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Design of a Simple Trickling Biofilter for Recirculating Aquaculture System (RAS)

Sarbari Dutta, B. Manojkumar* and Devika Pillai
Kerala University of Fisheries and Ocean Studies, Panangad - 682 506, Cochin, India

Abstract

Recirculating aquaculture system (RAS) is an environment friendly way of farming fish at a high density in a controlled environment. An efficient biofiltration system is the key for the success of any recirculating aquaculture system. Trickling filters are one of the oldest and widely used biofilter types in aquaculture. In the present study a simple trickling biofilter was designed and was tested with two types of filter media *i.e.,* river sand and charcoal for rearing *Litopenaeus vannamei* in a closed RAS. The experiment was carried out for a period of 90 days. Ammonia – nitrogen (NH3-N) was not detected from any of the tanks during the study. The mean pH and nitrite-nitrogen (NH3-N) of both the types' of filter tanks were 7.8 and 0.13 ppm respectively. There was no significant difference in pH and nitrite-nitrogen (NH3-N) between the charcoal based filter tanks (C1 and C2) and sand based filter tanks (S1 and S2). The sand based filter tanks (S1 and S2) showed significantly lower hardness than charcoal based filter tanks (C1 and C2). The average total hardness of sand based filter tanks and charcoal based filter tanks (C1 and C2) were 446.11 ppm and 504.78 ppm respectively. The mean length of the shrimp harvested at the end of 90 days from charcoal based filter tanks (C1 and C2) and sand based filter tanks (S1 and S2) were found to be 8.97 cm and 10.03 cm respectively. The average weight of the shrimp was 5.70 g in charcoal based filter tanks (C1 and C2) and 7.27 g in sand based filter tanks (S1 and S2). Statistical analysis shows that shrimp in sand based filter tanks (S1 and S2) shows higher growth rate than shrimp in charcoal based filter tanks (C1 and C2). The survival rate in sand based filter tanks (S1 and S2) was also higher *i.e.,* 47.5% and charcoal based filter tank (C1 and C2) was 39%.

Keywords: Recirculating Aquaculture System (RAS), Trickling biofilter, *Litopenaeus vannamei*

Introduction

According to FAO statistics global aquaculture production is rapidly increasing, reaching about 106 million tonnes in 2015 (FAO, 2017) when compared with total capture fisheries production where there is a stabilisation in production with 90-95 million tonnes since mid-90's. But there are some challenges for the aquaculture practices such as limitations in quality and quantity of water, availability and cost of land, limitations on water discharges and environmental impacts (Gutierrez-Wing & Malone, 2005). Adoption of environment friendly technologies such as recirculating aquaculture system (RAS) is a method to overcome these challenges. Recirculating systems, filter and clean the water for recycling back through fish culture tanks (Helfrich & Libey, 2003). The most prominent characteristic of these production systems is the presence of a biofilter, which reflects a commitment to internally treat water contaminated by dissolved organics and ammonia rather than discharge them, as in the case of net-pens and flow-through systems, or to treat them extensively as in the case of pond production (Gutierrez-Wing & Malone, 2005). Trickling filters are one of the oldest types of biofilters consisting of a fixed bed of media contained in a vessel which provides substrates for the growth of bacteria. Pre-settled wastewater is continuously 'trickled' or sprayed over the filter. As the water migrates through the pores of the filter, organics are aerobically degraded by the biofilm covering the filter material (Tilley et al., 2014). Among the various aquatic organisms, cultured shrimp occupy a prominent position and the culture of pacific white shrimp *L. vannamei* has gained much importance in

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*E-mail: manojkumarbnair@yahoo.com

India. Rearing of *L. vannamei* in RAS will provide year round production increasing the economic production and annual productivity. So the present study was conducted to design a simple trickling biological filter with river sand and charcoal as media for rearing *L. vannamei* in a recirculating aquaculture system (RAS).

Materials and Methods

Trickling filters with two types of media, river sand and charcoal was designed. The trickling filter was set in a rectangular plastic box (60 cm x 41 cm x 28 cm which is open at the top and having a hole the size of which is sufficient for a 2.54 cm 1" pipe to pass in the centre of its base. A poly vinyl chloride (PVC) pipe of 2.54 cm diameter and with height of 23 cm was fitted through the centre hole of the box, this acted as the central pipe for the passage for the filtered water, 18 cm of this central pipe was inside the filter tank and 5 cm was below the tank. Another pipe of 18 cm length and 3.175 cm (1.25") diameter and closed a tone end and with slots at the other end was used to cover the central pipe inside the filter tank. The end with slots of this suction pipe was in contact with the base of the filter tank.The portion of the central pipe below the base of the filter tank was fitted with an 'L' shaped outlet pipe for the discharge of filtered water (Fig. 1).

The trickling filter with river sand as media consists of 3 layers of bio-sponge, river sand and polyester staple fibre mat respectively. The bottom bio-sponge layer was of 11 cm thick consisting of 2 bio-sponge sheets of 5.08 cm thickness spread at the bottom of the filter tank. Above the bio-sponge 8 kg washed, cleaned and dried river sand was spread to form the sand layer of 7.62 cm height and this reached just below the top portion of the suction pipe. At the top, half part of the sand layer was covered with a layer of polyester staple fiber mat for the removal of solid waste.

The charcoal media trickling filter was also similar in construction of sand filter except that 8 kg of activated charcoal obtained by burning coconut shells was used instead of sand. The activated charcoal was properly washed, dried, weighed to 8 kg before spreading over the bio-sponge layer. The thickness of the charcoal layer was also 7.62 cm. Cross section of the trickling filter with sand and charcoal as a filter media is shown in the Fig. 1 and 2.

Four oval shaped fiber glass tanks of 1000 l capacity were fitted with two types of trickling biofilters. Two tanks C1 and C2 were fitted with charcoal based biofilter and two tanks S1 and S2 were fitted with sand based biofilter to form duplicates. The filter units were kept at the top of the tanks

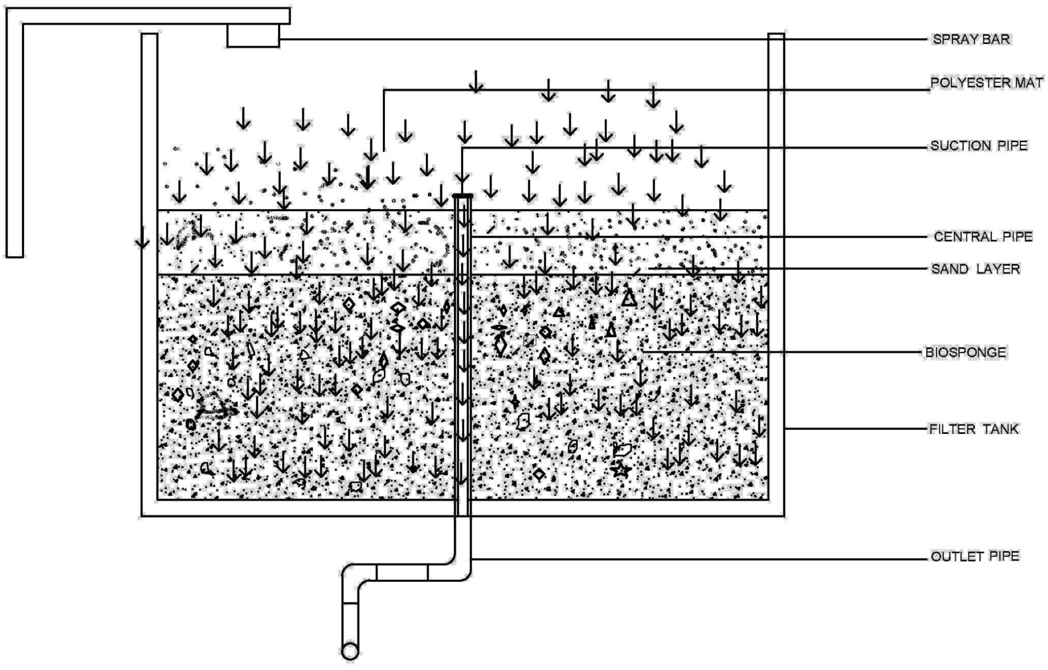

Fig. 1. Cross section of the trickling biofilter with sand as media

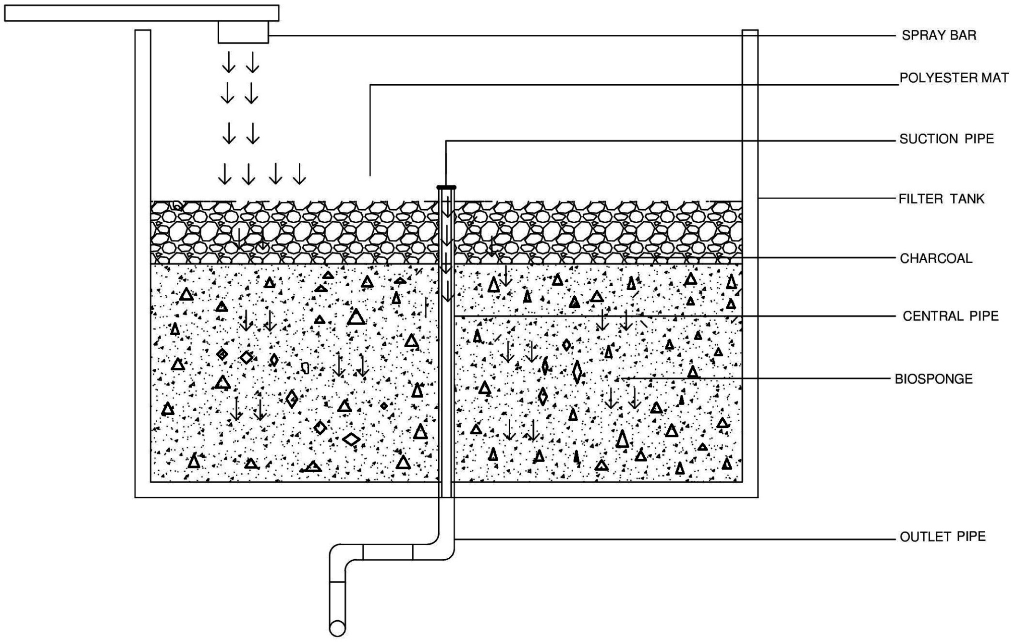

Fig. 2. Cross section of the trickling filter with charcoal as media

supported with steel rods and this was at a height of 93 cm from the water level in the tank. Stone aerator was used in each tank for aeration. A power head with a pumping capacity of 1600 l h^{-1} was used to pump water from the tank to the filtration unit. This submersible power head was connected with a 93 cm long flexible pipe with a spray bar made of PVC pipe kept at the top of the filter. For trickling of water into the filter media there were 8 holes in this spray bar.

The power head, pumps water from the tank through the flexible pipe and is dispersed on the top polyester mat (recron) layer of the filter by the spray bar. The water trickles and the solid waste settles over this polyester mat and when the water cascades down the filter media (sand/charcoal) ammonia is removed. The filtered water moves through the slots in the bottom of the suction pipe and reaches the top of the centre pipe and is discharged to the tank through the central pipe and the outlet pipe. Cross section of the water flow in the recirculatory system is shown in Fig. 3.

Two hundred numbers of specific pathogen free PL 20 of *L. vannamei* obtained from a private hatchery having license from coastal area authority were stocked in each tank on 26/01/16. The average length and weight at the time of stocking was 17 mm and 0.027 gm respectively.

The stocked shrimp were fed *ad libitum* four times a day, 6 am, 10 am, 2 pm, and 6 pm with commercial pelleted shrimp feed (Godrej Agrovet Limited). Initially for 15 days they were fed with juvenile starter feed followed by pelleted grower feed.

Throughout the experiment period the water quality parameters such as temperature, pH, salinity, ammonia – nitrogen (NH3-N), nitrite – nitrogen (NO_2-N) and total hardness were monitored every day at morning 6 am. Temperature was measured by using mercury thermometer daily three times 6 am, 10 am and 2 pm respectively. pH was monitored using universal indicator solution of Nice brand. Ammonia – nitrogen (NH3-N), nitrite – nitrogen NH3-N), total hardness were tested by using Aquacheck testing kit (Himedia). The salinity was measured by using a salinometer and was maintained to 5 ppt throughout the experiment.

Dead shrimp were removed and counted whenever observed. The duration of the experiment was for 90 days and at the end of the experiment the shrimp were harvested from all the tanks, counted and the length and weight were taken individually to study the final growth.

The water quality parameters of all the tanks (C1, C2, S1 and S2) and the length and weight of the

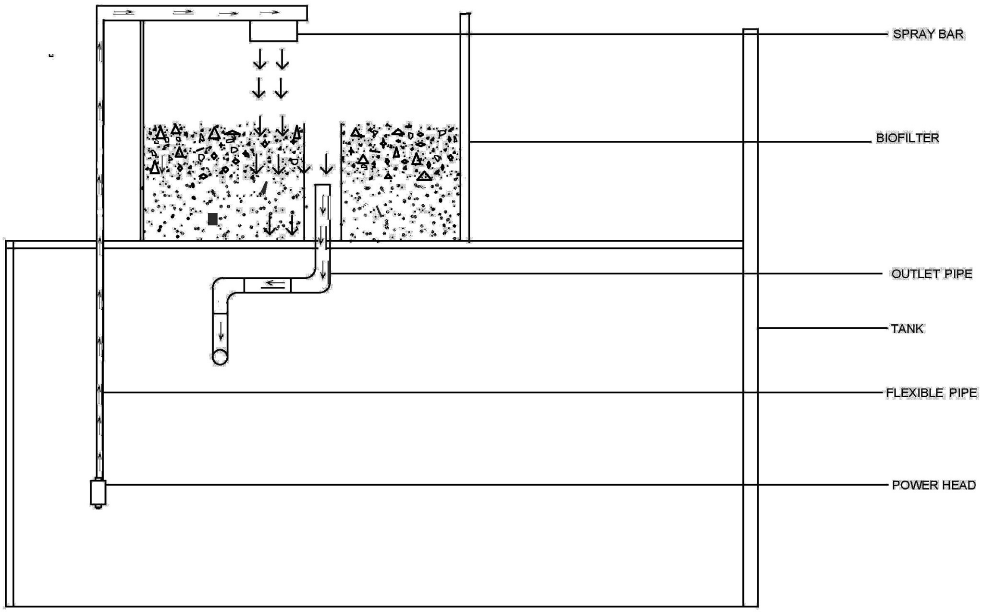

Fig. 3. Cross section of the water flow in the recirculatory system

shrimp harvested from all the tanks (C1, C2, S1 and S2) at the end of 90 days were subjected to statistical analysis using Student t- test by Statistical Package for Social Science (SPSS) Version 20 software to find out any significant difference in water quality parameters and growth rate of the shrimp between sand based filter tanks (S1 and S2) and charcoal based filter tanks (C1 and C2).

Results and Discussion

It was observed that the filter worked properly throughout the experiment. The suction part of the powerhead was covered with fine mesh mosquito cloth to prevent the post larvae stocked in the tank getting sucked up by the pump. The power head in the tank pumped water from the tank through the flexible pipe and dispersed it over the polyester staple fiber mat layer of the filter through the spray bar. The solid waste materials in the water were effectively removed by this layer. Water then percolated through the filter media and entered the suction pipe and once the water level reaches to the top of the central pipe the filtration process is completed.

The filtered water was discharged through central pipe and outlet pipe back into tank. The processes from pumping of water from the tank, filtration and the discharge of filtered water back into the tank are considered as one cycle of filtration. The total time taken for the completion of one cycle was observed to be 15 min.

The water quality throughout the experimental period was found to be conducive for the aquaculture operations. Throughout the present study ammonia-nitrogen was not detected in any of the tanks. Vanwyk, (1999) also observed no ammonia problems in his culture tanks for farming marine shrimp in freshwater aquaculture system. The range of ammonia concentration in the experimental tanks of Sichula et al. (2011) using maize cob activated carbon to remove ammonia from aquaculture water was 0.01 to 0.1 mg l^{-1}. The concentration of ammonia below the level of detection in the present experiment indicates the efficient performance of the filters used. Other parameters such as water temperature, pH, hardness and nitrite nitrogen were within the range suitable for aquaculture. There was no statistically significant difference between the values of water temperature, pH and nitrite – nitrogen between the culture tanks using sand based and charcoal based filters. The mean values of total hardness in charcoal based filter tanks was 504.77 ppm and that of sand based filter tanks was 446.11 ppm (Table 6 and Fig. 9). This difference was found to be significant statistically ($t = 6.47$ and $p < 0.05$). The total hardness averaged 400 mg l^{-1} in a study of farming marine shrimp *L. vannamei* in freshwater condition by Van Wyk (1999). The comparative analysis of three biofilter types treating waste water produced in fresh water recirculating aquaculture system by Hall (1999) showed that hardness ranged from 150 – 280 mg l^{-1} in all the systems studied. But in a study by Resley et al. (2006) on the growth and survival of juvenile Cobia at different salinities in a recirculating aquaculture system the total hardness ranged from 283 ppm in dechlorinated tap water to 5288.9 ppm in 30 ppt treatment. The total hardness above 400 ppm obtained in this experiment may be due to the fact the culture was carried out in water with a salinity of 5 ppt. The water temperature ranged from 24 to 30°C in all the tanks and there was no temperature difference between the tanks (Table 1, 2, 3 and Fig. 4, 5, 6). Kumlu & Turkmen, (2010) while studying thermal tolerance of *L. vannamei* observed that in culture conditions the temperature should never fall below 12ºC but the higher temperatures can go up to 34ºC. The average pH remained 7.83 in all the tanks which fall within the optimal range for aquaculture (Table 4 and Fig. 7). Hall in (1999) in his comparative analysis of three biofilters treating waste water produced in recirculating aquaculture system, the pH values in all systems typically ranged from 7 to 7.4. Only in a study by Lekang & Kleppe, (2000) there was a fall in in the pH in a recirculatory system where they studied the efficiency of nitrification in trickling filters using different media. They found out that over the first 7 weeks the pH value at the outlet of the trickling column showed little variability. After 7 weeks the value gradually fell for the remainder of the period, for all of the media. The pH fell below 6.5. The average nitrite nitrogen concentration in all the culture tanks was 0.13 ppm which was well below the lethal level (Table 5 and Fig. 8). Gross et al. (2004) reported that the exposure of white shrimp *L. vannamei* to a concentration of 4 mg l^{-1} for 2 days reduced their growth but did not affect their survival. They suggested a safe concentration of 0.45 mg l^{-1} for the pond culture of this species at a salinity of 2 g l^{-1}. Nijhof & Klapwijk, (1995) showed that reported nitrite ''accumulations'' in recirculation fish culture systems, in which trickling filters are applied stabilize around an equilibrium concentration at which all the nitrite produced is oxidized, indicating equal rates of total ammonical nitrogen (TAN) and NO_2-N removal.

The average length of the shrimp harvested after 90 days of the present study from charcoal based filter tanks and sand based filter tanks were 8.97 and 10.30 cm respectively. The sand based filter tanks (S1 and S2) showed significantly higher growth than the charcoal based filter tanks (C1 and C2) and rate of survival was also more in sand based filter tanks

Table 1. Average temperature in ºC at 10 days interval recorded at 6 am in all the tanks during the experimental period.

Days Treatment	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
C1	24.3	25.2	26.4	27.3	28.2	28.5	28.7	29.0	29.5
S1	25.4	25.2	26.4	27.3	28.2	28.5	28.7	29.0	29.5
C2	24.4	25.3	26.4	27.3	28.3	28.5	28.7	29.0	29.5
S2	24.5	25.3	26.4	27.4	28.3	28.6	28.7	29.0	29.5

Table 2. Average temperature in ºC at 10 days interval recorded at 10 am in all the tanks during the experimental period

Days Treatment	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
C1	24.6	26	26.7	27.7	28.7	28.7	29	29.2	29.8
S1	24.6	26	26.7	27.7	28.7	28.7	29.2	29.2	29.8
C2	24.6	26	26.7	27.7	28.7	28.7	29.2	29.2	29.8
S2	24.6	26.2	26.7	27.8	28.7	28.8	29.2	29.3	29.8

Table 3. Average temperature in ºC at 10 days interval recorded at 2 pm in all the tanks during the experimental period

Days Treatment	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
C1	27.2	28.4	29	29.0	30.0	30.1	30.3	30.5	30.7
S1	27.2	28.4	29	29.2	30.0	30.2	30.3	30.5	30.7
C2	27.2	28.4	29	29.2	30.0	30.2	30.3	30.6	30.7
S2	27.3	28.5	29	29.3	30.0	30.2	30.4	30.6	30.8

Table 4. Average pH values at 10 days interval recorded in all the tanks during the experimental period

Days Treatment	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	Highest value	Lowest value
C1	7.93	8.57	8.7	8	8	7.8	7	7.3	7.5	9	7
S1	7.59	7.02	7.8	7.9	8.7	8	8	7.9	7.5	8	7
C2	7.62	7.64	8.02	8.8	8	7.8	7	7.7	7.5	9	7
S2	7.59	7.72	7.75	7.9	8.8	8	8	7.8	7.5	8	7.5

Table 5. Average nitrite-nitrogen values at 10 days interval recorded in all the tanks during the experimental period

Days Treatment	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	Highest value	Lowest value
C1	0.13	0.2	0.1	0.1	0.16	0.13	0.18	0.11	0.1	0.2	0
S1	0.13	0.14	0.1	0.1	0.1	0.17	0.16	0.21	0.1	0.4	0
C2	0.18	0.2	0.14	0.16	0.16	0.12	0.11	0.1	0.1	0.8	0
S2	0.15	0.15	0.1	0.1	0.1	0.14	0.14	0.15	0.1	0.8	0

Table 6. Average total hardness at 10 days interval recorded at in all the tanks during the experimental period

Days Treatment	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	Highest value	Lowest value
C1	450	472.5	452.5	507.5	580	557.5	550	493	582.5	600	275
S1	385	300	497.5	542.5	465	425	530	492.5	492.5	600	225
C2	322.5	437.5	490	505	522.5	497.5	545	493	575	600	275
S2	277.5	360	337.5	462.5	497.5	482.5	582.5	490	497.5	575	200

than charcoal based filter tanks (Table 9). The mean weight of the shrimp harvested from sand based filter tanks (S1 and S2) was 7.27 g and charcoal based tanks (C1 and C2) was 5.70 g. Sand based filter tanks (S1 and S2) showed higher growth and survival rate. The general low growth of the shrimp in these tanks could be attributed to the low natural production in the experimental tanks. Otoshi et al. (2003) while comparing the growth rate of shrimp reared in bio securer circulating aquaculture system and flow through pond also attributed the slower growth rates in the RAS to the lack of natural productivity, which provides supplemental nutrition to the shrimp. Similar observations were made by Van wyk

Table 7. Summary statistics of water quality parameters of different treatments

Tank	N	Mean	Std. Deviation	Std. Error Mean
pH: 1 charcoal based filter tank	180	7.8356	.59052	.4401
2 sand based filter tank	180	7.8331	0.20093	0.01498
Nitrite				
1 Charcoal based filter tanks	180	0.1381	0.07210	0.00537
2 Sand based filter tanks	180	0.1341	0.08027	0.00598
Hardness				
1 Charcoal based filter tanks	180	504.7778	80.95765	6.03423
2 sand based filter tanks	180	446.1111	90.65336	6.75690

Table 8. Comparison of water quality parameters of different filter media by Student t-Test

	Levene's test for equality of variance		t- test for equality of means		P value
	F	Sig	T	Df	
pH: Equal variances assumed	98.642	0.000	0.054	358	0.957
Equal variances not assumed			0.054	219.899	0.957
Nitrite					
Equal variances assumed	0.162	688	0.490	358	0.624
Equal variances not assumed			0.490	353.957	0.624
Hardness					
Equal variances assumed	2.644	.105	6.476	358	0.00
Equal variances not assumed			6.476	353.515	0.00

Table 9. Survival and growth rate of the shrimp in all the tanks

	Number of shrimp stocked	Number of shrimp harvested	Survival percentage	Average initial weight	Average final weight	Average initial length	Average final length
C1	200	80	40%	0.027 g	6.56	17 mm	9.18 cm
S1	200	92	46%	0.027 g	6.98	17 mm	10.43 cm
C2	200	76	38%	0.027 g	5.83	17 mm	8.80 cm
S2	200	98	49%	0.027 g	7.54	17 mm	10.67 cm
CS	300	217	72.33%	0.027 g	7.8	17 mm	10.16 cm

Table 10. Summary statistics of growth parameters, length and weight of cultured shrimp *Litopenaeus vannamei* of different filter media

TANKS	N	Mean	Std. Deviation	Std. Error Mean
Length (cm)				
1. charcoal based filter tank	40	8.9700	1.07756	0.17038
2. sand based filter tanks	40	10.300	1.07322	0.16969
Weight (g):				
1. charcoal based filter tank	40	5.7000	1.23434	0.19517
2. sand based filter tanks	40	7.2683	1.10106	0.17409

Table 11. Comparison of growth parameters, length and weight of cultured *Litopenaeus vannamei* of different filter media by Student t- test

	Levene's test for equality of variances		t- test for equality of means		P value
	F	Sig.	t	Df	
Length Equal variances assumed	0.082	0.776	-5.531	78	0.000
Equal variances not assumed			-5.531	77.999	0.000
Weight Equal variances assumed	0.064	0.801	-5.996	78	0.000
Equal variances not assumed			-5.996	77.003	0.000

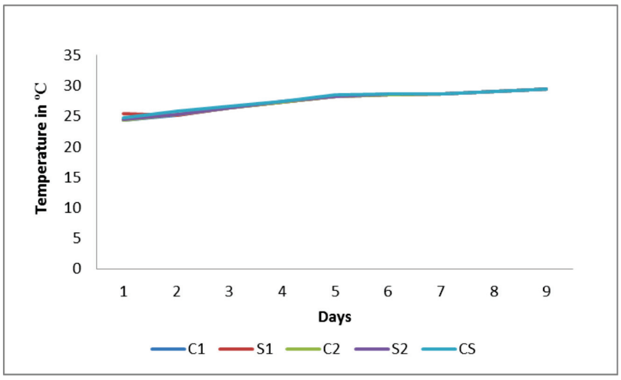

Fig. 4. Average temperature in °C at 10 days interval recorded at 6 am in all the tanks during the experimental period.

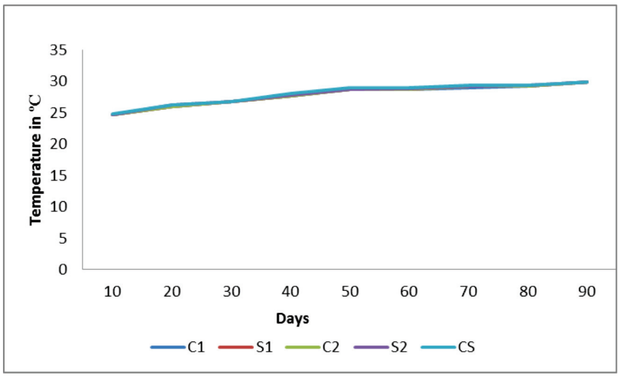

Fig. 5. Average temperature in °C at 10 days interval recorded at 10 am in all the tanks during the experimental period.

(1999) and Scura (1995) reported that *L. vannamei* grows well in ponds with high levels of natural productivity. In the present experiment algal growth was observed in the bottom and sides of all experimental tanks, this was cleaned manually but the algae grew again in tanks fitted with sand filter. This presence of algal growth may be the reason for the significantly better growth and survival observed in this treatment. Van wyk (1999) reported that survival rate varies from 40 to 80% in his comparison of three phase and single phase culture of *L. vannamei* fresh water recirculating system. In

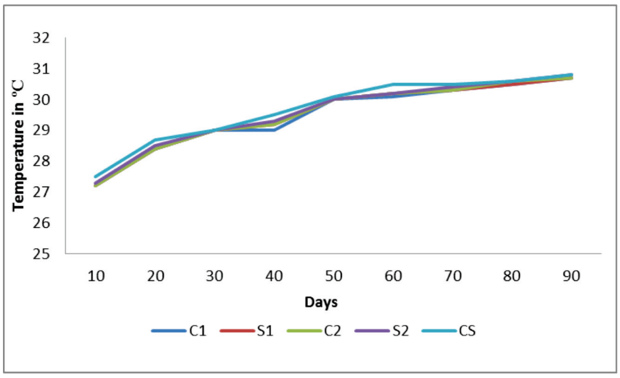

Fig. 6. Average temperature in °C at 10 days interval recorded at 2 pm in all the tanks during the experimental period

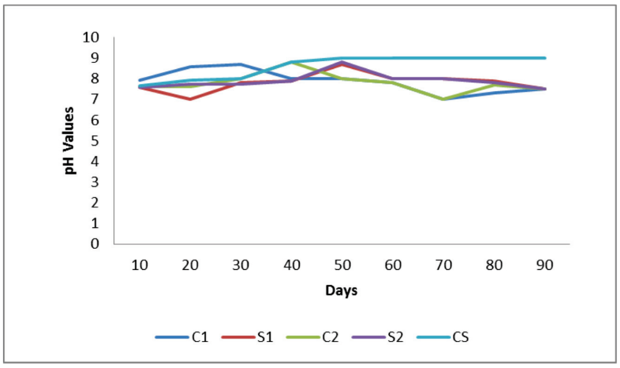

Fig. 7. Average pH values at 10 days interval recorded in all the tanks during the experimental period

the present study also the survival rate varied from 47.5% for sand filter and 39% for charcoal filter. He also attributed the low survival rate to the cannibalism exhibited by the species. Since there was no hiding place provided for the moulting shrimp in the present experimental tanks cannibalism must have taken place. Moreover most of the mortality was reported during the molting stage of the shrimp. From this experiment it can be concluded that even though recirculatory aquaculture systems can be used for the culture of shrimp and fishes, the success of such system will depend upon the presence of natural feed in the system. In order to obtain better growth and survival natural feed is essential. This system can be modified by introducing additional substrate in the tanks this will provide shelter for the shrimp during moulting and also enhance the productivity of the system by favouring the growth of periphyton in the tank. This will act as natural feed for the shrimp. The filtering rate should also to be studied and standardised so that the water flow will not dislodge the periphyton.

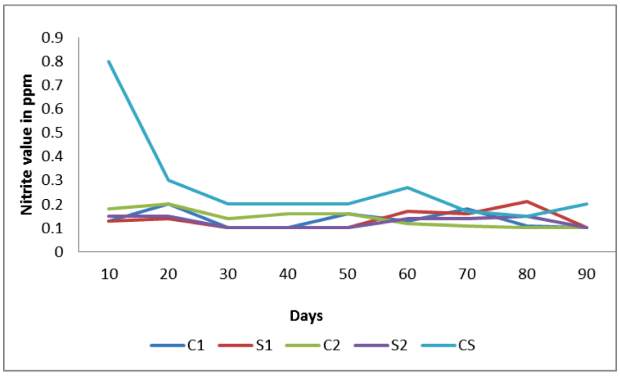

Fig. 8. Average nitrite- nitrogen values in ppm at 10 days interval recorded in all the tanks during the experimental period

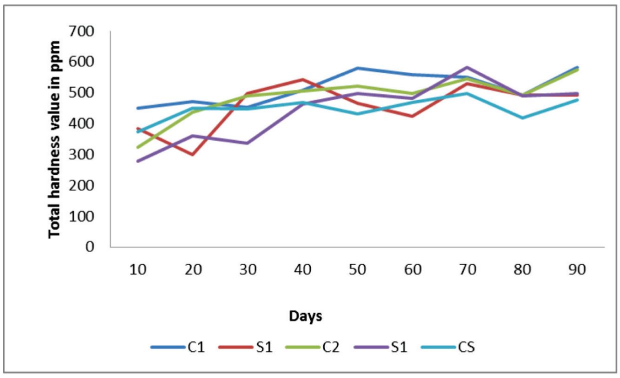

Fig. 9. Average total hardness values in ppm at 10 days interval recorded in all the tanks during the experimental period

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