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Sustainable growth of *Litopenaeus vannamei* in intensive raceways of biofloc technology

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Abstract*— Shrimp (Litopenaeus vannamei) culture and production at industrial have high value in India with higher export quantity. With the application of scientific research and development methodologies, aquaculture may be expanded to become a larger and more economically relevant industry. Furthermore, due to the high protein content of shrimps, this aquaculture has gained popularity among the general public. Despite all of these positive aspects, shrimp farming is plagued by diseases, which result in increased production losses. This present study provides Biofloc technology to cope with this specific problem which is considered to boost the development and survival of shrimp. This study was conducted in 3 different parts, namely, preparation of the tank, establishing the Raceway system, preparing the flocs in the tank, monitoring water quality, conducting microbiological tests, and managing the feed regularly. The study has found that there is significant growth in the test tanks as compared to the control tank. The test tanks had shown 30% more growth as compared to that of the control tank. The survival rate of shrimp is also significant in test tanks than in the control tank. The other findings, especially the condition of the water, are correlated and discussed at the appropriate place. The study found that Biofloc technology is effective in maintaining water quality, including hardness, minimising shrimp production loss, and enhancing shrimp culture average growth. This technology is the way shrimp culture will go in the future, and it should be applied across the country.

***Index Terms*— shrimp culture, biofloc technology, raceway system, aquaculture, litopenaeus**

I. INTRODUCTION

Shrimp culture and industrial production have high value in India with higher export quantity. Animal husbandry and fisheries are required to provide the increased need for durable food, particularly animal protein. This is also aided by the scientific development of techniques to expand aquaculture to grow it to a bigger industry. Capture fisheries are being overexploited, increasing aquaculture production. However, owing to overexploitation of fish meal and oil, as well as environmental contamination from the release of hazardous metabolites, aquaculture is now facing both environmental and economic unviability **[1]**. Despite the advent of various diseases, the development of scientific techniques in aquaculture has brought modification in the aquaculture industry and increased the production and export of shrimp **[2, 3]**. One such development in the aquaculture industry is Biofloc technology (BFT). It is a novel and cost-effective method that converts hazardous elements for fish and shellfish, such as nitrate, nitrite, and ammonia, into a valuable product, proteinaceous feed. It's a technique utilised in aquaculture systems with low or no water exchange, high stocking density, good aeration, and biota generated through biofloc. In the event of culture tanks exposed to the sun, biofloc culture will be fruitful **[4]**. This technique helps in maintaining the water quality by nitrogen uptake that are produced in-situ protein of micro-organisms, maintenance of nutrition and also helps in the competition of reproduction, growth, and survivability of the organism with the pathogens. Mainly fish and shrimp are grown by this BFT **[5, 6]**. BFT eliminates harmful metabolites from aquaculture ponds and helps to retain more nitrogen in the form of fish or shrimp biomass through microbial mass protein synthesis. BFT is a C/N ratio optimization method that requires additional understanding for optimum use **[7]**. Shrimp aquaculture gained importance due to its high protein value. But this aquaculture has suffered production loss due to the advent of various diseases, especially Acute Hepatopancreatic Necrosis Disease (AHND) leading to higher mortality among the shrimps **[8].** This diseased outcome is the result of a suboptimal growth environment along with poor nutrition. To prevent production loss, techniques like culturing shrimp by employing a Biofloc system are proved to be an efficient strategy for the successful production of shrimps. The fundamental of the Biofloc system is based on the regular recycling of nutrients, regulating the ratio of Carbon and Nitrogen (C/N) of the water by changing the amount of carbohydrate or carbon. The application of the Biofloc system has resulted in a significant increase in the growth and survival rate of shrimp **[9]**. It has also been found that the innate immunity of the shrimp has been positively stimulated by the Biofloc technique, hence, increasing the ability of the shrimp to prevent infection against pathogens **[10]**. Considering all these potential applications and advantages of the BFT, we have undertaken the research work to implement sustainable growth of *Litopenaeus vannamei* in intensive raceways of biofloc technology.

II. MATERIALS AND METHODS

This study was conducted in 3 different stages namely, preparation of the tank, establishing the Raceway system, preparing the flocs in the tank, monitoring water quality, conducting microbiological tests, and managing the feed regularly.

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A. Tank Preparation

The study was carried out at West Godavari District, Andhra Pradesh, India. PL was procured from the Kakinada hatchery. *L.vannamei* seeds were conformed negative for the white spot syndrome virus WSSV by PCR assay. The seeds were transported in oxygenated double-layered polythene bags with crushed ice packs between the inner and outer covers of the bags to maintain optimum temperature and in turn to reduce stress to the shrimp and the entire setup was packed in a carton. All the tanks were cleaned and disinfected using bleaching powder and dried for 2 days. Besides the above, there was a reservoir tank in the size of 250 sq.m. This reservoir tank was filled with 6ppt water and was treated with bleaching powder at 5 ppm and left for 3 days for dechlorination. The filter bags were checked properly, which was fitted in the inlet and outlet pipe, then the pumping was done to all tanks. The seeds were brought to the farm side and were treated with potassium permanganate, then the tank water was added slowly into the seed bags to adjust the salinity and pH subsequently the seeds were released slowly into the tanks.

B. Raceway system

Fig. 1 Raceway system developed during experiments

In this raceway system total of 9 tanks were confined for the experiment that is A1, A2, A3, B1, B2, B3, and C1, C2, C3. The representative raceway system is presented in **Figure 1**. Among 9 tanks C1, C2, C3 were taken as control i.e. without application of Biofloc, and A1, A2, A3, B1, B2, B3 were taken as a test which consist of Biofloc. All the tanks were supplied with aeration for a sufficient supply of oxygen which is necessary for the formation of Biofloc and also for shrimp. The size of the tanks of raceways was designed in an increasing manner i.e A1, B1, and C1 are 56 sq.m, A2, B2, and C2 are 112 sq.m, A3 B3 and C3 are 224 sq.m. At first, the PL was stocked in A1, B1, and C1 tanks with a stocking density of 120 PL/ sq.m. After 30 days they were transferred to A2, B2 C2 along with floc water with internal large pipes, and then after 90 days, they were transferred to A3, B3, C3 up to harvest i.e 120 days.

C. Floc preparation in the tanks:

Before introducing the seed, the Biofloc tanks (i.e A1, B1) were prepared as per the protocol described by Yoram Avnimelech. On the first day, ammonium chloride was added to initiate a nitrogen source in the system. On day 3 and day 5 carbon sources were added and on day 7 double the number of carbon sources was added. On day 9 PL-9 was introduced into the tanks. Due to the addition of carbon and nitrogen sources, the colour of the water changed to light brown indicating the formation of floc. This was done to acclimatize the animal to the microbial floc environment. Imhoff cones were used to measure the quantity of Biofloc. The cones have marked graduations on the outside that can be used to measure the volume of solids that settle from 1 liter of system water. The time interval was usually 10 to 20 min to settle the floc. The maximum volume of the floc can be from 10 to15ml /lit which will provide good functionality in Biofloc systems for *L.vannamei.* Initially, 0.1ml of floc was observed and it gradually increased.

D. Water quality parameters:

Throughout the study period, sampling of water was carried out daily up to harvest. Water quality parameters such as Temperature, Dissolved Oxygen (DO), pH, Ammonia, Nitrite, Nitrate, and Total alkalinity were recorded daily in the culture systems. Sampling was done usually between 6:00 to 9:00 during the morning hours and between 5:00 to 6:00 during the evening hours. The pH of the water was measured by using the laboratory model Elico pH meter. Modified/Winkler's titration method APHA (2005) was adopted to estimate the dissolved oxygen. Water temperature was measured using a mercury thermometer with an accuracy of 0.1^{o} C. Transparency of the water column was assessed with the help of a Secchi disk. Salinity was estimated with the help of a hand refractometer. Alkalinity, calcium, magnesium, total ammonia-N, nitrite-N, nitrate-N, and water hardness were determined as per the standard methods APHA (2005). The floc volume was determined by adopting the method explained by Avnimelech (2012).

E. Microbiological Test:

A microbiological test was also done regularly thrice a week. To observe the *Vibrio* count, the spread plate technique was used. 1ml of cultured water was serial diluted and 108, 109 ml of serially diluted culture water was spread on Thiosulfate citrate bile salts sucrose (TCBS) agar and incubated overnight at 37°C to find out the presence of *Vibrio* spp.

F. Feed management:

Up to PL 14, they were fed with PL feed and then with Blanca feed pellets (CP Aquaculture, India private limited) four times daily at 6 am, 10 am, 2 pm, 6 pm. The feed quantity was estimated depending on the floc volume. No water exchange

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was done during the culture period except the addition of water to refill the water levels after transferring the animal into A2, B2, and A3 B3 and after siphoning out the wastes. Sludge was removed regularly to control Nitrite and TSS in the system.

III. RESULTS

In our experiment, the quality of the water in the Biofloc system was monitored weekly for 120 days, and then it was compared with normal water (control). The quality parameters of the water in the Biofloc system had revealed that the salinity was 6-7 ppt, the temperature was determined at 28-29^{o}C, the pH was varied from 7.8 to 8.4. All the water quality parameters of the Biofloc system are given below (Table 1)

Table 1 The parameters of water quality in the biofloc system

Sr. No	Parameters	Range
1	Salinity (ppt)	6 – 7
2	Temperature (^{o}C)	28 – 29
3	pH	7.8 - 8.4
4	DO (mg/lit)	6.5 - 7.5
5	Transparency (cm)	13 – 14

We also monitored the other parameters including the concentration of ammonia, nitrite, and nitrate, and compared them to that of control (Table 2). The graphical representation is also presented in Figures 2-4 respectively.

Table 2: The parameters of water quality in Test tanks compared to control tank

Sr. No	Days	Ammonia average in 2 test tanks (mg/lit)	Ammonia average in control tank (mg/lit)	Nitrite average in 2 test tanks (mg/lit)	Nitrite average in control tank (mg/lit)	Nitrate average in 2 test tanks (mg/lit)	Nitrate average in control tank (mg/lit)
1	1	0.07	Nil	0.01	Nil	Nil	Nil
2	15	0.05	0.13	2.2	1.2	60	30
3	30	0.13	0.08	1.8	1.9	80	50
4	45	0.05	0.05	1.2	1.9	110	40
5	60	Nil	0.03	0.7	2.2	120	50
6	75	Nil	0.05	0.8	2.4	120	40
7	90	Nil	0.03	0.5	2	140	50
8	105	Nil	Nil	0.4	2	100	50
9	120	Nil	Nil	0.4	2.2	100	40

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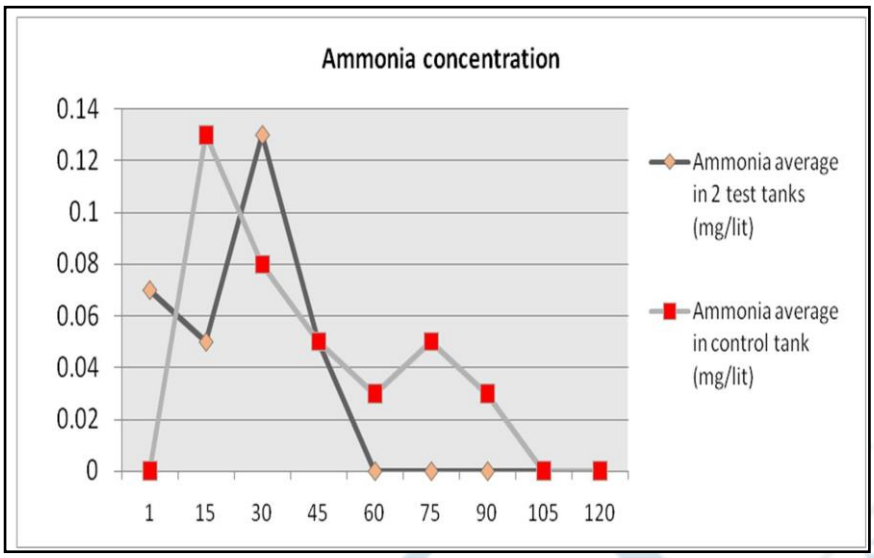

Fig. 2 The concentration of Ammonia in 2 test tanks and control tank and their variations with time

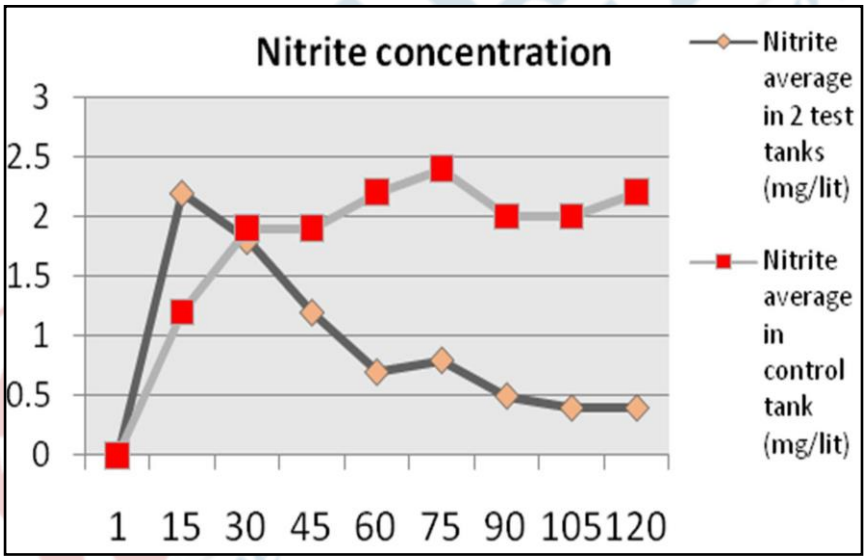

Fig. 3 The concentration of Nitrite in 2 test tanks and control tank and their variations with time

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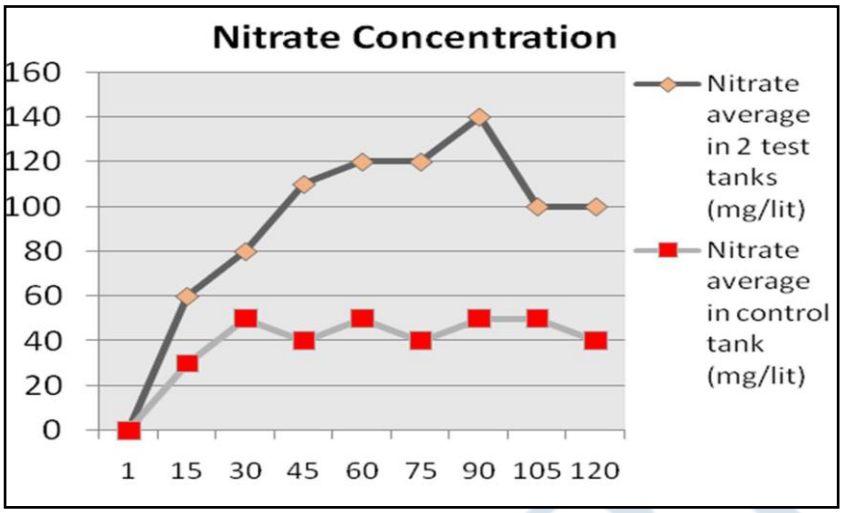

Figure 4: The concentration of Nitrate in 2 test tanks and control tank and their variations with time

The floc volume was measured for the full period of 120 days and was recorded (Table 3). The graphical representation is sown in Figure 5.

Table 3 The average floc volume in Test tanks

Sr. No	Days	Average floc volume in 2 tanks in ml/L
1	1	0.1
2	15	6
3	30	13
4	45	14
5	60	15
6	75	15
7	90	15
8	105	15
9	120	15

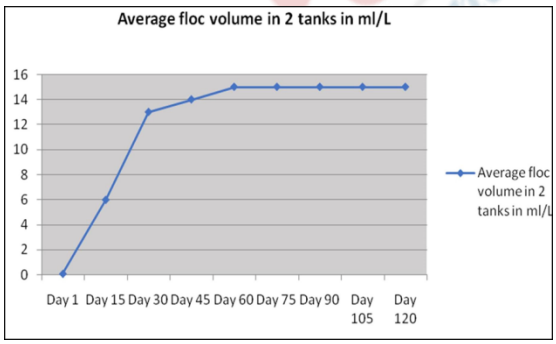

Fig. 5 The concentration of floc (ml/L) in 2 test tanks and its variations recorded with time

The measurement of the average concentration of Carbonate (CO3), Hydrogen Carbonate (HCO3), and Total Alkalinity in Test tanks are given below in Table 4.

Table 4 The average concentration of CO3, HCO3, and Total Alkalinity in Test tanks

Sr. No	Days	Co3 Average in 2 test tanks (mg/lit)	HCo3 Average in 2 test tanks (mg/lit)	Total Alkalinity Average in 2 test tanks (mg/lit)
1	1	20	80	100
2	15	25	80	105
3	30	35	100	135
4	45	30	110	140
5	60	20	140	160
6	75	30	140	170
7	90	35	155	190
8	105	20	145	165
9	120	25	135	160

The growth of *Litopenaeus vannamei* in two test tanks was also monitored and recorded on regular days and compared with the control tank. On Day 1, the growth of *Litopenaeus vannamei* was the same in the 2 tanks and the control tank. Later on, it revealed that the growth of *Litopenaeus vannamei* occurred much faster than that of the control tank. On the last day of our monitoring, it was found that there was a significant difference between the growth in the 2 test tanks as compared to the control tank. The final result has shown that the average growth of shrimp in the test tanks is 30% more than that of the control tank. Table 5 shows each of the results in 2 test tanks and control tanks at every DOC while Figure 6 and Figure 7 represent the growth variation in 2 test tanks and control tanks schematically.

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Table 5 The average growth of *Litopenaeus vannamei*in 2 test tanks and in the control tank

Sr. No	DOC	Average growth in 2 test tanks	Growth in the control tank
1	1	0.01	0.01
2	15	1.2	0.9
3	30	2.3	1.5
4	45	5.2	4.4
5	60	10.6	7.8
6	75	14	9.5
7	90	18.4	13.2
8	105	22.5	17
9	120	27.5	21.2

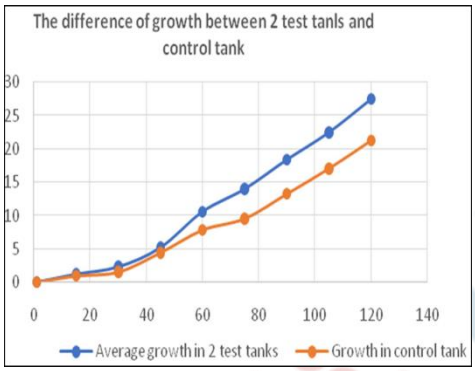

Figure 6: The average growth of *Litopenaeus vannamei*in 2 test tanks and in the control tank

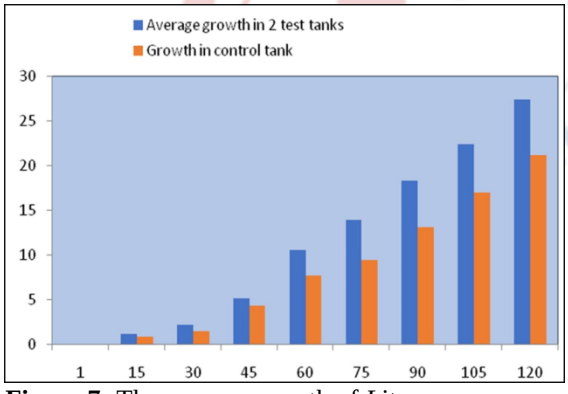

Figure 7: The average growth of *Litopenaeus vannamei*in 2 test tanks and in the control tank

The study also determined the hardness of the water in the biofloc system at the regular interval. The hardness ranged between 2400 ppm and 2600 ppm. The hardness of water was recorded each day of monitoring and is given below (Table 6 and Figure 8).

Table 6 The hardness of the water in the test tanks

Sr. No	Days	Calcium (ppm)	Magnesium (ppm)	Hardness (ppm)
1	1	245	441	2450
2	15	240	480	2600
3	30	250	426	2400
4	45	265	369	2200
5	60	250	426	2400
6	75	290	438	2550
7	90	245	441	2450
8	105	240	480	2600
9	120	250	426	2400

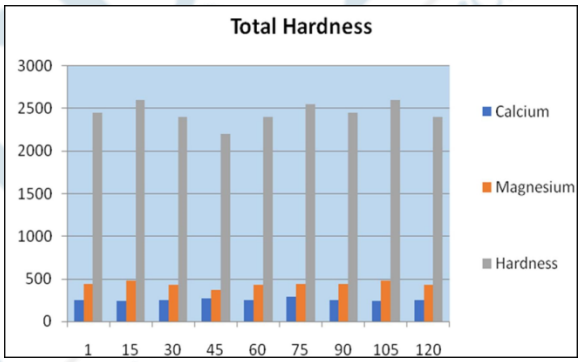

Figure 8: The hardness of water and concentration of calcium and magnesium 2 test tanks and in the control tank

It was found that 77% and 76% of the shrimp survived in tank 2 and tank 3 respectively while in the control tank, only 60% had survived (Figure 9). H_2S is nil during the culture period.

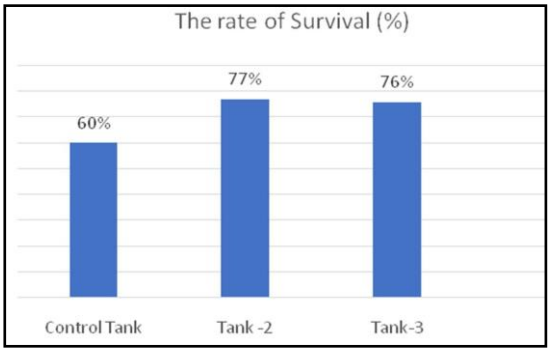

Fig. 9: The survival rate of shrimp in 2 test tanks and control tank

IV. DISCUSSION

Biofloc Technology (BFT) is seen as a new "blue revolution" since nutrients may be continually recycled and reused in the culture medium while minimising or eliminating the need for water exchange. BFT is an acronym for aquaculture approach based on in-situ microorganisms that is environmentally

friendly production. Biofloc is the aggregates of suspended growth in ponds/tanks live and dead particulate organic matter, phytoplankton, bacteria, and grazers of living and dead particle organic matter, phytoplankton, bacteria, and grazers of living and dead particulate organic the microbes It is the use of microbial activities within the pond/tank to do this. supply nourishment for cultured organisms while also acting as a water source remedy for treatment As a result, this system is also known as active suspension ponds or active suspension ponds. Green soup ponds, for example, are heterotrophic ponds. This study has presented a significant perspective of Biofloc technology in shrimp culture. BFT is a green aquaculture strategy that goes by a variety of names, including zero exchange autotrophic-heterotrophic system **[11, 12]**, active sludge or suspended bacterial based system, Single-cell protein production system, Microbial floc system, and so on. Biofloc is now mostly utilised as an alternative protein to fish meal, which is primarily generated in the form of microbial meal **[13]**. The nutritional acceptability of biofloc has been studied by several researchers. Biofloc technology can be used to bring a desirable result in shrimp culture by preventing the occurrence of diseases in the usual process. This is done by the maintenance of water quality, chemical composition, hardness of the water, reduction of ammonia, nitrite, and nitrate **[14]**. Biofloc technology, according to the study, is the best for closed system management and biosecurity in aquaculture. The periodic increase in total suspended solids, which causes clogging in shrimp and fish gills and necessitates more energy to fulfill the oxygen requirement, is one of the most dangerous aspects of this technique. The present study determined the concentration of ammonia, nitrite, and nitrate in 2 test tanks and compared it with that of the control tank. It was found that the ammonia concentration in the test tanks became nil since the 60th day of monitoring while it took 105th day in the control tank. A higher level of ammonia can inhibit chitinase expression, growth, phenoloxidase, and antimicrobial activity in shrimp **[15]**. Nitrite concentration has been reported to cause retarded growth and mortalities among the shrimp population. In our study findings, it revealed that nitrite concentration was significantly lesser than that of the control tank **[16]**. Nitrate can also affect the shrimp population by growth retardation. However, nitrate concentration in test tanks was higher than in the control tank, although it was much below the toxic level (which is 220 mg/l) **[17]**. The main finding of this study is that the average growth of *Litopenaeus vannamei* is significantly higher in 2 test tanks as compared to the control tank. This can be attributed to the optimum and favorable conditions provided in the test tanks as compared to the control tank. This is also evidenced by the significantly higher survival rate of *Litopenaeus vannamei* in the test tanks as compared to the control tank.

V. CONCLUSION

The study concludes that the Biofloc technology should be implemented in the shrimp culture to increase yield sufficiently by a significant increase in growth, maintenance of water conditions and imparting immunity to the shrimp. As shrimp culture is quite valuable in India both economically and nutritionally, further research on Biofloc technology should be conducted. Biofloc technology has increased the growth of the shrimp population significantly higher and along with imparting immunity and desirable water conditions, resulted in an increased survival rate compared to the natural method.

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