BOLETIM DO INSTITUTO DE PESCA
Scientific Article

Bacillus improves the health and growth performance of *Penaeus vannamei* in an intensive nursery without water exchange

Natalia Pereira[1]*, Amanda Dartora[2], Julia Delmonego Hess[2], Giovanni Mello[3], Adolfo Jatobá[1]

[1]Instituto Federal Catarinense – Programa de Pós-Graduação em Saúde Animal – Araquari (SC), Brazil.
[2]Instituto Federal Catarinense – Laboratório de Aquicultura – Araquari (SC), Brazil.
[3]Universidade do Estado de Santa Catarina – Laboratório de Aquicultura – Laguna (SC), Brazil.
*Corresponding author: nataliapereira.eng.pesca@gmail.com

ABSTRACT

The objective of this study was to investigate the effect of a bioremediator on the production of juveniles (*Penaeus vannamei*) in intensive nursery systems, focusing on zootechnical performance, water quality, and animal health. Two groups were used (control and bioremediation). In the bioremediation treatment, 0.1 $g{\cdot}m^{-3}$ of the product Arkhon Fix (*Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*) was applied daily at the concentration of 1×10^{-11} $CFU{\cdot}g^{-1}$. Physical-chemical parameters of the water were monitored, and health status was evaluated through presumptive analysis of the hepatopancreas tubule shape and filling, prevalence of dirt on the gills, quantification of *Vibrio* spp., and total heterotrophic bacteria in water and juveniles. The bioremediator led to a significant reduction in total dissolved solids (36.9%), settleable solids (68.9%), and organic matter presence on the gills (50.0%). It also resulted in an increase in final mean weight (28.3%) and yield (20.0%), as well as a reduction in feed conversion ratio (18.7%) compared to the control group. Despite higher *Vibrio* spp. concentration in the water due to bioremediation, it did not affect *Vibrio* spp. concentration in juveniles. In conclusion, the bioremediator improved culture water quality, thereby enhancing the growth performance of the shrimp.

Keywords: Shrimp farming; Water quality; Organic matter; *Bacillus* spp.; Growth performance.

Bacillus na melhoria do desempenho de crescimento e saúde de *Penaeus vannamei* em berçário intensivo sem troca de água

RESUMO

O objetivo deste estudo foi investigar o efeito de um biorremediador na produção de juvenis (*Penaeus vannamei*) em sistemas intensivos de berçário, focando no desempenho zootécnico, na qualidade da água e na saúde dos animais. Foram utilizados dois grupos (controle e biorremediação). No tratamento com biorremediador, foi aplicado diariamente 0,1 $g{\cdot}m^{-3}$ do produto Arkhon Fix (*Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*) na concentração de 1×10^{-11} $UFC{\cdot}g^{-1}$. Os parâmetros físico-químicos da água foram monitorados, e o estado de saúde foi avaliado por meio de análises presuntivas da forma e preenchimento dos túbulos do hepatopâncreas, prevalência de sujeira nas brânquias e quantificação de *Vibrio* spp. e bactérias heterotróficas totais na água e nos juvenis. O biorremediador proporcionou redução significativa nos sólidos totais dissolvidos (36,9%), sólidos sedimentáveis (68,9%) e na presença de matéria orgânica nas brânquias (50,0%). Também resultou em aumento no peso médio final (28,3%) e na produtividade (20,0%), além de reduzir a conversão alimentar (18,7%) em comparação ao grupo controle. Apesar da maior concentração de *Vibrio* spp. na água por causa da biorremediação, isso não afetou a concentração de *Vibrio* spp. nos juvenis. Em conclusão, o biorremediador melhorou a qualidade da água de cultivo, melhorando, consequentemente, o desempenho zootécnico dos camarões.

Palavras-chave: Carcinicultura; Qualidade de água; Matéria orgânica; *Bacillus* spp.; Desempenho de crescimento.

Received: March 30, 2024 | **Approved:** August 7, 2024
Section editor: Fabiana Garcia

INTRODUCTION

Aquaculture is a source of quality food and nutrients for millions of people (Natnan et al., 2021; FAO, 2022). The marine shrimp *Penaeus vannamei* has become important for its nutritional benefits and high economic value, which has sparked interest in large-scale production (Dayal et al., 2013).

In shrimp farming, intensification of the production system is a common strategy used to meet high demand (Edwards, 2015). Closed aquaculture systems, i.e., those without, or little, water renewal, enable greater production (Burford et al., 2003; Jatobá et al., 2019; Suantika et al., 2018). However, a low level of water renewal also leads to the accumulation of organic matter and increases total suspended solids (TSS). This results in reduced growth performance and feed efficiency rates, while promoting gill occlusion and making the environment more stressful. Closed systems also favor the emergence of pathogenic bacteria (Ray et al., 2010; Schveitzer et al., 2013; Vinatea et al., 2010). Thus, it is necessary to develop technologies able to manage the accumulation of organic matter, food waste, and toxic forms of inorganic nitrogen, such as ammonia ($NH_4^+ + NH_3$), nitrite (NO_2^-) and nitrate (NO_3^-) (Avnimelech, 2007). These nitrogenous compounds in inappropriate (high) concentrations for the reared fish species favor the emergence of diseases (Kuebutornye et al., 2019).

However, the introduction of bioremediators can help control organic matter and reduce pathogenic bacteria, such as *Vibrio* spp., while maintaining water quality at a low level of nitrogenous components that would otherwise be lethal to several aquatic species (Janeo et al., 2009; Patil et al., 2021). Bioremediation is the transformation or degradation of contaminants into less toxic substances. Its primary function is to degrade or convert pollutants, a process that can be carried out by bacteria, fungi, algae, and plants (Sharma, 2020). In particular, bacterial species of the genus *Bacillus*, such as *B. subtilis*, *B. licheniformis*, *B. cereus*, and *B. coagulans*, have been studied for aquaculture applications as probiotics, bioremediators and/or biocontrollers (Amin et al., 2015; Chen et al., 2013). These bacteria can perform bioremediation of the culture environment and effluents, among other benefits (Hlordzi et al., 2020). For example, *Bacillus* spp. can enhance innate immune response, enzyme production, resistance to pathogens, maintain water quality, reduce organic matter and nitrogenous waste in systems, and improve growth performance (Hoseinifar et al., 2018).

Nonetheless, the use of bioremediating bacteria in water applications still needs further testing and study. Therefore, this work aimed to evaluate the effects of the *Bacillus*-based commercial bioremediator Arkhon Fix (Gabbia Biotecnologia e Desenvolvimento, Itajaí, SC, Brazil) on the growth performance and health of juvenile Pacific white shrimp reared in an intensive nursery system without water exchange. We also evaluated water parameters, concentration of *Vibrio* spp., and total heterotrophic bacteria present in both water and animals.

MATERIAL AND METHODS

The experiment was carried out in the municipality of Laguna, located in southern Santa Catarina state, at Mar do Brasil Aquicultura (28°22'25.06" S 48°47'30.53" W). The experiment lasted 39 days and used 2,000 *P. vannamei* marine shrimp postlarvae (PLs). These PLs originated from the POND lineage (Aquatec, RN, Brazil), which is widely used in southern Brazil for their tolerance to low-temperature and high-saline environments.

The experiment included a treatment using the bioremediator Arkhon Fix, including *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, and *B. megaterium*, at manufacturer-guaranteed concentrations of 1×10^{-11} $CFU \cdot g^{-1}$.

Experimental design and management

Eight circular tanks made of polyethylene material were each filled with 250 L of water. The tanks were filled using a submerged pump with water from the farm's supply channel, which serves as a reservoir for the company's production nurseries. After tanks were filled, they were chlorinated with sodium hypochlorite at the concentration of 30 ppm and maintained with constant aeration until volatilization the sodium hypochlorite. Aeration of the experimental units was carried out using a 2.5 HP blower connected to microperforated hoses. Fresh water was used to replace the volume lost through evaporation.

Two thousand PLs with an initial average weight of 4 mg were distributed in eight tanks with 250 PLs each tank having a storage density of 1,000 $PL \cdot m^{-3}$ (1 $PL \cdot L^{-1}$). PLs were divided into two groups: treatment with bioremediator (Arkhon Fix), subjected to daily applications of 0.1 $g \cdot m^{-3}$, and control (without bioremediator). The experiment was performed in quadruplicate.

The feeding rate started at 40% of biomass and was reduced over the experimental weeks. At the end of the experiment, the daily feeding rate was 12% of biomass. Food in the form of commercial feed powder with 56% crude protein, 12% wet matter, and 10% ether extract was offered at 8 a.m., 11 a.m., 1 p.m., and 5 p.m.

Water quality analyses

Dissolved oxygen and temperature were measured at 8 a.m., 11 a.m., 1 p.m., and 5 p.m. (AKSO oximeter, model AK97). The pH was measured at 8 a.m. and 5 p.m. (AKSO, model AK90). Ammonia, nitrite and nitrate were measured twice a week, using a photocolorimeter (ALFAKIT, pH meter, model AT100 PII).

Orthophosphate and sulfide were analyzed with a photocolorimeter (ALFAKIT, model AT100 PII), alkalinity by volumetric titration (ALFAKIT commercial kit), and salinity with a refractometer, and all measurements were performed weekly.

The settleable solids (Imhoff cone, with a settling time of 20 min) and TSS (APHA, 1995) were carried out on day 0, 16, and 32 of the experiment.

Microbiological analyses

Microbiological analyses were performed to count *Vibrio* spp. and total heterotrophic bacteria in the control and bioremediation groups of juvenile shrimps at the end of the experiment and in water at the beginning and end of the experiment. One mL of water was sampled, serially diluted (factor 1:10) in sterile saline solution of 3% NaCl. Dilutions were carried out in tryptone soy agar (TSA) and thiosulfate citrate bile salts sucrose (TCBS) agar medium and incubated for 24 h at 30°C to count total heterotrophic bacteria and *Vibrio* spp., respectively. according to Jatobá et al. (2015). Shrimp juveniles in a sample weighing 1 g were washed in 70% alcohol solution, rinsed in distilled water, macerated, and then serially diluted (factor 1:10) in sterile saline solution of 3% NaCl. Dilutions were carried out in TSA and TCBS agar medium and incubated for 24 h at 30°C to count total heterotrophic bacteria and *Vibrio* spp., respectively. For water, the same process was used with 1 mL from each tank.

Growth performance and health status: presumptive analytics

Survival, feed conversion, and final biomass were evaluated at the end of the experiment. Ten juvenile shrimp were analyzed per experimental unit, following the modified methodology of Morales and Cuéllar-Anjel (2014). Health status of juveniles was evaluated by using a binocular biological microscope 100x (BIOPTIKA, model B10). Tubular deformity in the hepatopancreas was evaluated by the degree of severity, according to a classification system ranging from G0 to G4, in which:

- G0 = no tubular deformation or roughness;
- G1 = very low presence of tubular deformation (1-5/field/organism);
- G2 = moderate presence of tubular deformation (6-10/field/organism);
- G3 = high presence of tubular deformation (11-16/field/organism);
- G4 = a large number of deformed tubules (more than 16/field/organism).

The classification of presence or absence was also used to evaluate the double wall in the hepatopancreas, in the gills for melanization and necrosis; organic matter; and expansion of chromatophores (Table 1 and Fig. 1).

Table 1. Presumptive analyses carried out using the method of assessing severity from G0 to G4 and presence or absence.

Score	Organ	Clinical signs
Degree of severity (G0 to G4)	Hepatopancreas	Tubule deformity
Presence or absence	Hepatopancreas	Double wall
Presence or absence	Gills	Organic matter Melanization and necrosis Expanded chromatophores

Source: Adapted by Morales and Cuéllar-Anjel (2014).

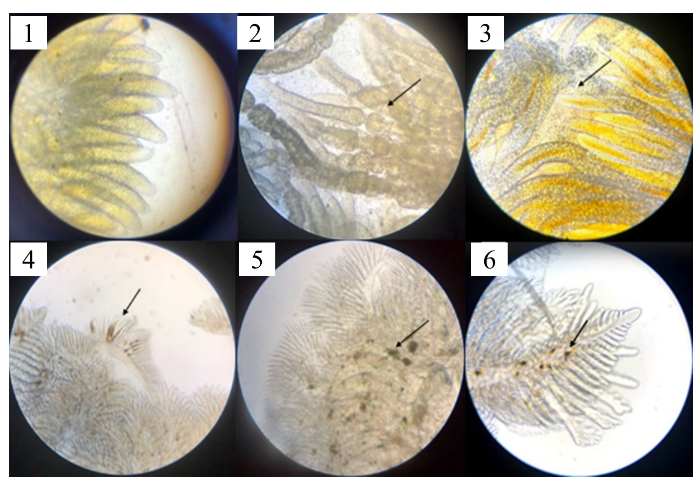


Figure 1. Microscopic images of shrimp samples of the species *Penaeus vannamei*. (1) Hepatopancreas without tubular deformities; (2) hepatopancreas with tubular deformities; (3) hepatopancreas with double wall; (4) sample of gills with melanization and necrosis; (5) gills with organic matter; (6) gills with expanded chromatophores.

Statistical analyses

The Kolmogorov–Smirnov test was used to test the null hypothesis that a set of data obtained was within the normality curve, and Levene's test was used to verify homoscedasticity of the data. If the data obtained met the prerequisites of normality and homoscedasticity, then a *t* test would be performed for separation of means. For presumptive analyses, the Mann–Whitney's test was performed. For dissolved oxygen, temperature, and pH, as well as microbiological count of water, a two-factor analysis of variance (treatment × time) and Tukey's test were respectively performed to separate the means. Assessment at $p > 0.05$ was considered significant (Zar, 2010).

RESULTS

Temperature and pH throughout the day showed significant differences in relation to time; the lowest values were observed at 8 a.m. for both variables. Dissolved oxygen did not differ between time and treatments (Table 2). No significant differences were observed between treatments for nitrogen, orthophosphate, sulfide and salinity (Table 3).

Total settable solids and TSS showed no differences on days 0 and 16; however, on the 39th day, the *Bacillus* group showed a significant reduction in these variables (Figs. 2 and 3, respectively).

Table 2. Dissolved oxygen, temperature, and pH of water in a closed cultivation system without water exchange in the nursery phase *of Penaeus vannamei*.

	Hour	Control	Bioremediation	Interaction	Treatment	Time
Dissolved oxygen ($mg \cdot L^{-1}$)	8 a.m.	8.27 ± 0.25	8.24 ± 0.27	NS	NS	NS
	12 a.m.	8.14 ± 0.14	8.43 ± 0.61	NS	NS	NS
	3 p.m.	8.22 ± 0.63	7.95 ± 0.39	NS	NS	NS
	6 p.m.	7.84 ± 0.75	7.60 ± 0.43	NS	NS	NS
Temperature (°C)	8 a.m.	25.09 ± 0.38^{a}	25.28 ± 0.22^{a}	NS	NS	*
	12 a.m.	27.37 ± 0.64^{b}	27.19 ± 0.5^{b}	NS	NS	*
	3 p.m.	28.21 ± 0.49^{b}	28.21 ± 0.64^{b}	NS	NS	*
	6 p.m.	27.63 ± 0.89^{b}	27.74 ± 0.59^{b}	NS	NS	*
pH	8 a.m.	7.84 ± 0.03^{a}	7.83 ± 0.04^{b}	NS	NS	*
	12 a.m.	NA	NA			
	3 p.m.	NA	NA			
	6 p.m.	8.06 ± 0.04^{b}	8.06 ± 0.04^{b}	NS	NS	*

*Significant differences ($p > 0.05$) in two-factor analysis of variance and Tukey's mean separation test; different letters indicate differences in values among times; NA: not assessed; NS: not significant.

Table 3. Water quality variables analyzed in a closed cultivation system without water exchange in the nursery phase of *Penaeus vannamei**.

Variables	Control	Bioremediation	Significance (*p*-value)
Ammonia (NH_3 $mg \cdot L^{-1}$)	0.12 ± 0.09	0.11 ± 0.09	0.429
Nitrite (NO_2 $mg \cdot L^{-1}$)	2.63 ± 2.17	3.03 ± 2.91	0.374
Nitrate (NO_3 $mg \cdot L^{-1}$)	43.31 ± 0.63	38.01 ± 0.39	0.433
Orthophosphate (PO_4 $mg \cdot L^{-1}$)	0.61 ± 0.29	0.81 ± 0.47	0.215
Sulfate (S^{2-} $mg \cdot L^{-1}$)	0.01 ± 0.01	0.01 ± 0.01	0.259
Alkalinity ($CaCO_3$ $mg \cdot L^{-1}$)	89.63 ± 8.15	89.13 ± 8.49	0.468
Salinity (ppt)	11.40 ± 1.14	11.40 ± 1.14	0.500

*Results presented as mean ± standard deviation.

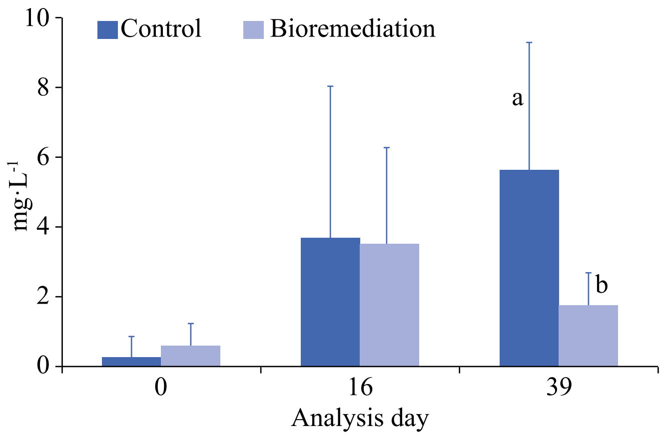


Figure 2. Variation (mean ± standard deviation) of settleable solids in a closed system for *Penaeus vannamei* with the addition of a bioremediator. Different letters indicate significant difference in the *t* test (5% significance).

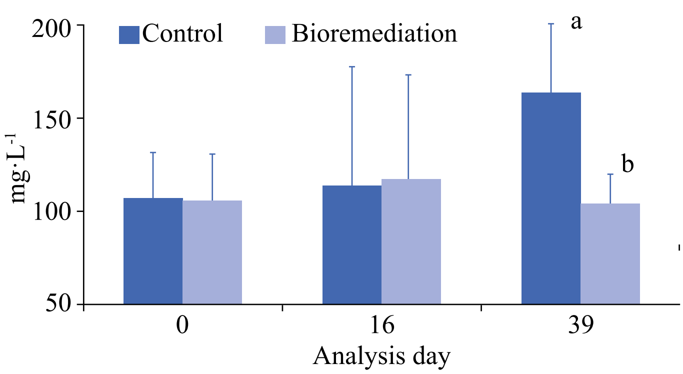
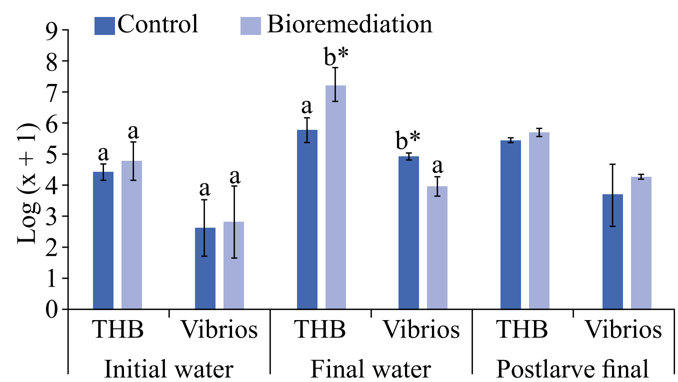


Figure 3. Variation (mean ± standard deviation) of total suspended solids in a closed system for *Penaeus vannamei* with the addition of a bioremediator. Different letters indicate significant difference in the *t* test (5% significance).

At the end of the experiment, higher level ($p < 0.05$) of total heterotrophic bacteria (THB) and of *Vibrio* spp. on water was observed when compared to the initial treatment values. THB concentrations were higher in the control treatment, while the content of *Vibrio spp*. had increased after treatment with the bioremediator. THB and *Vibrio* spp. bacteria counts did not differ in PLs body between groups (Fig. 4).



*Difference between groups ($p < 0.05$).

Figure 4. Variation (mean ± standard deviation) of total heterotrophic bacteria (THB) and *Vibrio* spp. present in initial and final water and in shrimp juveniles at the end of the experimental period in a closed *Penaeus vannamei* system with the addition of bioremediatiating bacteria. Different letters indicate significant differences ($p > 0.05$) in the two-way analysis of variance on the initial and final concentration of water bacteria.

Shrimp reared under bioremediation showed a lower amount of organic matter in the gills, as well as a lower presence of expanded chromatophores (Fig. 5 and Table 4). Melanization and necrosis in the gills and other analyses of the hepatopancreas did not differ between the groups (Table 4).

Table 4. Clinical signs (presumptive analysis) of *Penaeus vannamei* juveniles reared in a closed system without water exchange, using bioremediation.

Organ/tissue	Clinical signs	Control	Bioremediation	Significance (*p*-value)
Hepatopancreas	Tubule deformity	3.13 ± 0.29	3.2 ± 0.23	0.359
	Double wall	0.98 ± 0.05	0.98 ± 0.05	0.500
Gills	Organic matter	0.50 ± 0.14	0.25 ± 0.13*	0.020
	Melanization and necrosis	0.50 ± 0.08	0.58 ± 0.26	0.303
	Expanded chromatophores	0.40 ± 0.22	0.15 ± 0.10*	0.040

Data presented as mean ± standard deviation; *significant differences in the Mann-Whitney's test (5% significance).

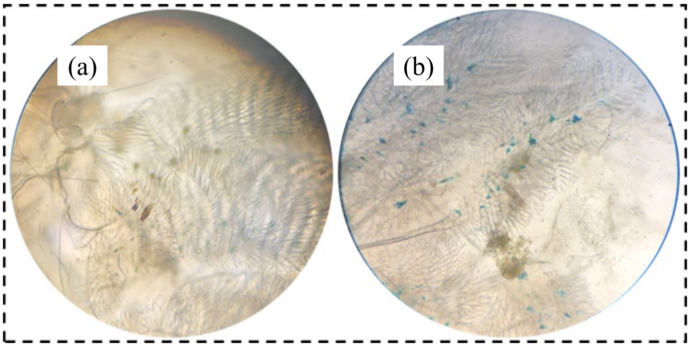


Figure 5. Gills of *Penaeus vannamei* juveniles maintained in a closed system with the addition of bioremediating bacteria. Optical microscope images at 20X magnification. (a) Gills in a cultivation environment using bioremediator. (b) Gills in a control culture environment.

Mean final weight and feed conversion were significantly better ($p < 0.05$) in the bioremediation group, whereas yield and survival showed no significant difference between the groups (Table 5).

Table 5. Growth performance (mean ± standard deviation) of *Penaeus vannamei* reared in closed system.

Variable	Control	Bioremediation	Significance (*p*-value)
Final mean weight (g)	0.53 ± 0.07	0.68 ± 0.10*	0.035
Yield ($kg \cdot m^{-3}$)	0.50 ± 0.08	0.60 ± 0.05	0.056
Food conversion	1.28 ± 0.19	1.04 ± 0.08*	0.048
Survival	93.5 ± 7.12	89.2 ± 9.36	0.259

*Significant differences by *t* test.

DISCUSSION

In aquaculture, bacteria used for bioremediation can enhance water quality by reducing nitrogen compounds and organic matter, thereby improving production and growth performance while mitigating potential environmental impacts (Hassan et al., 2022). However, most studies have focused on administering bacteria via food rather than through water (El-Kady et al., 2022).

In the present study, dissolved oxygen and pH were within acceptable limits for cultivation of the species (above 5 $mg \cdot L^{-1}$ and 6 to 9, respectively), while temperature and salinity remained close to the ideal range (from 28 to 32°C and 5 to 25 ppt, respectively), as recommended by some authors (Boyd, 2001; Boyd et al., 2011; Carbajal-Hernández, 2012). These variations did not compromise the survival of the animals since the experimental average was 91.35%. Dissolved oxygen is the most important water quality parameter for aquaculture. At low levels, dissolved oxygen make the animals more susceptible to diseases, and they do not feed well; consequently, growth decreases (Hlordzi et al., 2020). Dissolved oxygen levels in this study were unchanged by the continuous application of the bioremediator containing *Bacillus* spp. Similar results were observed by Kord et al. (2022), who tested several commercial probiotics containing *Bacillus* via water application in zero water exchange systems for Nile tilapia fingerlings.

Our results suggest that application of the bioremediator did not influence the pH value, corroborating the findings of other studies (Kord et al., 2022; Zhou et al., 2010). Temperature remained constant between the groups evaluated, as reported by other authors, indicating that *Bacillus* did not affect temperature, as it is a physical parameter (El-Kady et al., 2022). The difference in temperature among the times analyzed may have resulted from more pronounced thermal amplitude from the small volume of water in the experimental units and from the external environment (Mota et al., 2021; Vinatea, 2010).

The dynamics of nitrogenous compounds, such as ammonia, nitrite and nitrate, in aquaculture systems, especially in closed systems with zero or minimal water exchange, are especially important (Kord et al., 2022). In this study, the concentrations of nitrogenous compounds showed no significant difference between treatments. The same results were reported by Arias-Moscoso et al. (2018), who evaluated the use of water-administered probiotic in bio-floc technology (BFT) with zero water exchange during the nursery phase of *P. vannamei*. Our results align with other studies that found no reduction in nitrogenous compounds during the nursery phase, attributed to lower biomass and consequently reduced feed intake, as well as shorter cultivation periods of less than 45 days.

In this experiment, initial fertilizations were carried out in the treatment and control group, respecting the carbon to nitrogen ratio (C:N = 10:1), which may have favored the emergence of heterotrophic bacteria (Emerenciano, 2017). The recommended values for juveniles of the species for ammonia (NH_3) and nitrite (NO_2) are up to 0.12 and 6.1 $mg \cdot L^{-1}$, respectively, for a salinity of 15 ppt (Lin & Chen, 2001, 2003). The recommended nitrate (NO_3) value is up to 127 $mg \cdot L^{-1}$ at salinity of 10 ppt (Alves Neto et al., 2019; Lin & Chen, 2001, 2003). Thus, nitrite and nitrate presented values within the safe range for juvenile *P. vannamei*, and ammonia presented values close to those recommended, considering the salinity of 15 ppt.

Bacillus spp. plays a fundamental role in the removal of organic matter in cultivation systems (El-Kady et al., 2022). Those belonging to the gram-positive genus are more efficient in converting organic matter into carbon dioxide (CO_2) when compared to gram-negative bacteria, which convert it into sludge and bacterial biomass (Zorriehzahra et al., 2016).

Concentrations of TSS above the maximum recommended limit (500 $mg \cdot L^{-1}$) for *P. vannamei* (Samocha et al., 2007) can affect the branchial system in fish (Soaudy et al., 2023). In this work, TSS were below the recommended limit in both groups evaluated; however, a lower presence of organic matter was observed in the gills in the group subjected to bioremediation. The presence of organic matter in aquaculture systems, coupled with poor water quality, can lead to malformation of the opercula and ulceration of gill filaments (Hlordzi et al., 2020). Therefore, the higher presence of organic matter found in the gills of the control group may be associated with higher concentrations of settleable solids and TSS, potentially influencing the lower final weight observed in this treatment.

Bacillus can reduce the accumulation of particles (suspended and dissolved solids) (Jasmin et al., 2020) and organic carbon, acting to remove organic matter, thereby recycling nutrients and reducing the accumulation of sludge (Hlordzi et al., 2020). Decreased organic matter was reported in other studies with *P. vannamei* shrimp culture in systems without water exchange after application of *Bacillus* via water (Hassan et al., 2022) and in catfish culture through the use of *Bacillus* as bioremediating bacteria (Anggraini et al., 2019). Moreover, the phenomenon was seen in effluent treatment used in landscaping lakes, resulting in the appearance of clearer waters owing to the increase in transparency caused by *Bacillus* (Zhao et al., 2009).

The reduction in organic matter in water can be attributed to the ability of *Bacillus* to increase the activity of digestive enzymes responsible for breaking down and absorbing food (Hasan & Banerjee, 2020), including protease, which is known to catalyze the hydrolysis of protein peptide bonds; amylase, which is known to catalyze the hydrolysis of starch into sugars; and lipase, which is known to catalyze the hydrolysis of fat in shrimp (Amoah et al., 2019). Consequently, an increase in the appetite of animals, combined with an increase in the consumption of food (feed and SST), leads to lower loads of organic matter (Hlordzi et al., 2020).

Bacillus species can also enhance nutrient digestibility (Chien et al., 2020) by activating digestive enzymes in shrimp (Hassan et al., 2022) and stimulate better absorption and digestion of nutrients by increasing the host's intestinal villi (Xie et al., 2019). This could potentially explain the improved growth and feed conversion ratio observed in the bioremediation group.

Positive effects were reported by El-Kady et al. (2022), who cultivated tilapia using commercial *Bacillus* via application in water, and by Xie et al. (2019), who tested a mix of *Bacillus* via feed for juvenile *P. vannamei*. All these results corroborate those of the present study.

Several factors can cause stress in fish and shrimp, such as management, vaccination, water quality, transport, salinity, feeding, and high-stocking densities (Kuebutornye et al., 2019). In this study, a significant reduction in the expansion of chromatophores in the gills of shrimp in the bioremediation treatment was observed compared to the control group. The expansion of chromatophores can indicate illnesses, changes in water quality parameters, management, and stress (Morales & Cuéllar-Anjel, 2014). Similar results were observed with the use of *Bacillus* spp. in the diet, which resulted in reduced stress in *Oreochromis niloticus* caused by high-stocking densities (Telli et al., 2014), as well as yellow perch (*Perca flavescens*) exposed to air (Eissa et al., 2018).

Some studies have shown that *Bacillus* spp. can eliminate/reduce the pathogenicity of *Vibrio* spp. in shrimp farms (Barman et al., 2018) and, thus, prevent acute hepatopancreatic necrosis caused by *Vibrio parahaemolyticus*, or prevent the bacterium from acting as a gateway to other diseases (Amiin et al., 2023). In this work, a greater presence of *Vibrio* spp. was recorded in water of the bioremediation group, which is different from that observed by other authors (Hassan et al., 2022; Kord et al., 2022). However, this greater presence in the water was neither reflected in the higher body count of juveniles, nor affected the survival of the group, corroborating the study of Amoah et al. (2019), who reported that *Bacillus* spp. improved the immune response of shrimp by increasing resistance against some pathogenic *Vibrio* species.

The highest counts of TBH in the control at the end of the experiment corroborate findings in a previous report. In this study, the carbon source used at the beginning of the experiment and converted into organic matter can be used as an energy source for THB, stimulating their growth and metabolism, as well as helping the bacilli to remain in the system.

CONCLUSION

The bioremediator Arkhon Fix improved the cultivation environment by reducing THB, total dissolved solids, and settleable solids. Consequently, juvenile Pacific white shrimp showed improved health, evidenced by reduced or absent organic matter in the gills and decreased expansion of chromatophores. This enhancement in health translated to improved growth performance, as indicated by increased final weight and reduced feed conversion ratio when reared in a nursery without water exchange.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be available upon request from the author.

AUTHORS' CONTRIBUTION

Conceptualization: Pereira N; **Investigation:** Pereira N, Dartora A, Hess JD, Mello G, Jatobá A; **Data curation:** Pereira N, Jatobá A; **Formal analysis:** Pereira N, Jatobá A; **Writing – original draft:** Pereira N, Mello G, Jatobá A; **Writing – review & editing:** Pereira N, Jatobá A; **Final approval:** Pereira N.

FUNDING

Not applicable.

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

Alves Neto, I., Brandão, H., Furtado, P. S., & Wasielesky Jr., W. (2019). Acute toxicity of nitrate in *Litopenaeus vannamei* juveniles at low salinity levels. *Ciência Rural*, *49*(1), e20180439. https://doi.org/10.1590/0103-8478cr20180439

American Public Health Association (APHA) (1995). *Standad methods for examination of water and wastewater.* 19th ed. American Public Health Association.

Amiin, M. K., Lahay, A. F., Putriani, R. B., Reza, M., Putri, S. M. E., Sumon, M. A. A., Jamal, M. T., & Santanumurti, M. B. (2023). The role of probiotics in vannamei shrimp aquaculture performance: A review. *Veterinary World*, *16*(3), 638-649. https://doi.org/10.14202/vetworld.2023.638-649

Amin, M., Rakhisi, Z., & Zarei Ahmady, A. (2015). Isolation and Identification of i Bacillus i Species From Soil and Evaluation of Their Antibacterial Properties. *Avicenna Journal of Clinical Microbiology and Infection*, *2*(1), 23233. https://doi.org/10.17795/ajcmi-23233

Amoah, K., Huang, Q. C., Tan, B. P., Zhang, S., Chi, S. Y., Yang, Q. H., Liu, H.Y., & Dong, X.H. (2019). Dietary supplementation of probiotic Bacillus coagulans ATCC 7050, improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, *87*, 796-808. https://doi.org/10.1016/j.fsi.2019.02.029

Anggraini, S. I., Arfiati, D., & Nursyam, H. (2019). Effectiveness of Bacillus subtilis bacteria as a total organic matter reducer in catfish pond (Clarias gariepinus) cultivation. *International Journal of Biotech Trends and Technology*, *9*(2), 7-10. https://doi.org/10.14445/22490183/IJBTT-V9I2P602

Arias-Moscoso, J. L., Espinoza-Barrón, L. G., Miranda-Baeza, A., Rivas-Vega, M. E., & Nieves-Soto, M. (2018). Effect of commercial probiotics addition in a biofloc shrimp farm during the nursery phase in zezo water exchange. *Aquaculture Reports*, *11*, 47-52. https://doi.org/10.1016/j.aqrep.2018.06.001

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture*, *264*(1-4), 140-147. https://doi.org/10.1016/j.aquaculture.2006.11.025

Barman, P., Bandyopadhyay, P., Kati, A., Paul, T., Mandal, A. K., Mondal, K. C., & Mohapatra, P. K. D. (2018). Characterization and Strain Improvement of Aerobic Denitrifying EPS Producing Bacterium *Bacillus cereus* PB88 for Shrimp Water Quality Management. *Waste and Biomass Valorization*, *9*(8), 1319-1330. https://doi.org/10.1007/s12649-017-9912-2

Boyd, C. (2001). *Manejo na qualidade de agua na aquicultura e no cultivo de camarão marinho.* Associação Brasileira de Criadores de Camarão.

Boyd, C. E., Tucker, C. S., & Viriyatum, R. (2011). Interpretation of pH, Acidity, and Alkalinity in Aquaculture and Fisheries. *North American Journal of Aquaculture*, *73*(4), 403-408. https://doi.org/10.1080/15222055.2011.620861

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H., & Pearson, D. C. (2003). Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, *219*(1-4), 393-411. https://doi.org/10.1016/s0044-8486(02)00575-6

Carbajal-Hernández, J. J., Sánchez-Fernández, L. P., Carrasco-Ochoa, J. A., & Martínez-Trinidad, J. F. (2012). Immediate water quality assessment in shrimp culture using fuzzy inference systems. *Expert Systems with Applications*, *39*(12), 10571-10582. https://doi.org/10.1016/j.eswa.2012.02.141

Chen, X. H., Zeng, D. G., Chen, X. L., Xie, D. X., Zhao, Y. Z., Yang, C. L., & Wang, H. (2013). Transcriptome Analysis of *Litopenaeus vannamei* in Response to White Spot Syndrome Virus Infection. *Plos One*, *8*(8), e73218. https://doi.org/10.1371/journal.pone.0073218

Chien, C. C., Lin, T. Y., Chi, C. C., & Liu, C. H. (2020). Probiotic, Bacillus subtilis E20 alters the immunity of white shrimp, *Litopenaeus vannamei* via glutamine metabolism and hexosamine biosynthetic pathway. *Fish & Shellfish Immunology*, *98*, 176-185. https://doi.org/10.1016/j.fsi.2020.01.014

Dayal, J. S., Ponniah, A., Khan, H. I., Babu, E. M., Ambasankar, K., & Vasagam, K. K. (2013). Shrimps–a nutritional perspective. *Current Science*, *104*(11), 1487-1491.

Edwards, P. (2015). Aquaculture environment interactions: Past, present and likely future trends. *Aquaculture*, *447*, 2-14. https://doi.org/10.1016/j.aquaculture.2015.02.001

Eissa, N., Wang, H. P., Yao, H., & Abou-Elgheit, E. (2018). Mixed Bacillus Species Enhance the Innate Immune Response and Stress Tolerance in Yellow Perch Subjected to Hypoxia and Air-Exposure Stress. *Scientific Reports*, *8*(1), 6981. https://doi.org/10.1038/s41598-018-25269-z

El-Kady, A. A., Magouz, F. I., Mahmoud, S. A., & Abdel-Rahim, M. M. (2022). The effects of some commercial probiotics as water additive on water quality, fish performance, blood biochemical parameters, expression of growth and immune-related genes, and histology of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *546*, 737249. https://doi.org/10.1016/j.aquaculture.2021.737249

Emerenciano, M. G. C., Martínez-Córdova, L. R., Martínez-Porchas, M., & Miranda-Baeza, A. (2017). Biofloc technology (BFT): a tool for water quality management in aquaculture. *Water Quality*, *5*, 92-109. https://doi.org/10.5772/66416

Food and Agriculture Organization (FAO). (2022). *The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation*. FAO. https://doi.org/10.4060/cc0461en

Hasan, K. N., & Banerjee, G. (2020). Recent studies on probiotics as beneficial mediator in aquaculture: a review. *Journal of Basic and Applied Zoology*, *81*(1), 53. https://doi.org/10.1186/s41936-020-00190-y

Hassan, M. A., Fathallah, M. A., Elzoghby, M. A., Salem, M. G., & Helmy, M. S. (2022). Influence of probiotics on water quality in intensified *Litopenaeus vannamei* ponds under minimum-water exchange. *AMB Express*, *12*(1), 22. https://doi.org/10.1186/s13568-022-01370-5

Hlordzi, V., Kuebutornye, F. K. A., Afriyie, G., Abarike, E. D., Lu, Y., Chi, S., & Anokyewaa, M. A. (2020). The use of Bacillus species in maintenance of water quality in aquaculture: A review. *Aquaculture Reports*, *18*, 100503. https://doi.org/10.1016/j.aqrep.2020.100503

Hoseinifar, S. H., Sun, Y. Z., Wang, A. R., & Zhou, Z. G. (2018). Probiotics as Means of Diseases Control in Aquaculture, a Review of Current Knowledge and Future Perspectives. *Frontiers in Microbiology*, *9*, 2429. https://doi.org/10.3389/fmicb.2018.02429

Janeo, R. L., Corre, V. L., & Sakata, T. (2009). Water quality and phytoplankton stability in response to application frequency of bioaugmentation agent in shrimp ponds. *Aquacultural Engineering*, *40*(3), 120-125. https://doi.org/10.1016/j.aquaeng.2009.01.001

Jasmin, M. Y., Syukri, F., Kamarudin, M. S., & Karim, M. (2020). Potential of bioremediation in treating aquaculture sludge: Review article. *Aquaculture*, *519*, 734905. https://doi.org/10.1016/j.aquaculture.2019.734905

Jatobá, A., Borges, Y. V., & Silva, F. A. (2019). Biofloc: sustainable alternative for water use in fish culture. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *71*(3), 1076-1080. https://doi.org/10.1590/1678-4162-10309

Jatobá, A., do Nascimento Vieira, F., Silva, B. C., Mouriño, J. L. P., & Seiffert, W. Q. (2015). Influência da renovação e inclusão de naúplio de tanques em laboratório de camarões marinhos (Litopnaeus vannamei). *Brazilian Journal of Aquatic Science and Technology*, *19*(1), 77-80. https://doi.org/10.14210/bjast.v19n1.1984

Kord, M. I., Maulu, S., Srour, T. M., Omar, E. A., Farag, A. A., Nour, A. A. M., Hasimuna, O. J., Abdel-Tawwab, M., & Khalil, H. S. (2022). Impacts of water additives on water quality, production efficiency, intestinal morphology, gut microbiota, and immunological responses of Nile tilapia fingerlings under a zero-water-exchange system. *Aquaculture*, *547*, 737503. https://doi.org/10.1016/j.aquaculture.2021.737503

Kuebutornye, F. K. A., Abarike, E. D., & Lu, Y. (2019). A review on the application of *Bacillus* as probiotics in aquaculture. *Fish & Shellfish Immunology*, *87*, 820-828. https://doi.org/10.1016/j.fsi.2019.02.010

Lin, Y. C., & Chen, J. C. (2001). Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology*, *259*(1), 109-119. https://doi.org/10.1016/S0022-0981(01)00227-1

Lin, Y. C., & Chen, J. C. (2003). Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture*, *224*(1-4), 193-201. https://doi.org/10.1016/S0044-8486(03)00220-5

Morales, V., & Cuéllar-Anjel, J. (eds.). (2014). *Guía Técnica: Patología e Inmunología de Camarones Penaeidos* (2ª ed.). OIRSA.

Mota, V. L. R., Adolfo, J., & Silva, L. R. (2021). Caracterização da produção de Tilápia-do-Nilo *Oreochromis niloticus* em diferentes sistemas de cultivo no Sul de Santa Catarina. *Revista em Agronegócio e Meio Ambiente*, *14*(2), 455-468. https://doi.org/10.17765/2176-9168.2021v14n2e7906

Natnan, M., Mayalvanan, Y., Jazamuddin, F., Aizat, W., Low, C.-F., Goh, H.-H., Azizan, K. A., Bunawan, H., & Baharum, S. (2021). Omics Strategies in Current Advancements of Infectious Fish Disease Management. *Biology*, *10*(11), 1086. https://doi.org/10.3390/biology10111086

Patil, P. K., Antony, L., Avunje, S., Viswanathan, B., Lalitha, N., Jangam, A. K., Kumar, D., Solanki, H. G., Reddy, M. A., Alavandi, S. V., & Vijayan, K. K. (2021). Bioaugmentation with nitrifying and denitrifying microbial consortia for mitigation of nitrogenous metabolites in shrimp ponds. *Aquaculture*, *541*, 736819. https://doi.org/10.1016/j.aquaculture.2021.736819

Ray, A. J., Lewis, B. L., Browdy, C. L., & Leffler, J. W. (2010). Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. *Aquaculture*, *299*(1-4), 89-98. https://doi.org/10.1016/j.aquaculture.2009.11.021

Samocha, T. M., Patnaik, S., Speed, M., Ali, A.-M., Burger, J. M., Almeida, R. V., Ayub, Z., Harisanto, M., Horowitz, A., & Brock, D. L. (2007). Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering*, *36*(2), 184-191. https://doi.org/10.1016/j.aquaeng.2006.10.004

Schveitzer, R., Arantes, R., Costodio, P. F. S., Santo, C. M. D., Arana, L. V., Seiffert, W. Q., & Andreatta, E. R. (2013). Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. *Aquacultural Engineering*, *56*, 59-70. https://doi.org/10.1016/j.aquaeng.2013.04.006

Sharma, I. (2020). Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects. In Murillo-Tovar, M., Saldarriaga-Noreña, H., & Saeid, A. (eds.). *Trace Metals in the Environment - New Approaches and Recent Advances*. IntechOpen. https://doi.org/10.5772/intechopen.90453

Soaudy, M. R., Ghonimy, A., Greco, L. S. L., Chen, Z., Dyzenchauz, A., & Li, J. (2023). Total suspended solids and their impact in a biofloc system: Current and potentially new management strategies. *Aquaculture*, *572*, 739524. https://doi.org/10.1016/j.aquaculture.2023.739524

Suantika, G., Situmorang, M. L., Kurniawan, J. B., Pratiwi, S. A., Aditiawati, P., Astuti, D. I., Azizah, F. F. N., Djohan, Y. A., Zuhri, U., & Simatupang, T. M. (2018). Development of a zero water discharge (ZWD)—Recirculating aquaculture system (RAS) hybrid system for super intensive white shrimp (*Litopenaeus vannamei*) culture under low salinity conditions and its industrial trial in commercial shrimp urban farming in Gresik, East Java, Indonesia. *Aquacultural Engineering*, *82*, 12-24. https://doi.org/10.1016/j.aquaeng.2018.04.002

Telli, G. S., Ranzani-Paiva, M. J. T., Dias, D. C., Sussel, F. R., Ishikawa, C. M., & Tachibana, L. (2014). Dietary administration of *Bacillus subtilis* on hematology and non-specific immunity of Nile tilapia *Oreochromis niloticus* raised at different stocking densities. *Fish & Shellfish Immunology*, *39*(2), 305-311. https://doi.org/10.1016/j.fsi.2014.05.025

Vinatea, L. (2010). *Qualidade da água em aquicultura: princípios e práticas* (3ª ed.). Universidade Federal de Santa Catarina.

Vinatea, L., Galvez, A. O., Browdy, C. L., Stokes, A., Venero, J., Haveman, J., Leffler, J. W. (2010). Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: Interaction of water quality variables. *Aquacultural Engineering*, *42*(1), 17-24. https://doi.org/10.1016/j.aquaeng.2009.09.001

Xie, J. J., Liu, Q. Q., Liao, S., Fang, H. H., Yin, P., Xie, S. W., Tian, L. X., Liu, Y J., & Niu, J. (2019). Effects of dietary mixed probiotics on growth, non-specific immunity, intestinal morphology and microbiota of juvenile pacific white shrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, *90*, 456-465. https://doi.org/10.1016/j.fsi.2019.04.301

Zar, J. H. (2010). *Biostatistical analysis* (5ª ed.). Pearson Prentice Hall.

Zhao, S., Hu, N., Chen, Z., Zhao, B., & Liang, Y. (2009). Bioremediation of Reclaimed Wastewater Used as Landscape Water by Using the Denitrifying Bacterium *Bacillus cereus*. *Bulletin of Environmental Contamination and Toxicology*, *83*(3), 337-340. https://doi.org/10.1007/s00128-009-9684-x

Zhou, X., Tian, Z., Wang, Y., & Li, W. (2010). Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Fish Physiology and Biochemistry*, *36*(3), 501-509. https://doi.org/10.1007/s10695-009-9320-z

Zorriehzahra, M. J., Delshad, S. T., Adel, M., Tiwari, R., Karthik, K., Dhama, K., & Lazado, C. C. (2016). Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. *Veterinary Quarterly*, *36*(4), 228-241. https://doi.org/10.1080/01652176.2016.1172132