

Study on the Application of Bio-flocs Technology in Tail Water Treatment

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Abstract: In aquaculture, the deposition of biological manure and residual bait in water can cause water pollution. If the discharged aquaculture tail water is not treated in time, the surrounding water environment will be affected and the quality of aquaculture organisms will be reduced. Controlling inorganic nitrogen by controlling the carbon-nitrogen ratio is a potential aquaculture system control method. This method can effectively reduce the accumulation of inorganic nitrogen in ponds, convert ammonia and nitrite nitrogen in water into proteins for culture organisms, and inhibit the growth of harmful microorganisms. This paper discusses the research development of bio-flocs technology at home and abroad, how to treat tail water with maximum efficiency in order to solve the problems of debris and feed retention in aquaculture water and realize the reuse of bait, and purifying water quality.

1. Introduction

The 14th Five-Year Plan for National Aquatic Technology Promotion in China clearly proposes to improve the aquaculture environment, ensure the quality and safety of seafood products, and improve the management level of the marine aquaculture industry [1]. Aquatic animals require high concentrations of protein in their feed because protein oxidation and catabolism provide energy for aquatic animals. Ammonia is excreted during fish culture and may accumulate in ponds. In high-aeration ponds, ammonia is oxidized by bacteria into nitrite and nitrate species. Unlike carbon dioxide released into the air through diffusion or forced aeration, there is no effective mechanism to release nitrogenous metabolites out of the pond, which will cause an increase in the content of organic matters in the water body and eutrophication of the water body, which is one of the root causes of red tide [2].

In order to ensure the safety of aquaculture water [3], bio-flocs technology is considered an effective alternative technology to solve the environmental constraints and feed cost problems faced by the development of aquaculture industry. With this technology, the residues fed and the feces generated by aquaculture organisms can be decomposed during the aquaculture process of seawater ponds, and ammonia nitrogen and nitrite nitrogen in the water can be converted into protein for aquaculture organisms, while inhibiting the growth of harmful microorganisms [4]. By adjusting the carbon-nitrogen ratio in the water body, increasing the number of heterotrophic bacteria in the water body, and using

microorganisms to assimilate inorganic nitrogen, nitrogen-containing compounds such as ammonia nitrogen in the water body are converted into bacterial proteins to form biological flocs that can be directly ingested by filter-feeding animals, which can solve the problem of aquaculture water quality, improve the survival rate of aquaculture objects and increase the yield [5].

2. Research Significance

2.1 Definition of Bio-flocs Technology

With the expansion of aquaculture scale and the increase of aquaculture density, environmental pollution and economic constraints are becoming increasingly serious, which greatly restrict the development of aquaculture. Especially in the process of intensive aquaculture, a large amount of residual bait manure is discharged into the aquaculture water body, causing rapid accumulation of toxic substances such as ammonia nitrogen, resulting in deterioration of aquaculture water quality and potential environmental pollution. Bio-flocs technology stands out among various tail water treatment methods. In the aquaculture process, the carbon-nitrogen ratio in the water body is adjusted by artificially adding organic carbon sources, so that heterotrophic microorganisms can convert inorganic nitrogen in the water body into their own proteins while utilizing the organic carbon sources, thus being directly ingested by fish or shrimp, thus reducing the amount of water exchanged. Ammonia nitrogen, organic carbon source, dissolved oxygen and alkalinity

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are necessary for the formation of biological flocs [6]. Bio-flocculant formation is a process in which heterotrophic microorganisms in water use ammonia nitrogen and organic carbon sources added from outside to consume a certain amount of dissolved oxygen and alkalinity and convert them into heterotrophic microorganisms.

2.2 Significance of Bio-flocs

Compared with other aquaculture systems, the biological flocs aquaculture system has the advantage of reducing the discharge of a large amount of wastewater into the environment, thus effectively avoiding the pollution or even more serious damage to the surrounding environment of the aquaculture area, avoiding the eutrophication of the surrounding water environment and reducing the excessive waste of water resources. Due to the high concentration of ammonia nitrogen in most aquaculture industries, the addition of nitrifying bacteria converts ammonia nitrogen into nitrite nitrogen, resulting in excessive concentration of nitrite nitrogen in aquaculture water and secondary pollution [7]. In the bio-flocs technology, this aquaculture mode can solve the problems of traditional aquaculture mode such as abuse of antibiotics, low utilization rate of water resources and eutrophication of the surrounding environment caused by arbitrary discharge of aquaculture wastewater by little or no water change [8].

3. Research Progress at Home and Abroad

3.1 Research Progress Abroad

According to the study on the removal effect of inorganic nitrogen in water, the protein content of bio-flocs and the structure diversity of microbial community of bio-flocs, the carbon-nitrogen ratio plays a decisive role in the formation of biofloc. It has been demonstrated by ¹³C labeling that tilapia can use bacteria to culture single-cell proteins, and the best conversion rate of carbon to nitrogen ratio of 15:1 has been demonstrated under the conditions of sodium acetate and glycerol as carbon sources [9]. The ¹⁵N isotope labeling method was used to find that part of the nitrogen ingested by prawns in the *Litopenaeus vannamei* aquaculture system came from biological flocs and had a good removal effect on inorganic nitrogen [10]. Nowadays, bio-flocs technology has been widely used in large-scale shrimp farming in Asia, Latin America and Central America, as well as indoor greenhouse aquaculture in most countries.

3.2 Research Progress in China

There are many tail water treatment methods in China. One is the frequent replacement of pond water, which not only incurs excessive costs but also releases pathogens or nutrients into the environment. Another is to enhance nitrification of relatively inert nitrate species through the

use of biofilters. This is also costly and requires the treatment and digestion of large amounts of feed residue. Therefore, bio-flocs add carbonaceous material to the system to assimilate ammonium in water into microbial proteins, thereby removing ammonium from water [11]. This is both economical and efficient and represents a potential utilization of microbial protein as a source of feed protein for fish or shrimp. The utilization of biological flocs in Guizhou has promoted the sustainable development of pond aquaculture, improved the efficiency of tail water treatment and reduced the treatment cost [12]. In Fujian, bio-flocs were used to improve the ecological level of *Litopenaeus vannamei* seedling production, and different carbon sources were added to *Bacillus licheniformis*. There are also effective ecological technologies for the cultivation of crucian carp in China to promote the healthy development of crucian carp [13].

4. Analysis and Discussion of Typical Cases

4.1 Culture of Bacteria into Bio-flocs

Densely cultured bacteria tend to form biological flocs containing bacteria, other organisms and organic particles, usually 0.1-2 mm in diameter. Bacteria require nitrogen and oxygen, and heterotrophic bacteria and other microorganisms use carbohydrates, sugars, starches, and cellulose as food to produce energy and growth, i.e., proteins and new cells, using chemical energy in organic substrates [14]. Microorganisms degrade the waste. Some of this is converted to carbon dioxide and about 50% to microbial biomass, and we can control nitrogen in ponds by manipulating the activity of microorganisms. Nitrogen control is induced by feeding carbohydrates to bacteria and subsequently absorbing nitrogen from the water by synthesizing microbial proteins. By adding sucrose to the bacterial culture bio-flocs, the floc settling capacity (BFA) can reach 20 ml/L and the total suspended particulate matter (TSS) of the flocs can reach 355 mg/l [15].

4.2 Utilization of bio-flocs in aquaculture

The suitable carbon-nitrogen ratio required for the formation of biological flocs is added to the grass carp pond. The experiment shows that when the carbon-nitrogen ratio is 15:1, the biological flocs can effectively adjust the water quality, reduce the levels of ammonia nitrogen and nitrite nitrogen in the water body, and have a positive impact on the growth of grass carp [16]. The changes of fish growth and water quality in bio-flocs system of different carp aquaculture models were studied. The results showed that there was a significant positive correlation between the amount of bio-flocs formation and water temperature within a certain range in the three breeding models of bio-flocs system. In the whole biological flocculant aquaculture system, the mixed aquaculture mode conforming to biological principles can also effectively play the ecological function of the

aquaculture system and improve aquaculture efficiency [17]. The effects of different carbon-nitrogen ratios on the formation of bio-flocs, water quality and microflora structure in cultured waters of Chinese grass turtle were studied. Experiments have shown that the carbon-nitrogen ratio of 15:1 is the most suitable ratio for the formation of biological flocs, which not only promotes the formation of biological flocs, but also has a strong ability to regulate water quality [18]. Bio-flocs can fix ammonia nitrogen and nitrite nitrogen in water as their own organic nitrogen; The biological flocs contain the basic nutrients required for the growth of cultured organisms. Silver carp and *Litopenaeus vannamei* can fix the organic nitrogen of the biological flocs as their own proteins, and mixed culture of fish and shrimp can promote the feeding of *Litopenaeus vannamei* on the biological flocs. At the same time, the nitrogen removal by silver carp and *Litopenaeus vannamei* and the nitrogen fixation by biological flocs can promote the nitrogen circulation in aquaculture ponds.

4.3 Influence of Different Nitrogen Sources on Bio-flocs

Two nitrogen sources, ammonium chloride and feed, were selected to culture the bio-flocs. After 12 days of incubation, the biofloc formation of ammonium chloride group and feed group reached 7.2 mL/L and 8.8 mL/L respectively. Therefore, the effect of feeding artificial formula feed to culture biological flocs is better [19]. The relationship between the addition of carbohydrates, the reduction of ammonium, and the production of microbial protein depends on the microbial conversion coefficient, and the carbon content of the added substance. The addition of optimally calculated and quantitative carbohydrates in experimental prawn ponds and tilapia commercial aquaculture ponds can reduce inorganic nitrogen content and feed costs. The addition of carbohydrates is also a potential means of reducing inorganic nitrogen concentrations in intensive aquaculture systems [20].

4.4 Culture of Algae to Form Bio-flocs

Two water environments of bioflocculation culture tail water (BFW) and BG11 culture medium (BGW) were set up to discuss the high concentration of nitrate nitrogen and phosphate in the later stage of intensive culture mode with common green oscillating algae. The removal effect of *Spirulina platensis* on nitrogen and phosphorus in intensive culture tailwater and its growth status were also analyzed. The results showed that both *Spirulina platensis* SP1 and Oscillating algae could survive in trophic environments of BFW and BGW, and had good removal effects on nitrogen and phosphorus in water. Therefore, it is credible that *Spirulina* can be used as an alternative algae strain for intensive aquaculture tailwater purification in aquaculture applications [21].

4.5 Limitations on the Application of Bio-flocs

There are still some problems with bio-flocs technology, such as the complex microbial composition and poor stability of bio-flocs, the inability of bio-flocs to be eaten by shrimp in the late stage of cultivation, the accumulation of large amounts of flocculation, increasing oxygen consumption, affecting shrimp respiration, unstable water quality, high CO₂ content, low pH, aging, sedimentation, and decay of flocculation, etc [22]. Although there have been some achievements in the research of bio-flocs technology, there are also some difficulties in practical application, such as the acceptance of farmers, the stability of the technology, and the guarantee of production benefits[23].

5. Conclusion

Bio-flocs technology has been applied to the culture of *Tilapia*, *Litopenaeus vannamei*, *Macrobrachium rosenbergii* and *Penaeus monodon* abroad. The study of bio-flocs technology throughout the breeding cycle not only discusses the impact of aquatic animal growth, survival and water quality regulation, but also further analyzes the population diversity of microorganisms in the bio-flocs to determine the dominant flora of the bio-flocs [24]. In *Penaeus japonicus*, the presence of probiotics in the bio-flocs formed by the addition of sucrose inhibited the reproduction of pathogenic bacteria in culture waters to some extent. However, the cost of culturing bio-flocs by adding sucrose is too high to be suitable for large-scale popularization. Whether it is possible to try to ferment agricultural by-products (straw, wheat bran, soybean meal, peanut meal, corn meal, etc.) to produce bacillus, screen out the best fermentation medium composition through independent formula test, optimize the medium and fermentation conditions through single-factor sum positive test, establish a simple and feasible beneficial microbial fermentation process, and so on.

The important position of microorganisms in the ecosystem determines that biological flocs have a wide application prospect in aquaculture. By adding carbon sources, the carbon-nitrogen ratio in water can be adjusted, which is beneficial to the growth of biological flocs. Bio-flocs can effectively purify nitrogen-loaded aquaculture water, and also provide protein sources for the growth of fish or shrimp, which has a broader application prospect in aquaculture. However, in general, the application of bio-flocs technology in culture is still in the initial stage, and the technology is not mature. How to further optimize bio-flocs technology and develop applicable products through artificial regulation will provide technical support for aquaculture industry.

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