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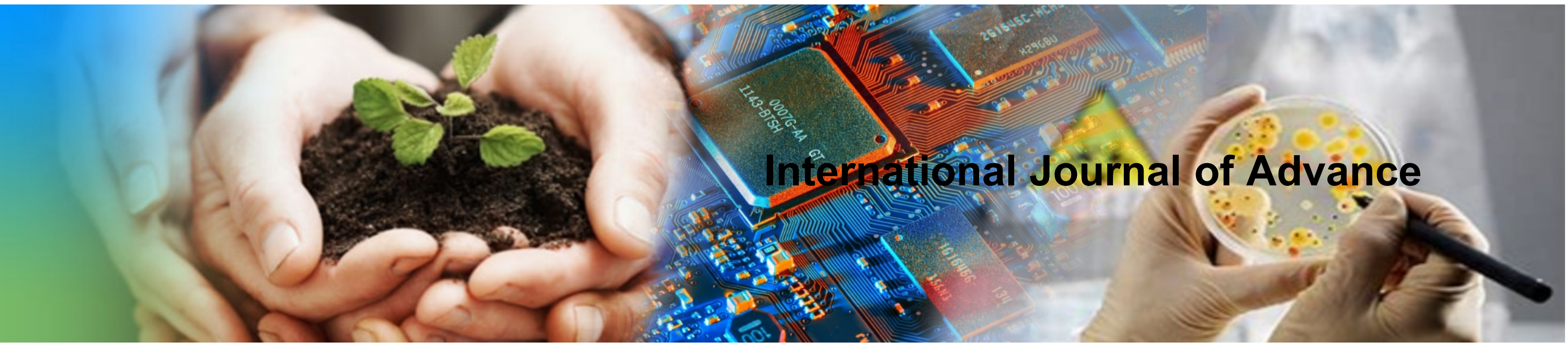

Biofloc Inoculum Preparation and Sustainable Aquaculture Practices: A Case Study of Rits Aqualife LLP, Maharashtra, India

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Abstract

Biofloc Technology (BFT) represents a modern and ecofriendly approach to aquaculture, enabling nutrient recycling, water quality improvement, and reduced feed costs. This research investigates the preparation and standardization of biofloc inoculum in Chhatrapati Sambhaji Nagar, Maharashtra, with a focus on largescale application using 20,000litre tarpaulin/HDPE tanks. Locally available carbon sources such as jaggery, rice bran, corn flour, and tapioca powder, combined with probiotics (*Bacillus spp.*, *Lactobacillus spp.*), were employed to initiate floc formation. Continuous aeration was maintained, and floc development was monitored over a 10day period. Key parameters including temperature (28–32 °C), pH (7–8), dissolved oxygen (>5 mg/L), and ammonia (<0.25 mg/L) were controlled to ensure microbial stability. Mature, odorless, and nutrientrich floc was achieved by day 8–10, suitable for stocking Pangasius fry. The study demonstrates that 20,000litre biofloc tanks provide a scalable model for highdensity fish culture, improving feed conversion ratio (FCR), survival rates, and profitability. By integrating scientific methodology with local resources, this research offers a replicable framework for rural aquaculture development in Maharashtra, contributing to sustainable fish farming and livelihood enhancement.

Keywords: Pangasius Fish, Indian Major Carps, Tilapia Aquaculture

Introduction

Aquaculture has emerged as one of the fastest-growing food production sectors globally, playing a crucial role in ensuring food security, nutritional sustainability, and livelihood generation. With increasing pressure on natural water resources and the rising demand for fish protein, conventional aquaculture practices are facing significant challenges, including water scarcity, disease outbreaks, environmental degradation, and high feed costs. These limitations have necessitated the development of innovative and sustainable aquaculture technologies. Among these, Biofloc Technology (BFT) has gained considerable attention as an environmentally sustainable and economically viable approach to intensive fish farming.

Biofloc Technology is a microbial-based system that promotes the growth of beneficial heterotrophic bacteria, which convert nitrogenous waste, particularly ammonia, into microbial biomass or "biofloc." These flocs serve as a natural supplementary feed for cultured species, thereby reducing dependency on commercial feed inputs. The principle of BFT is based on maintaining an optimal carbon-to-nitrogen (C:N) ratio, typically between 15:1 and 20:1, by adding external carbon sources such as jaggery, wheat flour, rice bran, or molasses. This process enhances nutrient recycling within the system, improves water quality, and minimizes the need for water exchange, making it particularly suitable for water-limited regions.

India, being one of the leading aquaculture producers in the world, has witnessed rapid expansion in fish farming activities over the past decade. However, traditional aquaculture systems in many parts of the country are still constrained by inefficient resource utilization and environmental concerns. In semi-arid regions such as Chhatrapati Sambhaji Nagar, where water availability is limited and climatic conditions can be challenging, the adoption of sustainable aquaculture practices becomes even more critical. Biofloc Technology offers a promising solution in such contexts by enabling high-density fish culture with minimal water usage and improved productivity.

One of the key components for the successful implementation of biofloc systems is the preparation of a stable and efficient biofloc inoculum. The inoculum acts as a starter culture containing beneficial microbial communities that initiate and accelerate floc formation in culture tanks. Proper inoculum preparation ensures rapid establishment of microbial equilibrium, reduces the risk of harmful pathogen proliferation, and enhances system stability. Despite its importance, there is limited standardized information available on inoculum preparation protocols, especially under local farming conditions in India. This highlights the need for location-specific studies that integrate scientific principles with locally available resources.

The present study focuses on the preparation and standardization of biofloc inoculum using locally accessible materials such as jaggery, rice bran, corn flour, and tapioca powder, along with probiotics containing bacterial strains like Bacillus spp. and Lactobacillus spp. These materials are not only cost-effective but also readily available to rural farmers, making the technology more accessible and scalable. The use of probiotics further enhances microbial diversity, improves digestion, and suppresses pathogenic organisms, contributing to healthier culture conditions.

In addition to inoculum preparation, the study emphasizes the importance of maintaining optimal water quality parameters for successful biofloc development. Factors such as temperature, pH, dissolved oxygen, and ammonia concentration play a critical role in determining microbial activity and system performance. Continuous aeration is essential to keep the flocs in suspension and to provide adequate oxygen for both fish and microbial communities. Maintaining dissolved oxygen levels above 5 mg/L and controlling ammonia below toxic levels are particularly crucial for ensuring fish health and survival.

The application of biofloc systems in large-capacity tanks, such as 20,000-liter tarpaulin or HDPE tanks, represents a scalable model for intensive aquaculture. These tanks are relatively easy to install, require less land area, and allow better control over environmental conditions compared to traditional pond systems. High-density stocking of species such as Pangasius, Tilapia, and shrimp can be effectively managed in such systems, leading to higher production per unit area. Pangasius, in particular, is well-suited for biofloc culture due to its tolerance to varying water conditions and its ability to utilize microbial protein efficiently.

This research is based on a case study of RITS AQUALIFE LLP, an aquaculture enterprise located in Village Jalgaon Baukud in Chhatrapati Sambhaji Nagar. The organization has been actively involved in promoting sustainable aquaculture practices, providing technical consultancy, and supporting rural entrepreneurs in adopting innovative fish farming techniques. By integrating scientific knowledge with practical field applications, RITS AQUALIFE LLP serves as a model for successful implementation of biofloc technology at the grassroots level.

The significance of this study lies in its practical approach to addressing real-world challenges faced by aquaculture farmers. By demonstrating the effectiveness of biofloc inoculum preparation under local conditions, the research provides a replicable framework that can be adopted by small and medium-scale farmers across Maharashtra and similar agro-climatic regions. The study also contributes to improving feed efficiency, reducing production costs, and enhancing overall farm profitability, thereby supporting rural livelihoods and economic development.

Furthermore, the environmental benefits of biofloc systems cannot be overlooked. By minimizing water exchange and recycling nutrients within the system, BFT reduces the discharge of waste into the environment, thereby mitigating the ecological impact of aquaculture activities. This aligns with the broader goals of sustainable development and responsible resource management.

In conclusion, Biofloc Technology represents a transformative approach to aquaculture that combines productivity, sustainability, and economic viability. The preparation of a robust biofloc inoculum is a critical step in ensuring the success of this system. Through this case study, the research aims to bridge the gap between theory and practice, providing valuable insights into the adoption of biofloc technology in rural India. The findings are expected to contribute to the advancement of sustainable aquaculture practices and to promote the widespread adoption of biofloc systems in regions facing resource constraints.

Materials and Methods

The study was conducted at RITS AQUALIFE LLP located in Village Jalgaon Baukud, District Chhatrapati Sambhaji Nagar, Maharashtra, India.

The experimental work was carried out over a defined period from 14 November 2025 to August 2026, covering multiple phases of biofloc inoculum preparation, system stabilization, and culture observations. This duration allowed for seasonal variation assessment and ensured the reliability and reproducibility of the biofloc development process under field conditions.

1. Study Area and Site Description

The study was conducted at RITS AQUALIFE LLP located in Village Jalgaon Baukud, District Chhatrapati Sambhaji Nagar, Maharashtra, India.

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The region is characterized by a semi-arid tropical climate with moderate rainfall and temperature fluctuations between 25 °C and 35 °C. Such climatic conditions are suitable for biofloc-based aquaculture due to enhanced microbial activity at higher temperatures.

The experimental facility consisted of a controlled aquaculture unit equipped with HDPE/tarpaulin tanks, aeration systems, and water quality monitoring instruments. The site was selected based on accessibility, availability of groundwater, and suitability for demonstrating scalable rural aquaculture practices.

2. Experimental Design and Tank Configuration

A completely randomized experimental setup was adopted using 20,000 L capacity circular biofloc tanks.

Fig 1: Field Visit

Fig 2: Biofloc Inoculum Preparation Step by Step

Fig 3: Recording the Parameters and noting it Down as Record

Fig 4: Field Inspection by Research Analyst

Fig 5: Rits Team Work by Satish Pawar Sir

Fig 6: Nitrogen Cycle in Biofloc Systems

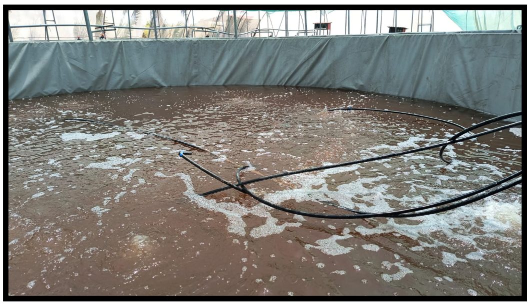

Fig 7: Biological Cycle & Inoculum Maturity

Fig 8: Fish Fingerlings Introduced in Biofloc Tank

Fig 9: Other Fish Seed IMC

Fig 10: Fish Health Pangasius

Fig 11: Fishes in Tank

Fig 12: Training Students and Fish Farmers

Tank Specifications

- Tank capacity: 20,000 liters
- Shape: Circular
- Material: HDPE tarpaulin supported by iron frame
- Effective water volume: 15,000–18,000 liters
- Tank color: Blue/black (to reduce algal bloom)

Aeration System

- Air blower capacity: 1–2 HP
- Aeration method: Continuous diffused aeration using PVC pipeline grid
- Diffusers: Fine bubble air stones evenly distributed
- Objective: Maintain dissolved oxygen and keep flocs in suspension

Water Source and Preparation

- Source: Borewell water
- Pre-treatment: Sedimentation and filtration
- Chlorine removal: Aeration for 24–48 hours before use

3. Raw Materials and Inputs

3.1 Carbon Sources

Carbon sources were selected based on local availability and cost-effectiveness to maintain the C:N ratio between 15:1 and 20:1, essential for heterotrophic bacterial growth.

Table 1: Carbon Sources Used in Biofloc Inoculum Preparation and Their Functions

Carbon Source	Function
Jaggery	Rapid carbon release, microbial activation
Rice bran	Slow-release carbon and nutrients
Corn flour	Energy source for microbes
Tapioca powder	Enhances floc structure

3.2 Probiotics and Microbial Inoculants

Commercial probiotics containing beneficial bacterial strains were used:

- Bacillus subtilis
- Bacillus licheniformis
- Lactobacillus spp.

Function

- Accelerate decomposition of organic matter
- Improve gut health of fish
- Suppress pathogenic bacteria
- Enhance floc formation

3.3 Additional Inputs

- Mineral mixture (Ca, Mg supplements)
- Agricultural lime (for pH stabilization)
- Salt (NaCl, optional for osmotic balance)
- Dechlorinated freshwater

4. Biofloc Inoculum Preparation Protocol

A standardized multi-step protocol was followed to develop biofloc inoculum:

Step 1: Tank Filling and Aeration

- Tanks were filled with 15,000–20,000 L of prepared water
- Continuous aeration was initiated immediately

Step 2: Carbon Source Addition

- Initial dose: 200–300 g jaggery per 1,000 L water
- Supplementary carbon (rice bran/corn flour) added daily
- Carbon input adjusted based on ammonia levels

Step 3: Probiotic Activation

- Probiotics were activated in a separate container
- Mixture: Water + jaggery + probiotics
- Incubation: 12–24 hours under aeration
- Activated culture added uniformly to tank

Step 4: Microbial Fermentation Phase

- Duration: 7–10 days
- Continuous aeration ensured microbial growth
- Carbon sources added regularly to maintain C:N ratio

Step 5: Floc Development Monitoring

Daily observations included:

- Water color change (clear → green → brown)
- Suspended floc particles
- Foam formation
- Odor assessment

Step 6: Inoculum Maturity Indicators

Biofloc was considered mature when:

- Brown-colored water with dense suspended particles
- Absence of foul smell
- Stable foam layer
- Floc volume within optimal range

5. Water Quality Monitoring and Management

Water quality parameters were monitored daily using standard aquaculture methods.

Table 2: Water Quality Parameters Maintained During Biofloc Inoculum Preparation

Parameter	Optimal Range	Method Used
Temperature	28–32 °C	Thermometer
pH	7.0–8.0	Digital pH meter
Dissolved Oxygen	> 5 mg/L	DO meter
Ammonia (NH_3)	< 0.25 mg/L	Test kit
Alkalinity	100–150 mg/L	Titration

Control Measures
- Low DO → Increased aeration
- High ammonia → Added carbon source
- Low pH → Lime application
- Excess floc → Partial water exchange or sludge removal

6. Biofloc Volume Estimation
Floc volume was measured using an Imhoff cone method:
- 1 L water sample collected
- Settling time: 15–20 minutes
- Floc volume recorded in mL/L

Optimal Range: 10–20 mL/L

7. Fish Stocking and Culture Practices
Species Selected
- Pangasius (*Pangasianodon hypophthalmus*)

Stocking Procedure
- Acclimatization for 20–30 minutes
- Gradual mixing of tank water
- Stocking density: High-density culture (depending on tank capacity)

Feeding Strategy
- Commercial floating feed
- Protein content: 24–28%
- Feeding frequency: 2–3 times daily
- Feeding adjusted based on biomass and floc availability

8. Growth Performance and Data Collection
The following parameters were recorded:
- Initial and final body weight
- Daily feed intake
- Survival rate (%)
- Feed Conversion Ratio (FCR)

FCR Calculation
FCR = Feed given (kg) Weight gain (kg) FCR =\frac {\text {Feed given (kg)}} {\text {Weight gain (kg)}} FCR = Weight gain (kg) Feed given (kg)

9. Statistical Analysis
- Data expressed as mean ± standard deviation
- Growth and water quality trends analyzed
- Graphical representation used for interpretation
- Comparative analysis performed with standard aquaculture benchmarks

10. Sustainability and Environmental Considerations
- Zero or minimal water exchange system
- Recycling of nitrogenous waste into microbial protein
- Reduced environmental discharge
- Cost-effective and farmer-friendly inputs

11. Replicability of Method
The methodology was designed using locally available materials and simple techniques, making it highly replicable for:
- Small-scale farmers
- Rural aquaculture entrepreneurs
- Semi-arid regions like Maharashtra

Results and Discussion
1. Biofloc Inoculum Development and Maturity
The biofloc inoculum developed successfully within 8–10 days under controlled conditions at RITS AQUALIFE LLP. A clear transition in water characteristics was observed during the fermentation phase:
- **Day 1–3:** Water remained relatively clear with initial microbial activation
- **Day 4–6:** Greenish coloration appeared due to phytoplankton and bacterial growth
- **Day 7–10:** Water turned dark brown, indicating mature biofloc formation

The Mature Inoculum Exhibited
- Dense suspended floc particles
- Stable foam formation
- Absence of foul odor (indicating healthy microbial activity)

Floc volume measured using the Imhoff cone ranged between 12–18 mL/L, which falls within the optimal range (10–20 mL/L). This confirms that the carbon supplementation strategy (jaggery, rice bran, corn flour, tapioca) effectively maintained the required C:N ratio and supported heterotrophic bacterial proliferation.

Table 3: Observed Water Quality Parameters during Biofloc Inoculum Development

Parameter	Observed Range	Optimal Range
Temperature	28–32 °C	28–32 °C
pH	7.0–7.8	7.0–8.0
Dissolved Oxygen	5.2–6.8 mg/L	> 5 mg/L
Ammonia	0.05–0.22 mg/L	< 0.25 mg/L
Alkalinity	110–145 mg/L	100–150 mg/L

The Results Indicate that
- Continuous aeration effectively maintained high dissolved oxygen levels
- Carbon addition successfully converted toxic ammonia into microbial biomass
- pH and alkalinity remained stable due to lime and mineral supplementation

These findings align with standard biofloc principles, confirming that minimal water exchange systems can maintain stable water quality when properly managed.

2. Fish Growth Performance (Pangasius Culture)
After stocking Pangasius (Pangasianodon hypophthalmus) fry in mature biofloc tanks, significant improvements in growth and survival were recorded:
- **Survival Rate:** 85–92%
- **Average Weight Gain:** High and consistent growth observed
- **Feed Conversion Ratio (FCR):** 1.2–1.5

The improved FCR indicates that biofloc acted as a natural protein source, reducing dependence on commercial feed. Fish exhibited:
- Active feeding behavior
- Uniform growth patterns

- Minimal disease incidence

The presence of beneficial microbes such as Bacillus spp. and Lactobacillus spp. contributed to improved digestion and immunity, enhancing overall fish health.

3. Economic and Operational Efficiency

The study demonstrated that the use of locally available carbon sources significantly reduced operational costs:

- Jaggery and agricultural by-products lowered feed and input expenses
- Reduced water exchange minimized pumping and water usage costs
- Improved FCR decreased feed expenditure by 15–25%

The 20,000 L HDPE Tank System Proved to be Highly Efficient for

- High-density fish culture
- Better monitoring and control
- Scalable commercial application

4. Sustainability and Environmental Impact

Biofloc technology showed strong environmental benefits:

- Zero or minimal water discharge, reducing environmental pollution
- Efficient recycling of nitrogenous waste into microbial protein
- Lower ecological footprint compared to traditional pond systems

This system is particularly suitable for semi-arid regions like Chhatrapati Sambhaji Nagar, where water conservation is critical.

Discussion

The findings of this study clearly demonstrate that standardized biofloc inoculum preparation plays a crucial role in the success of biofloc aquaculture systems. The rapid establishment of microbial communities within 8–10 days highlights the effectiveness of combining carbon sources with probiotic inoculants.

The observed water quality stability confirms that maintaining a proper C:N ratio (15:1–20:1) is fundamental for controlling ammonia toxicity and promoting heterotrophic bacterial growth. Similar results have been reported in previous biofloc studies, where carbon supplementation significantly improved nitrogen utilization efficiency.

Fish growth performance in this study, particularly the low FCR (1.2–1.5) and high survival rate (up to 92%), indicates that biofloc systems can outperform conventional aquaculture methods. The microbial flocs not only act as a supplementary feed but also enhance gut health and disease resistance, reducing the need for antibiotics and chemicals.

The use of 20,000 L tanks represents an important advancement in making biofloc technology accessible and scalable for rural farmers. Compared to traditional pond culture, tank-based systems offer:

- Better control over environmental parameters
- Higher productivity per unit area
- Reduced land and water requirements

Economically, the integration of low-cost inputs such as jaggery and rice bran makes the system farmer-friendly and profitable, especially for small and medium-scale entrepreneurs. The reduction in feed costs and improved growth efficiency directly contribute to higher net returns.

From an environmental perspective, the study supports the role of biofloc technology as a sustainable aquaculture solution, aligning with global goals of eco-friendly food production and resource conservation.

Conclusion

The case study of RITS AQUALIFE LLP confirms that:

- Biofloc inoculum can be reliably developed within 10 days using local resources
- Stable water quality can be maintained without frequent water exchange
- High-density Pangasius culture is feasible with improved growth and survival
- The system is economically viable, environmentally sustainable, and scalable

Overall, this research provides a practical and replicable model for adopting biofloc technology in rural Maharashtra and similar agro-climatic regions.

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