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STUDY OF BIOFILTER PLANTED WITH BASIL FOR REMOVAL OF AMMONIA IN AQUAPONIC WATER

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Abstract

This experimental investigation of a laboratory scale aquaponic system included testing of a biofilter with basil plant as the biofilter part on aquaculture water quality. Irridescent shark was selected as aquaculture species. The biofilter consist of natural biomaterials such as coconut husk, coco peat, and coarse aggregates. The experiment was carried out for three short water recirculation durations of 2, 3, and 4 h/day. The influent and effluent ammonia NH_3, total ammonia nitrogen (TAN), nitrites NO_2 and nitrates NO_3 levels were measured and analysed. The results showed that the biofilter effectively removed NH_3 (65 - 71 %), TAN (34 - 58 %), and NO_2 (60 - 67 %) from the aquaculture water. The dissolved oxygen (DO) levels were maintained between 3 - 7.0 mg/l during all the recirculation durations. The significance of water recirculation period was assessed by calculating the differences between the means of water quality parameters with a statistical test named one-way analysis of variance (ANNOVA) with significant level P taken as 5 %, i.e., $P \leq 0.5$. The effluent mean NH_3 levels 0.030 mg/l, 0.033 mg/l, and 0.022 mg/l exhibited significant difference at 4 h/d periods while effluent TAN levels 0.81, 0.77, and 0.77 showed no difference with varying periods.

Keywords:

Biofilter;
Ammonia;
Dissolved oxygen;
Recirculation;
Aquaponic.

1 Introduction

Aquaponics is an innovative agricultural production technique that combines two major components named aquaculture (fish tank) and hydroponics (soil less media) [1]. Unlike in hydroponics, the aquaponic method is capable of avoiding the complete dependence on nutrients and its sources [2]. It has the potential to solve the water scarcity problems and its hazardous effects [2]. The world's western part like US at Woods Hole Oceanographic Institute done strong developments in aquaponics since the 1960s [3]. Since then, the developed and developing countries like America and Australia adopted this system and carried out major research projects and programs on aquaponics [2]. These projects focused major objective of reducing the load of nutrient wastes and their environmental effect to increase the efficiency of nutrients for production of edible plants. The less availability of water is making the aquaculture production costlier and the only possible solution to overcome this problem is the effective recirculation of aquaculture wastewater to produce edible crops and fish [4]. The closed loop technique of aquaponic includes the recirculation of aquaculture water allowing the interaction of aquatic species, nitrifying bacteria and plants [5]. This method is capable of minimizing the need of micro and macronutrients essential for plant growth [4, 5]. The waste generated by fish (fish excreta) that contains nitrogen rich contents like urine and ammonia from the aquaculture unit is supplied to the hydroponic unit in this mechanism [1, 6]. Thus, the mechanism fulfills the nutrient need by minimizing its demand through hydroponic aquaculture integration [7]. The fish generated waste works as a fertilizer for edible plant growth and production in this mechanism [6]. The nitrogenous wastes generated by fish have major contents like the unionized ammonia NH_3, ammonium NH_4+, nitrite NO_2, and nitrate NO_3. The vital nutrients like Ammonia NH_3 are oxidized by nitrifying bacterias named *Nitrosomonas and Nitrobacter* into the forms like NO_2 and NO_3 which plants

can accept for their growth [1, 6]. Water is a limited resource in many places and therefore a remedy is essential to avoid environmental degradation by aquaponic effluents [7]. The methods like activated sludge process (ASP), and its applications named extended aeration and contact stabilization process along with the mechanisms like constructed wetlands are already in practice to treat the wastewater from different sources. Previous studies have shown that constructed wetlands are effective to remove major pollutants from effluents of shrimp, trout and catfish ponds when flow rates are between the ranges of 0.18 to 53.9 m^3/d [6, 8]. The additional advantage of treating aquaculture water with aquaponics is the phytoremediation capacity of the plants in the system which supports the filtration process. The available data in literature which can put light on the capacity of herbaceous plant as a part of biofilters to treat aquaculture water is limited [6]. Planted biofilters are capable to treat and minimize aquaponic wastes efficiently [9, 10]. The most common green leafy plants used for cultivation are mint, basil and lettuce [1]. The fast-growing basil *(Ocimum basilicum*) is known for its use in medicinal and aromatic purpose and has high recommendation among herbaceous plant species to grow as a biofilter part [11, 12]. Basil is widely tested crop and it showed 57 g/day/m^2 of production proving its sustainability in an aquaponic system at a feed input ratio of 99.6 g/day/m^2 [13]. Green leafy herbs such as spinach, lettuce, basil and watercress have low to medium nutritional needs while fruity vegetables like strawberries, tomatoes, peppers, and cucumbers have a higher nutritional need [14]. The ability of the herbs such as basil and mint as the part of biological filters were tested in aquaponic system and the results showed that the herbs could be used as part of the biological filters and that they remove significant concentration in nitrogen compounds and phosphates [15]. The water is allowed to pass through the media bed layers of the biofilter component transplanted with herbs and the phytoremediation takes place [16]. The root systems of such plants work as an effective fibrous network to uptake the contaminants or pollutants in contaminated substrates [17].

The aquaponic systems with less operational cost can be adaptable as food production systems in developing countries [18, 19]. Reduction in the water recirculation duration might be one of the effective solutions to adopt such systems economically. However, limited information is available on research on effect of low recirculation durations on water quality, plant growth and efficiency of biofilter [20]. More studies and experimental investigations are required to study the effect of low water recirculation and filtration periods on water quality in aquaponic systems at low recirculation periods [18, 19]. The major aim of this study was to examine the effects of different water recirculation durations on the DO, NH_3, TAN, NO_2, and NO_3 concentrations of aquaculture water. The second major objective of the study was to test how the biofilters with basil plant as a part of a biofilter in combination with iridescent shark as aquaculture, control the NH_3 and TAN concentrations in low DO conditions.

2 Materials and method

2.1 Aquaponic system

The components of aquaponic system is shown in Fig. 1. The laboratory setup of the system is shown in Fig. 2. The aquaponic system setup was done with a fish tank of 1.08 m^3 of volume and 1000 litres capacity as shown in Table. 3. One biofilter component was set with media bed layers and planted with basil plants to treat the recirculating water. The biofilter was supported by one hydroponic unit to provide additional filtration to the effluent water coming out from biofilter as shown in Fig. 4. PVC (polyvinyl chloride) pipelines of 20 mm diameter were used to bridge the biofilter and aquaculture components. A pump of 18 watts capacity was used to achieve the recirculation of the aquaculture water. Separate outlets were provided to the biofilter and hydroponic. Both the units were connected to the aquaculture unit with the separate outlets. The biofilter was provided with 0.5 m^2 of surface area. The biofilter was set up with 0.9 m length, 0.26 m depth and 0.55 m diameter. The coconut husk and coco peat material was used in the top biofilter layers shown in Fig. 5 and Fig. 6. Apart from the nutrients present in the wastewater, the coconut fibre contains several micronutrients such as Ca, K, Mg, and Na which influence the denitrification process [21]. Such advantages and easy availability of coconut coir makes it preferable than synthetic medium [21]. Several synthetic media like ceramic rings and PVC tubes are being used as the packing material for biofilter systems in general, but the biggest disadvantage is their relatively high cost [21].

The media layers in biofilter were set with 0.03 m of cocopit layer, 0.03 m of coconut husk below top layer, 0.06 m of sand particles as third layer, 0.03 m of gravel as fourth layer, and coarse aggregates at 0.01 m as bottom layer. The hydroponic component was filled with gravel of 0.7-40 mm size and porosity of 0.60. The porous plastic bottles of 1 liter capacity were set at the bottom of the

biofilter and hydroponic unit to carry filtered water to the fish tank through the connected pipe. Aquaculture water was recirculated by pumping it vertically to pass through the biofilter for particulate removal. The effluent water from biofilter was allowed to percolate through the hydroponic unit set at the lower level than biofilter which run under gravity through PVC pipes and falls directly to the fish tank again.

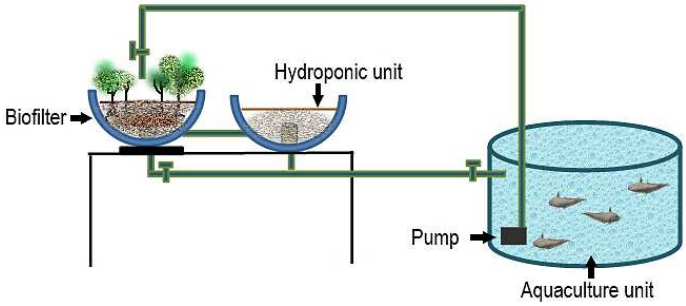


Fig. 1: Aquaponic system and components.



Fig. 2: Aquaponic system setup.



Fig. 3: Aquaculture tank.



Fig. 4: Planted biofilter and hydroponic unit.



Fig. 5: Coconut husk.



Fig. 6: Coco peat.

2.2 Hydraulic conditions and culture

The experimental investigation was carried out with three different recirculating durations for 15 days each to examine the effect on water quality and plant growth. The aquaculture component was stocked with the fish named iridescent shark *(Pangasianodon hypophthalmus* catfish) of the family Pangasiidae. The commercial status of iridescent shark has increased with its economic value and this fish has fixed its place in the markets of many Southeast Asian countries like India, Bangladesh, Thailand, and Malesia [22]. Irridescent shark has a stable market in almost 70 countries while in the countries such as Russia, USA and Europe, this fish is a popular food fish and has increased the trade [22, 23]. The reason behind the popularity of this fish in trade is its growing capacity up to 130 cm in length and 44 kg in weight [23]. The aquaculture unit was stacked with thirty fingerlings of 16 to 20 g weight. The fish was feed with the commercial floating pellets available in the market containing 4 % of fat, 32 % of protein and 10 % moisture. Manual feeding was done twice in the day. Basil (*Ocimum basilicum L*.) plant was selected as part of the biofilter and the plant for growth in the system. Past

experimental studies on hydroponic and aquaponic systems showed that basil is suitable plant for production in such systems [11]. Basil is used as a medicinal herb [24].

3 Sampling, data analysis and statistical analysis

As shown in Fig. 1, the biofilter was set at higher level than the hydroponic trough and the trough was fixed at a higher level than the aquaculture unit. The four weeks old basil seedlings were purchased from local sources. The samples of effluent water were collected from the hydroponic outlet. The samples were collected at varying recirculation durations of 2, 3, and 4 h. The recirculation for each duration was performed in span of 15 days. At the end of each recirculation duration 50 % of aquaculture water was replaced by freshwater and then the next recirculation was started. The effluent water from biofilter ran under gravity to the hydroponic unit. It continued its way until it falls and gets collected in the fish tank completing the cycle of recirculation. The NO_2, NO_3, and TAN content was measured with the colorimetric method. NH3 was calculated from the TAN values and the corresponding multiplication factor taken with respect to pH and temperature. The multiplication factor was taken as per [25]. DO content was measured with the water test kit. The Statistical Package for Social Sciences (SPSS) was used to calculate mean, standard deviation and one-way analysis of variance (ANOVA). The differences of means were evaluated by a range test of Tukey HSD with significant level P equal to or less than 5 %, (less than 1 in 20 chance of being wrong) i.e. $P \leq 0.5$.

4 Results and discussion

4.1 Hydraulic conditions and culture

As shown in Table 1, from the seedling stage, all the plants showed satisfactory growth. No disease or deficiency was observed during the experimentation stages. After three cycles of experimentation of 15 days each, the plant reached an average height of 22 - 25 cm. Fig. 2 shows the effect of recirculation period and flow rate on the growth of plant in terms of height.

Table 1: Average plant growth at different recirculation periods.

Sr. No.	Q [L/min]	Plant growth [cm/d]	Re-circulation period [h/d]
1	6.6	0.30	2
2	4.4	0.50	3
3	4.1	0.58	4

The first experimental turn started with recirculation duration of 2 h/d. The average plant height of 0.30 cm/d was recorded during the first experimental turn. In the next two turns, 0.50 cm/d and 0.58 cm/d were recorded respectively at 3 h/d and 4 h/d durations. It showed that the vertical growth of the plant increased at the different water recirculation durations. The plants showed healthy vertical growth at the duration of 4 h/d than the earlier recirculation durations. The satisfactory growth and count of leaves observed and the leaves looked equally fresh and good in colour as the leaves of locally grown plant. The additional filtration with hydroponic trough worked systematically and it controlled the water quality efficiently. Plants showed no mineral deficiency. A comparative growth of basil in the 45 days of experimentation is shown in Fig. 7. The growth pattern in the chart indicates faster growth of the plants with the increase in the recirculation period. The growth of the plants showed a steady rise at the duration of 3 h/d compared to the other two durations. The experimentation was carried out without the continuous flow of water to test the performance of the system in adverse conditions practically appear in water-scarce areas. The growth is shown for three different recirculation duration spans of 15 days each respectively.

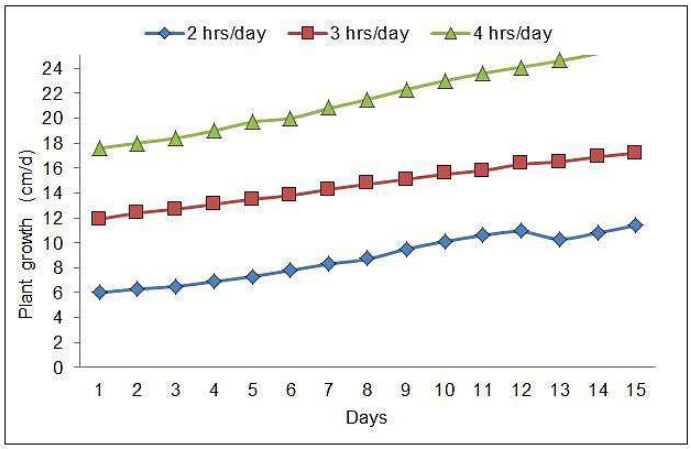


Fig. 7: Plants growth at varying recirculation period.

4.2 Water quality

The experiment was executed for 45 days for testing the basil plant as part of the biofilter. The important parameters of aquaculture water are shown in Table 2.

Table 2: Temperature, pH, and DO levels.

Sr. No.	Parameter	Levels (min-max)
1	Temperature [℃]	27-32
2	pH	7.8-8.2
3	DO before recirculation [mg/l]	3.0 – 5.0
4	DO before recirculation [mg/l]	5.0 – 7.0

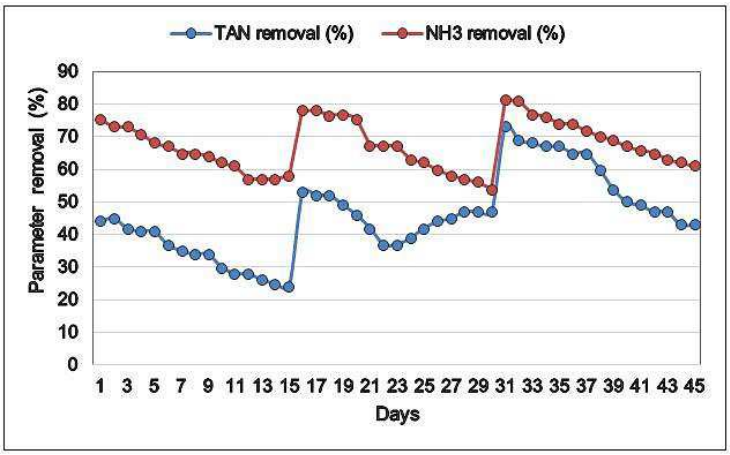


Fig. 8: Removal percentage of NH_3 and TAN at varying recirculation periods.

The DO levels in the fish tank water, before and after recirculation was affected by the tvariations in temperature. The temperature varied from 22 °C to 32 °C during the experimentation. The temperature and pH variations can affect fish, plants, and nitrifying bacteria. The suitable temperatures for the fish growth and nitrifying bacteria in warm water can be 25 °C – 30 °C [5]. Most of the plants prefer colder water temperatures between 20 °C – 25 °C [5].The indoor aquaponic setup in this research kept the temperature changes within the boundary conditions and prevent the fish and plant growth from any harmful effect. The hydroponic unit used as an additional filter for biofilter effluent, actually maintained the temperature of water passing through it. It is very difficult to detect the changes in the chemical reactions of elements in fish tank water. However, theTAN levels, the temperature changes along with pH changes affected the NH_3 concentration level of the aquaculture water. pH changes recorded in between 7.8 to 8.2. Fig. 8 shows the percentage of removal of NH_3 and TAN with varying circulation periods. Both curves showed an immediate rise with changed recirculation period. The curves further showed a gradual decrease until the next change is applied. There are two

possible reasons behind this reduced removal. The first is the addition of NH_3 in the filtered water to the fish tank water. The second is the increased food consumption by growing fish that released more NH_3 in the fish tank water. The effluent water from the planted biofilter travelled to the fish tank through the hydroponic trough which worked as an additional filter for effluent water. This double filtration efficiently controlled the concentration levels of NH_3 and TAN. A biofilm normally develops around the submerged plant roots of aquatic plant-based systems [26]. Higher nutrient levels in the wastewater are controlled by the the density and surface area of such submerged roots [26]. Barley was used in aquaponic units cultured with Arctic char for nutrient removal and removed 76 % of TAN [27]. The basil plant worked effectively as the part of the biofilter by consuming the essential amount of nutrients from the percolating water for the growth. Thus the plant made the purification process more effective. The filtered effluent water showed a significant difference in the influent NH_3 levels at different recirculation periods as shown in Table 3.

The mean values of influent NH_3 0.080 mg/l, 0.091 mg/l and 0.071 mg/l showed significant difference while operating at the durations of 2, 3 and 4 h/d. The percentage removal at 2, 3 and 4 hr/d durations was 65 %, 67 % and 71 %, respectively, as shown in Table 3.

Table 3: Mean values and percentage removal of water quality parameters at varying recirculation periods.

Parameter	Recirculation durations [h/d]	Influent [mg/l]	Effluent [mg/l]	Percentage removal [%]	Tukey HSD[a] subsets nfluent p ≤ 0.05	Tukey HSD[a] subsets effluent p ≤ 0.05
NH_3	2	0.080	0.030	65	1	1
	3	0.091	0.033	67	2	1
	4	0.071	0.022	71	3	2
TAN	2	1.20	0.81	34	1	1
	3	1.40	0.77	45	2	1
	4	1.78	0.77	58	3	1
NO_2	2	0.50	0.20	60	1	1
	3	0.40	0.12	67	1	2
	4	0.30	0.07	63	1	2
NO_3	2	17.0	7.7	53	1	1
	3	14.33	6.00	55	1	2
	4	17.67	6.30	60	1	2

Fig. 9 shows the influent and effluent levels of NH_3 and TAN. The mean values of effluent NH_3 content for the duration of 2 h/d (0.030), 3 h/d (0.033), and 4 h/d (0.022) showed significant difference $P \leq 0.05$ between the duration of 3 h/d and 4 h/d. It proved that the system responded to the variations in recirculation duration conditions and worked without any operational problem. The decreased mean value of the NH_3 level at 4 h/d duration showed that the system responded effectively at 4 h/d than at 3 hr/d with an increased removal percentage to 71 %. The presence of ammonia in water is one of the major reasons for functional problems in micro-organisms [9].

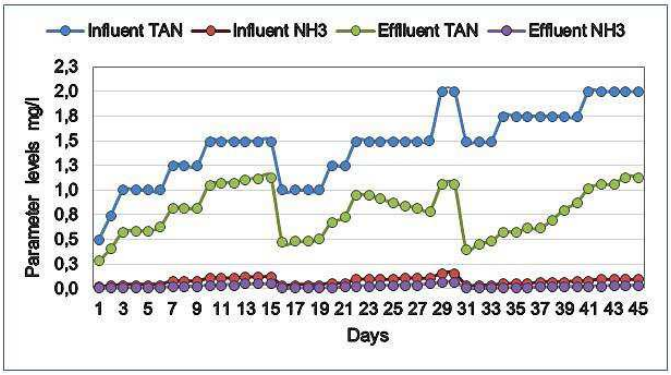


Fig. 9: Influent and effluent concentrations of NH_3 and TAN.

DO levels were observed in the range of 5.0 to 7.0 mg/l after recirculation and ranged in 3.0 to 5.0 mg/l before the next circulation as shown in Fig. 10. The DO levels dropped to 3 ppm due to the short-timed water recirculation durations.

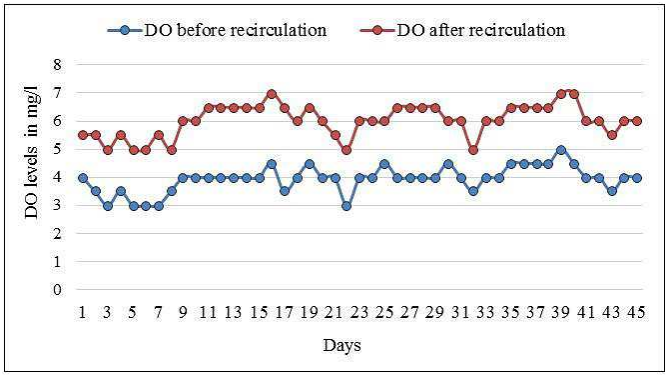


Fig. 10: DO levels before and after recirculation and influent TAN levels.

This system removed 65 % to 71 % NH_3 from the aquaculture water. The hydroponic trough worked on the basis of the principle of the attached growth process. The sufficient surface area of gravel allowed the growth of the micro-organisms for the biodegradation of wastes in the water. The TAN concentrations in the influent water from the fish tank did not differ much at 2, 3 and 4 hr/d durations 1.20, 1.40 and 1.78. No significant difference $P \leq 0.05$ was observed between the reciculation durations of 2, 3 and 4 h/d. The effluent mean values 0.81, 0.77, and 0.77 reflect that the system has responded positively to the increased TAN levels 1.21, 1.40, and 1.78 and improved its performance to 58 % removal at 4h/d. This experimental investigation showed the suitability of basil as a biofilter part in the system with hydroponic trough as a supporting filtration unit. Both basil and hydroponic unit effectively support the system in controlling the NH_3 level in this experiment.

There was a continuous increase in the NH_3 and TAN concentrations in the fish tank water due to the continuous generation of fish excreta resulted into more ammonia formation. At the same time, the filtered effluent added some percentage of NH_3 to the fish tank. As a result, the concentrations of both parameters kept rising gradually in the aquaculture unit. This system effectively removed 60 - 63 % NO_2 as shown in Table 3. The flow duration showed no significant effect on NO_2 generation but a significant effect on NO_2 effluent was observed at 2 h/d. Wastewater from an aquaponic unit with tilapia culture treated with hydroponically grown barley resulted in 98.1 % reduction of NO_2 [27]. The nitrification process occurs when aerobic bacteria like Nitrosomonas and Nitrobacter convert ammonium into nitrite NO_2 and then into nitrate NO_3 [6]. A pH range of 5.8 to 8.5 is suitable for the conversion of ammonium into nitrite [12]. The pH range in this experimental investigation was within this range. The average NO_2 concentrations in the effluent water were in the range of 0.07 - 0.20 mg/l. These concentrations are in accordance with the range of 0.4 - 1.5 mg/l published by previous studies [28]. The process of nitrification accumulates the NO_3 in the aquaculture systems [12]. This system achieved a 53 - 60 % reduction in the NO_3 levels. Barley, in previous studies showed 68.8 - 76.7 % of removal of NO_3 from the aquaculture wastewater in 21 days [27]. Whereas, the difference in the hydraulic loading rates in the past studies showed the poor performance of NO_3 removal. This experiment showed proportionate removal of nitrogen with the increased recirculation duration and recorded highest NO_3 removal of 60 % at 4 h duration..

4.3 Fish growth

The iridescent shark is used as fish species in this experiment. The habitat of fish was not changed. The growth of fish was never the objective of the study. But being an important part of the system, the fish growth in terms of length and weight was noted at the end of each recirculation duration. The average length and weight of the fish at the end of recirculation durations were as shown in Table 4. The reduced activity of the fish due to the periodic decrease in oxygen levels affected the length of fish. The fish become less activity to prevent energy loss and to reduce the oxygen demand. The percentage of weight gain by fish was 47 %, 52 %, and 56 %, respectively, at 2, 3, and 4 hr/d. These results showed that the fish growth was steady at increased recirculation duration and high levels of DO. But the fish can still easily adjust when DO drops below 4 mg/l. The levels were maintained above 3 mg/lit in the study by using an air bubbler whenever required. Some

of the fish gained more weight than some low active fish, but there was no fish death recorded during the study.

Table 4: Average length and weight gain at the end of each recirculation duration.

Sr. No.	Parameter	Flow duration		
		2 [h/d]	3 [h/d]	4 [h/d]
01	Number of fish	30	30	30
02	Avg. initial fish length [cm]	6	8	11
03	Avg. final fish length [cm]	8	11	15
04	Avg. initial weight [g/fish]	40	76	160
05	Avg. final weight [g/fish]	76	160	370

5 Conclusions

This experimental study showed that Basil grew well and suitable for production through aquaponic system. It can be concluded that the production of such herbs and medicinal plants is economic for production through such systems. Such productions are equally safe for human consumption as compared to traditionally produced herbs. The plant shown no disease and mineral deficiency. The average height of basil was in the range of 22 - 23 cm. Basil worked efficiently as a biofilter part for reduction of pollutants in aquaculture water stalked with the fish species such as iridescent shark. The recirculation periods showed efficient nutrient removal and controlled the major nutrients concentrations of NH_3 NO_2 and TAN. With 50 % replacement of fish tank water after every 15 days, the performance of the biofilter was steady without any operational difficulty at 2, 3 and 4 h/d recirculations. The aquaponic systems with low water recirculation periods can provide an effective technology for energy consumption. The concept of introducing a hydroponic trough as a supporting filtration unit in the process worked and helped in maintaining the system performance at the short recirculation periods. The reason behind such performance might be the quick development of pollutant removal processes in pilot-scale aquaponic systems compared to the other large scale systems. The NH_3 and TAN removal percentage by this system ranged in 65 - 71 % and 34 - 58 %, respectively. During the research process, all parts of the system including the biofilter material, the pump, air bubber, the pipes and fixtures worked well. Therefore, no part of the system need replacement for 45 days except 50 % of water replacement after 15 days. Depending upon further investigations of water quality and fish activity, the biofilter material could be replaced. However, more experimentation and investigation is needed on shorter recirculation periods along with the introduction of supporting units like sedimentation tanks and rock filters. The results of this study showed that use of basil as biofilter part for aquaculture water treatment is possible at low recirculation durations of 2, 3, and 4 h/d. With effective removal of nutrients, minimum energy consumption, satisfactory growth of plants, the system can work for 45 days without the daily replacement of fish tank water by fresh water. In areas suffering from water scarcity, such systems can be sustainable.

IAEC Committee approval

The study included fish an aquaculture component. Although the habitat of the species was not changed and there was no experiment or drug test done on the fish, still the authors of this study took the approval of IAEC Committee (Institutional Animal Ethical Committee). The proposal was approved by the IAEC (1211/PO/ac/08/CPCSEA) to conduct research work (Approval no. MES/COP/IAEC/01/ 2018-19).

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