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Dissolved oxygen, organic matter and nutrients in fish systems combined with bio-addition of friendly microorganisms

Dissolved oxygen, organic matter and nutrients in fish systems combined with bio-addition of friendly microorganisms

Oxígeno disuelto, materia orgánica y nutrientes en sistemas de peces combinados con bioadición de microorganismos amigables

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Abstract
Effluents from conventional intensive aquaculture, based on high water exchange, may strongly contribute to the acceleration of eutrophication processes, due to the significant nutrient load present in unconsumed foods and excreted waste. On the other hand, there are intensive and super-intensive systems for the cultivation of aquatic organisms that use water exchange associated with strong aeration and biological transformation of macronutrients. Such sustainable systems use the bio-addition of friendly microorganisms to increase the rate of degradation of macronutrients and maintain water quality. A mathematical adjustment was used in this study to verify the dynamics of organic matter, nitrogen and phosphate during the experiment period. A pulse dynamic at regular times occurred indistinctly for all indicators evaluated in this study, although the intensity of the pulses showed particularities among the different indicators of water quality. Although the behavior of regular pulses of the indicators occurred, this condition did not affect the growth of fish subjected to a simulated system of intensive production associated with the bio-addition of friendly microorganisms.
Keywords: Wastewater; Beneficial bacteria; Aquaculture; Mathematical adjustments.

Resumo
Efluentes da aquicultura intensiva convencional, baseados em altas trocas hídricas, podem contribuir fortemente para a aceleração dos processos de eutrofização, devido à significativa carga de nutrientes presente nos alimentos não consumidos e resíduos excretados. Por outro lado, existem sistemas intensivos e superintensivos de cultivo de organismos aquáticos que utilizam trocas de água associadas a forte aeração e transformação biológica de macronutrientes. Esses sistemas sustentáveis usam a bioadição de microorganismos amigáveis para aumentar a taxa de degradação dos macronutrientes e manter a qualidade da água. Um ajuste matemático foi utilizado neste estudo para

verificar a dinâmica da matéria orgânica, nitrogênio e fosfato durante o período de experimento. Uma dinâmica de pulso em horários regulares ocorreu de forma indistinta para todos os indicadores avaliados neste estudo, embora a intensidade dos pulsos apresentasse particularidades entre os diferentes indicadores de qualidade da água. Embora tenha ocorrido o comportamento de pulsos regulares dos indicadores, essa condição não afetou o crescimento dos peixes submetidos a um sistema simulado de produção intensiva associada à bioadição de microrganismos amigáveis.
Palavras-chave: Águas residuais; Bactérias benéficas; Aquicultura; Ajustes matemáticos.

Resumen
Los efluentes de la acuicultura intensiva convencional, basada en un alto recambio de agua, pueden contribuir fuertemente a la aceleración de los procesos de eutrofización, debido a la importante carga de nutrientes presentes en los alimentos no consumidos y los residuos excretados. Por otro lado, existen sistemas intensivos y superintensivos de cultivo de organismos acuáticos que utilizan intercambios de agua asociados a una fuerte aireación y transformación biológica de macronutrientes. Estos sistemas sostenibles utilizan la bioadición de microorganismos amigables para aumentar la tasa de degradación de macronutrientes y mantener la calidad del agua. En este estudio se utilizó un ajuste matemático para verificar la dinámica de la materia orgánica, el nitrógeno y el fosfato durante el período del experimento. Una dinámica de pulsos en tiempos regulares ocurrió indistintamente para todos los indicadores evaluados en este estudio, aunque la intensidad de los pulsos presentó particularidades entre los diferentes indicadores de calidad del agua. Si bien se presentó el comportamiento de pulsos regulares de los indicadores, esta condición no afectó el crecimiento de los peces sometidos a un sistema simulado de producción intensiva asociado a la bioadición de microorganismos amigables.
Palabras clave: Aguas Residuales; Bacterias beneficiosas; Acuicultura; Ajustes matemáticos.

1. Introduction

The water in intensive production of fish, become more concentrated in pollutants due to the drainage procedure and also to the synergism existing between water quality- storage density - feed rate, because depending on the conditions, the tank environment can become toxic. According to Liu et al. (2016), Leal et al. (2018) and Coldebella et al. (2018), aquaculture effluents cause variations in water quality such as: decreased dissolved oxygen concentration, increased biological oxygen demand, nitrogen and phosphorus forms, increase in total suspended solids (TSS), and increased algal biomass (bloom), this scenario, the environmental degradation caused by aquaculture effluents should be one of the points observed carefully. Boyd (2003) suggests the application of a set of standards and procedures that constitute the so-called good management practices (GMP) as a way to improve the quality and reduce the volume of effluents.

Regarding the environmental impact of effluents, the importance of friendly or beneficial microorganisms in aquaculture tanks is emphasized by Liong (2015) and Adel and Dawood (2021).

The use of bio-addition is justified when the need for rapid degradation of polluting compounds is necessary. According to Divya et al. (2015), Manzoor (2016) and Deng et al. (2021) the use of an immobilized bacterial consortium represents a viable alternative for the *in situ* removal of nutrient residues in aquaculture systems due to the low cost of its application.

The aim of this study was evaluating the behavior of the organic matter and nutrients in effluents of aquaculture systems when submitted to bio-addition of beneficial and friendly microorganisms.

2. Materials and Methods

The experiment was conducted following the methodology of Verdegem and Beristain (2005) and Chen et al. (2020), which were modified for this study. And the study was conducted in Piracicaba São Paulo, in the fish farming sector, linked to department of non-ruminant animal husbandry of the Escola Superior de Agricultura “Luiz de Queiroz” – ESALQ/USP, in a greenhouse in order to control environmental variables. The lot of fish was acquired from a fish farm. The species used in this study was *Oreochromis niloticus* (L.) (Nile tilapia). The tilapia was chosen due to this species' availability in the needed size for the beginning of the study.

2.1 Conduct of the study

In the reception of the tilapia juveniles' batch, we adopted a sanitary protocol. The fish were stored in six 1 m^{-3} boxes with a useful volume of 0.8 m^{-3} and submitted to malachite green solution at the concentration recommended by Brown (2000) (10 mg 100 L^{-1} for three days), combined with the administration of the antibiotic oxytetracycline incorporated into the food portion (30 mg kg^{-1} of portion) for one week. The mean weight of tilapia individuals was approximately 120 g. Then, the fish were then transferred and permanently stored in the experimental tanks.

Eighteen 1.0 m^{-3} polyethylene plastic boxes were used, with a useful volume of 0.8 m^3 $tank^{-1}$. The experimental tanks were individually equipped with constant aeration through air diffusers kept by a mechanical blower, and the oxygen rate was kept close to saturation. The system was provided with an individualized system of water inlet and outlet, supplied by a spring. The drainage system was composed of PVC pipes placed internally to the tanks that allowed the flow of wastewater. The water temperature was kept at ± 25 ºC.

For each experimental tank, 40 juveniles.m^{-3} were stored with initial mean weight of 120 g. Therefore, with a storage density for the useful volume of tanks corresponding to 32 fish m^{-3}. $tank^{-1}$, which is equivalent to an initial density of 0.025 fish.m^{-3} and a biomass of 4.8 kg.m^{-3} $tank^{-1}$. Commercial portion was fed to the fish, which was skinned and extruded with a content of 32 % crude protein. The food was offered during seven days of the week, twice a day throughout the experiment period. Food was offered to fish *ad libtum* up to satiety.

Food intake data were collected weekly during the experiment (weighing of the consumed food). From the weighing data, the following information on the zootechnical performance of fish was obtained: initial biomass; final biomass; initial mean weight; final mean weight; initial length; final length; weight gain ($GP = Pf - Pi$); feed conversion (amount of food/weight gain); condition factor ($Fc = \frac{100 \times GP}{total\ length^3}$); specific growth rate ($TCE = \frac{100 \times (lnPf - lnPi)}{days}$); food consumption index ($ICA = \frac{100 \times total\ consumption}{[(Pf+Pi)/2]}$); feed efficiency index ($IEA = \frac{GP}{CT}$) and survival rate (%).

The hydraulic retention time (HRT) of the tanks was 44.4 hours, the flow rate adopted during the test was 18 liters.h^{-1}. During the whole test we did not use the siphon for waste and unconsumed food.

2.2 The microbial inoculum

The composition of the inoculum, which we call the code AQ+ was: cereal bran 75 g^{-1}; salt 15 g; Protease 9.6 g^{-1}; Amylase 0.15 g; Lipase 0.07 g^{-1}; hemicellulose 0.13 g^{-1} and Beta-glucanase 0.05 g^{-1}. According to the supplier, the bacteria concentration present in the inoculum was 1.20×10^8 UFC.g^{-1}. The immobilized microorganisms that composed the microbial inoculum AQ+ were: *Bacillus subtilis*, *Bacillus liqueniformis* e *Bacillus polymyxa*. The viability of the microbial inoculum for use in the experiment was based on weighing, 20 g^{-1} of the material suspended in 80 mL^{-1} of distilled water, corresponding to a concentration of 0.25 mg mL^{-1}. The stock solution was then homogenized and filtered in Büchner funnel with 1 µm filter, by a vacuum pump coupled to a laboratory glass flask.

After the procedure of microbial inoculum viability for use in the experiment, it would have (according to the supplier) a concentration of 3.75×10^6 UFC g^{-1}. Combined with the inoculum AQ+, a solution composed of *Nitrosomonas spp.* e *Nitrobacter spp.* in the concentration of $1{,}5\times10^8$ UFC mg L^{-1} was added. At the time of inoculation, the experiment tanks received identical concentrations of the inoculum AQ+ and the nitrifying bacteria solution.

The pipetted volumes of the stock solution for composition of the concentrations of the treatments were: T1 (160 μL^{-1}), T2 (640 μL^{-1}), T3 (1280 μL^{-1}), T4 (2560 μL^{-1}) and T5 (3840 μL^{-1}), for the control, 160 μL^{-1} of the cereal filtrate was pipetted without the microbial inoculum, corresponding to 0.04 g L^{-1}. Then these volumes were diluted in 5 liters of water from the tanks and administered. The administration of the inoculums occurred from the 14 th day forward, being performed every two days.

2.3 Experiment design

The design used in the assay was completely randomized, consisting of six treatments and three replicates. The composition of the tested treatments had the following concentrations *T0* = control treatment (placebo), *T1* = 0,04 g L^{-1}, *T2* = 0,16 g. L^{-1}, T3 = 0,32 g L^{-1}, *T4* = 0,64 g L^{-1} and T5 = 0,96 g L^{-1}.

For the control (T0), we pipetted 160 μL^{-1} of the cereal filtrate without the immobilized bacterial consortium. Water samples were collected every fifteen days during the experiment period (75 days).

2.4 Water quality

The water samples were collected directly from the tank outlet using a siphon. The flow system of the experimental tanks was composed of a 3/4" PVC pipe covered by another 1" PVC pipe located inside each tank. The water samples for pH reading were performed by Orion Star potentiometer, the dissolved oxygen was determined by oxymeter. The electrical conductivity was determined by conductivimeter. The BOD was determined by ($DBO = \frac{ODi-ODf}{Incubation\ time \times 120}$). The total dissolved solids (TDS) were determined by conductivimeter. Total ammonia was determined by colorimetry using the salicylic acid addition method, and spectrophotometer read (640 nm wavelength).

Nitrate was determined using the diazotization method, and the reading was made in a spectrophotometer (543 nm wavelength). Nitrate was determined by the cadmium reduction method and the reading was performed by spectrophotometer (220 nm wavelength).

The phosphate was determined by the persulfate acid digestion method), and the reading was performed in spectrophotometer (680 nm wavelength). The phosphate was determined by the ascorbic acid method, and the reading was performed in spectrophotometer. The chlorophyll "*a*" (phytoplankton biomass index) was determined by chlorophyll extraction by filtration, and the reading was performed in spectrophotometer (663 nm wavelength) (Golterman et al., 1978).

2.5 Data analysis

An analysis of variance (ANOVA) was performed between treatments for both water quality parameters (physicochemical and nutrients) as well as for zootechnical performance parameters. When significant difference was detected between treatments, mean tests (Student's t test) were performed. The adopted significance level was 5% ($P < 0.05$).

2.6 Adjustment models

The calibration of a mathematical adjustment consists of adapting the parameters of the equation to the real world, considering the physical, chemical and biological variables of this environment, adjusting the estimated data in the adjustment with the data observed in the field (Simonovic, 2002). For the calibration of the mathematical model presented in this work, 10 empirical models from the specialized literature were evaluated and the mathematical model that best correlated the experimental data was the model presented in Equation 1. It is important to emphasize that the mathematical model of Equation 1 was selected because it is the only one to satisfactorily correlate dissolved oxygen, organic matter (*BOD*) and nutrients (NH_3, NO_2, NO_3 e PO_4).

$$y = e^{a+\frac{b}{Time}+c.\ln(Time)} \quad \text{Equation 1}$$

Where y: *DO*; *BOD*, NH_3, NO_2, NO_3 e PO_4.

2.7 Method for adjusting the coefficients of the mathematical model

The methodology for obtaining the three adjustable parameters of Equation 1 consisted of using the experiment data of dissolved oxygen concentration, organic matter (BOD) and nutrients: ammonia, nitrite, nitrate and phosphate in the experiment

system, as a function of the time for the decay of its rates. The coefficients *a*, *b* and *c* of Equation 1 were adjusted from the statistic software, using the mathematical method of adjustment of nonlinear functions of Levenberg-Marquardt.

The adjustment procedure consisted of an initial estimate for each of the three coefficients until adjusting them, using the highest value of the correlation coefficient R_2 and the absolute mean deviation as a decision-making criterion for the better optimization of these coefficients.

3. Results and Discussion

The individuals stored and kept under experimental conditions for 75 days had an average growth of 83% and survival of 100 % in all treatments against an average growth of 65 % and survival also of 100 % in control treatment (Table 1).

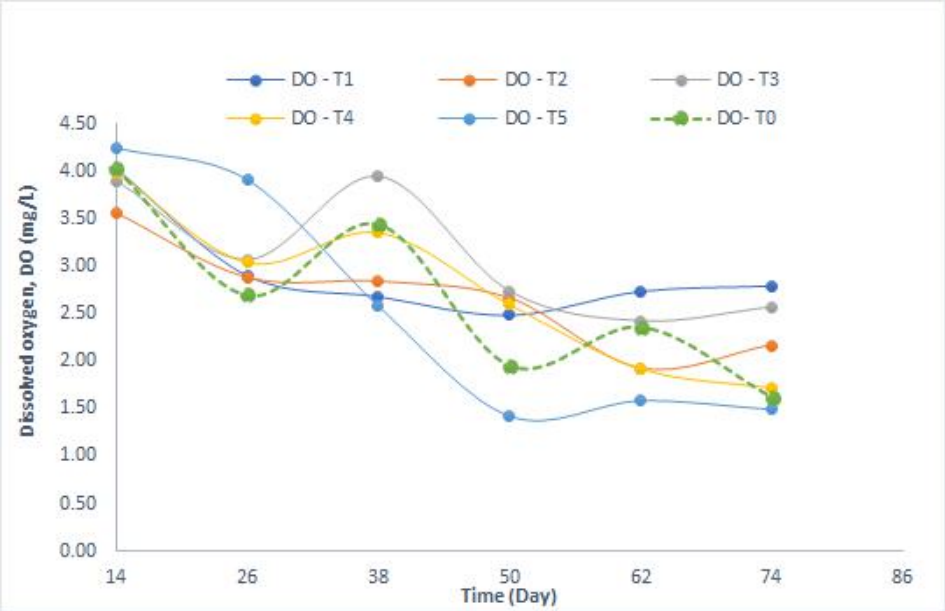
Table 1 - Performance of tilapia juveniles (*Oreochromis niloticus*) fed commercial portions and submitted to bio-addition of the microbial inoculum.

	Control	***T1***	***T2***	***T3***	***T4***	***T5***
Bi[1]	5094.69±906.36	5135.60±1.39	4728.40±684.1	4411.43±849.9	5091.26±1.17	4502.56±843.58
BF[2]	8362.23±1.96	9149.77±1.19	9262.11±1.87	8442.86±1.80	8974.06±1.35	9325.36±1.84
PMI[3]	127.37±22.66	128.39±34.99	118.21±17.10	116.12±110.29	127.28±29.39	112.56±21.09
PMF[4]	209.06±49.09	228.74±30.0	231.55±46.90	211.07±	224.35±33.77	233.13±46.24
CI[5]	18.43±0.85	18.04±1.57	17.96±0.76	17.26±0.80	17.87±1.14	17.68±0.98
CF[6]	22.47±1.71	23.49±0.85	23.63±1.79	22.76±1.60	23.56±1.14	23.14±1.62
GP[7]	103.49±64.83	107.32±47.94	95.69±27.86	92.14±73.68	83.07±64.62	98.13±55.91
CA[8]	0.66±0.71	0.50±0.03	0.50±0.19	0.64±0.31	1.10±1.23	0.60±0.46
FC[9]	0.78±0.43	0.79±0.38	0.45±0.42	0.73±0.37	0.58±0.38	0.84±0.42
TCE[10]	0.82±0.52	0.83±0.44	0.79±0.22	0.77±0.54	0.64±0.47	0.80±0.47
ICA[11]	0.52±0.08	0.59±0.06	0.69±0.12	0.70±0.07	0.63±0.09	0.62±0.03
IEA[12]	1.57±1.10	1.29±0.59	1.14±0.37	1.03±0.66	1.07±0.89	1.25±0.76
TS[13]	100	100	100	100	100	100

[1]Initial biomass (g); [2]Final biomass (g); [3]Initial mean weight (g); [4]Final mean weight (g); [5]Initial length (cm); [6]Final length (cm); [7]Weight gain (g); [8]Feed conversion day^{-1} (g); [9]Condition factor (%); [10]Specific growth rate (% day-1); [11] Food consumption index (% day-1 biomass); [12] Feed efficiency index; [13] Survival rate (%). Source: Authors (2021).

Pulses of dissolved oxygen gradients were verified at regular intervals (12 days) from the beginning to the end of the experiment period among all treatments. including control (Figure 1). After a fall in the first 12-day interval. Oxygen dynamics were stable only in *T5*. which maintained the dissolved oxygen gradients in the intervals between 2.5 and 3.0 mg L^{-1} (Figure 1, Table 2).

Figure 1 – Means of dissolved oxygen behavior between experimental treatments in the face of control as a function of time.



Source: Authors (2021).

Apparently *T3* presented the best behavior in the evolution of dissolved oxygen gradients over experiment time. It is important to mention that the biomass of fish stored in the different treatments started from an average weight of 4.8 kg m^{-3} and reached a final weight of 8.8 kg m^{-3}, a ratio twice higher than super-intensive aquaculture systems in Brazil. The hydraulic retention used in the system was 44.4 hours, which corresponds to a flow rate of 18 L h^{-1}.

Table 2 - Means and ± SD of water quality parameters in a simulated intensive aquaculture system with *Oreochromis niloticus* submitted to bio-addition of beneficial microorganisms.

Parameters	**Control**	***T1***	***T2***	***T3***	***T4***	***T5***
Temperature ºC	25.04±0.58	24.91±0.58	25.02±0.55	24.98±0.58	24.94±0.56	24.90±0.57
DO (mg/L)	2.69±1.12	3.06±0.91	2.63±0.86	3.11±0.74	2.77±1.07	2.54±1.35
pH	7.40±0.75	7.52±0.13	7.55±0.10	7.61±0.08	7.66±0.116	7.62±0.05
BOD (mg/L)	1.70±0.99	1.33±0.58	1.35±0.70	1.52±0.94	1.24±0.54	1.48±1.00
TDS (mg/L)	1398±64	1397±66	1400±63	1397±64	1395±67	1396±66
Clorofila "*a*"	1.11±0.66	0.78±0.40	1.17±0.66	0.81±0.55	1.02±0.62	0.86±0.39
P-Total (mg/L)	2.16±1.38	1.75±0.70	2.10±1.01	2.16±0.81	1.88±0.69	1.72±0.48
P-PO₄ (mg/L)	1.5±1.76	0.97±0.83	1.01±0.84	0.87±0.44	1.35±1.39	0.92±0.57
N-NH₃ (mg/L)	1.16±0.64	0.91±0.51	1.22±0.57	0.93±0.53	0.93±0.44	1.18±0.52
N-N0₃ (mg/L)	1.75±0.87	1.95±0.57	2.38±1.11	2.36±1.15	2.22±1.46	1.91±1.35
N-NO₂ (mg/L)	0.20±0.17	0.20±0.16	0.27±0.23	0.23±0.16	0.23±0.23	0.21±0.24

Source: Authors (2021).

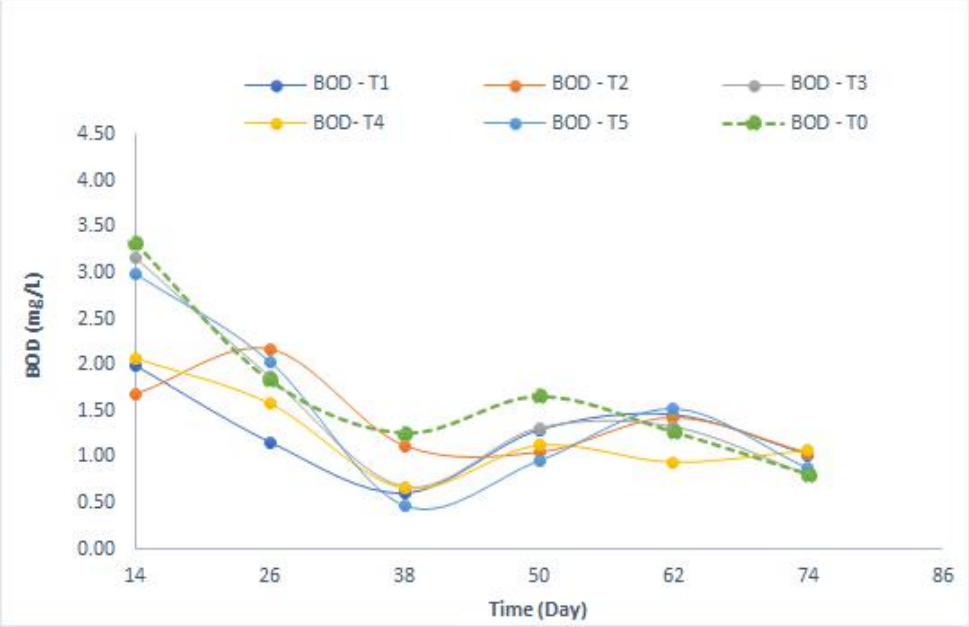
Regular pulses of dissolved oxygen at alternating times may be related to the nature of organic matter. The raw sewage load composed of unconsumed food and waste present in the experiment system presented a high fraction of carbon and assimilable nitrogen (high protein value) and when combined with temperature, accelerated the oxidation rate. The experimental condition allowed a higher coefficient of re-aeration due to both the ease of mixing and depth (Sperling, 2017), in addition to the greater surface turbulence induced by the mechanical aeration. Tables 3 and 4 show mathematical adjustment for the coefficients of dissolved oxygen, organic matter and nutrients.

Possibly, the stored fish (tilapia) by their foraging nature promoted an ammonia recharge in the water column, by re-suspending the thin layer of sediment formed at the bottom of the experiment tanks (Jalil et al., 2019; Li et al., 2017). In contrast, Hargreaves (1998) suggests that 25 to 33% of the ammonia transported to the water column comes from the sediment. Considering such conditions, the load of ammoniacal nitrogen present in the water column between treatments. For having presented a behavior in regular pulses of time intervals of 12 days, is similar to that verified by Nixon and Pilson (1983).

Altered water quality conditions promote increased resident microbial respiration. And also the benthic demand and nitrification can be significant enough to cause an imbalance of the dissolved oxygen rate in the system as a function of time. On the other hand, the re-aeration, verified over the experiment time, was probably more dependent on the mechanical aeration (that occurred in the system) than on the photosynthetic activity produced by algae and photosynthesizing bacteria.

To Yun et al. (2019) and Chen et al. (2020), the carbon load is particularly important due to its relationship with the BOD in the system. In this study, a low chlorophyll "*a*" gradient (0.94 mg m^{-3}) was found (Figure 2), according to the modified Carlson classification, thus indicating a non-dominance of photosynthetic algae. On the other hand, Samer (2015) suggest that under aerobic decomposition, 50% of metabolized organic matter is converted into bacterial cells and, therefore, as Luo et al. (2020) quotes, with the high load of intensive aquaculture systems, bacteria biomass is expected to be high.

Figure 2 – Means of the behavior of biological oxygen demand (BOD) between experimental treatments in the face of control as a function of time.



Source: Authors (2021).

The experiment system presented a dynamic for BOD in regular pulses from the 14th day until the end of the experiment period. The kinetics of BOD followed a first-order reaction pattern, an expected behavior for the decay of organic matter in aquatic environments. This evolutionary behavior verified in the experiment system was compatible, as a function of time, to self-purification zones, even under conditions of a BOD with low gradients due to the necessary mechanical aeration for the maintenance purposes of stored fish. Through mathematical modeling, Tables 3 and 4 indicate a high correlation for the BOD coefficient (R^2 = 0.98) regarding its decay in *T3*.

Although in super-intensive aquaculture systems (high storage density) there is often a reduced self-purification capacity of the system (Adel and Dawood, 2021; Li et al., 2019), considering the experiment conditions, it was observed the temporal

decay of organic matter in all treatments due to microbial activity, including the control the consortium of beneficial microorganisms inoculated at different concentrations in the treatments.

There was a synergistic evolution in time for nutrients, ammonia, nitrite and nitrate in the experiment system. A narrow and concentrated pulse was observed for nitrite (Figure 4) around day 26 of the experiment while for nitrate the pulse occurred with greater dispersion. Both pulses were observed in T5. For nitrite and nitrate, all treatments decayed in time, and for nitrite T4 and T5 presented an even greater decay than the control, while for nitrate only T5 showed greater decay in relation to the control. Tables 3 and 4 show high correlations in T4 for the decay of ammonia ($R^2 = 0.82$) and nitrite ($R^2 = 0.98$) due to the bio-addition of the microbial consortium.

Ammonia is the final product of aerobic or anaerobic mineralization of organic nitrogen produced by the respiration of different heterotrophic microorganisms (Esteves, 2011). In this study, non-ionized ammonia behaved temporally in cycles for all treatments. Treatments 1 and 4 showed greater dispersion over time in relation to other treatments and control.

Table 3 - Coefficients obtained through equation 1 for the mathematical modeling of the decay rate of organic matter and nutrients during the experiment period.

Nutrients	Treatment/ adjustable parameters																	
	T0			*T1*			*T2*			*T3*			*T4*			*T5*		
	a	***b***	***c***	***a***	***b***	***c***	***a***	***b***	***c***	***a***	***b***	***c***	***a***	***b***	***c***	***a***	***b***	***c***
DO	3.285	-4.488	-0.601	-1.046	18.735	0.420	2.735	-3.930	-0.452	3.181	-7.098	-0.504	4.652	-12.901	-0.896	8.695	-31.317	-1.895
BOD	0.789	13.395	-0.207	-5.063	39.687	1.105	4.877	-20.678	-1.074	-0.523	21.707	0.049	-0.907	17.622	0.148	-0.645	21.755	0.075
NH_3	-7.494	44.094	1.713	2.697	-13.161	-0.6404	2.705	-16.818	-0.552	-0.672	11.251	0.063	-6.247	45.294	1.300	-6.355	41.336	1.433
NO_2	12.370	-58.258	-3.436	7.360	-39.077	-2.163	8.637	-52.324	-2.294	2.938	-12.694	-1.124	16.597	-89.845	-4.272	50.155	-277.23	-12.231
NO_3	11.926	-68.008	-2.554	3.846	-22.689	-0.674	6.219	-37.172	-1.159	11.215	-71.825	-2.247	18.008	-116.94	-3.767	25.252	-167.27	-5.415
PO_4	158.828	-1836.56	-30.999	29.15	-277.993	-5.916	103.245	-1040.67	-20.889	7.173	-63.082	-1.493	158.808	-1616.4	-32.00	6.875	-69.245	-1.363

Source: Authors (2021).

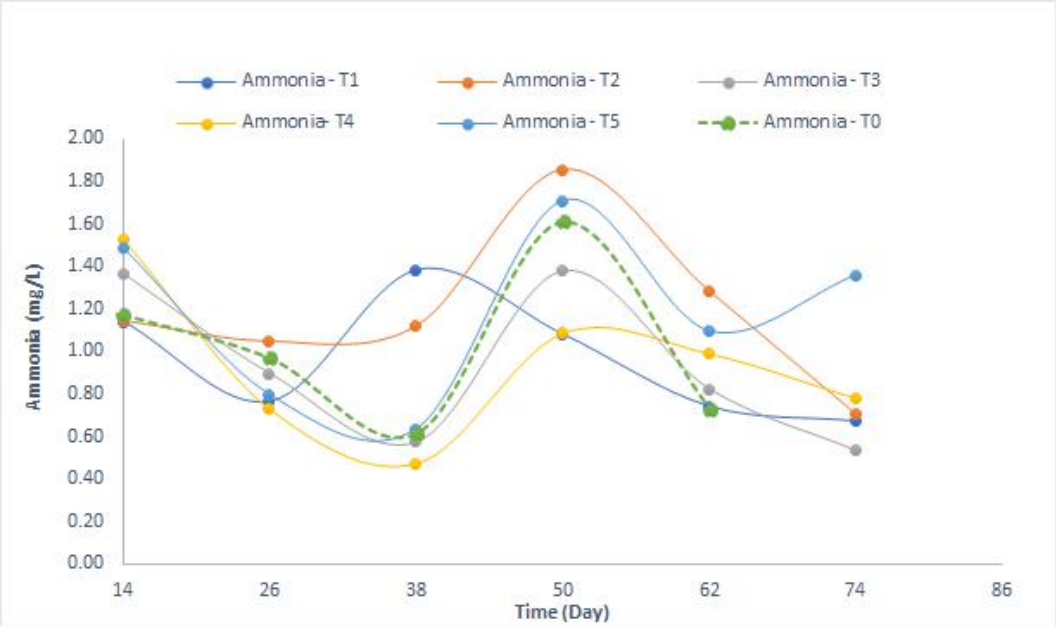
Table 4 - Correlation coefficients and absolute mean deviation obtained through the mathematical modeling for nutrient decay rate as a function of bio-addition of a microbial consortium.

Nutrients	Treatment/statistic data											
	T0		*T1*		*T2*		*T3*		*T4*		*T5*	
	R^2	***mean deviations***	***R^2***	***mean deviations***	***R^2***	***mean deviations***	***R^2***	***mean deviations***	***R^2***	***mean deviations***	***R^2***	***mean deviations***
DO	0.8542	0.6152	0.9891	0.1050	0.9349	0.2653	0.7711	0.5522	0.9319	0.4061	0.9717	0.3885
BOD	0.9639	0.3000	0.8077	0.3526	0.7183	0.4015	0.9278	0.4372	0.8751	0.3129	0.8634	0.5958
NH_3	0.5769	0.5171	0.4779	0.3190	0.2736	0.4725	0.5726	0.3957	0.8232	0.2670	0.6105	0.4220
NO_2	0.9595	0.0557	0.8686	0.0683	0.6735	0.1344	0.9234	0.0541	0.9864	0.0327	0.9633	0.0853
NO_3	0.8277	0.5694	0.7465	0.2833	0.8376	0.3704	0.8490	0.6095	0.8967	0.6867	0.9701	0.3925
PO_4	0.8990	0.7100	0.8829	0.3217	0.7602	0.5376	0.9059	0.1855	0.9317	0.5834	0.8731	0.2600

Source: Authors (2021).

The peak of ammoniacal nitrogen was observed at 50 days and the lowest value recorded at 38 days, around 0.40 mg L^{-1}. The trend to stabilize the ammoniacal nitrogen gradients was verified at 74 days with concentrations that fluctuated between 0.40 and 0.70 mg L^{-1}. The low concentration of chlorophyll "*a*" reveals that apparently the behavior of ammoniacal nitrogen (Figure 3) was governed by the activity of heterotrophic microorganisms.

Figure 3 - Mean ammoniacal nitrogen behavior between experiment treatments in the face of control as a function of time.



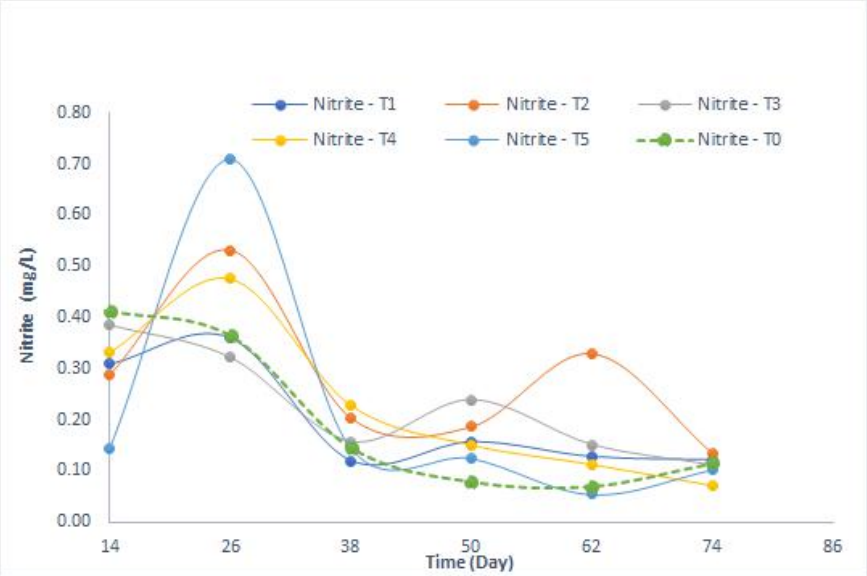
Source: Authors (2021).

This behavior may have occurred through the amonification-nitrification process, although phytoplankton is more efficient in ammonia transformation, when at low concentrations, as suggested by Hargreaves (1998), Klawonn et al. (2015) and Lukwambe et al. (2015). Unlike that verified by Hoang et al. (2018), there was no mortality and inhibition of the growth of stored fish in this study, during the experiment period, caused by the association of density with ammoniacal nitrogen gradients. Ammonia toxicity to fish in both natural and confined systems is a function of high pH and temperature and, which did not occur in this study, where the gradients remained neutral for pH, and the temperature had an average gradient of 24.9° C, which reveals a non-toxicity of ammonia to stored fish

In fish, ammonia toxicity leads to hyperactivity, convulsions, loss of balance, lethargy and coma, in aquaculture systems, ammonia toxicity is commonly expressed by reduced growth and suppression of immunocompetence (Boyd, 2017; Hargreaves, 1998; Pinto et al., 2016). The stored fish presented a final biomass yield compatible with the control, allowing us to infer that there was no toxicity from the ammonia gradients present in the system.

On the other hand, the temporal dynamics of nitrite and nitrate (Figures 4 and 5) was guided by an expected pattern because its higher concentrations during the study occurred when the dissolved oxygen consumption in the medium was at its highest. This dynamic evidenced the nitrification process, ruled by heterotrophic bacteria responsible for oxidizing ammonia, genus *Nitrossomonas* and bacteria for oxidation of nitrite, genus *Nitrobacter*. On the other hand, a lower plankton activity may also result in a lower rate of ammonia assimilation by algae, which would increase the load breathed by nitrifying bacteria.

Figure 4 - Means of nitrite behavior between experiment treatments in the face of control as a function of time.

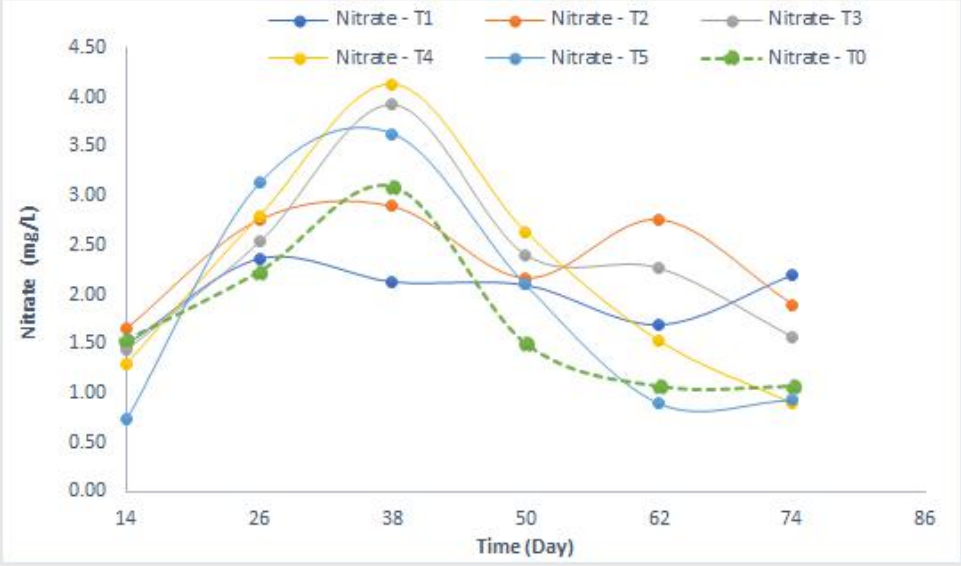


Source: Authors (2021).

Esteves (2011) notes that nitrification is an eminently aerobic process and only occurs in areas where oxygen is available. Throughout the experiment, however, it is observed the decay of nitrite in the medium, probably due to the breathing process, mediated by facultative anaerobic bacteria, in order to enable the oxidation of the remaining organic matter in the sediment, the denitrification process that may have occurred over the experiment period. Nitrite expresses its toxicity when hemoglobin is connected forming methemoglobin, a substance that does not have the capacity to carry oxygen.

Kroupová et al. (2018) infer that nitrite toxicity occurs mainly in intensive and super-intensive production systems and that among the clinical signs described for nitrite toxicity are lethargy, weakening of the escape reflex, muscle contractions, agony and death. However, during the experiment period, there were no clinical signs such as the mentioned above as a response to the use of bio-addition of the beneficial microorganism's consortium.

Figure 5 - Means of nitrate behavior between experiment treatments in face of the control as a function of time.



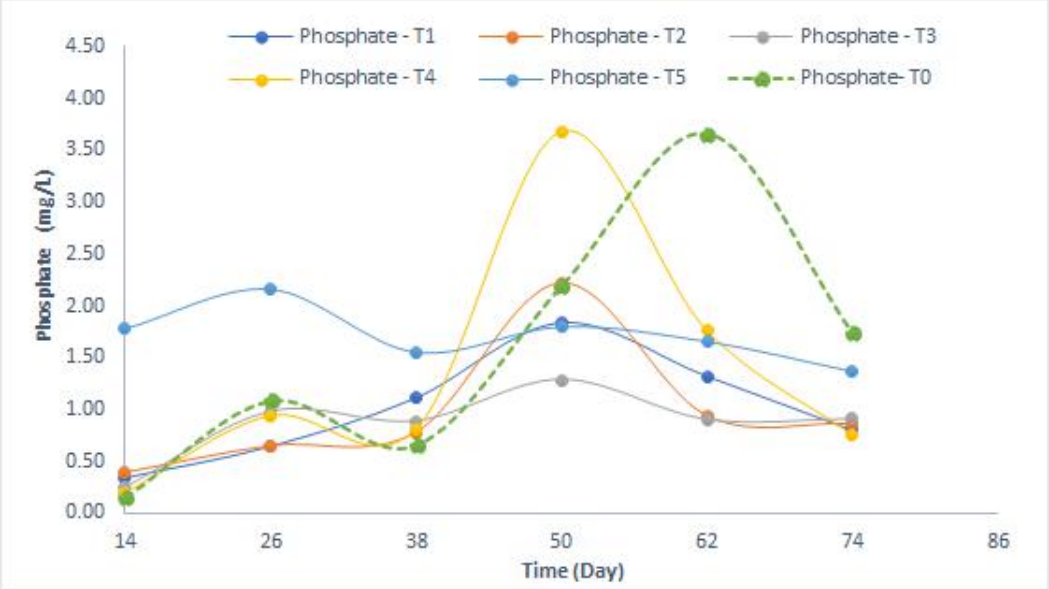
Source: Authors (2021).

The most prevalent inorganic form of phosphorus in wastewater and also in aquaculture systems is soluble phosphate or soluble reactive phosphorus. Phosphorus is an essential element for microorganisms responsible for the stabilization of organic matter (Sperling, 2017) and is primarily responsible for the cultural eutrophication of aquatic ecosystems (Esteves, 2011). The soluble phosphate gradients in this study showed a behavior close to linearity until day 38, that oscillated between 0.5 and 1.8 mg L^{-1} for treatments and control. In Tables 3 and 4, reactive phosphorus shows a high correlation (R^2 = 0.97) in *T5* due to its decay with the bio-addition of the microbial consortium.

On the other hand, pulses also occurred in all treatments and the control from day 38 of the experiment, except in *T5*. In *T4* and in the control, gradients between 4.0 and 5.0 mg L^{-1} were observed, respectively. Boyd and Tucker (1992) and Rubinos et al. (2011) observed strong interaction between high phosphate gradients at pH of up to 8.0.

While Xu et al. (2016) found that the bio-addition of *B. subtilis* reduced pH and soluble phosphate in aquaculture systems. In this study, the pH among all treatments, including control, remained neutral and presented an average gradient of 7.56. Excess phosphorus in diets offered to fish in intensive aquaculture systems is common and consequently generating high levels of phosphorus excretion. The behavior in the time of reactive phosphorus (Figure 5) in the system may be directly related to the performance of metabolic growth of tilapia juveniles (initial growth) as Verdegem and Beristain (2005) mentions.

Figure 6 - Means of reactive phosphorus behavior between experiment treatments in the face of control as a function of time.



Source: Authors (2021).

The thin layer of sediment deposited in the substrate of the tanks acted as a sink of phosphorus, resulting in the deposition of solids. Phosphorus gradients may have been increased at 50 days by the re-suspension process, Li et al. (2020) mentions that a significant fraction of mineralized organic matter in the substrate can occur in the water column from the foraging activity of fish. David et al. (2017) and Flickinger et al. (2020) observed in multitrophic cultivation that the evolution in phosphorus concentrations in water and sediment are highly correlated with the daily and corrected supply of food for growth of the stored biomass. Therefore, phosphorus exchanges between the water column and sediment contribute less to explaining phosphorus pulses in water than feeding.

4. Conclusion

A pulse dynamic at regular times occurred indistinctly for all indicators evaluated in this study, although the intensity of the pulses showed particularities among the different indicators of water quality. Although the behavior of regular pulses of the indicators occurred, this condition did not affect the growth of fish submitted to a simulated system of intensive production associated with the bio-addition of friendly microorganisms.

The mathematical adjustment proposed to represent pulses at regular time intervals. The temporal behavior of dissolved oxygen, organic matter and nutrients in a simulated intensive aquaculture system submitted to bio-addition of beneficial microorganisms responded satisfactorily to their adjustment with correlations greater than 0.8, that shows that the model can be considered a model that satisfactorily correlates to the experimental data. And as future suggestions it is expected that this work will be a guide for a greater use of friendly microorganisms in aquaculture to reduce cleaning costs and longer production cycles due to better water quality.

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