Biological strategy to improve decomposition of organic matter in tilapia pond

Estratégia biológica para incremento da decomposição de matéria orgânica em viveiros de tilápia

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Abstract: Aim: The increment of decomposition of organic matter in sediment samples from Nile tilapia farms was evaluated with the introduction of *Bacillus subtilis* and *B. licheniformis* bacteria. **Methods:** Sediment samples placed in 18L plastic boxes received single dose inoculum with the following concentrations: 1.21×10^6 CFU g^{-1} (equivalent to 75 g ha^{-1}), 2.41×10^6 CFU g^{-1} (equivalent to 150 g ha^{-1}), 4.82×10^6 CFU g^{-1} (equivalent to 300 g ha^{-1}) and 1.61×10^7 CFU g^{-1} (equivalent to 1000 g ha^{-1}), in addition to a control treatment with saline solution only. Organic matter content, total organic carbon (TOC) and oxidizable (OOC), total nitrogen (TN), ratios (TOC: N and OOC: N), clay content, pH in water, Shoemaker, McLean, Pratt index (SMP Index), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) contents, potential acidity (H + Al), cation exchange capacity (CEC) at pH 7.0, base saturation (V) and sum of bases (S). **Results:** The values of OM showed significant difference, between the lowest values (treatments 75, 150 and 300 g ha^{-1}) and the highest value, (control treatment). TOC, OOC, NT and their relationships (TOC: N and OOC: N) showed significant differences between the mean values of the control treatment and the other treatments. **Conclusions:** The addition of *Bacillus subtilis* and *B. licheniformis* bacteria increased the decomposition rate of organic matter in sediments samples from Nile tilapia farms.

Keywords: aquaculture; bioremediation; *Bacillus subtilis*; *Bacillus licheniformis*; sediment.

Resumo: Objetivo: Foi avaliado o incremento da decomposição da matéria orgânica em amostras de sedimento de viveiros de criação de tilápias-do-Nilo com a introdução das bactérias *Bacillus subtilis e B. licheniformis*. **Métodos:** Amostras de sedimento colocadas em caixas plásticas de 18L, receberam dose única de inóculos com as seguintes concentrações: $1,21 \times 10^6$ UFC g^{-1} (equivalente a 75 g ha^{-1}), $2,41 \times 10^6$ UFC g^{-1} (equivalente a 150 g ha^{-1}), $4,82 \times 10^6$ UFC g^{-1} (equivalente a 300 g ha^{-1}) e $1,61 \times 10^7$ UFC g^{-1} (equivalente a 1000 g ha^{-1}), além de um tratamento controle, com adição apenas de solução salina. Foram analisados teor de matéria orgânica, teores de carbono orgânico total (COT) e oxidável (COO), nitrogênio total (NT), relações de carbono: nitrogênio (COT:N e COO:N), teor de argila, pH em água, índce Shoemaker, McLean, Pratt (índice SMP), teores de fósforo (P), de potássio

(K), de cálcio (Ca) e de magnésio (Mg), acidez potencial (H+Al), capacidade de troca catiônica (CTC) a pH 7,0 e saturação por bases (V). **Resultados:** Os valores de MO apresentaram diferença significativa entre os menores valores (tratamentos 75, 150 e 300 g ha⁻¹) e o maior valor (tratamento controle). COT, COO, NT e suas relações (COT:N e COO:N) apresentaram diferenças significativas entre os valores médios do tratamento controle e os demais tratamentos. **Conclusões:** A adição das bactérias *Bacillus subtilis* e *B. licheniformis* incrementou a taxa de decomposição de matéria orgânica em amostras de sedimento de viveiro de criação de tilápias-do-Nilo.

Palavras-chave: aquicultura; biorremediação; *Bacillus subtilis*; *Bacillus licheniformis*; sedimento.

Fish production is currently one of the main sources of animal protein for human consumption, having reached a record total of 171 million tons in 2016 (FAO, 2018). Among the fish species produced worldwide, Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) is the fourth with the highest production volume and the main in Brazil, reaching 400,280 tons in 2018, with significant annual growth rates (Medeiros, 2019). However, this intensification of crops also increases the generation of organic waste. In excavated tanks, organic matter ends up at the bottom at a rate of approximately 1-2 cm year⁻¹ (Boyd et al., 2010). As the main fish breeding in Brazil, tilapia culture presents itself as an activity with great potential for eutrophication of farming environments, as well as surrounding areas (Coldebella et al., 2017).

A possible biological strategy for better management of accumulated organic matter in fish ponds is bioremediation, which is the transformation of contaminants, mediated by organisms and/or their enzymes, into non-toxic or less harmful substances (Karigar & Rao, 2011). Some bacteria have a recognized action on the decomposition of organic matter (De Marco et al., 2017), such as those of the genus *Bacillus* sp., which are producers of important hydrolytic enzymes in this process such as proteases (Degering et al., 2010), lipases. (Iqbal & Rehman, 2015), amylases (Raul et al., 2014), chitinases (Trachuk et al., 1996), cellulases (De Marco et al., 2017) and xylanases (Yoon, 2009; Yi et al., 2011).

The aim of this study was to evaluate on a laboratory scale the ability of *B. subtilis* and *B. licheniformis* to decompose organic matter from sediments of intensive fish farms. For this purpose, sediment samples were collected the day after the end of the production cycle of a fish farm (5,500 m²) located in Armazém, SC, Brazil (S 28° 10,942'-W049° 00,861').

The product tested in the present study was PureGro®, manufactured by the Netherlands-based company DSM Nutritional Products Inc., containing mineral oil, rice husk, calcium carbonate and probiotic bacteria *Bacillus subtilis* and *B. licheniformis*

at the estimated concentration of 1.12×10^9 CFU g⁻¹. The inoculum was diluted in saline solution (0.65% NaCl) at different concentrations, adjusting the fixed final volume of 30 mL per experimental unit for subsequent application on the sediment.

The experiment was carried out in experimental units consisting of 18 L plastic boxes, measuring $41 \times 34 \times 13$ cm (length x width x height), filled with sediment to the approximate height of 10 cm. The design used consisted of four treatments with the addition of bacteria inoculum at the following concentrations: 1.21×10^6 CFU g⁻¹ (equivalent to 75 g ha⁻¹), 2.41×10^6 CFU g⁻¹ (equivalent to 150 g ha⁻¹), 4.82×10^6 CFU g⁻¹ (equivalent to 300 g ha⁻¹) and 1.61×10^7 CFU g⁻¹ (equivalent to 1000 g ha⁻¹), plus a control treatment, with the addition of saline solution only. The experiment lasted nine days, with a single inoculum application on the first day, and with average sediment temperature in the experimental units of 24.08 °C ± 1.92.

To perform the physicochemical analyzes of the different treatments, samples of 800 mL of sediment were collected nine days after inoculum application and dried in an oven at 60 °C for 48 hours. The determination of the organic matter (OM) contents was made by the colorimetric method by potassium dichromate reduction (Silva, 2009). Total organic carbon (TOC) and oxidizable organic carbon (OOC) of the sediment were estimated based on the organic matter content through the expressions: TOC = 0.51 (MO - 1) + 0.48 and OOC = 0.51 (MO - 1) - 3.59, developed by Navarro et al. (1993). Based on the TOC, OOC and TN values (described below), carbon: nitrogen ratios (TOC: N and OOC: N) were determined. Total nitrogen (TN) was analyzed by the Kjeldahl method (APHA, 2005). The analysis of the clay contents was performed by shear with marbles and reading in densimeter, according to (Tedesco et al., 1995). The pH analysis in water was performed by the electrochemical method through the effective concentration of H⁺ ions in the sediment solution (Teixeira et al., 2017). Based on pH measurements in water, the SMP index was determined (Silva, 2009). Phosphorus, potassium,

calcium and magnesium contents were determined according to the methodology of Teixeira et al. (2017) and the potential acidity of the sediment (H + Al) was determined according to the methodology described by Campos et al. (2017). The sum of bases (S), cation exchange capacity (T) and saturation of potential acidity (V) were determined according to Teixeira et al. (2017).

Prior to data analysis, the Kolmogorov-Smirnov test was used to check for data normality and the Levene test for homogeneity of variance. In the present study, all the original datasets conformed to a normal distribution, and the potential differences among treatments were analyzed using one-way followed by Student-Newman-Keuls (SNK) test. One-way ANOVA was used to determine the differences among soil properties at P<0.05. All data in the figures and tables are presented with original data, and they are presented as mean ± SD (standard deviation).

The observed OM levels, ranging from 37.00 g kg^{-1} to 45.33 g kg^{-1} are within the range considered appropriate for fish farming (Boyd et al., 2002). Significant differences were observed between the mean values of the control treatment and the other treatments (Table 1). Treatments 75, 150 e 300 g ha^{-1} had the lowest content, indicating that the introduction of *Bacillus subtilis* and *B. licheniformis* increased the rate of decomposition of organic matter in the sediment samples.

Organic carbon is the major component of soil organic matter and energy source for heterotrophic microorganisms (Boyd et al., 2002; Madigan et al., 2010). In the present study, total organic carbon (TOC), oxidizable organic carbon (OOC) and their relationship to total nitrogen (TOC: N and OOC: N) showed significant differences between the mean values of the control treatment and the other treatments (Table 1). Treatments 75, 150 e 300 g ha^{-1} presented the lowest values in all the mentioned parameters, with reductions ranging from approximately 18 to 22%. The total nitrogen contents of the treatments 75, 150 and 300 g ha^{-1} were different from the control (Table 1).

Table 1. Chemical and physical variables of fish sediments treated with different concentrations of *B. subtilis* and *B. licheniformis in vitro* estimation model.

Treatment	0 g ha^{-1}		75 g ha^{-1}		150 g ha^{-1}		300 g ha^{-1}		1000 g ha^{-1}		P value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
OM g kg^{-1}	45.33[a]	1.53	41.00[bc]	1.73	37.00[c]	2.00	40.00[bc]	1.73	42.00[b]	2.00	0.003
TOC g kg^{-1}	23.09[a]	0.78	20.88[bc]	0.88	18.84[c]	1.02	20.37[bc]	0.88	21.39[b]	1.02	0.003
OOC g kg^{-1}	19.02[a]	0.78	16.81[bc]	0.88	14.77[c]	1.02	16.30[bc]	0.88	17.32[b]	1.02	0.003
TN g kg^{-1}	1.16[a]	0.05	1.32[b]	0.00	1.29[b]	0.05	1.29[b]	0.05	1.23[ab]	0.00	0.007
TOC:N	19.88[a]	1.47	15.81[bc]	0.67	14.63[c]	1.00	15.84[bc]	1.39	17.44[b]	0.83	0.002
OOC:N	16.39[a]	1.32	12.73[bc]	0.67	11.47[c]	0.92	12.68[bc]	1.25	14.12[b]	0.83	0.002
Clay g kg^{-1}	30.67	0.58	29.67	2.08	28.67	2.08	28.67	1.53	28.67	1.53	0.511
pH-Water 1:1	6.77	0.06	6.80	0.00	6.73	0.06	6.77	0.06	6.77	0.06	0.655
SMP Index	6.57	0.12	6.53	0.06	6.50	0.00	6.60	0.10	6.57	0.06	0.596
P mg dm^{-3}	390.20	13.36	381.10	2.13	379.40	3.30	368.10	7.07	377.50	9.27	0.082
K mg dm^{-3}	324.80[ab]	15.80	312.67[b]	7.57	318.27[b]	3.52	345.93[a]	12.07	323.67[ab]	6.91	0.022
Ca cmolc dm^{-3}	7.27	0.21	7.50	0.40	6.63	0.50	6.93	0.12	6.67	0.47	0.067
Mg cmolc dm^{-3}	3.23	0.45	3.07	0.06	3.03	0.12	2.97	0.21	2.77	0.21	0.302
Potential acidity H + Al cmolc dm^{-3}	2.33	0.29	2.33	0.06	2.40	0.00	2.20	0.10	2.33	0.15	0.630
CEC pH7.0 cmolc dm^{-3}	13.64	0.41	13.71	0.39	12.87	0.61	13.01	0.39	12.63	0.61	0.083
Base saturation - V	82.89	2.10	82.99	0.63	81.30	0.89	83.10	0.36	81.47	1.83	0.337
Sum of bases - S	11.31	0.45	11.38	0.39	10.47	0.61	10.81	0.30	10.30	0.71	0.096

Lower case letters indicate statistical difference between treatments (P <0.05). OM g kg^{-1} = Organic Matter content; TOC g kg^{-1} = Total Organic Carbon content; OOC g kg^{-1} = Oxidizable Organic Carbon content; TN g kg^{-1} = Total Nitrogen content; TOC:N = Total Organic Carbon: Nitrogen ratio; OOC:N = Oxidizable Organic Carbon: Nitrogen ratio; Clay g kg^{-1} = Clay content; pH-Water 1:1 = Soil pH in a 1:1 soil: water suspension; SMP Index = Shoemaker, McLean, Pratt index; P mg dm^{-3} = Phosphorus content; K mg dm^{-3} = Potassium content; Ca cmolc dm^{-3} = Calcium content; Mg cmolc dm^{-3} = Magnesium content; Potential acidity H + Al cmolc dm^{-3} = Potential acidity; CEC pH 7.0 cmolc dm^{-3} = Cation Exchange Capacity at pH 7.0; Base saturation – V = Base saturation; Sum of bases – S = Sum of bases; S.D = Standard Deviation.
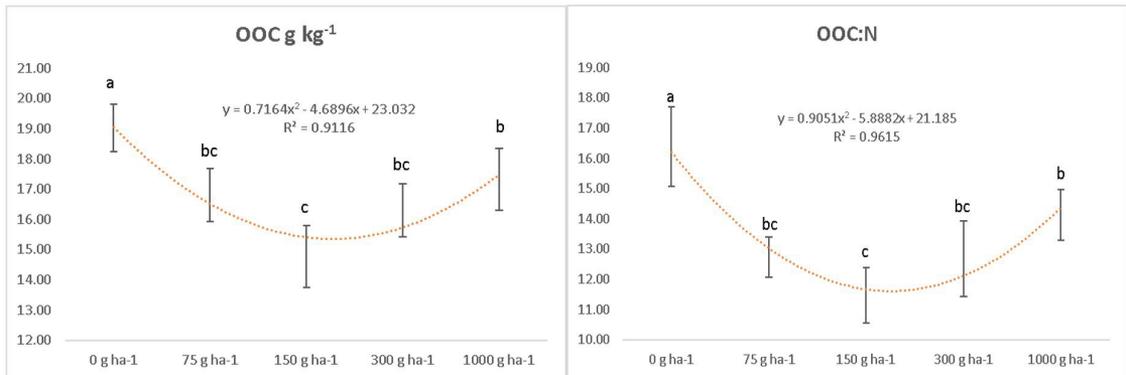
Figure 1. Estimation of a regression model of OOC (Oxidable carbon) and OOC:N (ratio of Total nitrogen and oxidable carbon) per different doses of *B. subtilis* and *B. licheniformis* in pond soil samples in an *in vitro* model.

The values of clay content, pH in water, SMP index, phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) contents, potential acidity (H + Al), exchange capacity cationic acid (CTC) at pH 7.0 and base saturation (V%) are within their normal range for fish pond sediments and can be seen in Table 1.

At Figure 1, can be observed that when the doses increased, at a mean level, no more degradation of the organic matter can be observed in this model. So, it can be concluded that the minimal dosage of use *B. subtilis* and *B. licheniformis* is 1,21 x 10^6 UFC g^{-1} soil pond.

Due to advantages of these model of evaluation, the use of *Bacillus subtilis* e *B. licheniformis* has been applied efficiently to detect the varying correlations between soil attributes and the different doses applied. We concluded that the addition of *Bacillus subtilis* and *B. licheniformis* at a minimum dosage of 75 g ha-1 is a way to increase the decomposition rate of organic matter in sediments samples from Nile tilapia farms, which can enhance the sustainability and an environmentally friendly biological approach to improve decomposition of organic matter in tilapia pond.

Acknowledgements

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