Aquaculture industry like any other human activity affects environment in one way or the other. Among the problems faced by aquaculture industry, treatment and release of farm effluents are of great concern. Farm effluents contain ammonia and nitrite that are generally generated as a consequence of aquatic animal excretion and microbial degradation of waste. The presence of these compounds in water above 1.0 mg/l can cause adverse health hazards in aquatic animals and create environmental concerns if effluents are not properly treated. Attempts have been made to find a solution to remove or reduce excess ammonia from intensive culture system. Many biological treatment systems have been developed to maintain ammonia and nitrite concentration in culture waters. Previously adopted methods viz. exchange and replacement of water, reduced feeding and establishing recirculating aquaculture system (RAS) are expensive, laborious and economically not feasible or may harm cultured animals. Biofloc technology (BFT) also called activated suspension technique was first used during early 1980s to solve water quality issues in aquaculture. By maintaining a high carbon: nitrogen ratio (C: N), toxic inorganic nitrogen species can be converted into microbial biomass. The microbial biomass will further aggregate with other microorganisms and particles suspended in the water forming "biofloc", which can be consumed by the cultured animals or harvested and processed as a feed ingredient. However, in the conventional open culture system, most of the unconsumed feed and nutrient rich faecal matter gets wasted and accumulate in the water which in turn deteriorates the water quality but this does not happen in biofloc ponds where microorganisms grow and multiply by consuming these nutrients. Application of BFT in aquaculture has received much attention in recent years. Recent studies have focused on the effects of aquaculture wastewater treatment and the availability of biofloc protein to shrimp and fish. The present work aim at the role of biofloc in nitrogen control and production performance of fingerlings of Labeo rohita in zero water exchange system.

MATERIAL AND METHODS

Experiment design: The experiment was conducted in three plastic troughs of 70 litres capacity, designated as T-1 (control), T-2 and T-3 and these were maintained for a period of 90 days. The fingerlings of Labeo rohita were procured from fish farm and acclimatised for one week under laboratory conditions. The experimental troughs were stocked with fingerlings at the rate of 20 fingerlings per trough.

Water Quality monitoring: Water samples were collected from each trough on weekly basis and analysed for the various physicochemical parameters viz. water temperature, pH, DO, FC0₂, nitrates, nitrites and ammonia. Various parameters were estimated following standard methods vide A. P. H. A.7.

Culture conditions: In T-1, fingerlings were fed with

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ON OF BINO - FLOC TECHNOLOGY IN THE NITROGEN CONTROL AND GROWTH PERFORMANCE OF FINGERLINGS OF LABEO ROHITA IN ZERO WATER EXCHANGE BIO - FLOC SYSTEM

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During the present study period of 90 days, fingerlings of Labeo rohita were cultured in three treatments units viz. T-1, T-2 and T-3. In T-1 i.e. control, fingerlings were fed with commercial feed, in T-2 i.e. first biofloc managed treatment unit, fingerlings were fed with commercial feed and bioflocs but the quantity of commercial feed was reduced to half and supplemented with bioflocs, whereas in T-3 i.e. second biofloc managed treatment unit, fingerlings were fed with only bioflocs. Bioflocs used in T-2 and T-3 were cultured ex-situ using waste water and molasses as carbohydrate source. The level of inorganic nitrogen species i.e. nitrates, nitrites and ammonia and water temperature, pH, DO and FC0₂ was recorded weekly in all experimental units. In the biofloc managed treatment units the level of total ammonia nitrogen (TAN) was found to be less as compared to the control. So far as the growth performance of Labeo rohita was concerned, when compared with control, it revealed efficient growth in T-2 (where both commercial feed and bioflocs were used) followed by T-3 where only bioflocs were used.
commercial feed pellets made up of *Spirulina* purchased from the market that comprised of 30% protein and 6% lipids. Feed was given at 2% body weight twice daily. In T-2, fingerlings were fed with a combination of commercial diet as used in T-1 and 100 ml biofloc suspension. In this treatment, artificial feed was reduced to half the amount used in T-1 and 100 ml biofloc suspension was added as the supplement for reduced artificial feed. In T-3, commercial feed was not used and fingerlings were fed entirely on the bioflocs and its concentration was doubled to that used in T-2 i.e. 200 ml.

**Fish growth analysis: done by calculating following parameters:**

- **Mean weight gain (MWG):** Mean final weight-Mean initial weight
- **Specific growth rate (SGR):** ln final weight-ln initial weight/No. of days x 100
- **Percent weight gain (%WG):** final weight-initial weight/initial weight x 100
- **Survival rate:** No. of fingerlings stocked -Died fingerlings/No. of fingerlings stocked

**Statistical analysis:** Fish yield parameters were compared using one way Analysis of Variance. Differences between the treatment means were considered significant (p<0.05).

**RESULTS AND DISCUSSION**

**Water quality:** Weekly monitored physico-chemical parameters of water in all experimental units revealed fluctuations as shown in Table-1. The results obtained from the study indicated that mean water temperature fluctuated between 16.5-25.5 °C in T-1, 16-25.5 °C in T-2 and 16.5-25.5 °C in T-3. Temperature in all experimental units remained within the biokinetics range of the fish as already suggested by Jhingran reported that carps thrive well between 18.3 °C and 37.8 °C. pH varied from slightly acidic to alkaline i.e. 6.9-8.1, 6.8-7.9 and 6.8-7.9 in T-1, T-2, and T-3, respectively. In the experimental units T-2 and T-3, pH remained within permissible limits required for growth of bioflocs as also observed by Mahanand et al., who recorded pH range from 6.94 to 8.65 as the desirable limit for biofloc growth.

Perusal of the Table-1 further revealed that Dissolved oxygen concentration varied from 5.0-5.6 mg/l, 5.0-5.4 mg/l and 5.0-5.4 mg/l in T-1, T-2 and T-3, respectively. These values were found to be within the permissible limits required for optimum aquaculture production as also reported by Banerjee who demonstrated that DO concentration between 3.0-5.0 ppm in ponds is unproductive and for average or good production it should be above 5.0 ppm. Present studies also recorded slightly low level of DO in T-2 and T-3 as compared to T-1 which may be attributed to the utilisation of oxygen by the growing heterotrophic bacteria, moreover dissolved oxygen found in these expt. Sets was found to be suitable for the growth of fingerlings and bioflocs. Free carbon dioxide concentration fluctuated from 6.0-11.2mg/l, 6.0-12.4 mg/l and 6.2-11.6 mg/l in T-1, T-2 and T-3, respectively. At the start of the experiment, its concentration was low but during the culture period it showed increasing trend due to its release during respiration by fingerlings of *Labeo rohita* and bacterial respiration. It was also observed that FCOC₂ concentration was slightly higher in T-2 which might be due to the decomposition of feed residues and bioflocs and also due to respiration of cultured animals.

During the experimental period, the level of ammonia, nitrate and nitrite showed varied fluctuations as shown in Table-1. Ammonia, the main nitrogenous waste product was found to be higher in T-1 i.e. 0.25 mg/l (±0.197) than T-2 i.e. 0.154 mg/l (±0.104) and T-3 i.e. 0.13 mg/l (±0.095). In T-1 (control) the concentration of ammonia showed an increasing trend throughout the culture period from 0.011 mg/l to 0.59 mg/l whereas its concentration increased from 0.011 mg/l to 0.29 mg/l in T-2 and 0.010 mg/l to 0.27 mg/l in T-3 upto 8th week. After attaining its peak value during 8th week, it declined till 10th week and thereafter remained static till the end of experiment (Fig:-1). This may be due to the utilisation of ammonia nitrogen for the production of protein by heterotrophic bacteria in biofloc managed treatment units T-2 and T-3, whereas T-1 (control) without bioflocs speaks of high ammonia concentration. Nitrate concentration also showed similar trend and in T-1 it increased from 0.020 mg/l to 0.72 mg/l throughout the experiment period. In T-2 and T-3 it increased initially from 0.019mg/l to 0.39 mg/l and 0.017 mg/l to 0.33mg/l respectively upto 8th week and after acquiring peak it slightly declined and then became static in both T-2 and T-3 till the end of culture period (Fig.-2). Nitrite showed similar trend as observed for nitrites. In T-1 (control) it increased from 0.004 mg/l to 0.069 mg/l till the end of the experiment whereas in T-2 it increased from 0.003 to 0.035 mg/l and in T-3 it increased from 0.001
Table 1. Water Quality parameters (min, max, mean±SD) recorded during 12 weeks of culture period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Min</th>
<th>max</th>
<th>Mean±SD</th>
<th>T-1</th>
<th>T-2</th>
<th>T-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>16.5</td>
<td>25.5</td>
<td>20.6±2.596</td>
<td>16.5±2.55</td>
<td>20.79±3.136</td>
<td>16.5±2.55</td>
</tr>
<tr>
<td>pH</td>
<td>8.9</td>
<td>8.1</td>
<td>7.44±0.437</td>
<td>8.8±0.79</td>
<td>7.4±0.434</td>
<td>8.8±0.79</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg/l)</td>
<td>5.0</td>
<td>5.6</td>
<td>5.28±0.158</td>
<td>5.0±5.4</td>
<td>5.13±0.130</td>
<td>5.0±5.4</td>
</tr>
<tr>
<td>Free carbon dioxide (mg/l)</td>
<td>6.0</td>
<td>11.2</td>
<td>8.26±1.799</td>
<td>6.0±12.4</td>
<td>8.95±2.26</td>
<td>6.2±11.6</td>
</tr>
<tr>
<td>Ammonia (mg/l)</td>
<td>0.011</td>
<td>0.59</td>
<td>0.26±0.197</td>
<td>0.011±0.29</td>
<td>0.154±0.104</td>
<td>0.010±0.27</td>
</tr>
<tr>
<td>Nitrates (mg/l)</td>
<td>0.020</td>
<td>0.72</td>
<td>0.35±0.262</td>
<td>0.019±0.39</td>
<td>0.2±0.137</td>
<td>0.017±0.33</td>
</tr>
<tr>
<td>Nitrites (mg/l)</td>
<td>0.004</td>
<td>0.069</td>
<td>0.04±0.021</td>
<td>0.003±0.035</td>
<td>0.022±0.010</td>
<td>0.001±0.030</td>
</tr>
</tbody>
</table>

Fig.-1. Weekly variation in ammonia during culture period

mg/l to 0.030 mg/l upto 8th week after which it showed slight decline and there after became stable at the end of the experiment (0.029mg/l in T-2 and 0.024 mg/l in T-3) (Fig.-3).

In the present experimental studies, the levels of ammonia nitrate and nitrite were found comparatively high in T-1 (control) than biofloc treatment units T-2 and T-3 which may be due to the absence of heterotrophic bacteria in the control set which utilise inorganic nitrogen for their growth and multiplication. Widanarni et al. also observed more fluctuated water quality parameters and highest nitrate and TAN in control treatment. Similar to present observations, Emerenciano et al. observed gradual increase in ammonia concentration in the control in which bioflocs were not used. In context to present observation, Mahanand et al. also recorded lower inorganic nitrogen concentration in biofloc tanks than T-1.

The results further showed that in T-1, ammonia nitrate and...
nitrite level increased throughout the experimental period while in biofloc treatment sets T-2 and T-3, their level increased initially but in small concentration and afterwards it declined and became constant at the end of experimental period which may be due to microbial assimilation of nitrogen which reduce ammonia, nitrate and nitrite concentration. The uptake of nitrogen from the bioflocs by fish was observed by Avnimelech and Kochba\textsuperscript{14} by using 15N isotope in biofloc suspension which confirms the presence of very low concentration of inorganic nitrogen in completely biofloc based system. Further observations revealed that in T-3, inorganic nitrogen concentration was comparatively lower than T-2. This may be due to the high concentration of flocculated particles used in T-3. Schweitzer et al.\textsuperscript{15} also observed that treatment with lower concentration of flocculated particles show higher ammonia concentration.

**Growth performance of fingerlings of *Labeo rohita***: Fish growth parameters recorded during the experimental period have been shown in Table-2:

At the start of experiment, initial mean weight of fingerlings of *Labeo rohita* was 3.66 ± 0.3281 g, 3.43 ± 0.0608 g and 3.90 ± 0.11 g in T-1, T-2 and T-3, respectively (Table-2). The final mean weight and mean weight gain attained by the fingerlings at the end of experimental period was 3.81 ± 0.037 gm and 0.15 ± 0.0152 gm in T-1, 3.56 ± 0.023 gm and 0.13 ± 0.0152 gm in T-2 and 3.48...
APPLICATION OF BIOFLOC TECHNOLOGY IN THE NITROGEN CONTROL

±0.058 gm and 0.08±0.0115 gm in T-3 (Table-2). The specific growth rate of fingerlings was 0.16±0.0153 in T-1, 0.15±0.0153 in T-2 and 0.09±0.0173 in T-3. Percent weight gain calculated was 3.99±0.1021 in T-1, 3.97±0.4007 in T-2 and 3.36±0.2536s in T-3. The results also showed 90% fish survival in T-1 and T-3, whereas fish survival was more in T-2 i.e. 95%.

Highest final mean weight, mean weight gain, specific growth recorded in control set i.e T-1, may be due to the use of artificial feed pellets made of Spirulina which acts as the preferred food for Rohu (Nandeesha et al.,)16. In biofloc managed treatment unit T-2, all fish yield parameters showed slightly lower values than control which may be due to the utilisation of bioflocs by the fingerlings in the presence of less artificial feed. The minor difference may be due to the time taken by the fingerlings for the acceptance of bioflocs during the initial period of experiment. Thus, the fingerlings of Labeo rohita can grow well in the presence of bioflocs and reduced artificial feed. Mahanand et al.,13 also reported optimum growth of rohu when fed with a feed mix containing 50% fish feed and 50% wet flocs. Similar to present observations, Guillaume17 while working on shrimps in high density zero exchange ponds, observed that by using the flocculated particles the protein requirement of the species can be reduced to half. In biofloc managed treatment unit T-3, the fingerlings of Labeo rohita attained least growth which was reflected by lowest final mean weight, mean weight gain and specific growth rate. This may be due to the absence of artificial feed and the total dependence of fingerlings on bioflocs. Fingerlings in this treatment set consumed only bioflocs and attained least growth but not efficient growth as obtained as in T-1 and T-2, because for efficient growth fingerlings of Labeo rohita require adequate nutrition and artificial feed plays an important role in intensive fish culture. The importance of supplementary feed in commercial carp polyculture has been well documented by Chaudhari et al.,18.

CONCLUSION

Biofloc technology was found to be helpful in four parts:

(i). Reducing total production cost by reducing artificial feed requirement.

(ii). Managing water quality and reducing the cost of pumping water.

(iii). Reducing burden on freshwater resources. and

(iv). Reducing the risk of disease outbreak.

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