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Addition Ammonia Assimilation Bacteria to a Biofloc System for Japanese Eel (*Anguilla japonica*) Farming, Comparison of Growth Performance and Water Quality

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Abstract: Biofloc technology (BFT) system is a renovated and promising aquaculture system which allows aquaculture animals to be farmed at a high density with little or zero water exchange. The research objective of this study was to investigate and compare the effect of BFT with and without exogenous ammonia assimilation bacteria supplementation on water quality and Japanese eel growth performance. Two biofloc treatments (BFT groups) with and without *Bacillus* sp. addition (Group A and B, respectively) and one control (Group C, traditional aquaculture) were created. Corn starch and sodium bicarbonate were added regularly to maintain C/N ratio and alkalinity of the biofloc treatments. Eels (30±1.2g) were stocked in each pond of 30m^3 for 60 days. The result showed that although all toxic nitrogen compound concentration in BFT groups were maintained at safe levels for eel culture during the experiment, bacteria addition could help the system maintain lower level of ammonia at a beginning period. The higher weight gain and specific growth rate were observed in BFT groups compared to control group. Especially, ammonia assimilation bacteria addition had a positive impact on water quality and eel production as the Group A showed the highest total biomass of 129.09 kg with the lowest FCR (feed conversion ratio) of 1.78. The present study revealed that Japanese eels can be reared effectively by biofloc technology with exogenous bacteria input.

Keywords: BFT, Japanese Eel Culture, Nitrogen Compound, Water Quality, *Bacillus* sp

1. Introduction

The eel has been regarded as a fish of high quality due to its high nutritious value and unique taste. Although there is a high demand for eel products all over the world, wild eel resources are limited in some areas. Therefore, high human demand could be possibly met by only relying on the aquaculture and in the real world and the majority of eels are being produced by fish farming [1]. However, large-scale eel production faces several problems such as a huge requirement of water, diseases spread] and high cost [2-4]. Thus, a renovated technology is needed to lower operating cost and ensure sustainable production in eel farming.

A biofloc technology (BFT) is an alternative and Eco-friendly aquaculture technology relied on microorganisms which can convert toxic ammonia and nitrite to non-toxic nitrate or mycoprotein. This technology enables not only to improve water quality by decreasing toxic nitrogen compound but also to form a biofloc as nutrient source for fish. It is also possible to realize minimal or zero water exchange in aquaculture system and increase fish production [5-7].

There are also some drawbacks such as high suspended solid concentration and instability of ammonia and nitrite concentration at the beginning period of a biofloc system [8, 9]. Therefore, suitable species like tilapia and shrimp which can tolerate poor water quality are widely cultured by BFT [10, 11]. However, limited data are available for eel farming with BFT. Eels are known to be very sensitive to high ammonia and nitrite concentration in water [12]. Thus, it is crucial to keep low ammonia and nitrite concentration in eel rearing water.

One strategy to eliminate ammonia in water is to make heterotrophic bacteria dominate in microbial community. Two main ammonia pathways exist in the biofloc system-nitrification by autotrophic bacteria and assimilation by heterotrophic bacteria [13]. Heterotrophic bacteria are referred to be more favorable because they can convert nitrogen compounds to microbial protein and their growth rate is 10 times greater than autotrophic bacteria [14].

From this point of view, we hypothesized that a biofloc system with exogenous ammonia assimilation bacteria input could provide better water quality environment and improve growth performance of eels. To our best knowledge, this is the first attempt to rear Japanese eels by BFT with ammonia assimilation bacteria supplementation. In this context, the present study aimed at comparison between biofloc treatment with and without exogenous bacteria introduction and control group where eels were reared by traditional aquaculture method.

2. Materials and Methods

2.1. Experimental Unit and Design

The experiment unit consisted of a round pond of $30m^3$ and one air stone with the air flow rate of 8 $L.min^{-1}$ to keep high dissolved oxygen concentration as well as water movement and circulation during the experiment period. The air stone was placed at the center of the pond and connected to air pump. Total nine ponds were divided into three groups (three replicates each)-group A (BFT + ammonia assimilation bacteria addition), group B (BFT without exogenous bacteria addition) and group C (Control, no BFT). No water exchange was performed in BFT treatments except compensation of evaporation water loss while 30% of pond water was daily replaced in group C. Water in group C was treated by sodium hypochlorite every ten days.

2.2. Ammonia-Assimilation Bacteria

Bacillus sp. that has a high ammonia assimilation activity was isolated from the other pond water where eels were cultured by traditional method at day 30 after culture. Culture media for ammonia assimilation bacteria composed of glucose (0.5%), NH_4Cl (0.025%), $Fe(NH_4)_2H(C_6H_5O_7)_2$ (0.01), NaCl (0.05%), $MgSO_4 \cdot 7H_2O$ (0.05%), $MnCl_2 \cdot 4H_2O$ (0.01%), K_2HPO_4 (0.1%), KH_2PO_4 (0.32%) and 1.5% agar [15]. This strain was scaled up using the medium containing 3% of corn powder and 0.19% of ammonia chloride for 24 hours at 35°C and pH 7.0. After culture, the bacterial concentration reached 35.8×10^8 $cfu.mL^{-1}$.

2.3. Culture Condition

Each pond was filled with $30m^3$ of fresh water treated by sodium hypochlorite. Carbon and nitrogen ratio was calculated from the previous report [16]. BFT ponds were provided with 0.02% of bean cake (nitrogen content 40%) per cubic meter and suitable amount of corn starch (C/N, 20:1) to fertilize the system. Then, group A was inoculated with 1L of ammonia-assimilation bacteria ($3\times10^9 cfu.mL^{-1}$) from the preliminary culture whereas group B was provided with 3 000L of natural bacteria (indigenous bacteria) in the pond water from previous culture. The BFT systems were managed to add some corn starch for maintaining C/N ratio of 20:1 and 1L of ammonia assimilation bacteria culture medium ($3\times10^9 cfu.mL^{-1}$) at three day intervals. Sodium bicarbonate was also added to manage the pH (7~7.4) of the system every three day [17]. We confirmed that proper biofloc system was developed by water quality analysis (ammonia concentration < 0.2ppm) and foam formation. Then, eels with average weight of 30±1.2g were stocked into each pond at a density of 2kg per cubic meter, which was set from our eel farming experience and practical conditions. The fish was fed with paste feed (32% protein) as 2% of body weight twice a day and the amount of feed was adjusted every week according to their weight gain. This study had been performed for two months.

2.4. Water Quality Analysis

Water samples were collected from each pond every five days. Ammonia and nitrate content were measured according to previous report [18].

2.5. Growth Performance and Survival Rate

To investigate eel growth performance and survival rate in different treatments, specific growth rate (SGR), feed conversion rate (FCR) and survival rate were calculated according previous method [19].

2.6. Proximate Analysis of a Biofloc

Proximate analysis was performed according to the previous method [20]. Briefly, bioflocs samples in BFT groups were collected by a 10-μm mesh nylon bag at the end of the experiment. All samples were dried in an oven until their weight did not change. Protein content was measured by Kjeldahl method, lipid was measured by Soxhlet method and ash was weighted after burning at 600°C for 4 hours.

2.7. Statistics Analysis

The data were analyzed by analysis of variance (ANOVA) and Tukey's HSD Test with a confidence level of 95% after confirmation of normality (Shapiro-Wilk test). All data were expressed as mean ± SD (standard deviation) and processed with Software Minitab 18.0 for windows version.

3. Results and Discussion

3.1. Water Quality Analysis

The changes of ammonia, nitrite and nitrate were shown in Figures 1, 2 and 3.

The concentration of ammonia in group A was stayed below 0.2ppm with some fluctuation, even lower than group C after day 20. However, it was risen up to 0.6ppm in group B until day 15, decreased rapidly and reached safe level below 0.3ppm after day 20. In group C, it was fluctuated at around 0.18ppm during the whole experimental period.

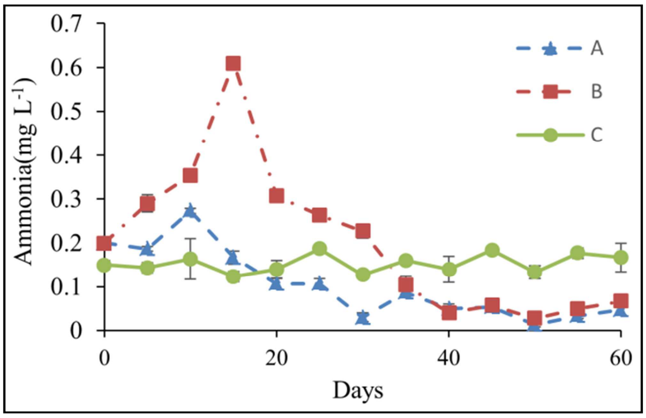

Figure 1. *Ammonia concentration in different groups during 60 days of the experiment.*

A: BFT group with exogenous bacteria addition, B: BFT group without bacteria addition, C: control group.

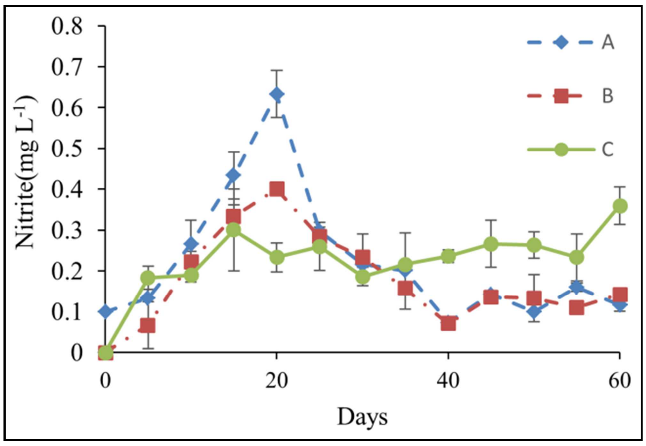

Figure 2. *Nitrite concentration in different groups during 60 days of the experiment.*

A: BFT group with exogenous bacteria addition, B: BFT group without bacteria addition, C: control group

Nitrite, which comes from ammonia oxidation, is also toxic nitrogen compound. Nitrite concentration in group A increased and reached about 0.7ppm at day 20 and decreased rapidly. It remained stable below 0.2ppm after day 30. In group B, it was firstly detected at day 5 and reached 0.4 ppm at day 20. After day 20, it showed similar pattern in both BFT groups. In control group, nitrite was firstly measured from the day 10 and maintained at around 0.2ppm from the day 20. Theoretically, nitrite should not be detected in group A because of ammonia assimilation by dominant heterotrophic bacteria but it was around 0.1ppm at a beginning and increased from day 5 to day 20. This may be attributed to chemical oxidation rather than nitrification. Although nitrite was somewhat high at day 20, eels showed high motility and a good appetite. It seemed that slight alkalinity (pH 7.5~8.5) of the system could minimize toxicity of nitrite.

Nitrate was firstly measured at day 10 in all groups and increased gradually. It reached 4.2ppm in group A and 3.9ppm in group B while 0.5ppm in control group. However, it decreased in BFT groups and remained at around 2.0~2.5ppm after day 40 whereas it showed no significant changes in control group.

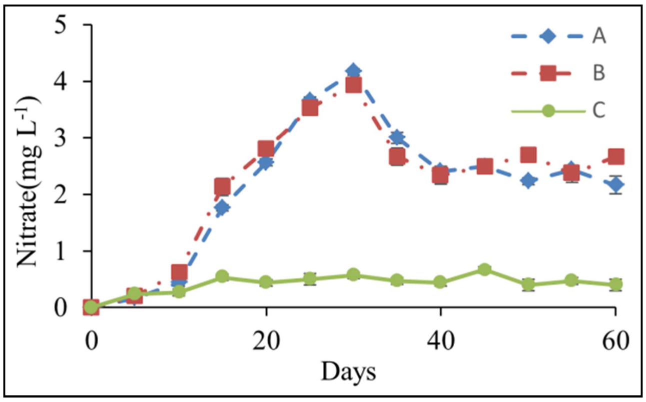

Figure 3. *Nitrate concentration in different groups during 60 days of the experiment.*

A: BFT group with exogenous bacteria addition, B: BFT group without bacteria addition, C: control group.

3.2. Growth Parameters and Survival Rate

The data on growth parameters and survival rate of eels in different groups are shown in Table 1. As can be seen, final average weight in group A and B was about 14 g and 11 g heavier, respectively, compared to control group ($P<0.05$) although initial weight was not significantly different among three groups ($P>0.05$). FCR in group A and group B was 1.78 and 1.8, respectively, which was approximately 0.4 lower than that in control group. SGR and survival rate were also higher in BFT groups. Overall, BFT groups showed positive results as total biomass in group A, B and C was 129kg, 125kg and 98kg, respectively. Growth performance and survival rate of BFT groups were much higher than control group. It seemed that less stress from water exchange in BFT groups could result higher growth performances and lower FCR. Stress from water exchange is the main factor affecting physiology, osmosis control, immune system, fertilization, feeding and growth [21, 22]. Higher survival rate may be attributed to no pathogen input due to zero water exchange.

Table 1. *Growth performances and survival rate in different groups.*

Parameters	Group A (BFT+Bacteria)	Group B (BFT)	Group C (Control)
Initial average weight (g)	29.6 ± 1.2	30.2 ± 1.4	29.6 ± 1.0
Final average weight (g)	65.1 ± 2.6[a]	62.2 ± 2.1[b]	51.5 ± 2.0[c]
FCR	1.78 ± 0.23[c]	1.90 ± 0.13[b]	2.21 ± 0.14[a]
SGR (% day^{-1})	1.31 ± 0.11[a]	1.27 ± 0.10[b]	0.91 ± 0.10[b]
Survival rate (%)	99.3 ± 0.2[a]	99.0 ± 0.3[a]	95.0 ± 0.2[b]
Total Biomass (kg)	129.09 ± 1.12[a]	125.84 ± 1.15[b]	98.04 ± 2.20[c]

Note: Values in same row with different superscripts mean significant difference ($P<0.05$). Data were expressed as mean ± SD (standard deviation).

3.3. Biofloc Composition

Proximate composition of biofloc with and without exogenous bacteria addition was shown in Table 2. The biofloc from group A contained more protein content (26.5 ± 1.2%) than that of group B (20.3 ± 1.4%). However, ash and carbohydrate content in group A were 3% and 4% lower than that of group B. Lipid and fiber content were similar in both groups.

Table 2. *Proximate analysis of a biofloc in BFT groups.*

Composition	A (%)	B (%)
Crude protein	32.5 ± 1.2[a]	30.3 ± 1.4[b]
Lipid	3.4 ± 0.2	3.3 ± 0.6
Fiber	10.5 ± 0.4[b]	13.5 ± 0.6[a]
Carbohydrate	29.6 ± 0.3	28.9 ± 0.7
Ash	13.8 ± 0.7[b]	14.8 ± 0.4[a]

Note: Values in same row with different superscripts mean significant difference ($P<0.05$). Data were expressed as mean ± SD (standard deviation).

4. Conclusion

This study proved that application of BFT with ammonia-assimilating bacteria supplementation has beneficial effects on eel growth performance and water quality improvement. The results of present study will be used as basic and preliminary data to rear eels by biofloc technology. However, further and more specific research is needed to investigate microbial composition of a biofloc in genetic level and optimize several factors to maximize eel production.

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Conflicts of Interest

The authors declare no conflicts of interest.

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