ActaPesca 22, 2025, 28-31
ISSN: 2357-8068
https://doi.org/10.46732/Actafish.22.28-31

ActaPesca
www.actapescanews.com

Artigo Open Access

Bacteria of the genus *Bacillus* are effective in reducing phosphorus in aquaculture pond sediments

Daniel Ferreira Rodrigues de Oliveira[1*], Vanessa Lewandowski[1], Renan Rodrigues Campos[1], Daniel Domingues[1], Guilherme Silveira Telli[1], Antonio Cesar Godoy[2] & Dacley Hertes Neu[1]

[1]Universidade Federal da Grande Dourados. Faculdade de Ciências Agrárias. Rodovia Dourados - Itahum, km 12. Cidade Universitária, Dourados/MS. Brasil.

[2] Departamento de Pesquisa em Recursos Naturais, Instituto Federal de Educação, Ciência e Tecnologia do Paraná. Assis Chateaubriand-PR, Brasil.

Received: 08 November 2024 / Accepted: 20 December 2024

Abstract

Biological products are commonly used in aquaculture to treat waste generated during production. In this context, the objective of the present study was to evaluate the efficiency of *Bacillus* bacteria in treating sediment from an aquaculture pond. For this purpose, 15 experimental units were used, arranged in a completely randomized design with five treatments and three replicates. The treatments were determined by different doses of a commercial bioremediatory composed of *Bacillus subtilis, B. licheniformis, B. amyloliquefaciens, and B*. pumilus, with a concentration of 3.5 x 109 UFC/g, at doses of 100, 200, 300, 400 and 500 g/ha. In each experimental unit, 5 cm of sediment from fishpond was added, followed by the addition of 15 liters of water. The bioremediator doses were added to the water in each experimental unit, and the study lasted for 15 days. Water and sediment chemical analyses were performed before and after the experimental period. The data were subjected to analysis of variance, followed by Tukey's test at a 5% significance level for mean comparison. No significant influence of the treatments was observed on water quality parameters, pH, effective cation exchange capacity, or base saturation of the sediment ($p > 0,05$). There was fluctuation in the organic matter percentage of the sediment, with the lowest value observed for the 300 g/ha ($p < 0.05$). The 400 g/ha dosage of the product resulted in a 60% reduction in sediment phosphorus. In conclusion, *Bacillus* bacteria, through the commercial bioremediatory, are efficient in reducing organic matter and phosphorus in fishpond sediments, and their action does not interfere with soil pH, cation exchange capacity, or base saturation.

Keywords: bioremediator, organic matter, fish farming, water quality.

Resumo - Bactérias do gênero *Bacillus* são eficazes na redução de fósforo em sedimentos de viveiros de aquicultura

Produtos biológicos são comumente usados na aquicultura para tratar resíduos gerados durante a produção. Nesse contexto, o objetivo do presente estudo foi avaliar a eficiência de bactérias *Bacillus* no tratamento de sedimentos de um viveiro de aquicultura. Para isso, foram utilizadas 15 unidades experimentais, dispostas em um delineamento inteiramente casualizado com cinco tratamentos e três repetições. Os tratamentos foram definidos por diferentes doses de um biorremediador comercial composto por *Bacillus subtilis, B. licheniformis, B. amyloliquefaciens e B. pumilus*, com uma concentração de 3,5 x 10^9 UFC/g, nas doses de 100, 200, 300, 400 e 500 g/ha. Em cada unidade experimental, foram adicionados 5 cm de sedimento de viveiro de peixes, seguidos da adição de 15 litros de água. As doses do biorremediador foram adicionadas à água em cada unidade experimental, e o estudo teve duração de 15 dias. Foram realizadas análises químicas da água e do sedimento antes e depois do período experimental. Os dados foram submetidos à análise de variância, seguida do teste de Tukey ao nível de 5% de significância para comparação das médias. Não foi observada influência significativa dos tratamentos nos parâmetros de qualidade da água, pH, capacidade de troca catiônica efetiva ou saturação por bases do sedimento ($p > 0,05$). Houve flutuação na porcentagem de matéria orgânica do sedimento, com o menor valor observado para a dose de 300 g/ha ($p < 0,05$). A dose de 400 g/ha

* Corresponding author: Oliveira, D. F. R., e-mail: danielferreira17@hotmail.com

do produto resultou em uma redução de 60% no fósforo do sedimento. Em conclusão, as bactérias *Bacillus*, através do biorremediador comercial, são eficientes na redução da matéria orgânica e do fósforo em sedimentos de viveiros de peixes, e sua ação não interfere no pH do solo, na capacidade de troca catiônica ou na saturação por bases.

Palavras-chave: biorremediador, matéria orgânica, piscicultura, qualidade da água.

Resumen - Las bacterias del género *Bacillus* son eficaces para reducir el fósforo en la acuicultura

Los productos biológicos se utilizan habitualmente en acuicultura para tratar los residuos generados durante la producción. En este contexto, el objetivo del presente estudio fue evaluar la eficacia de bacterias *Bacillus* en el tratamiento de sedimentos de un estanque de acuicultura. Para ello, se utilizaron 15 unidades experimentales, dispuestas en un diseño completamente aleatorizado con cinco tratamientos y tres repeticiones. Los tratamientos se determinaron mediante diferentes dosis de un biorremediador comercial compuesto por *Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens* y *B. pumilus*, con una concentración de 3,5 x 109 UFC/g, a dosis de 100, 200, 300, 400 y 500 g/ha. En cada unidad experimental se añadieron 5 cm de sedimento de estanque piscícola, seguidos de la adición de 15 litros de agua. Las dosis de biorremediador se añadieron al agua de cada unidad experimental, y el estudio duró 15 días. Se realizaron análisis químicos del agua y los sedimentos antes y después del periodo experimental. Los datos se sometieron a un análisis de varianza, seguido de la prueba de Tukey a un nivel de significación del 5% para la comparación de medias. No se observó ninguna influencia significativa de los tratamientos sobre los parámetros de calidad del agua, pH, capacidad efectiva de intercambio catiónico o saturación de bases del sedimento ($p > 0,05$). Hubo fluctuaciones en el porcentaje de materia orgánica del sedimento, observándose el valor más bajo para la dosis de 300 g/ha ($p < 0,05$). La dosis de 400 g/ha del producto dio lugar a una reducción del 60% del fósforo del sedimento. En conclusión, las bacterias *Bacillus*, a través del biorremediador comercial, son eficientes en la reducción de materia orgánica y fósforo en sedimentos de estanques piscícolas, y su acción no interfiere con el pH del suelo, la capacidad de intercambio catiónico o la saturación de bases.

Palabras clave: biorremediador, materia orgánica, piscicultura, calidad del agua.

Introduction

The freshwater animal production accounts for 44.6% of global aquaculture, with fish farming being the primary activity (FAO 2024). In Brazil, fish farming also takes the lead in aquaculture and is continually evolving and growing. In 2023, the production reached 887, 029 tons, representing a 3.1% increase compared to 2022 (PeixeBR, 2024). Tilapia farming stands out, and the country ranks as the 4th largest producer of Nile tilapia (*Oreochormis niloticus*), with a total production of 579,080 tons (PeixeBR, 2024). This activity relies on intensive production, utilizing specific feeds and equipment such as aerators and controlled feeding systems.

Intensification is essential for increasing production to meet the demand for fish consumption and enable the economic viability of the activity. The development of aquaculture benefits the regional economy by generating employment across the entire production chain (Verdegem et al., 2023). However, animal density and large-scale production bring numerous challenges, particularly in terms of management and environmental control. In fish production, leftover feed and feces are the primary sources of water in aquatic environments, and excess of these residues negatively impacts water quality and, consequently, animal development (Cyrino et al., 2010; Lopes et al., 2020).

Excessive nitrogen and phosphorus compounds from production waste can lead to eutrophication in water (Wang et al., 2019). Eutrophication promotes excessive phytoplankton growth, resulting in alterations in water quality, particularly regarding fluctuations in dissolved oxygen concentration and pH over a 24-hour interval (Boyd, 2020). Another crucial factor in intensive production is the organic load generated, which exceeds the natural microbial mineralization capacity of the system, leading to residue accumulation in pond sediments. Among the negative effects of organic enrichment in sediment are the development of pathogenic bacteria (Chávez-Crooker and Obreque-Contreras, 2010), decreased oxygen levels in the water column, and the release of toxic metabolites such as ammonia and nitrite (Robinson et al., 2016).

One of the ways to address issues caused by excessive organic matter is to empty the fishpond at the end of cultivation. This exposes the sediment to air, and subsequently, it is covered with lime to enhance microbial activity (Nimrat et al., 2008). Recently, new waste treatment methods have been employed in aquaculture, with a notable focus on the use of biological products. These products have gained popularity due to their low capital and operational costs as well as reduced energy consumption (John et al., 2020). These biotechnological products, known as bioremediators, contain a variety of bacteria, primarily heterotrophic ones. These bacteria mineralize organic matter and utilize the resulting products as a carbon source to build non-harmful microbial mass within the aquatic system (Jasmin et al., 2020).

Bioremediators primarily consist of bacteria from the *Bacillus* genus, which play a crucial role in organic matter mineralization and also contribute to the nitrification process (Reddy et al., 2018). In light of this, the objective of the current study was to evaluate the efficiency of *Bacillus*-derived bacteria, using a commercial bioremediatory, in treating the sediment of a fishpond that had undergone a Nile tilapia (*O. niloticus*) production cycle.

Material and Methods

The study was conducted at the Aquaculture Production Laboratory of the Faculty of Agricultural Sciences at the Federal University of Grande Dourados over a 15-day period. Sediment was collected from an aquaculture fishpond used in intensive fish farming, following a production cycle of Nile tilapia (*O. niloticus*). The fishpond is located in the city of Itaporã, Mato Grosso do Sul, Brazil (UTM Coordinates: 726134.25E and 7565315.21S). Initially, a physical, chemical, and organic matter analysis of the sediment was performed using the methodology outlined by Embrapa (2017) (Table 1).

Fifteen experimental units were used, consisting of black-colored aquariums with dimensions of 44.0 x 27.0 x 35.0 cm (length x width x height). A sediment-water mesocosm was set up, with a 5 cm layer of sediment placed at the bottom of the aquariums, followed by addition of 15 liters of water (Table 1).

Fifteen experimental units were used, consisting of black-colored aquariums with dimensions of 44.0 x 27.0 x 35.0 cm (length x width x height). A sediment-water mesocosm was set up, with a 5 cm layer of sediment placed at the bottom of the aquariums, followed by addition of 15 liters of water (Table 1).

In a completely randomized design, five treatments were tested with three repetitions. These treatments involved five levels of a commercial bioremediatior containing 3.5 x 109 UFC/g. The bioremediatory was composed of the following bacterial species: *Bacillus subtilis, B. licheniformis, B. amyloliquefaciens, and B. pumilus*. The study evaluated the effects of these treatments at the following concentrations: 100, 200, 300, 400, and 500 g/ha. The experiment lasted for 15 days, during which the application of the bioremediatory was carried out once, directly into the water on the first day of the study. At the end of the experimental period, the following water and sediment quality parameters were assessed, consistent with the measurements taken at the beginning of the study: pH, dissolved oxygen (mg/L), electrical conductivity (µS/cm), and total solids dissolved (mg/L). These parameters were evaluated using a multiparameter device (Akso model SX836). The alkalinity (mg/L) and water hardness (mg/L) were determined through titration methods: phenolphthalein indicator for alkalinity and EDTA titration for hardness. Furthermore, the concentrations of toxic ammonia (mg/L), nitrite (mg/L), orthophosphate (mg/L), and total phosphorus (mg/L) were analyzed using laboratory kits and a spectrophotometer.

At the end of the experimental period, soil samples from the units were sent for analysis of pH, phosphorus (mg/dm^3), effective (t) and potential (T) cation exchange capacity (CEC) (cmolc/dm3), base saturation (V) (%) and aluminum saturation (m) (%). In addition to the chemical parameters, the amount of organic matter (g/dm) was also determined. Soil analyses were performed following the procedures established by (Embrapa, 2017).

The statistical analyses were conducted using the data obtained at the end of the study. Firstly, they underwent tests for normality and homogeneity of variances. Subsequently, an analysis of variance (Anova) was performed, and upon detecting statistical differences, the means were compared using the Tukey test at a 5% probability level. The statistical procedures were carried out using Statistica 7.0 softwares.

Results and e Discussion

No influence of the treatments on the evaluated water quality parameters was observed ($p > 0.05$) (Table 2). It is worth noting that the water used in the study was within the ideal conditions for fish production (Boyd, 2020), as the main objective of the study was to assess the effect of the bioremediatory on the sediment, not specifically on the water. However, an increase in water quality parameters was observed compared to the initial values at the beginning of the experiment. This is due to the interaction that occurs between soil/sediment and water, involving ion exchange and other elements, as demonstrated by (Li et al., 2013). These authors showed soil/sediment and water leads to an increase in pH, alkalinity, and hardness. The initial water did not contain detectable phosphorus or orthophosphate, but after 15 days of the study, these parameters increased in the water, indicating that some of the phosphorus and related particles may be released from the sediment into the water.

Table 1. Initial parameters of water sediment used in the study.

Parameters	**Sediment**
pH	5.90
Organic matter (%)	1.45
CEC effective ($cmol_c/dm^3$)	5.15
CEC potential ($cmol_c/dm^3$)	6.77
Base saturation (%)	76.70
Phosphorus ($cmol_c/dm^3$)	1279.80
	Water
pH	6.66
Dissolved oxygen (mg/L)	6.20
Electrical conductivity (µS/cm)	52.40
Total solids dissolved (mg/L)	37.30
Alkalinity (mg/L)	50.00
Hardness (mg/L)	53.00
Toxic ammonia - NH_3 (mg/L)	0.46
Nitrite - NO_{2-} (mg/L)	0.03
Ortophosphate - PO_4^{3-} (mg/L)	0.00
Total phosphorus - P (mg/L)	0.00

Note: CEC = Cation exchange capacity.

In relation to sediment, no influence of the treatments on pH, effective cation exchange capacity, potential, and base saturation was observed ($p > 0.05$) (Table 3). Cation exchange capacity indicates how much the soil can adsorb and release cations, while base saturation indicates the proportion of soil exchange sites occupied by basic cations such as Ca2+, Mg2+, and K+ (Brady and Weil, 2013). These soil/sediment chemical parameters directly influence water pH, alkalinity, and hardness. The higher the observed value in the soil/sediment, the greater the values observed in the water (Li et al., 2013). Consequently, it can be observed that the inclusion of the bioremediatory based on *Bacillus* genus bacteria does not affect these sediment parameters, which aligns with the results obtained by (Lopes et al., 2020), who found the same effect when evaluating *B. subtilis and B. licheniformes* species in sediment treatment.

Tabela 2. Chemical water variables after 15 days of including a bioremediatory for fish farming sediment treatment.

Parameters	**Bioremediator doses (g/ha)**				
	100	**200**	**300**	**400**	**500**
pH	7.43±0.06	7.20±0.20	7.27±0.21	7.20±0.10	7.20±0.10
DO (mg/L)	3.53±0.68	1.90±1.39	1.77±1.17	1.23±0.45	1.57±0.29
EC (µS/cm)	158.30±3.80	152.44±12.60	152.13±11.56	155.47±26.96	151.27±40.60
TDS (mg/L)	112.67±2.52	108.40±8.92	108.33±8.02	110.17±19.28	107.87±28.71
Alkalinity (mg/L)	83.33±3.06	80.00±6.00	80.33±6.66	80.67±17.01	80.67±25.40
Hardness (mg/L)	88.33±2.89	84.67±5.77	83.00±8.19	80.00±11.14	78.67±18.58
NH_3 (mg/L)	0.08±0.09	0.54±0.49	0.62±0.49	0.92±1.08	0.40±0.43
NO_{2-} (mg/L)	0.08±0.04	0.11±0.04	0.11±0.05	0.10±0.03	0.08±0.04
PO_4^{3-} (mg/L)	0.19±0.18	0.32±0.21	0.48±0.26	0.50±0.34	0.26±0.27
P (mg/L)	0.06±0.06	0.11±0.07	0.16±0.08	0.16±0.11	0.09±0.09

Note: DO = Dissolved oxygen; EC = Electrical conductivity; TDS = Total dissolved solids; NH3 = Toxic ammonia; NO2- = Nitrite; PO43-= Orthophosphate; P = Phosphorus.

However, oscillation in organic matter percentage in the sediment was observed, with a lower value for the 300 g/ha ($p > 0.05$). The same effect was observed by (Lopes et al., 2020), where organic matter reduction occurred, but at higher doses, this effect was not observed.

Tabela 3. Chemical water variables after 15 days of including a bioremediatory for fish farming sediment treatment.

Parameters	Bioremediator doses (g/ha)				
	100	200	300	400	500
pH	5.67±0.06	5.83±0.12	5.87±0.06	5.97±0.15	5.93±0.15
Organic matter (%)	2.41±0.22ab	2.38±0.87ab	1.28±0.11a	3.08±1.32ab	4.40±0.87b
CEC effective cmol$_c$/dm^3)	5.02±0.13	5.62±0.40	7.09±2.40	7.74±1.40	6.54±1.00
CEC potential cmol$_c$/dm^3)	6.54±0.16	7.15±0.40	8.61±2.38	9.31±1.39	8.10±1.06
Base saturation (%)	76.70±0.28	78.59±1.37	81.40±4.86	82.91±2.72	80.55±1.70

The most prominent result observed in the study was the decrease in phosphorus concentration in the sediment when the bioremediatory was added ($p < 0.05$) (Figure 1). The application of 400 g/ha of the product resulted in a 60% reduction in sediment phosphorus content in the fish farm. At the dosage of 300 g/ha, where there was greater reduction of organic matter, a 48% decrease in phosphorus was observed compared to the initial value. This result demonstrates the efficiency of using *Bacillus* bacteria for removing this component, which is directly related to eutrophication and water quality decline. Phosphorus is rarely studied in the context of bioremediation, and this regard, (Reddy et al., 2018) conducted a study evaluating *Bacillus* bacteria in water quality and found a reduction in phosphate, but the author did not assess the compound's action in the sediment.

These bacteria use phosphorus to compose their biomolecules, and furthermore, they can store a significant amount of phosphorus beyond their metabolic needs (Wang et al., 2019). Controlling phosphorus concentration in sediment and water within aquaculture ponds of great importance. This control aims to ensure better aquatic quality, increased productivity, and compliance with regulatory requirements regarding effluent characteristics generated by fish farming (Coldebella et al., 2017). According to these authors, during fish Harvest, sediment in the ponds gets disturbed, causing the particulate phosphorus to be suspended in the water, resulting in an elevated concentration in the effluent above the levels recommended by environmental agencies. In order to improve the quality of fish farm effluents, various measures are adopted, such as improving nutritional management and efficient use of fertilizers. The results of this study demonstrate that the use of bioremediators composed of Bacillus bacteria can be a practical, safe, and cost-effective tool for significantly reducing phosphorus concentration in sediment.

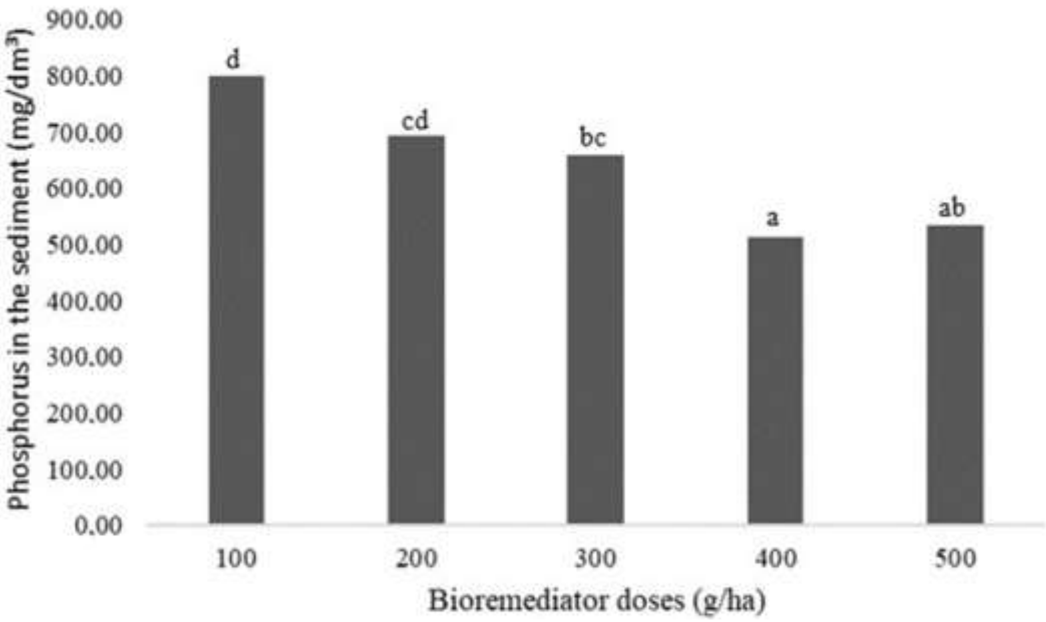

Figure 1. Phosphorus in fish farm sediment after 15 days of treatment with bioremediator.

The development and growth of heterotrophic bacteria are governed by environmental conditions such as pH, temperature, and oxygen. Consequently, these factors influence the efficiency of the bioremediation process (Lananan et al., 2014). In the present study, oxygen incorporation into the water was solely due to atmospheric exchange, without supplementary aeration. A reduction in oxygen plays a significant role in sediment bioremediation by accelerating the oxidation and decomposition of pollutants, while also meeting the consumption needs of heterotrophic bacteria (Wang et al., 2019). Therefore, the lack of more prominent results regarding organic matter reduction may be attributed to the oxygenation system used in the study.

The bacterial species *Bacillus subtilis, B. licheniformis, B. amyloliquefaciens, and B. pumilus* are effective for phosphorus reduction in fish farm sediments, and their action does not interfere with soil pH, cation exchange, and base saturation.

Acknowledgments

The authors thank the Federal University of Grande Dourados for the financial support.

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Como citar o artigo:

Oliveira, D.F.R., Lewandowski, V., Campos, R.R., Domingues, D., Telli, G.S., Godoy, A.C. & Neu, D.H. (2025). Bacteria of the genus *Bacillus* are effective in reducing phosphorus in aquaculture. *Actapesca*, 22, 23-29. DOI 10.46732/Actafish.22.28-31