

## Use of a bacterial preparation in the formation of a bacterial composition in Biofloc technology

Aktamqulova M.A and Mullabayev N.R \*

*Tashkent State Agrarian University.*

World Journal of Advanced Research and Reviews, 2026, 30(02), 1702-1706

Publication history: Received on 11 April 2026; revised on 18 May 2026; accepted on 20 May 2026

Article DOI: <https://doi.org/10.30574/wjarr.2026.30.2.1435>

### Abstract

This study is devoted to investigating the possibilities of intensive cultivation of African catfish (*Clarias gariepinus*) using bioflick technology under the conditions of Uzbekistan. The experiment was conducted in 700-liter tanks, and the bacterial preparation "Doctor Robik" was used to form activated sludge, or biofloc. During the study, the bacterial composition of the water was identified, and the presence of *Citrobacter freundii*, *Fictibacillus barbaricus*, and *Plestiodon's shigelloides* was recorded. The results showed that two of these bacteria had beneficial effects, while one had a negative effect, indicating the risk of uncontrolled bacterial proliferation in the biofloc system. Therefore, the formation of a positive bacterial composition in biofloc systems is of particular importance.

**Keywords:** Biological Flocculation; Floc; Flocculant; Water Clarity; Microorganisms; Bacterial Composition; African Catfish; Heterotrophic System

### 1. Introduction

For Uzbekistan, located in the center of Eurasia, the only way to increase the volume of fish production is to improve the productivity of inland water bodies, that is, to transition aquaculture from an extensive system to an intensive one.

In industrial aquaculture, there are two main systems for cultivating aquatic organisms: flow-through systems, including ponds, cages, and tanks, and recirculating systems, including recirculating aquaculture systems (RAS), closed water supply systems, biofloc technology (BFT), and green water systems/aquaculture. Among recirculating systems, zero-water exchange biofloc systems are used for cultivating certain aquatic organisms under conditions of limited water and land resources, as they make it possible to achieve nearly 100% recirculation, maintain high industrial stocking densities, and keep the feed conversion ratio at a consistently low level [1].

The concept of cultivating aquatic organisms in a zero-water exchange system is based on raising fish, such as tilapia, catfish, and carp species, or crustaceans, such as shrimp, together with microorganisms in a tank without water exchange. These microorganisms perform the main water purification function, meaning that the water does not need to circulate through several filtration systems as in a conventional closed water supply system [2].

This aquaculture system is based on strict control of the number of bacteria in the water. Under production conditions, monitoring was carried out using an Imhoff settling cone. Over time, the number of flocs in the water increased. When the flocs reached a critical level capable of clogging the gills of catfish fry, it became necessary to replace part of the water in the tanks. In this case, the used water was discharged into a settling tank without aeration, where the sediment settled. The settling period ranged from 20 minutes to 4 hours. After sedimentation, the clarified water was returned to

\* Corresponding author: Mullabayev N.R

the tank where the fish were being grown. The floc sediment could then be utilized as fertilizer or as a protein source in compound feed. The use of simple settling tanks makes it possible to regenerate water almost indefinitely [3].

At a time when industrial aquaculture is characterized by a tendency toward a high level of production intensification, controlling a complex of abiotic and biotic factors plays an important role in achieving high productivity. In this process, particular importance is given to hydrochemical parameters, temperature, water exchange, and biological water purification, which is managed through various microbiota.

For microorganisms to effectively assimilate total ammonium nitrogen (TAN), they require an easily digestible carbohydrate source, such as sugar, molasses, starch, or glycerin. Since the water layers in the tanks are fully mixed with air, there is no need for separate equipment to additionally introduce atmospheric or liquid oxygen into the water or to remove carbon dioxide.

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## 2. Literature Review

Bio floc technology (BFT) is recognized as a highly effective method for controlling water quality in modern intensive aquaculture. This technology originated in the 1970s from experiments on tilapia cultivation conducted by the Aquacop research group in France and later became widely used around the world [1].

The main principle of the BFT system is that, under conditions of zero or minimal water exchange, heterotrophic bacteria assimilate nitrogenous wastes, mainly ammonia and nitrites, and convert them into microbial biomass known as biofloc. This biomass, in turn, serves as an additional protein source for fish. A number of studies have shown that biofloc particles contain 25–40% protein, 0.5–15% lipid, and essential amino acids, which determines their nutritional value as a supplementary feed source for fish [2].

For the effective operation of a biofloc system, several important conditions must be maintained: the dissolved oxygen level should be above 4 mg/L, the C:N ratio should be kept within the range of 12–15:1, the floc volume in an Imhoff cone should remain between 15 and 50 mL/L, and the pH should be maintained within the range of 7–8. If these parameters become imbalanced, undesirable microorganisms may proliferate in the system and pose a risk to fish health [3].

African catfish (*Clarias gariepinus*) is considered one of the most promising fish species for cultivation using biofloc technology, as it is characterized by tolerance to low dissolved oxygen levels and high stocking densities. Although this species is of African origin and adapted to tropical and subtropical climates, experiments have shown that its cultivation can also be effective during the hot summer season in Uzbekistan.

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## 3. Research Materials and Methods

The experiment was conducted from February to May 2025. African catfish were reared during the growth period from an initial weight of 2.5 g to 100 g. round plastic tanks with a diameter of 135 cm, a height of 65 cm, and a total capacity of 700 liters were used in the experiment. In each tank, four porous stone diffusers with a diameter of 12 cm were installed by attaching them to a cord stretched across the upper part of the tank. The water level varied between 45 and 50 cm.

The concentration of flocs was measured using an Imhoff settling cone. Other hydrochemical parameters, including total ammonium nitrogen (TAN), nitrites, and nitrates, were monitored using drop titration tests. The water temperature was maintained within the range of 26–28°C, which is considered favorable for the optimal growth of African catfish.

To form activated sludge, or biofloc particles, the initial bacterial culture “Doctor Robik” was used. This preparation is a composite of beneficial bacteria specially produced for septic tanks and toilet pits. It is available in powder form, with one package containing 75 g. One package was added to 500 liters of water to form the bacterial composition.

The experimental tanks were divided into three groups: the first group served as the control group with a conventional water supply; the second group was maintained as a biofloc system using “Doctor Robik”; and the third group was maintained as a biofloc system supplemented with probiotics. Fifty fry were stocked in each group, and the experiment lasted for 90 days.

Samples for microbiological analysis were collected on the 30th, 60th, and 90th days of the experiment. The samples were collected in sterile plastic containers, transported to the laboratory at 4°C, and analyzed within 24 hours. Selective culture media, including MacConkey agar, Blood agar, and TCBS agar, were used for bacterial identification. The obtained colonies were identified based on their morphological and biochemical characteristics.

#### 4. Results and Discussion

The composition of the “Doctor Robik” product is not publicly disclosed; it is described as a standardized mixture of six specially selected *Bacillus* bacterial strains. These bacteria were developed by scientists to decompose even substances that are difficult to break down and are not easily degraded by biological agents.

In the experimental tank, flocs reached equilibrium within 7–10 days. On May 20, 2025, the bacterial composition of the biofloc tank was analyzed, and the following bacteria were identified: *Citrobacter freundii*, *Fictibacillus barbaricus*, and *Plesiomonas shigelloides*.

**Table 1** Bacteria identified in the biofloc tank and their characteristics

Bacterial species	Characteristics	Role in biofloc	Type of effect
<i>Citrobacter freundii</i>	Facultative anaerobic, Gram-negative enterobacterium	Nitrogen degradation, denitrification	Conditionally beneficial / potentially harmful
<i>Fictibacillus barbaricus</i>	Aerobic, Gram-positive, spore-forming bacterium	Decomposition of organic matter	Beneficial
<i>Plesiomonas shigelloides</i>	Facultative anaerobic, Gram-negative aquatic bacterium	No beneficial role identified	Negative / pathogenic

##### 4.1. *Citrobacter freundii*

*Citrobacter freundii* is a facultative anaerobic, Gram-negative bacterium belonging to the Enterobacteriaceae family. Some strains participate in nitrogen degradation and may function as nitrate-reducing bacteria in biofloc systems. This bacterium is also actively involved in the decomposition of organic residues, which plays an important role in biological water purification. However, pathogenic strains also exist and may cause disease in fish with weakened immunity. Fish at the fry stage are particularly susceptible to the negative effects of this bacterium. Therefore, its presence in a biofloc system should be carefully monitored.

##### 4.2. *Fictibacillus barbaricus*

*Fictibacillus barbaricus* is a Gram-positive, spore-forming, aerobic bacterium that belongs to a relatively poorly studied species. It may have the ability to decompose organic matter and reduce biosludge accumulation. Its spore-forming structure allows it to survive under unfavorable conditions, including high temperature, dryness, and chemical exposure. As an environmental cleaning bacterium, it may be useful in decomposing complex organic compounds such as cellulose, lipids, and proteins. However, the role of this bacterium in biofloc systems has not yet been sufficiently described in the scientific literature, which indicates the need for further research.

##### 4.3. *Plesiomonas shigelloides*

*Plesiomonas shigelloides* is a Gram-negative, facultative anaerobic bacterium commonly found in aquatic environments. This bacterium is rarely considered beneficial. It is more commonly known as a toxic and opportunistic pathogen that can cause enteritis and other infections in fish. It is often found in warm and polluted waters; therefore, its presence in the tank may indicate a deterioration of sanitary and hygienic conditions. The detection of *P. shigelloides* suggests the formation of an undesirable microbial environment in the biofloc system.

During the experiment, the hydrochemical parameters were as follows: total ammonium nitrogen (TAN) ranged from 0.2 to 0.8 mg/L, nitrites from 0.05 to 0.15 mg/L, nitrates from 5 to 20 mg/L, dissolved oxygen from 5.5 to 7.2 mg/L, and pH from 7.2 to 7.8. Overall, these parameters remained within a satisfactory range for a biofloc system; however, the presence of *P. shigelloides* indicated a potential weakness in the system.

Preliminary results on fish growth showed that catfish in the biofloc system demonstrated an 18–22% higher growth rate compared with the control group. This improvement is presumed to be mainly due to the use of biofloc particles as an additional protein source. However, due to the presence of *P. shigelloides*, further experiments require careful monitoring of fish health.

### *Recommendations*

The results of the study showed that only bacterial strains with beneficial effects should be used in biofloc systems. To achieve this, several important measures are recommended.

First, before introducing an initial bacterial culture, it should be tested under laboratory conditions, and only certified strains should be used. Particular attention should be paid to the use of products not intended for consumption, such as septic system preparations, in aquaculture.

Second, regular microbiological monitoring should be carried out in biofloc systems every 2–4 weeks. As part of this monitoring, the presence of major pathogenic bacteria, including *Aeromonas* spp., *Pseudomonas* spp., and *Plesiomonas* spp., should be checked.

Third, the use of probiotic strains such as *Bacillus subtilis*, *Bacillus licheniformis*, and *Lactobacillus* spp. can help maintain a beneficial bacterial environment. These strains compete with pathogenic bacteria and limit their proliferation.

Fourth, maintaining the C:N ratio at approximately 15:1 ensures the effective assimilation of nitrogen by heterotrophic bacteria and restricts the development of pathogenic microorganisms. For this purpose, it is recommended to add sugar, starch, or other carbon sources to the system.

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## **5. Conclusion**

The bacteria identified in the experimental tank — *Citrobacter freundii*, *Fictibacillus barbaricus*, and *Plesiomonas shigelloides* — included two conditionally beneficial species and one species with a negative effect. This indicates that uncontrolled bacterial proliferation may occur in biofloc systems.

The “Doctor Robik” preparation, which is intended for septic systems, is not optimal for aquaculture purposes, as its bacterial composition has not been certified according to aquaculture requirements. Although this preparation showed partial effectiveness in forming biofloc, it also increased the risk of introducing pathogenic microorganisms into the system.

Therefore, in biofloc technology, it is advisable to promote only potentially beneficial bacterial compositions. Otherwise, other bacterial strains may also proliferate and negatively affect fish health and productivity. Future studies should test certified probiotic complexes specifically designed for aquaculture and develop a standardized protocol for biofloc technology under the conditions of Uzbekistan.

The study demonstrates that special attention should be paid to microbial control when introducing biofloc technology in Uzbekistan. It also highlights the need to develop technological protocols adapted to local conditions. This, in turn, may contribute to increasing fish productivity in inland water bodies, producing import-substituting products, and addressing the issue of fish product supply in the domestic market.

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## **Compliance with ethical standards**

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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## **References**

- [1] Bossier P., Ekasari J. Biofloc technology application in aquaculture to support sustainable development goals // *Microbial Biotechnology*. 2017. 10 (5). P. 1012—1016.

- [2] Biofloc technology in aquaculture: Beneficial effects and future challenges / R. Crab [et al.] // *Aquaculture*. 2012. Vol. 356—357. P. 351—356.
- [3] Daniel N., Nageswari P. Exogenous Probiotics on Biofloc based Aquaculture: A Review // *Current Agriculture Research Journal*. 2017. Vol. 5 (1). P. 88—107.
- [4] Avnimelech Y. *Biofloc Technology — A Practical Guide Book*. 2nd ed. Baton Rouge: World Aquaculture Society, 2012. 272 p.
- [5] de Schryver P., Crab R., Defoirdt T., Boon N., Verstraete W. The basics of bio-flocs technology: The added value for aquaculture // *Aquaculture*. 2008. Vol. 277 (3—4). P. 125—137.
- [6] Kumar V., Roy S., Meena D.K., Sarkar U.K. Application of probiotics in shrimp aquaculture: importance, mechanisms of action, and methods of administration // *Reviews in Fisheries Science & Aquaculture*. 2016. Vol. 24 (4). P. 342—368.