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SELECTION OF INDIGENOUS MICROBIAL STRAINS FOR APPLICATION IN SHRIMP FARMING USING BIOFLOC TECHNOLOGY IN QUANG TRI PROVINCE

Le Cong Tuan* and Le Thi Ha Thanh

University of Sciences, Hue University, 77 Nguyen Hue, Hue city, Thua Thien Hue province, Vietnam, 530000

*Corresponding author: **lctuan@hueuni.edu.vn**

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ABSTRACT

The biomass culture of biofloc for initially screening indigenous microbial strains recorded that most of the water quality parameters were within the appropriate growth thresholds for shrimp, the survival rate reached over 83% after 48 days of experiment. The method of bacteria isolation on selective media and molecular identification by sequencing the 16S rRNA gene segment discovered 04 isolates of metabolizing nitrogen, including 03 strains belonging to the species *Nitratireductor kimnyeogensis* (NQ1, NQ2, and NQ4), 01 strain highly similar to *Hyphomonas polymorpha* (NQ3), and 04 isolates relevant of creating biofloc were all the *Bacillus* genus (*Bacillus* sp. BQ1, *B. velezensis* BQ2, *B. subtilis* BQ3, and *B. subtilis* BQ4), with similarity from 98 to 100%. The highest transformation of ammonium and nitrate was strain NQ3, 96.1% and 83.7%, respectively. Strain NQ1 had the best nitrate removal efficiency (87.5%). Interestingly, the two strains BQ1 and BQ2 had good biofloc formation (78.3% and 55.8%), and simultaneously showed high proportions of ammonium metabolism (88.8% and 92.7%). Besides, the isolated strains could degrade organic substances and inhibit pathogenic bacteria. Particularly, strain BQ3 was able to decompose starch and cellulose, and strain BQ2 could decompose cellulose components and against Vibrio parahaemolyticus. As a result, 04 strains that met the criteria for application in shrimp farming using biofloc technology as NQ1, NQ3, BQ1 and BQ2 has been sellected.

Keywords: Bacteria, nitrogen transformation, antagonism, vibrio

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1 Introduction

In 2020, aquaculture production reached 87.5 million tons and generated an economic value of 264.8 billion USD, with 70% concentrated in the Asia region [1]. The socio-economic value of the aquaculture sector not only impacts the economies of countries and territories but also plays a crucial role in food security and social development. For shrimp, global aquaculture production reached a record high of 9.4 million tons in 2022, with whiteleg shrimp accounting for over half of the global shrimp market [2]. The rapid growth in shrimp culture scale, accompanied by unsustainable farming practices, creates significant pressure on the environment, leads to disease outbreaks, and affects product quality. The green revolution in shrimp farming is marked by a shift towards environmentally friendly practices and aimed at enhancing production efficiency.

Biofloc technology is proposed as an alternative solution for wastewater treatment and feed reuse, which is an innovative approach with a simple operation process and relatively low cost [3, 4]. In general, biofloc technology develops microbial communities, maintains them in a suspended state, and enhances their metabolic capabilities to assimilate pollutants and convert them into biomass that can be consumed by the cultured shrimp [5]. The microbial community is a crucial factor involved in the conversion of nitrogen compounds (NH4+, NO2-) into less toxic or harmless forms, the decomposition of organic matter, and the enrichment of protein sources to enhance shrimp growth [6, 7]. Min Deng et al. (2021) successfully isolated the strain *Pseudomonas* sp. DM02 from aquaculture environments, which showed efficiency in nitrogen processing [8]. Furthermore, the extracellular polymer production activity of microorganisms serves as a bioadhesive to bind cell fragments and suspended particles, which is considered the key to biofloc formation [9]. In Che Hashim's study (2019), several strains of bacteria were isolated from the biofloc technology-based whiteleg shrimp farming system, capable of producing bioadhesive substances, such as *Halomonas venusta, Bacillus cereus, B. subtilis, B. pumilus, Nitratireductor aquimarinus* and *Pseudoalteromonas* [9]. In the mechanism of biofloc formation, prioritizing the selection of indigenous microbial strains helps ensure adaptability, leading to higher efficiency and reducing the opportunity for bacterial invasion. Juan M. Pacheco-Vega's study (2018) analyzed the capability to regulate water quality and inhibit the growth of *Vibrio* spp. by the two strains, *Schizochytrium* sp. and *Lactobacillus plantarum* (T19 bacilli), isolated from the California Gulf water source and the digestive system of outdoor biofloc shrimp farming. The results demonstrated better effectiveness and competitive potential compared to commercial products [10].

This study focuses on isolating microbial strains from the water environment of shrimp farming ecosystems in Quang Tri province and assessing their capability in nitrogen and organic matter transformation, flocculation efficiency, and inhibition of pathogenic bacteria. These results serve as a testing scale for successful application in local shrimp farming models using biofloc technology in practice.

2 Materials and methods

2.1 Materials

The water samples for the experiment were collected from two locations: brackish water in the Thach Han River (Mai Xa bridge) with a salinity of 12 ppt and coastal surface water at Trieu Lang beach with a salinity of 28 ppt.

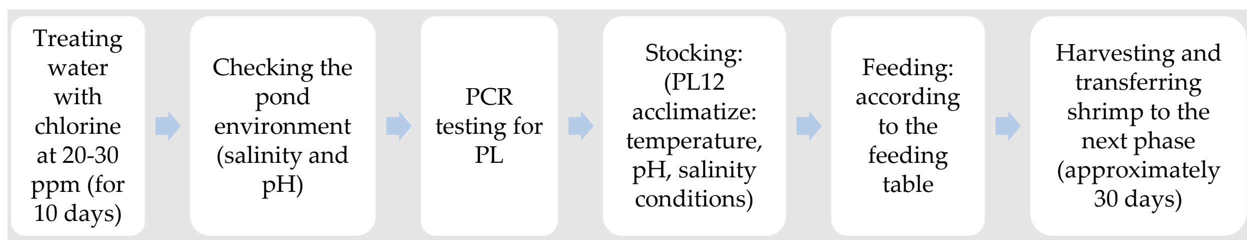
Whitelegs shrimp juveniles, size PL12, with an initial weight of 0.01 mg/individual, were stocked at a uniform stocking density of 1000 PL/m^2. Six composite tanks were designated as B1, B2, B3 for water from the coastal area, and S1, S2, S3 for water from the river mouth area (each tank had a water volume of 1 m^3, height of 1.0 m, equipped with a bottom aeration system and a covering net).

2.2 Experiment Design for cultivating natural biofloc

The water samples were filtered through cloth bags to remove suspended solids and sand. Filtered coastal surface water and brackish water from the river mouth area were allocated to tanks B1-B3 and tanks S1-S3 respectively. The juvenile shrimps were introduced directly into six rearing tanks, all following the same feeding regimen, commercial feed. Carbon supplementation is added by molasses or golden cane sugar to sustain the growth of beneficial bacteria with a ratio (C:N, 15:1). Environmental parameters such as pH, temperature, dissolved oxygen (DO), salinity, NH4-N, and NO2-N were monitored daily, conducted at the Environmental Laboratory, Faculty of Environmental Sciences, Sciences university, Hue University.

The principle of environmental control in shrimp farming using biofloc technology: The experiment design aimed to maintain oxygen levels in the water above 5mg/l and continuously agitate the water in the farming system. Daily monitoring of environmental parameters was conducted, and materials were used to maintain stable pH levels in the pond at 7-8, with alkalinity greater than 100mg/l. Only treated freshwater was added to compensate for evaporative water loss, and experimental water is not replaced.

Summary of the main steps of the biofloc cultivation process are as follows:



Samples of the floc were collected for isolation at the Laboratory of biotechnology, Hue University every 10days (Table 1). Shrimp were fed three times a day (7:00 AM, 12:00 PM, and 5:00 PM daily). The amount of feed was calculated as a percentage of body weight. Throughout the experiment, no sediment removal, antibiotics, or any other chemicals were used. Shrimp were checked every 10 days, with a sample size of over 30 individuals to assess growth in length, weight, and survival rate. The cultured shrimp data was analyzed with the following parameters:

+ Feed conversion ratio: FCR = Amount of feed used / (Harvested shrimp weight - Initial shrimp weight) (1)

+ Survival rate: SR (%) = (Number of shrimp at harvest / Number of shrimp stocked)*100% (2)

+ Daily growth rate in weight: DGR (g/day) = (W2 - W1) / N (3)

+ Daily growth rate in length: DGR (cm/day) = (L2 - L1) / N (4)

Where: W1, W2 were respectively the shrimp weight at the initial time and at the sampling time (g); L1, L2 were respectively the shrimp length at the initial time and at the sampling time (cm); N: the number of days from the initial time to the sampling time in the experiment.

Table 1. *Analysis methods for environment, biofloc and shrimp*

Indicator	Sampling time	Sampling interval	Analysis method
The environmental parameters			
Temprature (^{o}C)	8-10 hours	1 time/day	HANNA – HI 9142
pH	8-10 hours	1 time/day	EcoSense
DO (mg/L)	8-10 hours	1 time/day	HANNA – HI 9142
Salinity (ppt)	8-10 hours	1 time/day	HACH – sension 156
NH_4-N (mg/L)	8-10 hours	1 time/day	OPP method
NO_2-N (mg/L)	8-10 hours	1 time/day	Photometric method
NO_3-N (mg/L)	8-10 hours	1 time/ week	SMEWW 4500 NO^-_3 E: 2005

Floc sample			
Fresh biomass (mg/L)	8-10 hours	1 time/ 10 days	Measuring by using an Imhoff cone and weighing fresh biomass
Dry biomass (mg/L)	8-10 hours	1 time/10 days	Drying at 100°C and weighing the dry weight
Shrimp sample			
Daily growth rate in weight	Weighing shrimp before and after the experiment		Digital scale precision to the nearest 0.1 gram
Daily growth rate in length	Measuring shrimp length before and after the experiment		Measuring ruler
Survival rate	Shrimp survival rate after the experiment		Counting the number of remaining shrimp in each tank

2.3 Method for isolating bacterial strains capable of nitrogen transformation and floc formation

Sample collection: When flocs appeared, water samples were collected from the rearing tanks using Imhoff cones to assess biomass and isolate bacteria (Figure 1). The water samples containing flocs were stored at 4°C until the microbial strains were isolated.

Isolation of nitrogen-transforming bacterial strains: The group of nitrogen-transforming microorganisms was isolated based on the method described by Qiu et al. (2012) with some modifications [11]. Water samples were cultured and enriched in a medium with the following composition: 0,5 g/L ammonium sulphate, 2,17 g/L sodium succinate và 50 mL/L solution containing 5 g/L K_2HPO_4, 2,5 g/L $MgSO_4 \cdot 7H_2O$, 2,5 g/L NaCl, 0,05 g/L $FeSO_4 \cdot 7H_2O$ và 0,05 g/L $MnSO_4 \cdot 4H_2O$, pH 7,2 for 7 days at 30°C, with shaking at 180 rpm. Then, 100 µL of the cultured solution was spread onto a filtration medium containing: 1 g/L KNO_3, 1 g/L KH_2PO_4, 0.5 g/L $FeSO_4 \cdot 7H_2O$, 0.2 g/L $CaCl_2$, 1 g/L $MgSO_4 \cdot 7H_2O$, 8.5 g/L sodium succinate, 20 g/L agar, and 1 mL/L bromothymol blue solution (1% in alcohol, w/v), pH 7.0 ~ 7.3, and incubated at 30°C for 7 days. Colonies exhibiting a blue color were selected for investigating nitrogen transformation properties.

Isolation of bacterial strains capable of floc formation: The water sample was diluted at a 10^{-5} ratio, then 50 µL of the diluted solution was spread onto Marine agar plates. Colonies of bacteria capable of producing mucilage on their surface were selected and streaked onto new Marine agar plates to obtain pure bacterial strains. The microorganisms then were enriched and cultured on liquid Marine medium. Biochemical tests were used to examine the composition of flocculant substances secreted by the microorganisms.

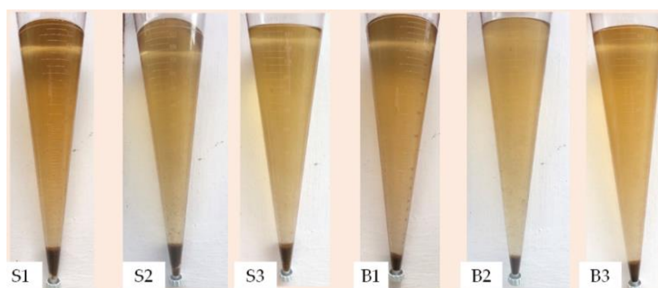


Figure 1. *Floc samples were evaluated using Imhoff*

2.4 Method of bacterial identification

The identification steps were conducted as follows: Bacterial colonies selected were cultured in 5 mL of liquid LB at 30°C with shaking at 150 rpm. The bacterial culture tubes were shaken overnight, and cell biomass was harvested to isolate total DNA using the AquaPure Genomic Isolation kit (catalog number

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732-6340) (Bio-Rad) following the manufacturer's instructions. PCR amplification of the 16S rRNA gene segment was performed using primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The thermal cycling program consisted of initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute 30 seconds. Finally, PCR reaction was incubated at 72°C for 10 minutes. PCR products were checked by electrophoresis on a 1.2% agarose gel. The nucleotide sequences of PCR products were determined by Firstbase (Malaysia) using the Sanger method. The 16S rRNA sequences of the bacterial strains were compared to the nucleotide database on GenBank using BLAST software.

2.5 Surveying the nitrogen transformation ability, organic matter transformation, and Vibrio resistance of the selected bacterial strains

Surveying the abitily of nitrogen transformation

The single bacterial colonies were cultured in 5 mL of medium comprising 0,84 g/L $NaNO_3$, 1g/L KH_2PO_4, 1g/L $MgSO_4{\cdot}7H_2O$, 4,16 g/L glucose, 0,05 g/L $FeSO_4{\cdot}7H_2O$, và 0,02 g/L $CaCl_2$, pH 7 at 30°C, with agitation at 180 rpm for 24 hours. The bacterial cell biomass was then recovered and transferred to culture medium supplemented with components for nitrate reduction1 g/L KH_2PO_4, 1 g/L $MgSO_4{\cdot}7H_2O$, 2,8 g/L sodium succinate, and 0.6 g/L $NaNO_3$. The nitrate reduction ability was tested using the single colony culture technique on liquid medium with the following components: 3.38 g/L Sodium succinate, 0,33 g/L $(NH_4)_2SO_4$, 0,1 g/L peptone, 0,08 g/L NaCl, 0,023 g/L $CaCO_3$, 0,023 g/L KH_2PO_4, 0,094 g/L $MgSO_4$, 0,065 g/L $NaHCO_3$, 0,2 mg/L $FeSO_4{\cdot}7H_2O$, 0,2 mg/L $MnSO_4{\cdot}H_2O$, 0,2 mg/L $CuSO_4{\cdot}5H_2O$ và 0,2 g/L $CoCl_2{\cdot}6H_2O$. Changes in nitrogen forms in the medium were analyzed using standard methods [12]. The nitrogen transformation ability of the isolated bacteria was determined based on changes in nitrogen content in the culture solution.

Surveying the capacity of organic matter transformation

The bacterial strains capable of forming flocs were cultured in optimal conditions at 30°C, 200 rpm. Centrifugation was performed at 13000 rpm for 30 minutes to collect the supernatant. 6 mm diameter holes were drilled on the surface of compatible agar plates. Then, 100 µL of centrifuged bacterial suspension was dispensed into each well. The plates were then incubated at 37°C for 24 hours to assess the capability of organic matter transformation by determining the activity of cellulase, protease, and amylase using the diffusion method on agar plates.

Evaluate the antagonistic ability of the selected bacterial strain against Vibrio strains causing disease in shrimp

The bacterial strains capable of nitrogen transformation and floc formation were cultured in an optimal environment at 30°C, 200 rpm, and centrifuged at 13,000 rpm for 30 minutes to collect the cell-free supernatant. The bacterial strain used for resistance testing was V. parahaemolyticus KS02, stored in the laboratory of the Department of Biotechnology, Faculty of Science, Hue University. This strain was cultured in alkaline peptone medium (2% peptone, 2% NaCl, pH=8.6) at 30°C for 24 hours. Then, 100 µL of the V. parahaemolyticus solution was evenly spread on the surface of TCBS agar plates (Himedia, India). Holes with a diameter of 6 mm are punched on the agar surface. 100 µL of the centrifuged solution of selected bacterial strains is added to each hole and incubated at 37°C for 24 hours to test the bacterial resistance circle [13].

2.6 Statistical analysis

The data was calculated and processed using Microsoft Excel 2016 and IBM SPSS Statistics 20 software (One-way ANOVA and Paired Samples T-test).

3 Results and discussion

3.1 The effectiveness of indigenous bacterial biofloc culture

Fluctuations of water quality in the biofloc rearing tank

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In the experimental shrimp farming system using coastal surface water, the water paramaters were water temperature (28.1 ± 1.35°C), pH (7.1 ± 0.09), salinity (27.9 ± 0.40 ppt), while shrimp raised in brackish water ponds in estuarine areas have temperature (28.1 ± 1.27°C), pH (8.19 ± 0.09), salinity (12.4 ± 0.29 ppt). They were similar in terms of temperature ($p>0.05$), but statistically different in terms of pH and salinity between the two ecosystems ($p<0.05$). However, the values of these parameters still fell within the suitable range for whiteleg shrimp farming in tropical regions. Dissolved oxygen concentration in the water was equivalent between the two groups of experimental rearing tanks, coastal surface water (4.5 ± 0.15 mg/L) and brackish water in estuarine areas (4.4 ± 0.14 mg/L). The DO concentration was evaluated to be lower than the minimum threshold required for shrimp growth (5-10 mg/L) according to Whetstone's study (2000), enhancing the aeration process was required to improve oxygen concentration in the water [14]. Maintaining optimal DO in the biofloc system was at least from 5 mg/L to limit competition for oxygen demand between aquatic organisms and farmed shrimp.

The ammonium concentration showed continuous fluctuations over time ($p<0.05$). NH_4-N concentrations ranged from 0.05 to 2.03 mg/L (1.0 ± 0.53 mg/L) in the coastal surface water shrimp rearing tank and from 0.05 to 2.35 mg/L (1.0 ± 0.51 mg/L) in the brackish water shrimp rearing tank, with no significant difference between the two environments ($p>0.05$). According to Whestone (2000), ammonium levels should be controlled below 2 mg/L to limit risks for stable shrimp growth [14]. It can be observed that NH4-N concentrations in the experimental models mostly remained within the safe range, except for the 17th day of the rearing cycle, where a significant increase was observed, possibly due to the influence of supplementary feed and shrimp waste yet to be decomposed.

The nitrite concentration in the water varied daily and there was a difference between the two shrimp rearing ecosystems ($p<0.05$). The NO_2-N concentration in the coastal surface water shrimp rearing tank and the estuarine brackish water shrimp rearing tank ranged from 0.01 to 2.32 mg/L (0.93 ± 0.67 mg/L) and 0.01 to 2.56 mg/L (0.86 ± 0.67 mg/L), respectively. Chen and Chin's study (1988) published the safe nitrite concentration for shrimp larvae as 4.5 mg/L [15]. Generally, the nitrite levels in the experiments always remained within the suitable range for shrimp growth (Figure 2)

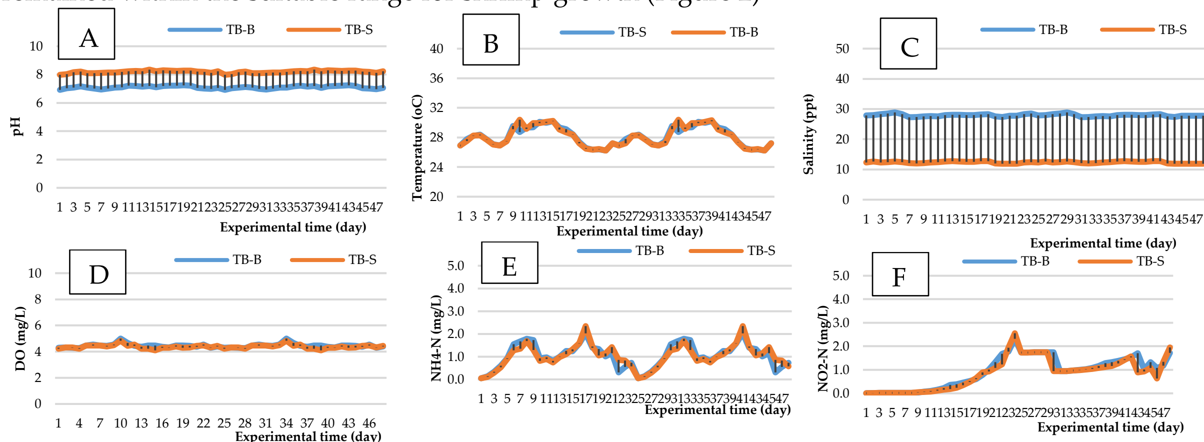

Figure 2. Fluctuation of water quality over time in the biofloc rearing tanks

Biofloc indicators

The fresh and dry biomass showed no significant difference between the two experimental shrimp rearing environments ($p>0.05$). The mean value of fresh biomass was 2461.4 ± 688.10 mg/L in the coastal surface water rearing tank and 2564.5 ± 575.72 mg/L in the brackish water estuarine rearing environment. The dry biomass in the first week of the experimental rearing cycle was recorded below the optimal threshold according to Azim and Little's study (2008), which was 16.6–560 mg/L [16]. The dry biomass increased gradually and fluctuated continuously. The average dry biomass in the coastal surface water and

the brackish water estuarine rearing tanks were 31.6 ± 12.85 mg/L and 31.79 ± 9.68 mg/L respectively (Figure 3).

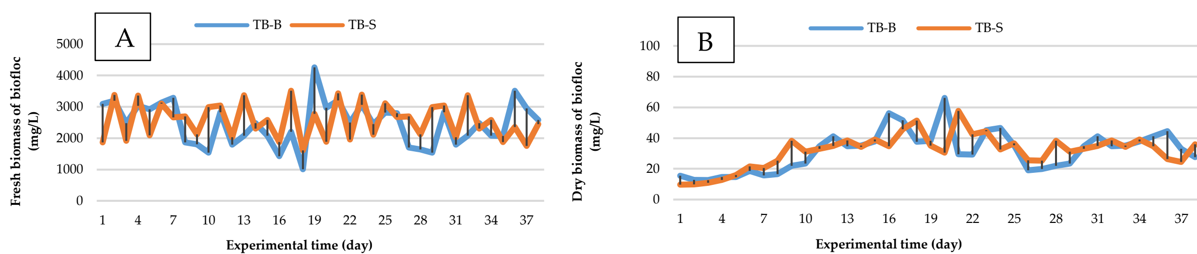

Figure 3. Dynamics of biofloc biomass over time

3.2 Growth rate of whiteleg shrimp in biofloc rearing tanks

Shrimp in the biofloc rearing tank showed comparable results in length and weight between the two water sources, the estuarine and coastal areas ($p>0.05$), with an average shrimp size after the experiment at 2.0 cm/shrimp, with a growth rate of 0.08 cm/day (Table 2). Regarding weight gain, the growth rate reached 0.05 g/day, and the shrimp weight at the end of the study was 1.17 ± 0.06 g/shrimp in the coastal surface water rearing system and 1.20 ± 0.10 g/shrimp in the estuarine brackish water rearing system, with no statistically significant difference ($p>0.05$). The shrimp yield in the coastal surface water rearing tanks was higher than that in the estuarine brackish water rearing tanks, but this difference was not statistically significant ($p>0.05$).

The survival rate of shrimp after 48 days of rearing was relatively high (over 83%) and there was no difference between the two experimental systems ($p>0.05$). The survival rate was lower compared to Khanjani et al.'s study (2022) on silver shrimp farming using the biofloc system at the same initial stocking density of 1000 individuals/m^3 (95.55%).

The feed conversion ratios in both experimental rearing systems were relatively high, with an average of 1.4. In the study by Nguyen Khac Huong and colleagues (2007), under pond shrimp farming conditions, the feed conversion ratios ranged from 1.1 to 1.3 [17].

Table 2. Growth rate of whiteleg shrimp juveniles in the biofloc rearing tank

Indicators	TB-S	TB-B
Harvested shrimp weight (g)	856,7 ± 35,12^a	833,3 ± 20,82^a
Average harvested shrimp weight (g/individual)	1,17 ± 0,06^a	1,20 ± 0,10^a
Average size of harvested shrimp (cm/individual)	2,0 ± 0,0^a	2,0 ± 0,0^a
Survival rate (%)	85,7 ± 3,51^a	83,3± 2,08^a
FCR	1,4 ± 0,16^a	1,4 ± 0,18^a
Daily growth rate in weight (g/day)	0,05 ± 0,0^a	0,05± 0,0$^{a\ a}$
Daily growth rate in length (cm/day)	0,08 ± 0,0^a	0,08 ± 0,0^a

Note: Values in the same row with the same characters do not have statistically significant differences (p>0.05)

3.3 Isolation and identification of selected bacterial strains

From the water samples collected from the experimental shrimp rearing systems, four bacterial strains capable of nitrogen transformation were isolated, designated as NQ1, NQ2, NQ3, and NQ4 [11]. The colonies of these isolated bacteria were round, with smooth edges, ranging in color from milky white to cream, with convex surfaces on LB agar medium (Figure 4).

Floc particles formed during the biofloc rearing process in the composite tanks, with 4 bacterial strains capable of producing slime on Marine agar plates, designated as BQ1, BQ2, BQ3, and BQ4. The floc-forming efficiency of strain BQ1 reached a relatively high level of 78.3%, while strains BQ2, BQ3, and BQ4 had lower floc-forming abilities, at 55.8%, 14.6%, and 29.7%, respectively. The floc-forming bacterial strains

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were subcultured onto LB agar medium to observe the morphology of the colonies, which appeared creamy white, round, smooth, large-sized, and some irregularly shaped, flat, and rough-surfaced (Figure 4).

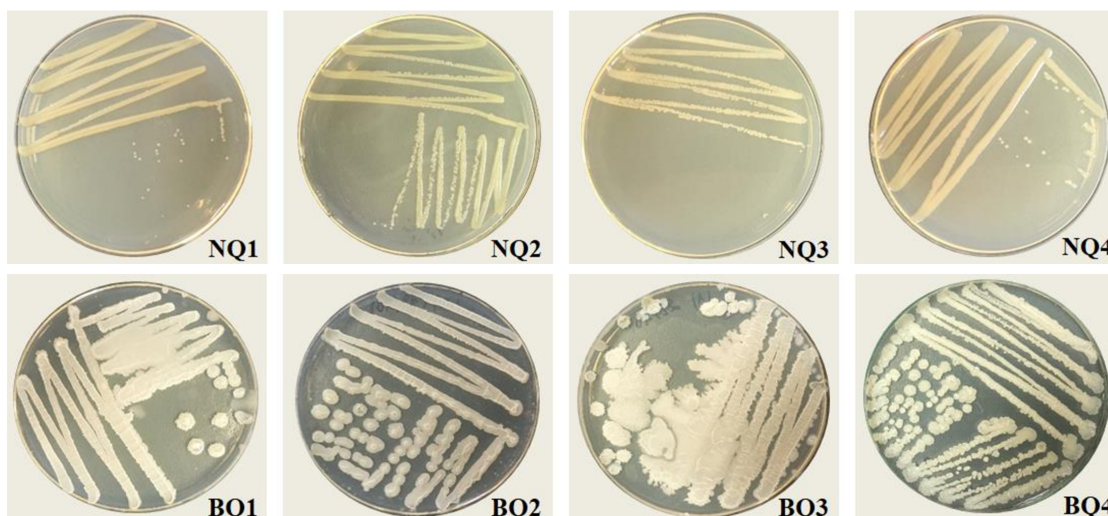

Figure 4. Morphology of nitrogen-transforming bacteria and floc-forming bacteria on LB agar medium

The comparison results of the 16S rRNA sequences of nitrogen-transforming bacterial strains with GenBank data showed that strains NQ1, NQ2, and NQ4 were over 99% similar to *Nitratireductor kimnyeogensis*, while strain NQ3 had a high level of similarity (>98%) to *Hyphomonas polymorpha*. For the floc-forming bacterial strains, sequence similarity searches using BLAST revealed that all four isolated strains belonged to the genus *Bacillus*. Strain BQ1 showed 99.57% similarity to *Bacillus* sp., similarly, strain BQ2 exhibited 99.86% similarity to *Bacillus velezensis*, while strains BQ3 and BQ4 both showed 100% similarity to *Bacillus subtilis*. The 16S rRNA gene sequences of these bacterial strains have been registered on GenBank with accession numbers presented in Table 3. *Bacillus* strains have been evaluated for their ability to produce bioadhesive substances isolated from various sources including aquaculture water, wastewater, soil, and crude oil. *Bacillus cereus* and *B. pumilus* strains isolated from seawater, *B. licheniformis* and *B. thuringiensis* obtained from intensive shrimp farming systems, have been identified as biofloc-producing bacterial strains.

Table 3. GenBank accession numbers of selected bacterial strains

Strain	GenBank accession numbers	Reference bacterial strain		
		Strain	Similarity (%)	Genbank ID
NQ1	PP829908	*Nitratireductor kimnyeogensis* KY 101	99,70	NR_042613
NQ2		*Nitratireductor kimnyeogensis* KY 101	99,18	NR_042613
NQ3	PP829909	*Hyphomonas polymorpha* DSM 2665	98,18	NR_025325
NQ4		*Nitratireductor kimnyeogensis* XTF-62	99,78	OP835952
BQ1	PP829910	*Bacillus* sp. JQ.GSRS-2	99,57	OQ450510
BQ2	PP829911	*Bacillus velezensis* FZB42	99,86	ON041103
BQ3		*Bacillus subtilis* TIMMAY	100	OQ327039
BQ4		*Bacillus subtilis* M13	100	MF187645

3.4 Initial nitrogen transformation capability

In the environment containing ammonium, nitrification occurred robustly with 78.5% to 96.1% of NH_4^+ being removed after 8 days of cultivation, with strain NQ3 having the highest transformation capability. Additionally, two floc-forming bacterial strains (BQ1 and BQ2) also exhibited very high ammonium transformation capabilities (88.8% and 92.7%) (Figure 5A). There was no nitrite in the culture environment, but nitrate formation was observed, ranging from 2.66 to 6.21 mg NO_3-N/L, with the highest efficiency recorded in strain NQ3 (Figure 5B). This result suggests that most of the nitrite formed from ammonium was converted into nitrate, and the bacteria might utilize nitrate compounds as nutrients for growth.

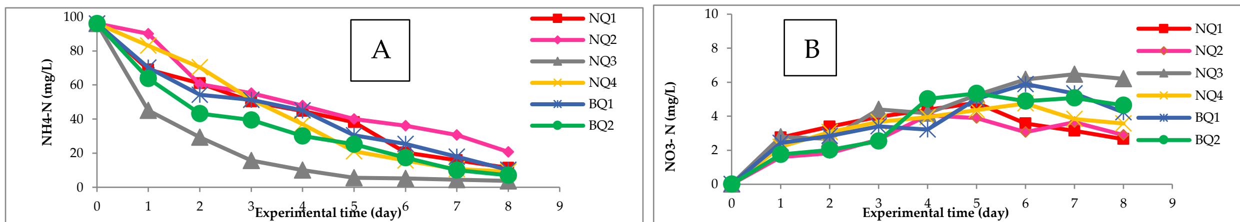

Figure 5. Amonium reduction capability of the selected bacterial strains in an ammonium-containing environment

The selected bacterial strains were cultured in an environment containing nitrite, 56.9 - 87.5% of the NO_2^- in the medium was removed during the second stage of the nitrification process, with strain NQ1 showing the best conversion capability (Figure 6A). The nitrate content in the medium increased rapidly and reached its peak on the fourth day of cultivation, ranging from 10.5 to 16.3 mg NO_3-N/L, with the highest nitrate concentration detected in strain NQ4. The NO_3^- concentration tended to decrease from the fifth day to the eighth day of the cultivation period. NO_3^- is considered non-toxic to aquatic organisms if the nitrate concentration is controlled below the threshold of 20 mg/L [15]. Water changes were required when nitrate levels became too high. From Figure 6, it can be seen that the bacterial strains converted nitrite into nitrate, which is a less toxic compound compared to nitrite and ammonium [18], [19], [20], [21].

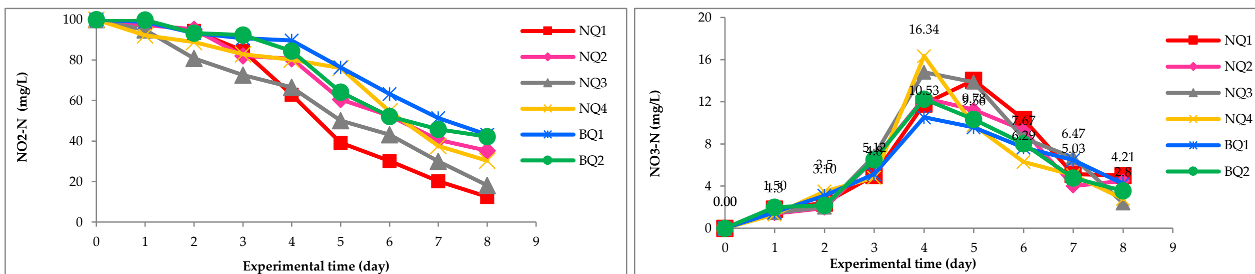

Figure 6. Nitrite reduction capability of the selected bacterial strains in a nitrite containing environment

Figure 7 shows that the nitrate reduction process was most effective in the presence of strain NQ3, with a nitrate reduction efficiency of up to 83.7%. The nitrate reduction efficiency of the two strains characterized by their ability to form flocs, BQ1 and BQ2, was insignificant (around 17%). Additionally, no nitrite formation was detected in the culture environment, possibly because the bacteria had completed the reduction of NO_3^- to N_2 gas and H_2O, thereby ending the nitrogen conversion cycle.

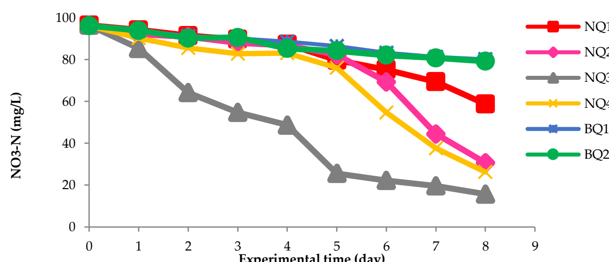

Figure 7. Nitrate reduction capacity of selected bacterial strains in a nitrate-containing environment

3.5 Organic matter metabolism capacity

One of the benefits of biofloc technology is leveraging the microorganisms in the rearing system to decompose organic matter from leftover feed and animal waste, while also converting harmful substances into a food source. Therefore, bacteria strains capable of metabolizing organic matter are prioritized for isolation and application. From the selected bacteria strains, cellulase, protease, and amylase activities were investigated. The results showed that strain BQ3, identified as *Bacillus subtilis*, could produce amylase and cellulase enzymes to break down starch and cellulose (Figure 8). This is consistent with Jingcheng Dai's (2020) study, where *B. subtilis* ZIM3 demonstrated the ability to degrade starch and cellulose with an efficiency of 30-48%. The selected *B. subtilis* BQ4 strain also exhibited starch-degrading capability, while *B. velezensis* BQ2 showed cellulose-degrading ability.

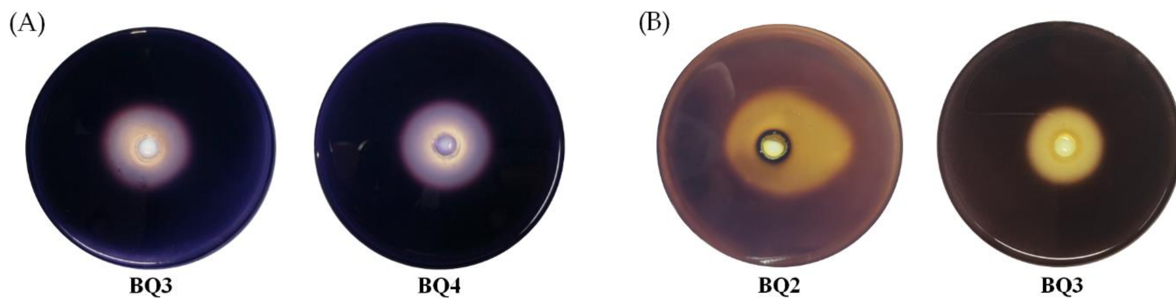

Figure 8. Starch decomposition ability (A) and cellulose decomposition ability (B) of the selected bacterial strains

3.6 Antagonistic ability against Vibrio strains pathogenic of selected bacterial strains

Aquaculture brings substantial economic benefits but always faces numerous risks, especially diseases. *Vibrio* strains are considered common pathogenic bacteria encountered at all stages of shrimp development [21], [22]. The ability of bacteria residing in the pond ecosystem to compete with and antagonize pathogenic *Vibrio* strains is crucial. The survey recorded that *Bacillus* sp. BQ1 and *Nitratireductor kimnyeogensi* NQ2 strains have the ability to resist *Vibrio parahaemolyticus* KS02 (Figure 9). In the study by Werasan Kewcharoen (2019), *Bacillus* spp. isolated from the whiteleg shrimp culture system showed effective antagonism against *V. parahaemolyticus* [23], [24].

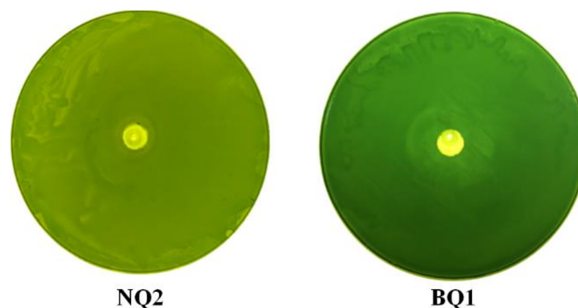

Figure 9. Antagonistic ability of selected bacteria against the pathogenic *V. parahaemolyticus* KS02

4 Conclusions

The water quality parameters and biofloc indicators during the indigenous bacteria biofloc rearing process generally remained within safe limits for shrimp growth. After the biofloc rearing period, the shrimp attained a weight of 1.17-1.20 g/shrimp, an average size of 2.0 cm/shrimp, with a survival rate above 83% at an initial stocking density of 1000 individuals/m^3.

The four isolated strains capable of nitrogen conversion included strains NQ1, NQ2, and NQ4 identified as *Nitratireductor kimnyeogensis*, and strain NQ3 as *Hyphomonas polymorpha*. Additionally, four strains capable of biofloc formation, all belonging to the *Bacillus* genus, were isolated including BQ1 (*Bacillus* sp.), BQ2 (*B. velezensis*), BQ3, and BQ4 (*B. subtilis*), with ranging from 98-100%.

The isolation of indigenous microbial strains from water sources and the biofloc system in experimental shrimp rearing has yielded promising results in the early stages of white shrimp culture. Strain BQ1 demonstrated the highest flocculation efficiency (78.3%). Regarding nitrogen conversion assessment, strain NQ3 exhibited high capability in ammonium and nitrate conversion, with rates of 96.1% (NH_4^+) and 83.7% (NO_3^-), respectively. Strain NQ1 showed the best nitrite conversion efficiency, reaching 87.5%. Strains BQ1 and BQ2, characterized by their biofloc-forming ability, also exhibited high rates of ammonium conversion (92.7% and 88.8%). Additionally, strain BQ3 demonstrated starch and cellulose degradation capabilities, while strain BQ2 showed cellulose degradation ability and resistance against the shrimp pathogen *V. parahaemolyticus*.

The study identified four strains of NQ1, NQ3, BQ1, and BQ2, that effectively met the criteria for nitrogen conversion, biofloc formation, and pathogen resistance. They could be determined for the subsequent experimental steps in shrimp farming using biofloc technology in Quang Tri province.

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Conflict of interest

The authors declare that there are no conflicts of interest related to the publication of this article.

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