

# Comparative Assessment of Static, Aerated, and Probiotic-Assisted Biofloc Systems for Intensive Indoor Rearing of *Clarias batrachus* (Linn.)

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**Abstract**—*Clarias batrachus*, also known as the walking catfish, is a significant fish species in South and Southeast Asia. Despite challenges like water quality, low cost, and disease outbreaks, Biofloc Technology (BFT) is proving to be an effective solution for improving growth performance, utilization, and environmental stability in aquaculture. The objective of this study was to determine the effectiveness of BFT under indoor rearing *C. batrachus* through various culture conditions in terms of growth, survival, feed utilization and water quality. Experiment of eight weeks was carried out with three treatments which include static water (control, T1), BFT with aeration (T2) and BFT with probiotics (*Bacillus subtilis* and *Lactobacillus plantarum*) (T3) all replicated three times. Stocking density of 120 fish/m<sup>3</sup> of juveniles (5-10 g) was done in 500 L tanks. The molasses or wheat brane was added to ensure the C: N biofloc of about 15:1. Growth (weight, specific growth rate, FCR), water quality, biofloc volume and survival were monitored at 2-week intervals. Eight-week experiment was conducted on three treatments that are: static water (control, T1), BFT with aeration (T2) and BFT with probiotics (*Bacillus subtilis* and *Lactobacillus plantarum*) (T3) each replicated thrice. In 500 L containers the stocking density of juveniles (510 g) of 120 fish/ m<sup>3</sup> was performed. The molasses or wheat brane was added to provide the C: N biofloc of 15:1 that was approximate. The monitoring of the growth (weight, specific growth rate, FCR), water and the biofloc volume and survival was implemented after each 2 weeks. An environmentally sustainable, affordable and ecologically friendly solution of indoor farming of *C. batrachus* consists of probiotic-enriched BFT. It enhances growth rates, decreases the number of feeds and limits the exchange of water and thus applicable in intensive aquaculture systems.

**Index Terms**—Biofloc Technology, *Clarias batrachus*, Indoor Aquaculture, Growth Performance, Probiotics, Water Quality.

## I. INTRODUCTION

### 1.1 Overview of Biofloc Technology and *Clarias batrachus*

#### 1.1.1 Biofloc Technology:

A better solution is given by Biofloc Technology (BFT) whereby microbial biotechnology is used to manage waste and recycle nutrients as fish feed supplements. In a BFT system, heterotrophic bacteria oxidize toxic nitrogenous compounds as microbial biomass, which feeds the cultured organisms as a protein-rich floc [1].

The major strengths are: [2].

- Better quality of water through recycling of nitrogen internally
- Additional nutritional support which cuts down on feed expenses provided by biofloc.
- Improved survival, immune response, and efficiency in production over conventional systems.

BFT has been shown to be effective in several fish species viz; tilapia, shrimp, carp and *Clarias* with significant improvement in terms of growth/performance, survival and efficiency towards utilization of resources.

A recent study was directed to catfish in modified BFT system using carbonation and bio-balls, with the maximum floc volumes of 76- 88mL L<sup>-1</sup>, SGR 0.058- 0.066, FCR 0.93- 1.10, and 84- 88% survivals. Another study demonstrated that the combination of probiotics (*Bacillus* NP5) and flocs (biofloc) was

advantageous by showing an improved growth, immune status and disease resistance of *Clarias* sp. fry of the model under pathogen challenge [3].

#### 1.1.2 *Clarias batrachus*:

In South and Southeast Asia, *Clarias batrachus* or the walking catfish is of great nutrition, economic, and ecological importance [4]. Being protein and vitamin rich, as well as full of minerals and polyunsaturated fatty acids, it helps food security and the livelihoods of the locals especially in India and Bangladesh. The low-dissolved oxygen, high stocking density and fluctuating water quality that Clariid catfish tolerate makes it ideal to intensive and low-input systems [5]. Catfish grows in a short duration and are sold at high prices in the market, and it is thus economically feasible, too small for medium scale farmers [6].

#### 1.2 Global Demand for Sustainable Aquaculture

The share of aquaculture in the global production of seafood has reached Branch of learning over 50 percent now, and the trend will be likely to carry on through 2025 and past [7]. Traditional intensive systems are susceptible to water exchange and external feeds that have led to nutrient pollution, great consumption of energy, and high cost of production. Eco-friendly solutions, such as bioflocs technology (BFT) and recirculating aquaculture systems also have been frequently demanded to cope with these economic and environmental problems [8].

#### 1.3 Challenges in Intensive Farming [9]

Although it is adaptable, there are a few problems that are subsisting with the indoor and intensive culture of *C. batrachus*:

- The build-up of ammonia, nitrite, and solids leading to deterioration of water quality and retarding the growth and even killing the fish.
- Dependent on high food (protein-based commercial diets form over 50 percent of production costs).
- The effects on the environment such as the release of nutrient-laden effluent and ineffective utilization of water in the traditional culture procedures.

These issues highlight the necessity to develop resource-saving, low-discharge culture systems that would allow reducing the consumption of feed time, preserving water qualities, and increasing the sustainability of production.

1.4 Catfish and Probiotic- Augmented BFT Research Probiotics (i.e. *Bacillus* spp. and *Lactobacillus* spp.) are commonly conjoined with BFT, to further maximize microbial multivalence, disease resistance, and nutrient uptake. In research on *Clarias gariepinus*, the use of *Bacillus* probiotics with BFT produced specific growth rate (SGR) up to 2.4 per cent/ day, survival rate close to 99 per cent and FCR as low as 0.95 in larger pond culture [10]. The report of another experiment on modified BFT systems (e.g. carbonation, physical solids management) indicated that the biofloc volume was 7688 mL/L, SGR was 0.0580.066/day, FCR was 0.931.10, and survival rates were 84 and 88% [11]. Studies on *Heteropneustes fossilis* as well as *Pangasius* species also demonstrated enhanced digestion in probiotic-enhanced BFT conditions, hematology, and immune response-but less conclusive evidence is available regarding *C. batrachus* [12].

#### 1.5 Knowledge Gap and Rationale

Despite this, the majority of the BFT research with *Clarias* species have focused on pond and semi-intensive systems or other fish such as *C. gariepinus*. Fixed indoor studies of comparison of static culture and aerated BFT and BFT + probiotics particularly on *C. batrachus* are not present. Also, not a lot of information is available about:

- Biofloc performance in farm tanks, plots of floc volume and water-quality.
- Comparison of BFT techniques in controlled environment.
- Bettering the forward and dose of probiotics in shaking off microbial stability and productivity effectiveness.

RESEARCH OBJECTIVE: This study compares the effectiveness of three indoor culture regimes; control (static water), aerated BFT and the use of probiotics in the BFT, in the development performance, survival, and water conversion, water quality, and biofloc formation of *Clarias batrachus* juveniles kept under these regimes over an eight-week period.

## II. REVIEW OF LITERATURE

Bajracharya S et al., (2025) compares the effect of the culture activity of Pacific white shrimp *Litopenaeus vannamei* at the various stocking densities under the recirculating indoor biofloc and

outdoor mixotrophic system. It carried out two separate growth experiments. The former used a recirculating aquaculture system, based on biofloc, and working indoors with a thirty-two 150-L tank system. The second test was done on an out-of-door recirculating mixotrophic with-twenty 800- L tanks. What was also found in both systems is significant differences in FCR and growth (biomass, mean weight, weight gain) of different stocking densities [13].

Kalaiselvan P et al., (2024) determines survival, growth and digestive ontogeny of *C. striatus* larvae used on different experimental diets between 4 dph and 32 dph at three-day time intervals. The twenty four thousand larvae, which consisted of sixteen hundred larvae per tank, in triplicates, and a mean weight at 4 days post hatch (dph) of  $0.64 + 0.01$  mg were exposed to five early weaning diets these diets were as follows; *Artemia nauplii* (T1), co-feed diet (*Artemia nauplii* and formulated micro diet) (T2), formulated micro diet (T3), formulated micro diet supplemented with protease (T4) [14].

Solanki S et al., (2023) carried out a twenty-day factorial experiment in 100 L HDPE experimental tanks, to determine the impacts of the C/N ratio and stocking density on *Gibelion catla* spawn nursery rearing within the indoor biofloc system. The carbon source of manipulating C/N ratios was rice bran. Water parameters indicated that an increment of C/N ratio of 10 to 20 changed greatly ( $P < 0.05$ ) in TAN and nitrite nitrogen (NO<sub>2</sub>-N) and nitrate nitrogen (NO<sub>3</sub>-N) in the water [15].

Kumar V et al. (2023) examined different microbial inoculum composed of useful microbes that possessed the immunostimulatory, probiotics and flocs development and bioremediation characteristics would result in the making of optimum bio floc development. Three group-mix-of microbes, viz., group 1 [*Bacillus subtilis* (AN1)+*Pseudomonas putida* (PB3)+*Saccharomyces cerevisiae* (ATCC-2601)], group 2 [*B. subtilis* (AN2)+*P. fluorescens* (PC3)+*S. cerevisiae* (ATCC-2601)], and group 3 [*B. subtilis* (AN3)] [16].

Xu W et al., (2021) conducted a research project which was to design an efficient and environment sustainability commercial scale tank-based shrimp biofloc system. Eight identical and independent tanks were randomly sampled, and they were ready to undergo a trial, four of them were indoors and the

remainder outdoors. A full-scale experiment was carried out on the super-intensive culture of *L. vannamei* in both indoor and outdoor environment at the same time [17].

BWOGA J. A (2021) performed an experiment between December 2017 and May 2018 with a mission of experiencing stocking density and seasonality as the determinants of monogenean versus digenean trematodes parasitism in *Oreochromis niloticus* in cages at Uhanya Beach in Lake Victoria, Kenya. The systematic random sampling procedure was used to sample a total of 600 fish during the rainy and dry seasons. The monogenean *Dactylogyrus*, the digeneans; *Tylodelphys*, *Clinostomum* and *Neascus* were some parasites isolated from the studied fish. The fish had predominant abundance of *Dactylogyrus* sp. in all the 10 cages [18].

Verma, A et al., (2016) assess biofloc based rearing technique of *Labeo rohita* fingerlings. Four biofloc systems were used to indicate their C/N coefficient (15) using indirect and long-term carbon sources and a control (C). Every group was composed of three triplicate tanks, and each of the tanks was stocked with 50 fingerlings ( $4.80 \text{ g} \pm 0.12$ ) of *L. rohita*. Fish were inoculated against *A. hydrophila* 60 days after rearing and the percentage survival (RPS) was observed after 14 days of inoculation [19].

### III. MATERIALS AND METHODS

#### 3.1 Study Area and Duration

The experiment was done in a controlled environment aquaculture system [Department of Zoology, Jai Prakash University], [Chapra, Bihar India]. The facility was kept under control in condition, and a lot of attention was paid to temperature ( $27\text{-}29$  °C) and photo period (12 hours of light and 12 hours of darkness). All the experiments were conducted under the ethical management of animal research of the institution.

#### 3.2 Experimental Design

The effects of three biofloc-based cultures with one control of juveniles of *Clarias batrachus* (Linn.) in triplicates were compared with respect to growth performance and water quality:

- T1 (Control): Stagnant water system in absence of bio-floc development (change of water regularly).

- T2 (BFT-A): Biofloc Technology-Continuous aeration.
- T3 (BFT-P): Probiotics (*Bacillus* sp. and *Lactobacillus* sp.) supplementation of biofloc technology.

The embryos of catfish (mean weight 5 to 10 g) were stocked with a density of 120 per m<sup>3</sup>. The circular tanks (500 L, 0.8 m deep, 1.2 m in diameter) were taken, where each tank had its own aeration system (air stones and central blower). Biofloc suspension in BFT treatments was a continuous aeration (0.5 L/min and 0.7 L/min per tank).

### 3.3 Sample Selection Criteria

#### 3.3.1 Inclusion Criteria

- Juvenile healthy *Clarias batrachus* (Linn.) with mean body weight of 5 to 10 g, without the presence of external injury or anatomical deformity.
- Fish should be obtained to use a certified hatchery to guarantee standard genetic and health condition.
- Those that have acclimatized (at least 7 days in laboratory environment) prior to stocking are taken.

- The tanks and the biofloc systems were kept under controlled temperatures indoors (27-29 Celsius, PH 6.5-8.0).

#### 3.3.2 Exclusion Criteria

- Fish with disease-looking fish, skin parasites, or deformities before being stocked.
- People with abnormal feeding behavior or stress signs during acclimatization.
- There were tanks where the water quality parameters were outside of critical levels as the result of system failure.
- Mortalities resulting either through handling or non-experimental sources of stress, and wherein the growth and survival data were not considered.

#### 3.4 Biofloc Preparation and Management

To sustain a carbon-to-nitrogen (C: N) ratio of 15:1, Biofloc was created with the use of a carbon source (molasses or wheat bran). Daily supplements were made depending on the total ammonia nitrogen (TAN) values. In T3, bacterial probiotic mixture, consisting of *Bacillus subtilis* (1 x 10<sup>8</sup> CFU/g) and *Lactobacillus plantarum* (1 x 10<sup>8</sup> CFU/g) were topically applied at the rate of 1 g/m<sup>3</sup> every day to increase microbial diversity and inhibition of pathogens.

Table No.1: Biofloc Preparation and Management Protocol Used for Indoor Rearing of *Clarias batrachus*

Parameter	Details
Carbon Source	Molasses (primary) or Wheat bran (alternative)
C: N Ratio Maintained	~15:1 (Carbon: Nitrogen)
Carbon Addition Rate	Total Ammonia Nitrogen (TAN) levels (10–20 g carbon per g TAN)
Probiotic Supplement (for T3)	<i>Bacillus subtilis</i> and <i>Lactobacillus plantarum</i> (1 × 10 <sup>8</sup> CFU/g each)
Probiotic Dosage	1 g/m <sup>3</sup> every three days (broadcast directly into tank water)
Aeration	Continuous aeration (0.5–0.7 L/min per tank) using air stones connected to blower
Settling Prevention	Regular stirring (manual once daily) to keep floc particles suspended
Biofloc Volume Monitoring	Imhoff cone (weekly; 5–15 mL/L considered optimal)
Water Exchange	Minimal (≤10% per week; only for sludge removal or salinity)
Floc Maturation Period	10–14 days before stocking fish to establish stable microbial community
pH and Alkalinity Control	Maintained between 7.0–8.0 and 100–150 mg/L CaCO <sub>3</sub> using lime

#### 3.5 Feeding and Husbandry

Daily feeding of fish was twice a day (09.00 and 17.00 h) using a commercial floating pelleted feed with the 28-32% crude protein level. The intake of

natural biofloc filled up about 1015 percent of the dietary protein demand and as such there is consequent reduction in artificial feed input. The

amount not eaten was observed to adjust the food every day.

### 3.6 Data Collection

#### 3.6.1 Growth Performance:

- The fish was also sampled on a biweekly basis with its weight and length being measured.
- Standard formulae were used to calculate Specific Growth Rate (SGR, %/ day), Weight Gain (%) and Feed Conversion Ratio (FCR).

3.6.2 Survival Rate: The last survival (%) was established after the experimentation.

3.6.3 Water Quality Monitoring: Parameters, including dissolved oxygen (DO), pH, temperature, ammonia, nitrite, nitrate, and alkalinity were measured three times a week using standard methods.

3.6.4 Biofloc Volume: Determined in ml/L weekly by the Imhoff cone method (turbidity and settleable solids in mL/l after 15 min).

#### 3.7 Ethical Considerations

- The work was performed in accordance with the recommendations of the Committee of Purpose

of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and Institutional Animal Ethics Committee (IAEC).

- All the fish were treated with care to reduce stress during stocking, sampling and measuring biometric variations.
- Sampling took place with the help of anesthetics (clove oil, 2040 mg/L) to avoid stress caused by handling.

## IV. RESULTS

### 4.1 Growth Performance of *Clarias batrachus*

There was also a difference in the growth parameters between different treatments ( $p < 0.05$ ). The best final body weight, specific growth rate (SGR), and survival were observed in fish reared under BFT with probiotics (T3) than in control (T1) (Table 5). Feed Conversion Ratio (FCR) was drastically reduced (improved) in T3 than in other treatments.

Table No. 2: Growth Performance of *Clarias batrachus* under Different Culture Methods

Parameter	T1 (Control)	T2 (BFT-Aeration)	T3 (BFT + Probiotics)
Initial Body Weight (g)	5.2 ± 0.4 (ns)	5.1 ± 0.3 (ns)	5.3 ± 0.4 (ns)
Final Body Weight (g)	45.3 ± 3.1 <sup>c</sup>	65.2 ± 3.8 <sup>b</sup>	72.5 ± 4.2 <sup>a</sup>
Specific Growth Rate (SGR, %/day)	2.5 ± 0.2 <sup>c</sup>	3.1 ± 0.1 <sup>b</sup>	3.3 ± 0.1 <sup>a</sup>
Feed Conversion Ratio (FCR)	1.6 ± 0.1 <sup>a</sup>	1.2 ± 0.05 <sup>b</sup>	1.1 ± 0.04 <sup>b</sup>
Survival Rate (%)	85 ± 3 <sup>c</sup>	92 ± 2 <sup>b</sup>	95 ± 2 <sup>a</sup>
Production (kg/m <sup>3</sup> )	4.2 ± 0.3 <sup>c</sup>	6.5 ± 0.4 <sup>b</sup>	7.2 ± 0.5 <sup>a</sup>

Different superscripts within rows indicate significant differences ( $p < 0.05$ , Tukey test).

### 4.2 Water Quality Parameters

Water quality was kept in the optimal range in all the treatments but with significant improvement in the BFT groups ( $p < 0.05$ ). The concentration of ammonia and nitrite was lowest and that of dissolved oxygen was high in T3 as compared to T1.

Table No. 3: Water Quality Parameters Across Treatments

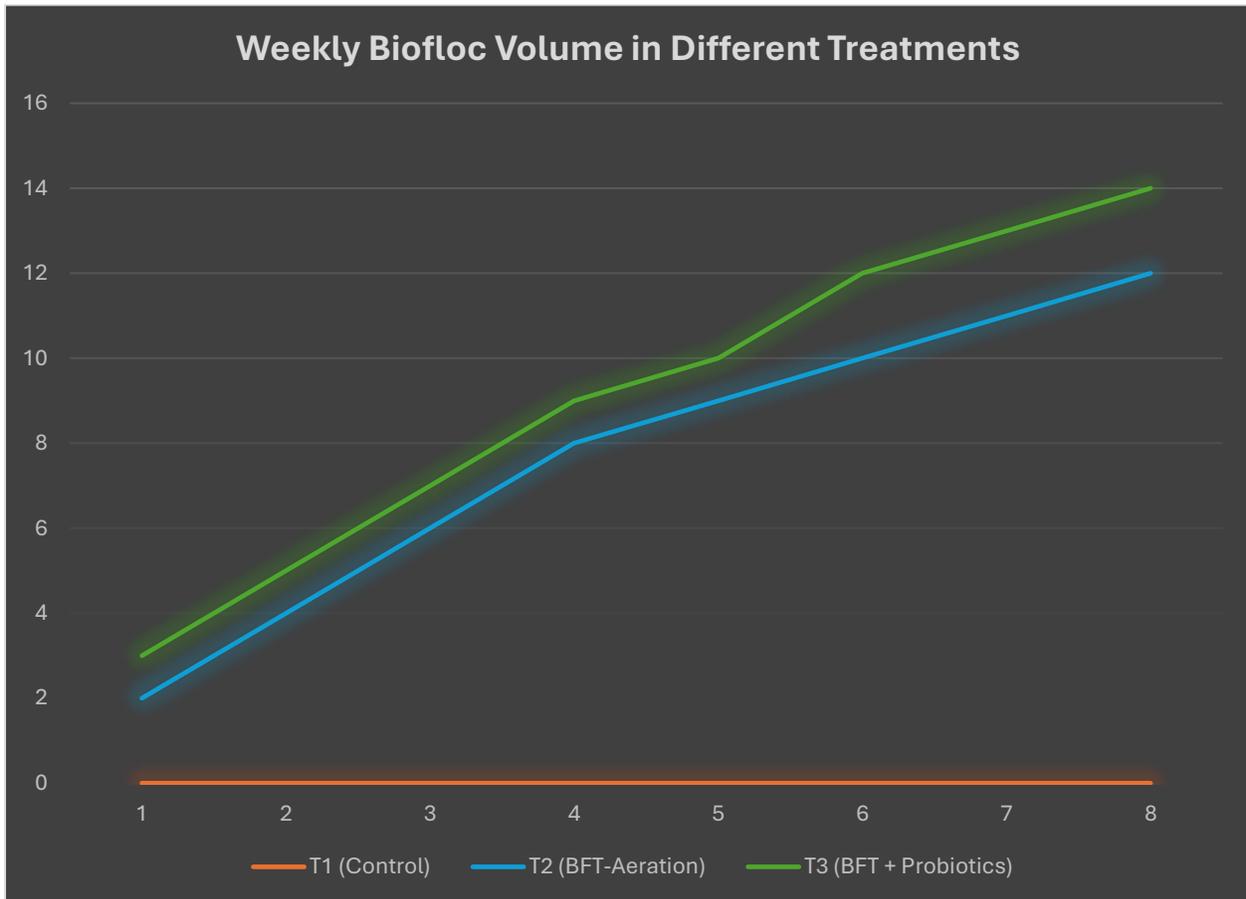
Parameter	T1 (Control)	T2 (BFT-Aeration)	T3 (BFT + Probiotics)
Temperature (°C)	28.1 ± 0.3 (ns)	28.4 ± 0.2 (ns)	28.5 ± 0.2 (ns)
Dissolved Oxygen (mg/L)	3.8 ± 0.5 <sup>c</sup>	5.6 ± 0.4 <sup>b</sup>	5.8 ± 0.3 <sup>a</sup>
pH	6.5 ± 0.2 <sup>c</sup>	7.2 ± 0.3 <sup>b</sup>	7.4 ± 0.3 <sup>a</sup>
Ammonia (mg/L)	1.5 ± 0.3 <sup>a</sup>	0.6 ± 0.2 <sup>b</sup>	0.4 ± 0.1 <sup>c</sup>
Nitrite (mg/L)	0.4 ± 0.1 <sup>a</sup>	0.2 ± 0.05 <sup>b</sup>	0.1 ± 0.05 <sup>c</sup>
Alkalinity (mg/L as CaCO <sub>3</sub> )	80 ± 5 <sup>c</sup>	120 ± 8 <sup>b</sup>	125 ± 7 <sup>a</sup>

### 4.3 Biofloc Volume Dynamics

The volume of biofloc (weekly measurement) went up steadily during the culture phase in BFT treatments. T3 had a higher floc volume compared to T2 but by a small margin thus the effect of probiotics on microbe clumping was observed.

Table No. 4: Weekly Biofloc Volume (mL/L) in Different Treatments

Week	T1 (Control)	T2 (BFT-Aeration)	T3 (BFT + Probiotics)
1	0	2	3
2	0	4	5
3	0	6	7
4	0	8	9
5	0	9	10
6	0	10	12
7	0	11	13
8	0	12	14

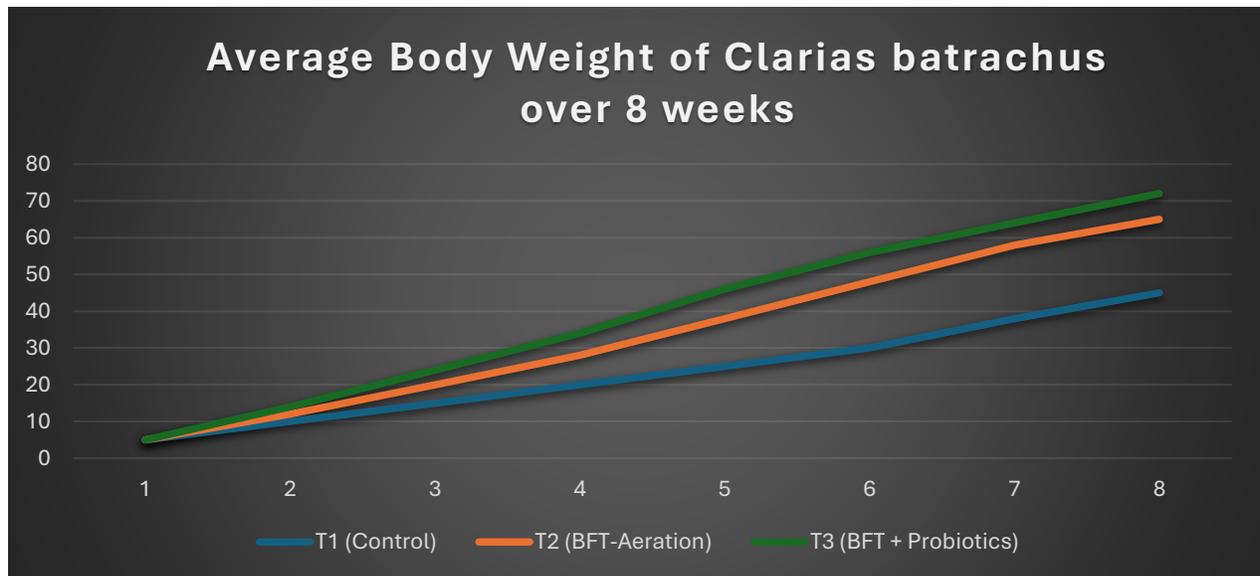


### 4.4 Comparative Growth Trends

The growth trend of fish cultured under BFT with probiotics (T3) was the steepest followed by BFT with aeration (T2) whereas the control (T1) showed the slowest growth. The trend in growth shows that very good nutrient availability and water quality in biofloc systems using probiotics was established and, hence, faster and non-hierarchical weight gain curves were observed than the conventional techniques.

Table No. 5: Average Body Weight (g) of *Clarias batrachus* over 8 weeks

Week	T1 (Control)	T2 (BFT-Aeration)	T3 (BFT + Probiotics)
1	5	5	5
2	10	12	14
3	15	20	24
4	20	28	34
5	25	38	46
6	30	48	56
7	38	58	64
8	45	65	72



## V. DISCUSSION, CONCLUSION AND FUTURE ASPECTS

### 5.1 Discussion

The current investigation shows clearly that biofloc technology (BFT), particularly with the incorporation of probiotics, plays a remarkable role in increasing the growth performance, survival and feed efficiency in *Clarias batrachus*. Despite the fact that there is not enough similar studies on *C. batrachus*, some similar studies conducted on *Clarias gariepinus* support a similar advantage: the supplementation of *Bacillus* sp. probiotics into a biofloc system has led to maximum specific growth rates of up to ~2.4 %/day,

survival rates of nearly 99 %, and FCRs of as low as 0.95 in a pond-scale culture [20].

There are various processes that underline these ameliorations. BFT systems enhance heterotrophic bacteria to fully oxidize the nitrogenous waste into microbial biomass and feedstuff to the fish and simultaneously keep up the water quality with augmentation of nutrients and reduction in effective feed input (i.e., decreased FCR and enhanced nutrient retention) [21]. In these systems, when probiotics are included, especially *Bacillus* and *Lactobacillus* species, they enhance growth, immunity, and pathogen their resistance to catfish even more [22]. Our findings concluded that the inclusion of probiotics or prebiotics in BFT leads to a

considerable increase in innate immunity, fish health, and growth parameter levels, as opposed to biofloc or conventional systems. Similarly, changes in BFT protocols, including sustaining proper C:N ratio and upsurge of microbial aggregation, have also been indicated to discard floc volume, feed utilization performance, as well as survival (~8488%) of cultured catfish in small-scale studies [23].

Further, the trends we have observed, with T3 (BFT + probiotics) performing better than T2 (BFT + aeration) and the latter being more effective than T1 (static control) demonstrate that probiotics have a potential to synergize with biofloc systems (aerated ones). The same results were observed in *Clarias gariepinus* in which the doses (e.g. 5 days) of the probiotic showed a significant result in the tendency of the treatment with respect to growth and feed conversion [24].

In general, the increased performance in T3 is a testimony of the combination of direct nutrient commendation through the microbial biomass, the amelioration of water quality by stabilization as well as stimulation of host digestive and the immune processes by probiotics organisms. This validates the inclusion of BFT + probiotic as a strong, sustainable aquaculture in intensive indoor culture of *C. batrachus*.

### 5.2 Conclusion and Future Aspects

Biofloc used in closed systems holds great potential as it has been observed to promote the growth, feed conversion, water quality stability, and increase survival in the cluster farming of the *Clarias batrachus*. Biofloc, enriched with probiotics (*Bacillus* sp. and *Lactobacillus* sp.) seem to improve dramatic growth, elevate feed conversion, water quality stability, and enhance survivability in the juvenile cone farming of *Clarias batrachus*.

BFT with an aeration system ensured moderate improvement in performance compared to conventional static system whilst addition of probiotics generated the most significant change proving the synergistic effect of the latter.

These results endorse the idea that probiotic-supplemented biofloc technology is an economical, environmentally sustainable, and expandable method of indoor rearing walking catfish. With all these benefits of water savings, reduced environmental impact, and good fish health, the given approach can be highly effective when implemented by smaller-

scale producers and medium-size aquaculture enterprises.

To continue in the future, it is advisable to:

- Minimize number of probiotic species and treat frequencies based on local conditions,
- Define the dynamics of microbiome and its immunomodulator properties,
- Trial to grow-out farm conditions and varying stocking densities.

This paper contributes to a good step towards embracing contemporary sustainable aquaculture industry in the Indian and Southeast Asia catfish fish farming systems.

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