Efficiency of bioaugmentation in the removal of organic matter in aquaculture systems

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(With 5 figures)

Abstract

Several techniques are currently used to treat effluents. Bioaugmentation is a new bioremediation strategy and has been employed to improve effluent quality by treating the water during the production process. This technology consists basically of the addition of microorganisms able to degrade or remove polluting compounds, especially organic matter and nutrients. The objective of this study was to assess the effects of bioaugmentation on some parameters of organic matter and on the performance of juvenile tilapias in an intensive aquaculture production system. The combination of two bacterial consortiums in a complete randomized design was employed in a factorial analysis with two factors. Statistical differences between treatments were analyzed by the analysis of variance (ANOVA) and Tukey test at the 5% level. One of the treatments, heterotrophic bacterial supplementation, was able to reduce biochemical oxygen demand (BOD) by 23%, dissolved organic carbon (DOC) by 83.7% and phytoplanktonic biomass by 43%. On the other hand, no damage was done to either the physical-chemical indicators of water quality or to the growth performance of juvenile tilapias assessed in this study.

Keywords: water quality, fish, heterotrophic bacteria.

Eficiência da bioadição na remoção de matéria orgânica em sistemas aquaculturais

Resumo

Existem diversas tecnologias para tratamento de efluentes, o processo de bioadição consiste em uma vertente da biorremediação e tem sido empregado na melhoria da qualidade dos efluentes através do tratamento da água de produção. Esta tecnologia consiste basicamente na adição de microrganismos com a capacidade de degradar ou remover compostos poluentes, especialmente matéria orgânica e nutrientes. Este estudo objetivou avaliar os efeitos da suplementação de composto bioativo sobre alguns parâmetros de matéria orgânica e de desempenho de juvenis de tilápias em um sistema intensivo de produção aquacultural. Foi empregada a combinação de dois consórcios bacterianos em delineamento inteiramente aleatorizado, em um esquema fatorial com dois fatores. As diferenças estatísticas entre os tratamentos foram analisadas por meio da análise de variância (ANOVA) e do teste de Tukey ao nível de 5%. Verificou-se neste estudo, que a bioadição heterotrófica foi capaz de reduzir em 23% a demanda bioquímica de oxigênio (DBO); em 83,7%, o carbono orgânico dissolvido (COD); e em 43%, a biomassa fitoplanctônica. Por outro lado, não se observou nenhum prejuízo com relação aos parâmetros físico-químicos de qualidade de água bem como ao desempenho de crescimento para juvenis de tilápias avaliados neste estudo.

Palavras-chave: qualidade de água, peixe, bactérias heterotróficas.

1. Introduction

The expansion of aquaculture in Brazil has been evident over this last decade. According to the Food and Agriculture Organization (FAO, 2009), Brazil has today, in global terms, the fifth annual growth rate in the production of aquatic organisms. The Ministry of Fisheries and Aquaculture of Brazil believes that aquaculture production will have a decisive role in the coming decades to balance extractive fishing production with the increasing demand for aquatic products.

Fish-farming systems are characterized mostly by high-density storage in a small area which often limits their capacity for self-purification. These systems are considered by Henry-Silva and Camargo (2008) as thermodynamically open because they have low stability and continuous exchange of matter and energy in order to decrease their internal entropy. Organic matter, generated in the production process, needs to be managed properly. Modern aquaculture has faced increasing environmental demands both from domestic and foreign agencies. An example is the need to operate at high production rates under critical resource conditions. In some cases, water is the critical resource (biomass/L/year) while in others, space is vital (biomass/m^2/year) (Piedrahita, 2003). High intensification brings advantages, but it also creates a series of environmental liabilities, among these, the increase of effluent discharges. Yoo and Boyd (1994) state that the receiving bodies of water are able to assimilate organic matter and nutrients discharged from aquaculture systems; however, this is obviously related (Piedrahita, 2003) to the concentration of organic compounds released and to their capacity of dilution.

Recently, in Brazil, much attention has been paid to effluent emissions of aquaculture (Ostrensky and Boeger, 2008), which might be attributed to the annual growth of the activity and the demands imposed by environmental agencies. In several countries, aquaculture is considered a polluting production system that requires control of its waste disposal (Davis, 1993). There are several techniques used both directly and indirectly to improve the quality of effluent emissions. The methods adopted involve physical, chemical and biological procedures and often a combination of all three. Among the indirect methods (bioremediation) used for wastewater treatment is the process of heterotrophic microbial supplementation, or bioaugmentation, which is seen as a viable alternative (Rittmann and Whiteman, 1994; Hovanec et al., 1998). Although recent (Janeo et al., 2009), it has been practiced in China for about a decade (Zhou et al., 2009) for disease control and water quality improvement. Bioaugmentation is based on the addition of single or combined strains of bacteria to strains of synergistic and catalyst action, whether endogenous or not. The role of this bioaugmentation of beneficial bacteria is to improve the capacity of nutrient cycling through the detritus route and to enhance the conditions of an environment of low stability, subjected to high stress, which characterizes the tanks on fish farms (Ehrlich et al., 1998; Shariff et al., 2001).

The aim of this study was to assess the supplement of a bioactive microbial consortium regarding its capacity to remove organic matter and its effects on the performance of juvenile tilapias in an intensive aquaculture production system.

2. Material and Methods

This study was conducted in a greenhouse in the Fishing Agricultural Sector of ESALQ-USP, in Piracicaba, São Paulo, Brazil. Prior to the experiment, sanitary procedures for disinfecting the tanks and sanitizing the fish batch with malachite green were carried out (Scott, 2000). These procedures were combined with the administration of oxytetracycline in the diet for a week. After this, the fish were transferred to and kept permanently in experimental tanks.

We used 16 tanks of 1.0 m^3 with a usable volume of 0.8 m^3 and 40 juvenile fish of the species *Oreochromis niloticus* (Linnaeus, 1758) (average weight 122.7 g) were kept in each tank The fish were fed until apparent satiation with commercial feed (protein content 32%), twice a day, throughout the experimental period of 90 days. The water supply for the tanks was provided by vertical submerged pump and maintained with continuous mechanical ventilation by four air diffusers per tank attached to a 4-hp compressor. A drainage system was installed to allow the removal of bottom water. Throughout the test there was no siphoning of waste out of the tanks. The hydraulic detention time of the tanks was 44.4 hours, and the flow rate adopted was 18 $L.h^{-1}$.

We assessed the consortium now known as AQ+, consisting of a consortium of heterotrophic bacteria *Bacillus subtilis* (Cohn), *Bacillus licheniformis* (Weigmann) and *Bacillus polymyxa* (Prazmowski). The physical and biochemical composition of the consortium AQ+ was as follows: cereal flour (75%), salt (15%), protease (9.6%), lipase (0.07%), hemicellulose (0.13%) and β-glucase (0.05%). The addition of this consortium was combined with a compound of chemoautotrophic bacteria, *Nitrosomonas* spp. (Winogradsky) and *Nitrobacter* spp. (Winogradsky), denominated CN. The density of the consortium AQ+ was 3.75×10^6 $CFU.g^{-1}$ and of the nitrifying bacteria compound CN was 21×10^6 $CFU.mL^{-1}$. The preparation of AQ+ started with the weighing of the immobilized compound on semi-analytic scales. Next, 20 g of the compound was diluted in 80 mL of distilled water to prepare the stock solution. The solution was then filtered through filter paper of 1 µm on a Buchner funnel under suction by a vacuum pump. The inoculum was prepared just before each administration. The pipetted volumes of stock solution were then diluted in 5 L of water from the tanks and applied in combination with the commercial consortium.

The treatments with AQ+ had the following concentrations: T0 = control treatment (placebo), T1 = 0.04 $g.m^{-3}$, T2 = 0.16 $g.m^{-3}$, T3 = 0.32 $g.m^{-3}$,

$T4 = 0.64\ g.m^{-3}$ and $T5 = 0.96\ g.m^{-3}$. To obtain these concentrations, these volumes of stock solution were pipetted: for T1 (160 µL,), T2 (640 µL), T3 (1280 µL,), T4 (2560 µL) and T5 (3840 µL). For the control (T0), 160 µL of the cereal filtrate were pipetted without the immobilized bacterial consortium. Volumes of the CN+ consortium were identical to those adopted for the AQ+ consortium. The administration of consortiums occurred on the fourteenth day of the experiment. The consortiums were applied every two days.

Samples of the fish population were weighed and measured at the beginning and the end of the experiment. To facilitate the handling of fish, prior to the biometric procedure, the fish were immobilized with 3 g of benzocaine (ethyl aminobenzoate) dissolved in alcohol and administered in 40 L of water. Data on weight of feed consumed were collected weekly during the trial. From the biometric data, the following information on fish growth performance was obtained: initial biomass, final biomass, initial weight (Wi), final weight (Wf), initial length, final length, weight gain (WG = Wf – Wi); feed conversion (amount of feed/weight gain), condition factor ($CF = 100 \times WG/\text{total length}^3$), specific growth rate (SGR = 100 × (lnWf – lnWi)/days; feed consumption rate (FCR = 100 × total consumption (g)/ [(Wf + Wi) / 2] / days); feeding efficiency (FE = WG/TC (g)) and survival rate (%).

The parameters of water quality, viz. temperature, pH, dissolved oxygen, electrical conductivity and total dissolved solids, were monitored fortnightly. To determine the pH, we used an Orion Star potentiometer; to read the temperature and dissolved oxygen, we used a YSI 55 oxymeter. The total dissolved solids and conductivity were read with a YSI EC 300 conductivity meter.

Fortnightly, water samples were collected directly from the drainage system of tanks by siphoning. The biochemical oxygen demand ($BOD_{5\ days}$) was determined by calculating BOD = [ODi] – [ODf]/incubation time (in hours) × 120. The dissolved organic carbon (DOC) was determined by wet oxidation, readings being taken with a total carbon analyzer (TCA) (Eaton et al., 1995). Thin and thick sediment in suspension (FCS) was determined by the ABNT (1989) gravimetric method. Chlorophyll-*a* (index of phytoplankton biomass) was determined by chlorophyll extraction through filtering and the reading was carried out in a spectrophotometer HACH DR-5000 (wavelength 663 nm) (Golterman et al., 1978).

The assumptions of ANOVA were applied, using the family of Box and Cox (1964) transformations and the test of Hartley (1950) to check homogeneity of variance. The design used in this study was a completely randomized factorial analysis with two factors (treatments and sampling events), there being three replicates per treatment and six replicates per sampling event. The treatments consisted of different concentrations of AQ+ combined with CN, as mentioned above, while the sampling events referred to six observations, SS1 ... SS6, of the behavior of the bacterial consortium during the experimental period. Once the assumptions of ANOVA were verified, the *F* test ($p < 0.05$) was used to assess possible differences between treatments. When a difference between treatments was confirmed, the Tukey test ($p < 0.05$) for comparison of means was carried out. The statistical program SAS / STAT 9.1 (2003) was used in the analysis of results.

3. Results

Among the physical-chemical parameters of water quality monitored, only the dissolved oxygen and conductivity did not differ among treatments. The other measurements showed significant differences in the *F* test ($p < 0.05$). Average temperatures observed for the treatments were between 24.91 and 25.02 °C; the pH ranged from 7.40 to 7.66; the dissolved oxygen ranged between 2.54 and 3.11 mg/L; electrical conductivity was between 2055.0 and 2152.11 $\mu S\ cm^{-2}$; total alkalinity between 1136.39 and 1383.60 $CaCO_3$ mg/L; hardness ranged from 66.11 to 72.72 $CaCO_3$ mg/L and finally the total suspended solids content fluctuated between 1347.20 and 1400.44 mg/L.

The ANOVA (Table 1) showed that the organic matter variables, biochemical oxygen demand (BOD), dissolved organic carbon (DOC), chlorophyll-*a* and thin sediment (TS) were different ($p < 0.05$) between treatments, sampling events and time versus treatment interaction, while thick sediment (ThS) was not. Averages for BOD

Table 1. Summary of ANOVA results expressed as *F*-statistics: treatment factors (TR), sampling events (SE), interaction of treatment v. sampling events (TR × SE) and respective coefficient of variation (CV) for organic matter parameters in water of aquaculture systems: biochemical oxygen demand (BOD), dissolved organic carbon (DOC), chlorophyll-*a*, thin sediment (TS) and thick sediment (ThS).

			***F*-statistics**		
Factors	**BOD(mg/L)**	**DOC (mg C/L)**	**Chlorophyll-*a* (mg/L)**	**TS (mg/L)**	**ThS (mg/L)**
TR	7.42*	2.73*	5.75*	2.73*	0.52
SE	80.98*	4.92*	20.96*	4.92*	7.04*
(TR × SE)	3.37*	1.75*	3.04*	1.75*	1.19
CV (%)	23.9	42.19	17.08	42.19	16.73

*Significant at 5% level, ^{ns}not significant at 5% level.

among treatments were between 1.24 and 1.70 mg/L. The highest BOD was found in the control group while the lowest occurred in treatment T4 (Table 2). Among the sampling events, the highest value was recorded in the first collection and the lowest in the third collection (Table 3). The control was different from other treatments, by the Tukey test ($p < 0.05$), except for T2 and T3. Among the sampling events, the first collection was significantly different from all others. BOD was fitted to a cubic curve with respect to time (Figure 1) for all treatments, except for treatment T4, where the linear regression fitted best. For this variable, the control as well as the other treatments showed a tendency to decrease over time.

Concentrations of DOC varied, in the treatments, between 4.18 and 10.0 mg/L, while among the sampling events the range was between 2.50 and 11.71 mg/L. The highest concentration was in the control group, while the highest removal was obtained in T1. Among the sampling events, the highest concentration was observed in the second collection, while the highest removal occurred in the third collection. Averages show a significant difference only between the control and T2. As a time profile for DOC, the best fitting curve was quadratic (Figure 2). Compared to the control, concentrations of DOC tended, in time, to be situated between stabilization and reduction and treatments T3, T4 and T5 clearly show this trend.

The mean values observed for chlorophyll-*a* lie, among the treatments, between 0.78 and 1.11 mg/L, while among the sampling events the amplitude was between 0.65 and 1.63 mg/L. The highest concentration was observed in T2, while the largest sequestration occurred in T1. Among the sampling events, the highest concentration occurred in the fifth collection and the lowest was obtained in the fourth collection. Averages show significant differences between the control and the other treatments, while significant differences were also observed among the first, third, fourth and fifth sampling events ($p < 0.05$) (Table 3). The curves that best fitted the behavior of chlorophyll-*a* over time were cubic, except for the control (Figure 3). Phytoplankton biomass represented by the chlorophyll-*a* revealed, in the control, a continuous growth throughout (Figure 3); T2 and T5 produced similar behavior, although these show a plateau between the second and fifth collection. The other treatments led to a tendency of reduction or stabilization of the phytoplankton biomass.

Results found for thin sediment in the various treatments lay between 0.03 and 0.04 mg/L, while among the sampling events the content was between 0.02 and 0.04 mg/L. The average values for thick sediment were, in the treatments,

Table 2. Comparison between treatment means and their respective standard deviations for the variables: biochemical oxygen demand (BOD), dissolved organic carbon (DOC), chlorophyll-*a*, thin sediment (TS) and thick sediment (ThS).

	Means				
Treatments	**BOD (mg/L)**	**DOC (mg C/L)**	**Chlorophyll-*a* (mg/L)**	**TS (mg/L)**	**ThS (mg/L^{1})**
T0	1.70^{a} ± 0.99	10.00^{a} ± 14.35	1.11^{ab} ± 0.66	0.04^{a} ± 0.02	10.11^{a} ± 7.59
T1	1.33^{b} ± 0.58	4.18^{ab} ± 1.94	0.78^{b} ± 0.40	0.04^{ab} ± 0.02	11.05^{a} ± 9.28
T2	1.38^{ab} ± 0.70	5.33^{b} ± 3.24	1.17^{a} ± 0.66	0.03^{b} ± 0.02	12.40^{a} ± 11.75
T3	1.52^{ab} ± 0.94	6.22^{ab} ± 6.22	0.81^{b} ± 0.55	0.03^{ab} ± 0.03	10.47^{a} ± 10.47
T4	1.24^{b} ± 0.54	5.26^{ab} ± 2.69	1.02^{b} ± 0.62	0.04^{ab} ± 0.02	8.30^{a} ± 5.37
T5	1.48^{b} ± 1.00	6.24^{ab} ± 4.64	0.86^{ab} ± 0.39	0.04^{ab} ± 0.02	16.84^{a} ± 28.22

Means followed by the same letter in columns do not differ significantly by Tukey ($p < 0.05$).

Table 3. Comparison between the means of collection events and their standard deviations for the variables: biochemical oxygen demand (BOD), dissolved organic carbon (DOC), chlorophyll-*a*, thin sediment (TS) and thick sediment (ThS).

	Means				
Sampling events	**BOD (mg/L)**	**DOC (mg/L)**	**Chlorophyll-*a* (mg/L)**	**TS (mg/L)**	**ThS (mg/L)**
SE1	2.66^{a} ± 0.90	2.50^{a} ± 0.53	0.78^{c} ± 0.35	0.04^{a} ± 0.01	4.88^{c} ± 2.83
SE2	1.78^{b} ± 0.44	11.71^{ab} ± 12.42	0.87^{bc} ± 0.36	0.04^{ab} ± 0.01	17.96^{a} ± 25.25
SE3	0.75^{e} ± 0.34	3.39^{b} ± 1.36	1.21^{ab} ± 0.68	0.02^{b} ± 0.02	8.72^{bc} ± 7.74
SE4	1.16^{cd} ± 0.37	8.52^{ab} ± 3.47	0.65^{d} ± 0.68	0.04^{ab} ± 0.02	16.03^{ab} ± 11.17
SE5	1.39^{c} ± 0.47	4.89^{ab} ± 1.94	1.63^{a} ± 1.63	0.03^{ab} ± 0.02	10.04^{ab} ± 4.56
SE6	0.89^{de} ± 0.29	ND	0.82^{bc} ± 0.38	ND	ND

Means followed by the same letter in columns do not differ significantly by Tukey ($p < 0.05$); ND: not determined.

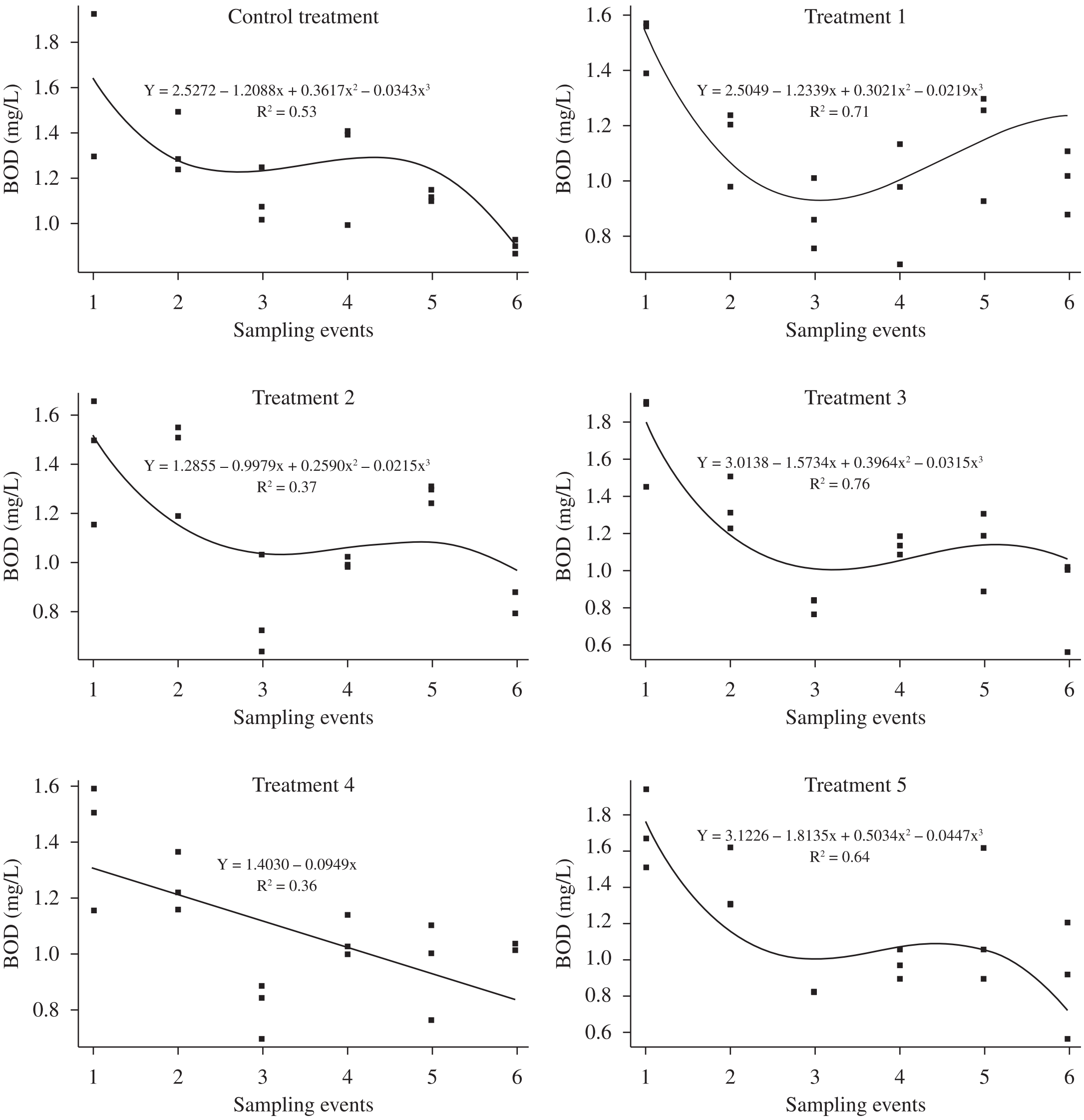

Figure 1. Curves relating the effects of different treatments with the bacterial consortium AQ+ on the behavior of biochemical oxygen demand (BOD) to sampling events.

between 8.30 and 16.84 mg/L, while among sampling events this range was between 4.88 and 17.96 mg/L. The means contents of thin sediment in suspension did not reveal any significant difference for either treatments or sampling events. The same behavior was seen for thick and thin sediments, among treatments. On the other hand, differences among the sampling events were noticeable ($p < 0.05$) between the first, second and fourth collections. Curves fitted to the thin sediment data were quadratic (Figure 4) and to the thick sediment, linear (Figure 5), except for T1 and T4. The suspended particulate organic matter, represented by thin and thick sediment (Figures 4 and 5), reveals that the respective controls showed a tendency to grow; however, it is noted, among the other treatments, that the thin sediment falls during the sampling events, while the curves fitted to model the behavior of the thick sediment show greater stability throughout the experimental period.

The mean values for zootechnical performance at the beginning and end of the experiment were as follows (Table 4): final average weight ranged from 216.08 to 238.83 g; final biomass ranged from 8643.20 to 9433.20 g; the final length was between 22.87 and 23.40 cm; weight gain oscillated from 93.38 to 118.08 g, being higher than that of the control in all treatments; feed conversion showed a range between 0.73 to 1.00 g/day; the condition factor was between 0.70 and 0.98 (%); feeding efficiency ranged from 0.99 to 1.38 (%), feed conversion ratio was from 0.56 to 0.69 (%) and finally the specific growth rate

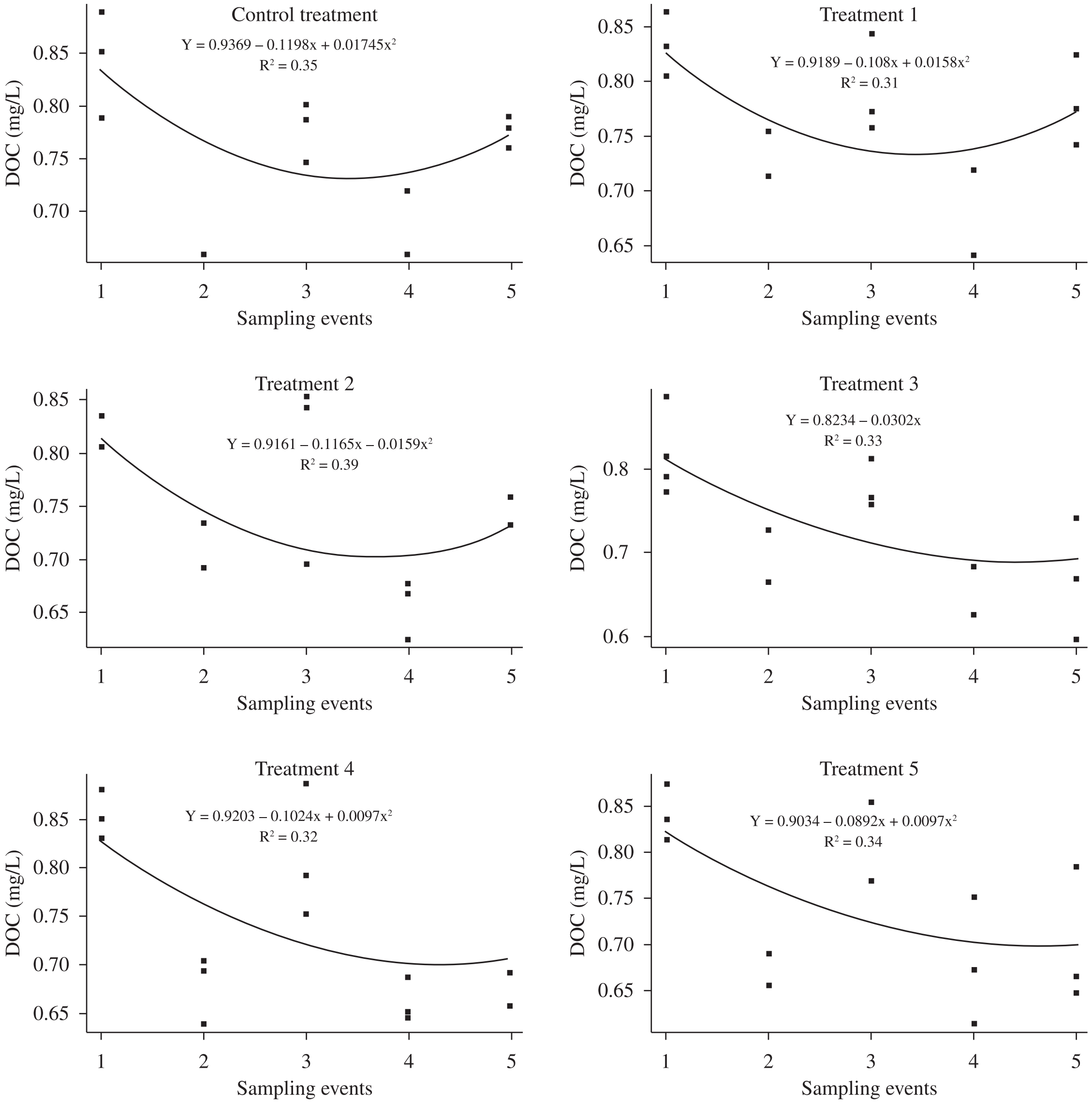

Figure 2. Curves relating the effects of different treatments with the bacterial consortium AQ+ on the behavior of dissolved organic carbon (DOC) to sampling events.

ranged from 0.62 to 0.97 (%). During the experiment, the survival rate was 100%.

4. Discussion

Although the analysis of the physical-chemical quality parameters showed, for the most part, significant variations among the treatments regarding time, they remained within tolerable levels for the growth of tilapia, and apparently they were not affected by either the application or the frequency of application of bioaugmentation.

For comparison, the BOD of fish farming effluents falls between those of river water and household sewage (Pillay, 1992). However, the intensification of aquaculture may worsen this scenario. Results on parameters for organic matter obtained in this study do not corroborate those found by Queiroz and Boyd (1998), where the values of BOD were higher than in the control. Furthermore, Boyd (1990) believes that the BOD deriving from fish farming tanks is much more related to plankton respiration than to the decomposition process. Even though Brune et al. (2003) emphasize that the BOD contained in specific feeds for aquaculture accounts for roughly 60% of the feed dry weight, these values can vary significantly with the amount of bacterial and fecal matter present in the

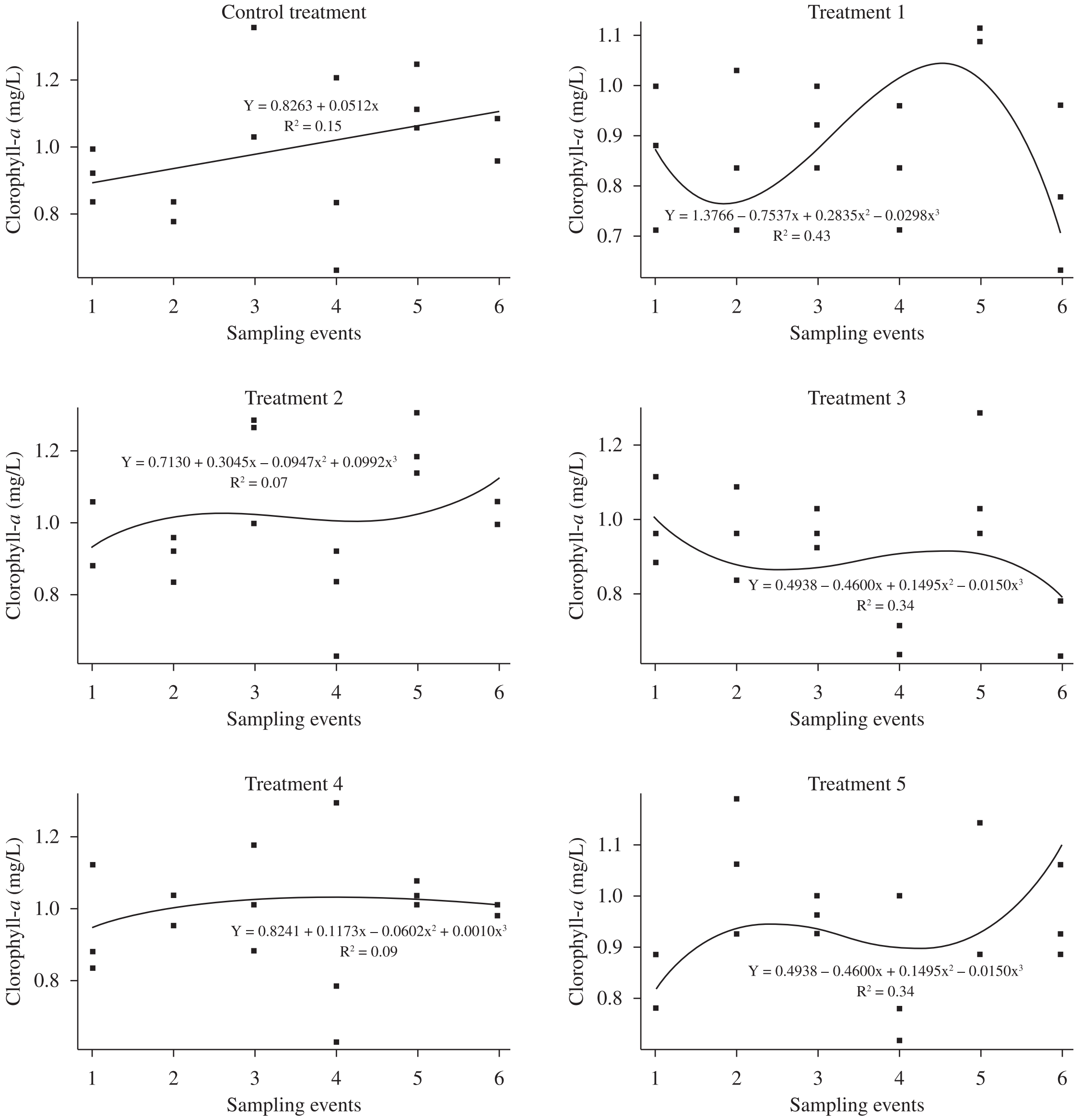

Figure 3. Curves relating the effects of different treatments with the bacterial consortium AQ+ on the behavior of chlorophyll-*a* to sampling events.

system. Once in this study, we adopted non-siphoning of the waste and the BOD concentration then corresponded to the maximum concentration expected, which may have favored the biosynthesis of heterotrophic bacteria added to the medium. The result obtained in the control group approaches the minimum value cited by Pillay (1992). Unlike the bio-flocks technique, bioaugmentation does not require organic matter to control ammonia concentration (Hargreaves, 2006), which possibly makes the process of organic matter oxidation more efficient. Our results show that the average BOD removal rate observed in the control was about 23%. Although the values obtained for BOD prove to be highly significant (Table 2), the fitted curves for BOD did not show, relative to the control, differences over time, which may be explained by the diversity of organic matter variables in fish-farming systems (Tucker and Hargreaves, 2003).

The source of DOC is the phytoplankton biomass. On the other hand, phytoplankton biomass is an important nutrient source for heterotrophic bacteria in the detritus chain, owing to the presence of vitamins in its composition (Esteves, 1988). Ebeling et al. (2006) reported that organic carbon is related to the BOD, especially when the body of water requires additional aeration. The concentrations of DOC

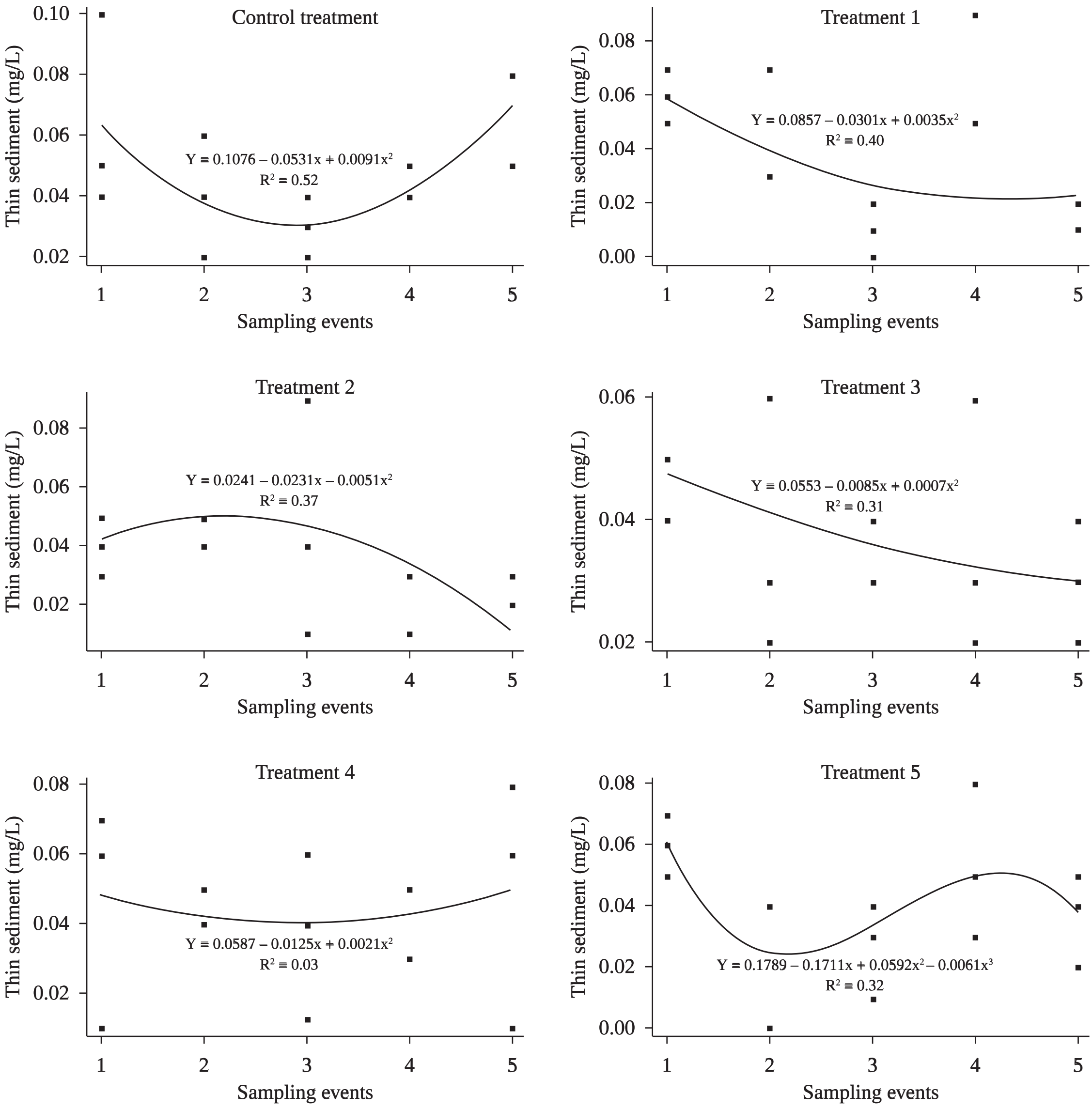

Figure 4. Curves relating the effects of different treatments with the bacterial consortium AQ+ on the behavior of thin sediment to sampling events.

in this study, among the inoculated tanks, showed a clear decay relative to the control, indicating the trophic use of this material by heterotrophic bacteria (bacterioplankton) (Suhett et al., 2006). The average DOC removal rate, among the treatments inoculated with the bioactive consortium, was 87.6%. This result apparently indicates that heterotrophic augmentation of the medium was more effective than microbial carbon (Avnimelech, 1999). This result is corroborated by that of Ebeling et al. (2006), who state that the heterotrophic bacteria have a significantly higher growth rate than the nitrifying bacteria.

The maximum removal rate for chlorophyll-*a* was about 43%, obtained in T1, which may indicate the rapid consumption of nitrogen and phosphorus in the medium. The bioaugmentation removed, on average, about 23% of phytoplankton biomass, probably by means of competitive exclusion related to foraging and phosphorus bioavailability. On the other hand, Wang and Kang (2007) used *Acinetobacter calcoaceticus* as the inoculum and achieved a removal of 83.7%.

Presumably, the suspended particulate matter contributes to the increase in biochemical oxygen demand (Kelly, 1992). The results of this study show that suspended solids, especially thicker particulate matter, exhibit two peaks, in the second and fourth collection. These high concentrations may have occurred, according to Hargreaves

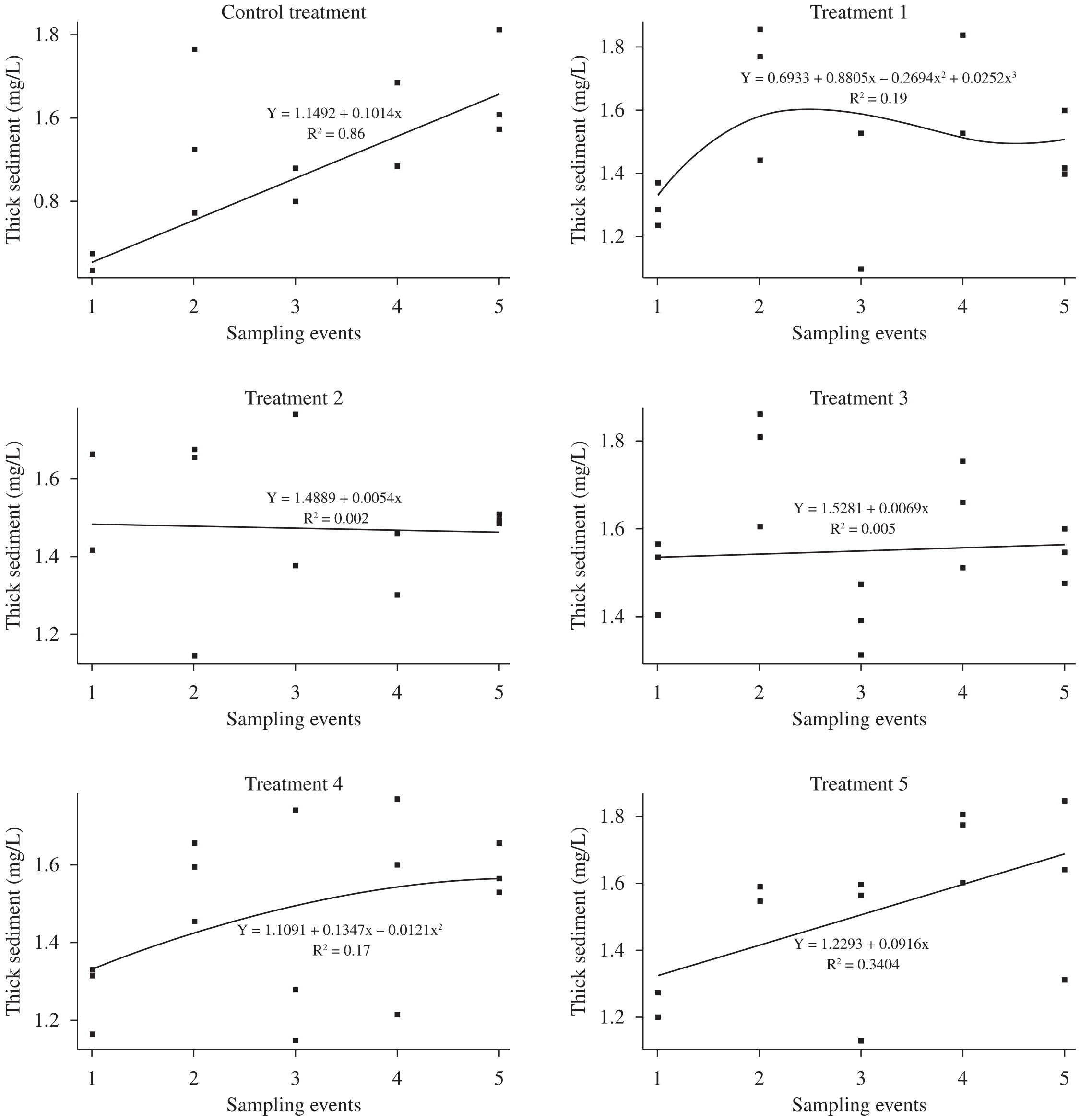

Figure 5. Curves relating the effects of different treatments with the bacterial consortium AQ+ on the behavior of thick sediment to sampling events.

(2006), by aeration. The stocking density used, the bioavailability, due to the continuous allochthonous feed the phytoplankton biomass and factors associated with the hydrodynamic conditions (water residence time) (D'Aquino et al., 2006) probably contributed to the high concentrations of suspended sediment.

Among the zootechnical performance variables determined for juveniles of the species *Oreochromis niloticus* during the experimental period, there was no significant difference, by *F* test, between the control and the other treatments inoculated with the microbial consortium, except for the weight gain. The feed conversion rate in this study, although low, is of the same order of magnitude as that observed by Furuya et al. (2006), although those authors worked with fries. Comparatively, no influence, other than the response to the diet supplied and to the frequency of feed provision used in this experiment, could be related to the performance levels observed.

We conclude that the results show the ability of bioaugmentation to reduce organic matter, especially BOD, organic carbon and chlorophyll-*a*. These results demonstrate the feasibility of adding bioactive heterotrophic compounds to fish-farming systems, with no impairment of either physical or chemical parameters of water quality, or of the performance of the juvenile tilapia tested in this study.

Table 4. Performance of juvenile tilapia (*Oreochromis niloticus*) fed with commercial feed and subjected to bioaugmentation of bacterial inoculum.

	Control	T1	T2	T3	T4	T5
IB[1]	4908^{a} ± 608,1	5223.7^{a} ± 827.3	4577.4^{a} ± 278.6	4372.6^{a} ± 454,0	5312.2^{a} ± 237.5	4377.0^{a} ± 817.8
FB[2]	8643.2^{a} ± 327.9	9647.7^{a} ± 210.3	9300.6^{a} ± 155.6	8547.8^{a} ± 1536.5	9094.8^{a} ± 755.2	9038.6^{a} ± 780.0
IW[3]	122.7^{a} ± 15.20	131.5^{a} ± 20.68	144.4^{a} ± 6.9	109.3^{a} ± 11.3	132.8^{a} ± 5.9	109.4^{a} ± 20.4
FW[4]	216.0^{a} ± 8.2	236.6^{a} ± 5.2	232.5^{a} ± 3.8	216.2^{a} ± 38.4	227.3^{a} ± 18.8	225.9^{a} ± 19.5
IL[5]	17.9^{a} ± 0.4	18.3^{a} ± 0.9	17.6^{a} ± 0.4	17.2^{a} ± 0.4	18.2^{a} ± 0.2	17.2^{a} ± 0.9
FL[6]	23.4^{a} ± 0.6	23.5^{a} ± 0.4	22.9^{a} ± 1.1	22.8^{a} ± 1.8	23.2^{a} ± 1.0	23.3^{a} ± 0.6
WG[7]	93.3^{a} ± 7.6	105.3^{a} ± 25.1	118.0^{b} ± 9.7	106.8^{a} ± 49.0	94.5^{a} ± 14.9	116.5^{b} ± 16.4
FCD[8]	0.7^{a} ± 0.1	0.8^{a} ± 0.2	0.7^{a} ± 0.1	0.8^{a} ± 0.3	0.8^{a} ± 0.1	0.7^{a} ± 0.07
FC[9]	0.7^{a} ± 0.1	0.7^{a} ± 0.1	0.9^{a} ± 0.1	0.8^{a} ± 0.2	0.7^{a} ± 0.07	0.9^{a} ± 0.1
SGR[10]	0.7^{a} ± 0.1	0.8^{a} ± 0.2	0.9^{a} ± 0.07	0.8^{a} ± 0.3	0.7^{a} ± 0.07	0.9^{a} ± 0.1
FCR[11]	0.5^{a} ± 0.1	0.6^{a} ± 0.0	0.6^{a} ± 0.07	0.6^{a} ± 0.1	0.5^{a} ± 0.07	0.6^{a} ± 0.1
FE[12]	1.3^{a} ± 0.3	1.2^{a} ± 0.4	1.2^{a} ± 0.1	1.3^{a} ± 0.7	1.1^{a} ± 0.1	1.3^{a} ± 0.1
SR[13]	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Means followed by the same letter in columns do not differ significantly by Tukey ($p < 0.05$); [1]initial biomass (g); [2]final biomass (g); [3]initial weight (g); [4]final 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]feed consumption rate (% biomass day^{-1}); [12]feeding efficiency; and [13]survival rate (%).

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