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SCREENING COMMERCIAL PROBIOTIC PRODUCTS FOR ENHANCING FLOC MATURATION IN BIOFLOC SYSTEM

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Abstract

The global population is steadily increasing, including in Bangladesh, and this necessitates the exploration of new technologies to meet the future demand for fish and biofloc technology holds great potential. Unfortunately, there is a dearth of information regarding biofloc culture technology in Bangladesh. Therefore, the purpose of this study was to identify a suitable probiotic for achieving rapid floc maturation. The research was taking place at the Pran Fisheries Project located in Razapur village, under the Sreemangal Upazilla in the Moulvibazar district, from 01 October to 15 October 2021. Thus, four different probiotics, namely Everfresh-Pro, PondCare-3S, Profs, and MI Plus, were tested and each one exhibited distinct mean value. Throughout the floc maturation process, consistent conditions were maintained, including a temperature range of 27-30°C, dissolved oxygen (DO) levels of 6 ppm, pH of 7.8, total dissolved solids (TDS) concentration of 980 ppm, and salinity of 0.8 ppt for all probiotics. The results revealed flocculation measurements of 28 mL for Everfresh-Pro, 23 mL for PondCare-3S, 20 mL for Profs, and 15 mL for MI Plus, as determined by the Imhoff Cone. The utilization of Everfresh-Pro probiotic results in a shorter FCO (Fermented Carbon Organic) production time among the other probiotics within biofloc system. These findings emphasize the significant potential of employing suitable probiotics in FCO making process, as it can substantially reduce maturation time and enhance the quality of floc, ultimately leading to more efficient and effective aquaculture practice through biofloc technology.

Key words: Biofloc, Everfresh-Pro, Floc maturation, Imhoff Cone, Probiotic.

Introduction

The exponential growth of the global human population has resulted in a significant surge in the demand for food production to meet the escalating needs. Consequently, there is an urgent and imperative necessity to increase the production of animal protein, which serves as the primary source of food intended for human consumption. Aquaculture presents the most efficient and cost-effective method for the rapid production of animal proteins. To ensure its expansion aligns with sustainable practices, three primary objectives must be pursued: the development of environmentally friendly aquaculture systems that safeguard the environment (Naylor et al. 2000), increased aquaculture output without excessive utilization of water and land resources, and the establishment of economically and socially sustainable systems with a balanced cost-benefit ratio (Avnimelech 2009). Biofloc technology fulfils all three requirements for sustainable aquaculture development and represents an environmentally friendly approach (Khanjani et al. 2021a,b,c).

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Biofloc technology operates primarily on the principle of recycling waste nutrients, particularly nitrogen, by converting them into microbial biomass. This biomass can be utilized by the cultured animals in situ or can be harvested and processed into feed ingredients (Avnimelech 2009, Kuhn et al. 2010, Kamruzzaman et al. 2023). In this system, the addition of carbonaceous organic matter to the water column facilitates the formation of heterotrophic bacteria while maintaining an optimal carbon-to-nitrogen ratio (Haslun et al. 2012, Panigrahi et al. 2019, Minabi et al. 2020, Khanjani and Sharifnia 2021).

The formation of biofloc relies on effectively regulating the C:N ratio within the culture system, accompanied by minimal or no water exchange and vigorous aeration (Khanjani and Sharifnia 2020 & 2021). Maintaining a high carbon-to-nitrogen ratio is crucial for facilitating the optimal growth of heterotrophic bacteria (Emerenciano et al. 2012). This surplus energy is utilized for cellular maintenance, growth, and reproduction. Molasses, being a simple sugar (Azhar 2013), is readily assimilated by bacteria, promoting their development and proliferation. The introduction of a carbon source into the culture medium stimulates bacterial growth and serves as a supplementary feed for fish. Bacteria play a crucial role in utilizing nitrogenous wastes derived from leftover feed and the excretion of cultured fish for their own development. This process leads to the formation of biofloc, which serve as an additional source of nutrition for domestic fish. According to Avnimelech (2012), a recognized expert in the field, the addition of molasses as a carbon source in aquaculture can elevate the C/N ratio of water. Consequently, this increase promotes the reduction of inorganic nitrogen levels by stimulating the growth of heterotrophic bacteria. The heterotrophic bacteria play a crucial role in the formation of biofloc, which can be utilized as a high-protein fish feed to enhance growth. The microbial biomass, along with other microorganisms present in the flocs, serves as a valuable food source for aquaculture fish (Luo et al. 2020). Achieving the appropriate carbon-to-nitrogen ratio is vital for biofloc development, with recommended ratios ranging from 10:1 to 20:1 (Asaduzzaman et al. 2010, Khanjani et al. 2017). In fish culture systems, a C:N ratio exceeding 10:1 is considered optimal for maximizing biofloc production while minimizing ammonia regeneration.

Corn starch is commonly utilized in biofloc ponds due to its probiotic properties and its ability to promote optimal ratios during both the initial and maintenance phases of biofloc technology (BFT). Tapioca, a flour derived from cassava, enjoys popularity in various regions worldwide, including Brazil and Mexico. It stands as one of the most abundant naturally carbohydrate-rich food products and is easily accessible, particularly in the Americas. Rice bran, sourced from rice, a widely consumed staple food, serves as a valuable carbon source. The implementation of biofloc technology has demonstrated promising outcomes in fish production, leading to significant cost savings in commercial food production and aiding in the controlled administration of medicines and chemicals for disease treatment. Moreover, it contributes to reduced water exchange, which positively impacts the environment by limiting pollution resulting from aquaculture effluent discharges (Avnimelech 2007, Azim and Little 2008).

On the other hand, the use of probiotic additives in aquaculture has increased as an alternative to the use of chemicals and antibiotics for disease control. The term "probiotics" was first coined by Parker in 1974, who defined them as organisms and substances that contribute to the balance of gut microbial communities (Parker 1974). Probiotics are live bacterial preparations that harness various beneficial microorganisms and specialized processes to enhance animal immunity (Flores-Valenzuela et al. 2021). In aquaculture, there are three types of probiotics: soil, feed, and water probiotics, although the majority of usage is observed with feed and water probiotics (Ringo and Birkbeck 1999). Probiotics play a significant role in preserving the health and disease resistance of aquaculture organisms. They compete with pathogenic bacteria for nutrients and attachment sites, produce antimicrobial substances, and modulate the host's immune response. Numerous studies have demonstrated the efficacy of probiotics in safeguarding aquaculture species against bacterial, viral, and fungal infections (Gatesoupe 1999, Balcazar et al. 2006, Ringo et al. 2010).

Biofloc refers to a diverse mixture of suspended particles, extracellular polymeric substances, and various microorganisms, including bacteria, fungi, invertebrates, and detritus. The formation of matrix flocs, which are agglomerations of microbial cells, is governed by intricate flocculation processes influenced by physical, chemical, and biological factors, as described by De Schryver et al. (2008). The matrix of these flocs primarily comprises extracellular polymeric structures, which act as microbial capsules, binding the biofloc components together (De Schryver et al. 2008, Suresh et al. 2018). Polysaccharides, proteins, humid compounds, nucleic acids, and lipids are the major constituents of the flocs, predominantly produced as slime or capsule layers under nitrogen-limited conditions (Basuvaraj et al. 2015). While some flocs can be observed with the naked eye, most of them are microscopic in size, typically ranging from 50 to 200 microns (Ray and Mohanty 2020).

To enhance the cost-effectiveness of the biofloc system, it is crucial to identify suitable probiotics for implementation during culture. While there are limited studies available on probiotics in the culture system (Table 1), there is a lack of specific research on probiotics suitable for rapid floc maturation. As a result, this current research study was undertaken to address this gap and select the optimal probiotic for achieving rapid flocs maturation in BFT. The objective of this study was to promote rapid floc maturation and compare the effectiveness of various probiotics in improving water quality. The study's findings will provide valuable insights for the selection of the most appropriate probiotic for biofloc technology.

Table 1: Available study on probiotic in different culture system.

Aspects	Technology	References
Effects of probiotics on the water quality, growth, & intestinal flora	Biofloc Technology System	Qiu et al. (2023)
Effect of different probiotic diets on microbial gut	Do	Vazquez-Euan et al. (2022)
Effect of commercial probiotics on the phytoplankton diversity	Do	Kurniawati et al. (2021).
Risk of giving different commercial probiotics	Do	Nugroho et al. (2019)
Effect of two probiotics on bacterial composition	Do	Kathia et al. (2018)
Exogenous Probiotics	Do	Daniel and Nageswari (2017)
Effectiveness of using Commercial Probiotics	Biofloc System	Hartono and Barades (2022)
Probiotics used for fish and crustacean	Do	Kathia et al. (2017)
Application of probiotics	Unknown	Subedi and Shrestha (2020)

Materials and Methods

Study area and duration

The current study took place at the Pran Fisheries Project, located in Razapur Village within the Sreemangal Upazilla of the Moulvibazar district (Fig. 1). The experiment was carried out over a duration of 15 days.

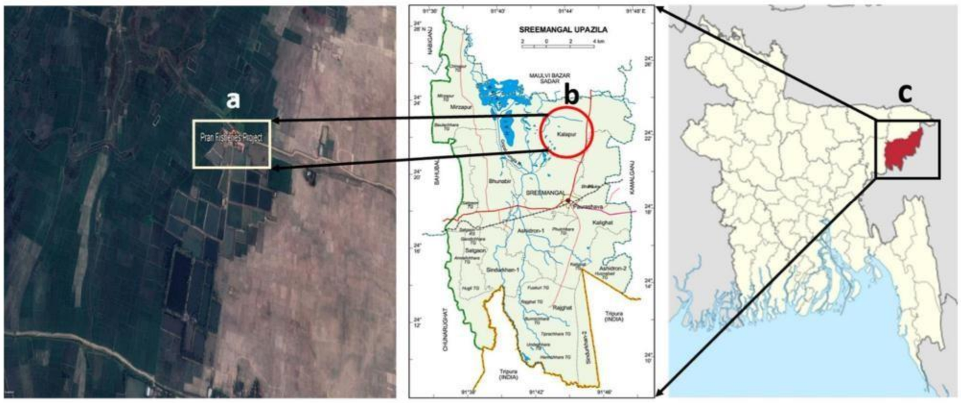

Fig. 1: Map of the experimental site, where the experiment was conducted (www.google.com/earth).

Commercial probiotic ingredients

Everfresh-Pro, which contains *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, and *Bacillus pumilis*. PondCare-3S contains *Bacillus subtills*, *Bacillus licheniformis*, *Bacillus polymyxa, Bacillus pumillus, Bacillus megaterium, Aspergillus niger*, *Aspergillus oryzae.* Profs is a combination of beneficial bacterial strains including *Bacillus* sp., *Pediococcus* sp., *Nitrosomonas* sp. and *Nitrobacter* sp.

Experiment producers

In this study, the water in the tank was prepared by maintaining continuous aeration for a duration of two days. At first, molasses was taken from the sugar mill. It is the by-product and only carbon source in our experiment. Here we used four probiotics from different company. After that taken underground water and removing iron by bleaching treatment and also all kind of bacteria. Following this, 10 g of probiotic and 50 g of molasses were added per 40 liters of water to promote microbial growth. A total of four commercially available probiotics, as shown in Fig. 2, were obtained for experimentation.

Fig. 2: Probiotics used for floc preparation.

The names of the probiotics and their respective companies can be found in Table 2. Floc measurement was carried out by observing the Imhoff Cone after a period of 15 days.

Table 2: List of probiotics used for selecting suitable molasses and probiotic dose for rapid floc maturation.

Probiotic name	Company name	Remarks
Everfresh-Pro	Blue weight Biotech LLP	India
PondCare 3S	Eskayef Animal health Division	Abode Biotec India Pvt. Ltd.
Profs	EON Animal Health Product Ltd.	Biostadt India
MI Plus	ACI Animal Health	Biovac (Thailand) Co., Ltd.

A biofloc maturation time experiment was conducted using various probiotics in separate containers under identical conditions to produce Fermented Carbon Organic (FCO). The different steps of making FCO-

Take clean tub/can with 40 Liters of water and continue vigorous aeration

+

50 g of carbon source (Molasses)

+

10 g of probiotic (*Bacilus* sp., with a total concentration of 10 x 10^9 CFU/g to 10x10^{12} CFU/g)

+

15 days need to develop floc

Water quality monitoring

In this study, various water quality parameters were assessed, including pH, temperature, dissolved oxygen (DO), total dissolved solids (TDS), and salinity. Temperature, DO, and pH were monitored on a weekly basis using specialized instruments. Temperature measurements were obtained using a thermometer, while a portable DO meter (Lutron D5510, Taiwan) and a pH meter (Hanna 981,017, USA) were employed for measuring dissolved oxygen and pH levels, respectively. To ensure accurate measurements, the sample collection and preservation procedures during the DO and TDS assessments adhered to the guidelines outlined in APHA (2005).

Data recording and statistical analysis

After a timeframe of 15 days, the mean values of dosage of each treatment were computed to evaluate the efficacy of the probiotic products. Throughout the experiment, daily monitoring of the floc formation and maturation process took place, and all relevant data was meticulously recorded. The meticulous monitoring methodology adopted enabled a thorough examination of the experimental outcomes, thereby facilitating a comprehensive assessment of the probiotics' efficacy.

Following the data collection phase, the collected data was systematically organized, summarized, and analyzed to align with the objectives of the study. To facilitate the analysis of the gathered information, a tabular method was employed. Data analysis was carried out using MS Excel and GraphPad Prism-6, enabling the generation of more robust outcomes from the experimental results. The statistical technique was applied to determine any significant differences in the floc formation and maturation process, as well as the water quality parameters, across the various probiotics.

Results

Assessment of probiotic condition

The experiment comprised of four different treatments: Everfresh-Pro, PondCare-3S, Profs, and MI Plus. The results demonstrated that all four probiotics effectively promoted floc formation and maturation. However, significant variations were observed in their effectiveness (Table 3). Based on the mean values, Everfresh-Pro exhibited the highest efficacy among the different probiotics (Fig. 3).

Table 3: Descriptive analysis of probiotic dosage.

Probiotics	Minimum	Maximum	Mean ± SD	Confidence level (95%)
Everfresh-Pro	0	16	4.38 ± 5.14	1.54 - 7.22
PondCare-3S	0	10	2.89 ± 3.23	1.10 - 4.68
Profs	0	6	1.17 ± 1.69	0.23 - 2.10
MI Plus	0	7	1.38 ± 1.95	0.30 - 2.46

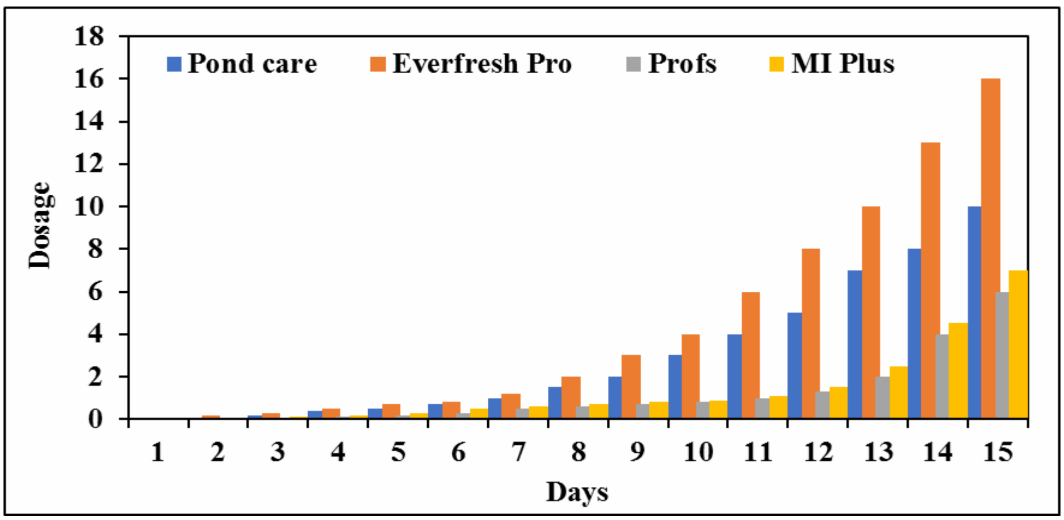

Fig. 3: The dosage of applied probiotic in 15 days.

Floc measurement

Following a 15-day experimental period, the measurements taken in the Imhoff cone yielded flocculation volumes of 28 mL for Everfresh-Pro, 23 mL for PondCare-3S, 20 mL for Profs, and 15 mL for MI Plus (Table 4 and Fig. 4).

Table 4: Biofloc flocculation measurement after 15 days of experiment.

Probiotic name	Company Name	Flocculation time	Imhoff cone measurement
Everfresh-Pro	Blue weight Biotech LLP	15 days	28 mL/**L**
PondCare 3S	Eskayef Animal health Division	15 days	23 mL/L
Profs	EON Animal Health Product Ltd.	15 days	20 mL/L
MI Plus	ACI Animal Health	15 days	15 mL/L

The recommended range for floc volume in the Imhoff cone for fish is ideally between 25-35 mL/L (NFDB). Notably, the floc volume obtained with Everfresh-Pro within this ideal range, indicating the high effectiveness of this probiotic in facilitating rapid floc maturation. Moreover, the study revealed that floc formation and maturation could be achieved in a shorter timeframe when utilizing the FCO making technique with aeration, as compared to traditional pond floc preparation.

Fig. 4: Measurement of floc using Imhoff cone measurement during experiment.

Water quality

According to Nontji (2007), various climatic factors such as precipitation, evaporation, air humidity, air temperature, wind speed, and solar radiation impact the surface temperature of water. In the current study, temperature readings were collected daily over a period of 15 days. The results indicated that the temperature ranged between 27°C and 30°C, with an average of 28.26°C. Dissolved oxygen (DO) measurements were conducted once during the experimental period, yielding an average DO value of 6.0 ppm. The amount of dissolved oxygen in water is influenced by temperature and plays a crucial role in the metabolism of both the microbial community and the cultured species, thereby affecting fish growth parameters (Boyd 1982). The pH value recorded during the experimental period was 7.8. Additionally, the total dissolved solids (TDS) value was measured at 980 ppm, and the salinity level was 0.8 ppt. The average measurements of water quality parameters throughout the study are presented in Table 5.

Table 5: Water quality values during the study.

Parameters	Value	Optimum level (Bhatnagar et al. 2013, Setiadi et al. 2019)
Temperature (°C)	28.26	26–32°C
Dissolved oxygen (ppm)	6.0	≥3 ppm
pH	7.8	6-9
TDS (ppm)	980	<1000 ppm
Salinity (ppt)	0.8	Depends on cultured species

Discussion

The current literature offers limited information regarding the effect of probiotics on promoting floc maturation in Biofloc systems. Hence, this study was designed with four distinct probiotics (Everfresh-Pro, PondCare-3S, Profs, and MI Plus) to identify the most effective probiotic for enhancing floc maturation within a 15-day period in biofloc technology.

The experiment included a total of four probiotics (Everfresh-Pro, PondCare-3S, Profs, and MI Plus) conducted over a period of 15 days. At the end of the study, Everfresh-Pro showed the highest mean value among the treatments. This result indicates that the use of probiotics can significantly increase floc volume within a shorter period of time compared to traditional pond floc preparation methods. Everfresh-Pro, which contains *Bacillus* subtilis, *Bacillus licheniformis, Bacillus megaterium,* and *Bacillus pumilis,* is known to generate antimicrobial peptides that provide immune-stimulatory effects to animals (Barbosa 2005). These findings are consistent with previous studies that have demonstrated the effectiveness of probiotics in promoting floc formation and maturation in aquaculture systems (Wang et al. 2015, Kuhn et al. 2018). In contrast, Kurniawati et al. (2021) conducted a study on the effect of commercial probiotics, where they implemented three treatments using different commercial probiotics for floc formation. Treatment A involved biofloc formation without the use of probiotics, Treatment B utilized commercial probiotics (*Bacillus* spp., lactic acid, *Lactobacillus* spp., *Saccharomyces* spp.) in a 50/50 feed mixture, and Treatment C utilized commercial probiotics (a native microbial consortium) in a 50/50 feed mixture. Interestingly, the highest diversity index value was observed in Treatment C, which utilized a native microbial consortium containing active ingredients such as *Lactobacillus* spp., *Nitrosomonas* spp., *Bacillus subtilis,* and *Bacillus* spp. Overall, these observations highlight the choice of probiotics and their composition can play a crucial role in achieving optimal results.

The recommended floc volume in an Imhoff cone for fish is 25-35 mL/L according to the National Fisheries Development Board (NFDB). In our experiment, we obtained an ideal floc volume of 28 mL/L using the FCO making technique with the Everfresh-Pro probiotic. However, the volumes obtained with the other three probiotics used in the experiment were 23 mL/L for PondCare-3S, 20 mL/L for Profs, and 15 mL/L for MI Plus, which are not ideal for fish. Avnimelech (1999) reported that floc dominance typically occurs after approximately 30 days in pond floc preparation, with floc volumes ranging from 2-10 mL/L. In our study, we generated floc in a tank for FCO making with continuous and intense aeration over a 24 hours period. As a result, the floc volume increased more rapidly compared to the traditional pond floc preparation method. Unfortunately, we could not find any additional references regarding floc maturation to make direct comparisons with our results. Consequently, we were unable to compare our findings with those of other studies.

In this experiment, molasses was chosen as the carbon source for the biofloc system. Molasses, classified as a simple carbohydrate, is easily metabolized by bacteria, which necessitates frequent additions to maintain optimal activity of heterotrophic bacteria. Conversely, complex carbohydrates like starch grains and cellulose are broken down at a slower rate by bacteria, leading to more stable carbohydrate levels and longer reaction times (Khanjani et al. 2017, Khanjani and Sharifnia 2020). Molasses, known for its cost-effectiveness, is commonly used as a bacterial growth promoter in shrimp culture (Khanjani et al. 2017). Overall, the selection of molasses as the carbon source in this experiment offers a cost-effective and efficient approach for promoting bacterial growth in the biofloc system.

Throughout the experiment, meticulous monitoring of water quality parameters was carried out, ensuring strict adherence to desired conditions. The temperature was consistently maintained within the range of 27°C to 30°C, providing favorable thermal conditions for the process of floc maturation. The dissolved oxygen (DO) level was closely monitored and consistently measured at 6 ppm, indicating sufficient oxygen availability to support the microbial activity essential for floc development. The salinity, total dissolved solids (TDS), and pH values were recorded as 7.8, 980, and 0.8 ppt, respectively, suggesting a conducive environment for floc maturation. It is worth noting that despite the comprehensive monitoring of water quality parameters in this study, limited specific information is available in the existing literature regarding water monitoring during the process of floc maturation. As a result, making direct comparisons with other studies is currently constrained.

Overall, the use of Everfresh-Pro probiotic resulted in higher floc formation with reduced maturation time, and leading to improved floc quality. However, it is important to note that there is a lack of existing literature on the selection of suitable probiotics for rapid floc maturation, making this study the first of its kind in the field of enhancing floc maturation in biofloc technology. The findings of this research will serve as a valuable reference for future studies aiming to identify the optimal probiotic for fish culture using biofloc technology.

Conclusion

This experiment revealed the suitable probiotic that responsible for rapid floc maturation in biofloc technology. Based on our findings Everfresh-Pro considered as ideal among other three probiotics and recommended for BFT. Ideal probiotic enhances profitability in biofloc system. This strategy holds promise for the future of aquatic species, considering potential fisheries health risks. In conclusion, optimizing probiotic dosage in biofloc systems, along with technological advancements, offer a promising approach to enhance profitability.

Conflict of interest: The authors assert that this research is devoid of bias.

Contribution: Authors contributed uniformly in the research and writing of this article.

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