calcium alginate–immobilized *C. vulgaris* was higher than that in blank alginate bead.

The TP removal efficiency of the algal beads increased during 30 days of the test because of the increase in their uptake capacity from 0.12 to 0.32 % and from 0.33 to 1.72% in 0.5 and 5 mg L^{-1} of TP, respectively. This result probably was due to the growth and multiplication of microalgae cells throughout the experiment. These results indicate that the synthetized algal beads could maintain their TP removal efficiency for a long time (30 days). The uptake capacity of alginate beads increased overtime from 0.02 to 0.03% and from 0.06 to 0.24% which resulted in a slight increase in their TP removal efficiency in 0.5 and 5 mg L^{-1} of TP, respectively. The TP removal efficiency of alginate beads only reached up to 10.31% and 8.10% at the end of the experiment in 0.5 and 5 mg L^{-1} of TP, respectively. These findings revealed that the sodium alginate beads had no great potentiality in removing TP from water. The uptake capacity of both alginate and algal beads for TP increased with a 10-fold increase in TP concentration from 0.5 to 5 mg L^{-1}, while the TP removal efficiency of both biosorbents decreased. It was shown that absorption capacity of phosphate ions by alginate-calcium carbonate composite beads increased with increasing phosphate initial concentration (Mahmood et al. 2015). Shawky et al. (2015) also reported that removal efficiency of microalgae *S. quadricauda* for phosphate decreased from 86 to 61% with increasing phosphate concentration from 1 to 5 mg L^{-1}.

In this study, the potentialities of using alginate and algal beads as biofilter in a RAS were investigated. The survival rate and growth performance of cultured fish in a RAS with algal beads were improved. The survival rate, weight gain, and FCR values of fish cultured in the RAS containing algal beads increased by 23.8%, 25.58%, and 48.42% compared to those cultured in the RAS without biosorbent (control), respectively. Indeed, the enhancement of these indices could be due to the improvement of water quality in the RAS. It is well known that deterioration of water quality in RAS adversely influence on the growth and health of fish (Timmons et al. 2002). Application of microalgae in RAS is not prevalent, and water quality is often maintained by autotrophic and heterotrophic bacteria (Ebeling et al. 2006; Ramli et al. 2017). In the current study, the fish of each RAS were fed approximately with 0.5 g of diet containing 42% crude protein daily. The TAN produced daily in each RAS due to fish feeding was approximately 0.3 mg/L. The concentration of ammonium ions in water is approximately more than 50% of TAN (Kikuchi et al. 1995), which can be absorbed by the algal and alginate beads. The use of the algal beads decreased the TAN concentration by 40%, 63.44%, and 42.85% in comparison to the control on days 10, 20, and 30 of the experiment, respectively. These values were recorded as 10%, 37.24%, and 28.57% for the alginate beads. These results indicated that the sodium alginate beads could be considered as an effective biosorbent in RAS and their TAN removal efficiency could be improved by immobilization of microalgae cells. Ramli et al. (2017) stated microalgae *Stigeoclonium nanum* immobilized in sodium alginate absorbed TAN more efficiently than NO_3-N and is a suitable approach for TAN removal in RAS. The removal efficiency of NH_4^+-N and NO_3^--N by microalgae membrane bioreactor (MMBR) from marine wastewater aquaculture reached 85% and 52% after 40 days, respectively (Ding et al. 2020). The results of the current study showed that the nitrate concentration in RAS containing the alginate and algal beads was not significantly different from the control group, indicating no absorption of nitrate by biosorbents during experimental period. Dortch (1990) explained that the algae are capable to uptake ammonium ions more efficiently and with less energy consumption than nitrate. In the presence of both ammonium and nitrate, microalgae preferentially uptake ammonium ions (Domingues et al. 2011; Hii et al. 2011). Ramli et al. (2017) also reported that TAN uptake by *Stigeoclonium nanum* immobilized in sodium alginate beads was more efficient than nitrate uptake.

In the current study, the concentration of TP increased gradually in the RAS due to feeding the fish and excretion of phosphorus into water. Phosphorus (P) excretion rate is influenced by many factors, including fish diet, fish growth rate, and fish size (Verant et al. 2007). The TP concentration in RAS containing the algal beads decreased by 71.53% and 44.90% compared to those containing the alginate beads and the control at the end of cultivation period, respectively. These findings indicated higher TP removal efficiency of the algal beads than the alginate beads in the RAS. Ding et al. (2020) reported that microalgae membrane bioreactor (MMBR) decreased PO_4^{3-}-P concentration by 80% from marine wastewater aquaculture after 40 days. The use of alginate beads had no significant effect on the reduction of TP, which indicates this biosorbent is not very suitable in removing TP from the RAS. The water TP concentration in the RAS without biosorbent was lower than the RAS with the alginate beads, probably the results of more fish mortality and subsequently less food consumption and phosphorus secretion.

Today, discharge of nutrient-rich effluents from aquaculture farms into aquatic environments is a serious environmental problem. Aquaculture farms that use the RAS discharge 10% of nutrient-rich water into environment per day. According to national standards (Vice Presidency for Strategic Planning and Supervision of Islamic Republic of Iran 2010), permissible concentrations of total phosphorus, ammonium, and nitrate in effluent for discharge to surface waters and groundwaters are 6, 2.5, 50, 6, 1, and 10 mg/L, respectively. Based on the international permissible limits for discharge of wastewater to streams (WHO 2006), the maximum concentrations of total phosphorus, ammonium, and nitrate are 30, 5, and 45 mg/L, respectively. The findings of the present study showed that the concentration of TP, TAN, and nitrate in the RAS with two types of biosorbents on different days of the experiment were lower than the national and international permissible limits.

Conclusion

The present study demonstrated the capability of both sodium alginate–immobilized *Scenedesmus* spp. and *Chlorella* spp. and sodium alginate beads without microalgae for removal of TAN and TP from water solution. The TAN and TP removal efficiency of algal beads was more efficient than that of the alginate beads. The TAN removal efficiency of both biosorbents increased with increasing TAN concentration from 0.5 to 5 mg L^{-1}. Our results showed that the use of sodium alginate–immobilized microalgae as a biofilter in the RAS resulted in a significant reduction in water TAN and TP concentrations and thus improved the survival rate and growth performance of African cichlid (*L. lividus*) fingerlings. However, further studies should investigate the reusability, stability, and life span of the alginate and algal beads under aquaculture water conditions for continuous removal of nitrogen and phosphorus compounds. Furthermore, the leakage of algal cells from alginate-immobilized microalgae beads should be investigated under different water conditions (e.g., water flow rate, water speed) for practical application in the RAS.

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Author contribution Mehrdad Sarkheil: Supervision, conceptualization, methodology, funding acquisition, project administration, writing—original draft preparation. Maryam Ameri: Conceptualization, methodology, writing—review and editing. Omid Safari: Writing—review and editing. All authors read and approved the final version.

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Code availability Not applicable

Declarations

Statement of animal rights Ferdowsi University of Mashhad (FUM) animal ethic rights was applied to all experiments on fish.

Ethics approval Ferdowsi University of Mashhad (FUM) animal ethic rights was applied to all experiments on fish.

Consent to participate Not applicable

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Conflict of interest The authors declare no competing interests.

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