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Use of *Spirulina platensis* in treatment of fish farming wastewater[1]

Uso de *Spirulina platensis* no tratamento de efluentes de piscicultura

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ABSTRACT - Each year, the amount of fish produced around the world increases, which contributes to several environmental impacts such as the disposal of effluents without treatment in the environment. This scientific work had as main objective the development of Spirulina platensis in fish effluents, a low cost medium for the production of biomass, in order to reduce the levels of some inorganic nutrients to reach the allowed parameters by the Brazilian environmental standards for effluent disposal and to enable reuse of the water. Nile tilapia (*Oreochromis niloticus*) fingerlings were produced in fresh water. The effluent generated by the culture was transferred to a pool where seawater was added until the salinity of 10 ‰ was reached. A strain of cyanobacteria, *Spirulina platensis*, was inserted into the mixture in order to remove the nutrients dissolved on the fish culture effluent. The abiotic parameters analyzed were: absorbance, pH, dissolved oxygen, temperature, salinity and concentrations of ammonia, nitrite, nitrate and phosphate. The results revealed that the maximum cellular density of *S. platensis* resulted in the production of 0.22 g L^{-1} of dry biomass and maximum productivity of 0.03 g L^{-1} day^{-1}. The concentration of ammonia, nitrite, nitrate and phosphate got lowered by more than 94.8%, maintaining the nutrient levels within the standards those required by Brazilian environmental standards. Hence, this effluent has become adequate for reuse in fish production or could be safely disposed in nature.

Key words: *Oreochromis niloticus*. Microalgae. Cyanobacteria. Bioremediation. Nutrients.

RESUMO - A cada ano a quantidade de peixes produzidos no mundo é maior, podendo ocasionar sérios impactos ambientais, como o despejo de efluentes no meio ambiente sem tratamento prévio. Este trabalho científico buscou desenvolver a *Spirulina platensis* em efluentes de piscicultura, um meio de baixo custo para a produção de biomassa, a fim de reduzir os níveis de alguns nutrientes inorgânicos até parâmetros permitidos pelas normas ambientais brasileiras para o descarte de efluentes e que possibilite o reuso desta água. Alevinos de tilápia do Nilo (*Oreochromis niloticus*) foram produzidos em água doce. O efluente gerado nestes cultivos foi transferido para uma piscina onde foi adicionada água do mar até atingir a salinidade 10‰. Uma cepa da cianobactéria *Spirulina platensis* foi inoculada na mistura com o objetivo de remover os nutrientes dissolvidos no efluente da piscicultura. Os parâmetros abióticos analisados foram: absorbância, pH, oxigênio dissolvido, temperatura, salinidade e as concentrações de amônia, nitrito, nitrato e fosfato. Os resultados revelaram que a densidade celular máxima de *S. platensis* resultou na produção de 0,22 g L^{-1} de biomassa seca e produtividade máxima de 0,03 g L^{-1} dia^{-1}. As reduções das concentrações de amônia, nitrito, nitrato e fosfato foram superiores a 94,8%, deixando os níveis dos nutrientes dentro dos padrões exigidos pelas normas ambientais brasileiras. Assim, este efluente se tornou apto para reuso na própria produção dos peixes ou ser descartado com segurança na natureza.

Palavras-chave: *Oreochromis niloticus*. Microalgas. Cianobactérias. Biorremediação. Nutrientes.

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INTRODUCTION

The aquaculture is an activity that requires a large volume of water because every year millions of tons of fish are produced around the world (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2012). Therefore, a considerable amount of residual waters is produced (TURCIOS; PAPENBROCK, 2014).

Because it is an expanding activity, the aquaculture has caused several environmental problems, such as: soil and marine habitats degradations; chemical pollution; danger to biodiversity by the introduction of exotic species; reduction of the immunobiological resistance of the fishes; spread of diseases; mortality of aquatic organisms; several changes in water bodies; as well as economic damages in fishing industry (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2012).

These impacts are mainly caused by intensive aquaculture, which turns the water rich in organic and inorganic compounds resulting from fish excreta and decomposition of unconsumed feeding ration, that can generate many negative impacts to aquatic environments, making them susceptible to eutrophication (BENEDITO *et al.*, 2014).

Physical, chemical and biological methods (sedimentation basins, biofilters and phytoplankton treatment) used in conventional wastewater treatment have been adopted in aquaculture systems which promote the treatment and reutilization of the aquatic environment (TURCIOS; PAPENBROCK, 2014).

Among the biological methods, the use of microalgae and cyanobacterias stands out for its growth capacity using solar energy and large quantities of nutrients. And, the use effluents rich in inorganic pollutants, such as nitrogen and phosphate, are a good alternative for its large scale production with low cost (MARKOU; GEORGAKAKIS, 2011).

Currently, several species of microalgae and cyanobacterias are used in many biotechnological applications like in the production of biofuels (MATA; MARTINS; CAETANO, 2010); in agriculture as soil biofertilizers (ARAÚJO *et al.*, 2011); in diets in aquaculture (JAIN; SINGH, 2013); in the production of various components, such as, colorants, antioxidants, emulsifiers, enzymes, etc. (ALONSO *et al*., 2012). Thus, there is a noble destination for subproducts of the effluent treatment.

Among the species of microalgae, the cyanobacterias of the genre *Spirulina* are excellent candidates for cultivation in effluent because they produce a good amount of biomass and can be harvested easily due to their size and structure, low cost of cell concentration, easy production, high strength and other factors (MARKOU; GEORGAKAKIS, 2011).

Several studies have used *Spirulina* to evaluate direct and indirect positive effects on aquatic organisms of economic interest, for example, in the dietary supplementation of trout (GÜROY *et al*., 2011); to increase the growth and coloration of the rainbow trout *Oncorhynchus mykiss* (TEIMOURI; AMIRKOLAIE; YEGANEH, 2013); as well as in the activation of the immune defense system of the white shrimps *Litopenaeus vannamei* (TAYAG *et al*., 2010).

There are accounts of its use for water quality control and for treating effluents in shrimp farming (CHUNTAPA; POWTONGSOOK; MENASVETA, 2003), pig farming (CHEUNBARN; PEERAPORNPISAL, 2010), industries (CHINNASAMY *et al*., 2010) and fish farming (WUANG *et al*., 2016), proving the versatility of this species of microalgae.

Spirulina platensis when used in the pharmaceutical industry and in the food and cosmetic industry (COSTA *et al*., 2016) has high production costs. However, the use of this microalgae in effluent treatment reduces production costs (PUMAS; PUMAS, 2016). And the *S. platensis* have been employed in the treatment of wastewaters from the most diverse mediums (MEZZOMO *et al*., 2010). Therefore, due to the ability to treat effluents and produce inputs demanded by the industry with low cost, the use of *Spirulina* in research and industrial applications has been increasing.

Even with all these benefits, the literature about the treatment of effluents from fish farming by cyanobacteria is very scarce. Hence, this study had as main objective the treatment of residual water from the *Oreochromis niloticus* production, one of the most produced fish in the world, with *Spirulina platensis*, in order to reach the allowed parameters by the Brazilian environmental standards for effluent disposal.

MATERIAL AND METHODS

Acquiring the Spirulina platensis

The strain with 300 mL of *S. platensis* was acquired from a plancktology laboratory at Aquaculture Biotechnology Center (CEBIAQUA/UFC) and its volume was increased on a daily basis to a maximum of 50 L by the addition of the modified Jourdan medium (JOURDAN, 2012) with salinity of 10 ‰ (Table 1), remaining under constant aeration and artificial lighting of 40 µE m^{-2} s^{-1}, termed stock culture.

Table 1 - Chemical composition of the modified Jourdan medium

Reagent	Concentration (g L^{-1})
Urea (CH_4N_2O)	0.07
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.2
Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$)	0.005
Potassium sulfate (K_2SO_4)	1.0
Ammonium phosphate (NH_4HPO_4)	0.1
Potassium nitrate (KNO_3)	2.0
Sodium chloride (NaCl)	10.0
Sodium bicarbonate ($NaHCO_3$)	8.0

Source: Adapted from Jourdan (2012)

Optical density and cell density

The monitoring of the growth of *S. platensis* cultures was performed daily by measuring the absorbance (optical density - OD) of a sample with 0,025 L of water from the culture in a spectrophotometer HACH DR2500, with a wavelength of 680 nm (OD_{680nm}). To obtain the cell density (CD), the linear regression equation below was used, a result from a calibration curve, whose correlation coefficient was equal to 0.97:

CD (trichomes/mL) = [(OD + 0.127)/0.179].10

Determining the yield and productivity of the S. platensis culture

To determine the yield and productivity of the *S. platensis* cultures it was necessary to separate the biomass from the culture medium. In order to do this, five liters of the culture, obtained from two different phases of the growth curve (exponential growth and reduced growth), were filtrated through a 60 µm. Subsequently, the biomass was dried in a laboratory oven for 24 h with air renewal at 40 °C.

Experimental design

The cultivation system was made in a covered environment, in triplicate, and each unit consisted of two 500 L boxes for the fish production and a pool of 2,400 L (3m²) for the biological treatment of the effluents by the cyanobacterias. The pools were provided with continuous artificial illumination, utilizing HQI lamps of 200 W with an illuminance of 440 µE m^{-2} s^{-1}. The aeration system was made using compressors of 800 L h^{-1} and the circulation of the water in the pools were aided by a submerged pumps with a flow rate of 1,000 L h^{-1}.

The fishes were continuously produced in 500 L boxes with fresh water at a density of 0.1 fish L^{-1} and were initially 6.35 ± 0.86 cm in size and 9.19 ± 3.53 g in weight. The animals were fed with commercial ration, containing 40% of crude protein, 2,800 Kcal kg^{-1} and 0,001 m of diameter. The feeding rate was 5%, with adjustments according to the weight of the fish.

These animals received appropriate care in accordance with the instructions provided in the directive 2010/63/EU of the European Parliament, in 22nd September 2010, on the protection of animals used for scientific purpose (EUROPEAN COMMISSION, 2010).

The effluents generated by a week of fish production (approximately 1,200 L) were transferred to their respective pool and the water salinity was increased to 10‰ by the addition of seawater with a salinity of 45‰. After transferring the effluents, the *S. platensis* stock cultures, maintained in a Jourdan medium, were inoculated into the pools. After 24 hours, the volumes of the pools were increased to 1,500 L with the addition of more effluent from the fish culture and, the growth of cyanobacteria and the nutrients levels in the waters were monitored every day.

Abiotic parameters

The abiotic parameters were determined according to the Standard Methods for the Examination of Water and Wastewater (AMERICAN PUBLIC HEALTH ASSOCIATION, 2012), monitoring in triplicate. The methods were: power of hydrogen (pH) - method 4500 H^+B; temperature; dissolved oxygen - method 4500 O; salinity; ammonia - NH_3 (method 4500 B/E); nitrite (NO_2^-) - method 4500 B/C, nitrate (NO_3^-) - method 4500 B/C; and phosphate (PO_4^{-3}) - method 4500 B/E.

The percentages of nutrients removal (NR) presented in the effluents treated by *Spirulina platensis* were calculated using the following equation:

NR(%) = 100 - [100.concentration of nutrient in treated effluent/concentration of nutrient in influent]

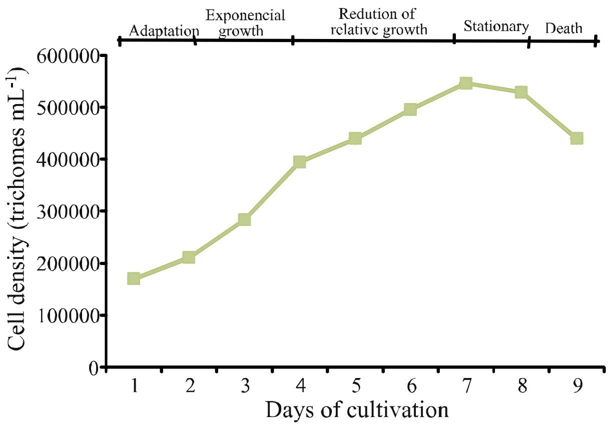
Statistical Analysis

The results were submitted to simple analysis of variance tests (ANOVA) ($p < 0.05$) and in the case of significant differences, the means were compared by the Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

The growth curve of *S. platensis*, constructed during the treatment of the tilapia culture's effluent, has exhibited five distinct phases (Figure 1).

Figure 1 - *Spirulina platensis* growth curve during treatment of tilapia wastewater



Source: The authors own

The cultures began with an approximate average of 170,000 trichomes mL^{-1} and remained in adaptation stage for just a day, when it reached the average of 200,000 trichomes mL^{-1}. Next, the cultures entered in the exponential growth stage and doubled their densities, reaching approximately 400,000 trichomes mL^{-1} at the end of this phase, on the fourth day of growth. After this, the population growth rate was reduced and the cultures entered in the reduction of relative growth phase. After three more days of growth, the cultures reached the maximum of 550,000 trichomes mL^{-1}, reaching the stationary phase, at which the number of growing cells is close to the number of the dying ones.

In another study, the *Spirulina* culture in wastewaters from pigs, was able to obtain production of 178,000 trichomes mL^{-1} in 12 days (CHEUNBARN; PEERAPORNPISAL, 2010).

As non-renewal of the environment is a characteristic of stationary cultures, the cultures quickly entered in decline and, after nine days of fish farming, they were terminated due to the death of the algae cells. A typical algae growth curve, in a stationary cultivation, can present up to five stages of growth, as was observed in this experiment (LAVENS; SORGELOOS, 1996).

In aquaculture, the nitrogen and the phosphate are the main residuals capable of causing eutrophication of the water. However, this effect can be minimized by the use of sedimentation, biofilters and phytoplankton treatment, thereby mitigating the environmental impacts of the effluents from this activity.

Beyond this, the growth of the cyanobacteria in this kind of environment promotes the recuperation of the effluent and, concurrently, also produces biomass, which can be used for various purposes, such as animal feed, energy production, pigment production, polysaccharides, carotene, sterols, vitamins and polyunsaturated fats, among others (MEZZOMO *et al*., 2010).

At the instant of the culture's highest cellular density, on the seventh day, a production of 0.22 g L^{-1} of *S. platensis* dry biomass, which resulted in maximum productivity of 0.03 g L^{-1} day^{-1}, was obtained. Autotrophic cultures with *S. platensis* using molasses and achieved a biomass and maximum productivity of 2.83 ± 0.13 g L^{-1} ^{and} 0.098 ± 0.015 g L^{-1} day^{-1}, respectively, utilizing 0.50 g L^{-1} of liquid molasses (ANDRADE; COSTA, 2008). It is important to highlight that the nutrient levels employed in these two cultures were well above those employed in our experiment.

The elements that most limit the growth of microalgae are the nitrogen, mainly in the form of nitrate and ammonia, and phosphorus in the form of phosphate. The production of *S. platensis* biomass increases with the increase of nitrogen concentration in the cultivation environment, at which ammonia is the compound most easily incorporated by microalgae and cyanobacteria. However, when ammonia is no longer available, these microorganisms utilize other sources of nitrogen as nitrite, nitrate and nitrogen gas (MARKOU; GEORGAKAKIS, 2011).

There were statistical difference ($p < 0.05$) between the initial and final phase of the experiment for all the nutrients analyzed (Table 2). It was noted that, after nine days of growth, the *S. platensis* were capable of reducing the nitrite, nitrate and phosphate levels by 100; 98.7 and 94.8%, respectively. Recently, it has been reported the efficacy of *S. platensis* on the treatment of wastewater from fish farming, indicating its ability to remove the concentrations of ammonia and nitrate from the water's culture (WUANG *et al*., 2016).

Table 2 - Values of mean concentrations ± standard deviation, initial, final and total reduction, obtained during the tilapia's residual water treatment; and maximum values admissible by Brazilian Resolution Nº. 357/2005 of CONAMA

Abiotic Parameter	Initial Concentration (mg L^{-1})	Final Concentration (mg L^{-1})	Reduction (%)	CONAMA Brackish Water Class I* (mg L^{-1})
Ammonia	0.26 ± 0.00 a	0.21 ± 0.01 b	19.8	0.40
Nitrite	10.91 ± 0.34 a	0.00 ± 0.00 b	100	0.07
Nitrate	28.33 ± 0.49 a	0.37 ± 0.01 b	98.7	0.40
Phosphate	0.96 ± 0.01 a	0.05 ± 0.00 b	94.8	0.124

*Brackish water standards presenting: recreational use; aquatic community protection; aquaculture and seasonal fishing activities; manual feeding following conventional or advanced treatment; and fishing or farming of organisms for intensive consumption, different letters represent statistical difference ($p<0.05$)

As the concentration of ammonia was already very low, probably due to rapid transformation to nitrite and nitrate, its level remained practically unchanged, being reduced by approximately only 20%.

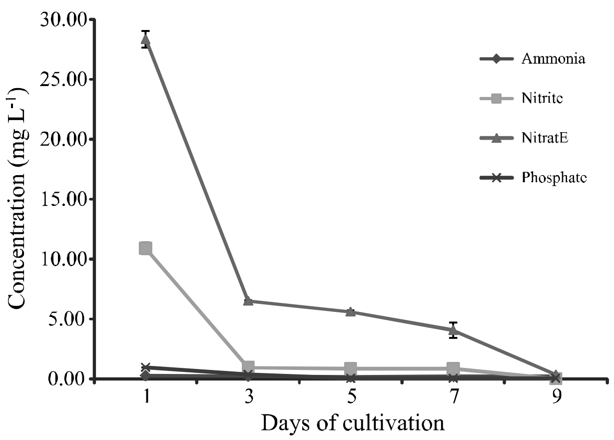
Besides the action of the cyanobacteria, it is probable that the reductions of ammonia and nitrite were aided by the action of nitrifying bacteria, responsible for the nitrification process where the ammonia is oxidized to nitrite (NO_2^-) and then nitrate (NO_3^-) when in oxygenated waters (CHUNTAPA; POWTONGSOOK; MENASVETA, 2003). As in this experiment, in which the dissolved oxygen concentration was above 6 mg L^{-1}. There is also the possibility that fractions of some of these elements have been adsorbed or precipitated in the sediment.

The total reductions obtained in this experiment allowed the inclusion of treated water within the current environmental standards in Brazil, according to the resolutions of the National Environmental Council (CONAMA), Nº. 357/2005 (BRASIL, 2005) and Nº. 430/2011 (BRASIL, 2011), that determine the conditions and standards for effluent discharge.

The greatest nutrient level reductions occurred, rightly, during the adaptation and exponential growth stages, which lasted from the first to the third day of culture (Figure 2), in other words, culture growth was provided by cyanobacteria nutrient assimilation. It is known that there is a direct relation between cellular multiplication and the reduction of the nutrients present in cultivation water, which is caused due to the absorption by those microorganisms (VIDOTTI; ROLLEMBERG, 2004).

On the other hand, nutrient level reduction also resulted in decreased cell density of cyanobacteria, characterizing death stage, as they were a stationary type of culture, and to maintain the cyanobacteria and microalgae cultivation it is necessary to establish a state of equilibrium between the biomass level and nutrients concentration at the water, so that the assimilation is as large as possible, without reducing them enough to expressively affect the growth rate (LEFEBVRE *et al*., 2004).

Figure 2 - Ammonia, nitrite, nitrate and phosphate concentration curves during tilapia effluent treatment by *Spirulina platensis*



Source: The authors own

Currently, in order to keep nutrient levels within acceptable amounts for commercial fish culture in tanks or ponds, it is necessary to provide water renovation, as it is not possible to remove the excess cyanobacteria prior to their death.

Given that light supply for *S. platensis* was continuous, it was possible to accelerate its development and a rapid nutrient removal. A study analyzed the influence of luminous intensity on *S. platensis* cultivation and observed that the maximum cell concentration was obtained 15 days after cultivation, utilizing a photosynthetic active radiation of 38.7 µE m^{-2} s^{-1} (CONVERTI *et al*., 2006). Furthermore, *S. platensis* may vary its growth rate accordingly to the cultivation medium utilized and the environmental conditions (CHAIKLAHAN *et al*., 2010), also modifying its biochemical composition (HSIEH; WU, 2009).

The removal and separation of *Spirulina* biomass from the water can be performed by filtering using a screen with a 60 µm opening due to its large dimensions (MARKOU; GEORGAKAKIS, 2011).

Table 3 - Mean values ± standard deviation, initial and final, of temperature, pH, dissolved oxygen and salinity during the tilapia's effluent treatment with *Spirulina*

Parameter	Initial values	Final values
Temperature (ºC)	30,1 ± 0,1 a	31,2 ± 0,3 b
pH	8,2 ± 0,1 a	8,9 ± 0,2 b
Dissolved oxygen (mg L^{-1})	6,4 ± 0,1 a	6,1 ± 0,1 a
Salinity (‰)	10,0 ± 0,0 a	11 ± 0,0 a

Source: The authors own; Different letters represent statistical difference ($p<0.05$)

Hence, *Spirulina* can build a viable alternative in wastewater treatment in integrated systems with water recirculation, or in multi-crop systems with fish or shrimp, utilizing a semi-continuous collection of the biomass in excess, without the need for water renewal and the disposal of the effluents, thereby guaranteeing water quality maintenance for aquatic animals, as well as avoiding the risk of eutrophication.

The values for temperature and pH presented significant differences ($p<0.05$) during the effluent treatment by *S. platensis* (Table 3). The temperature were a little higher was due to the heat emitted by the HQI lamp. While the increase in pH occurred due to carbon dioxide (CO_2) fixation and the production of oxygen during photosynthesis of microalgae.

Already, the salinity and dissolved oxygen (DO) did not show significant differences ($p<0.05$) between the initial and final values of the treatment. However, all four levels were kept within the satisfactory ranges for cyanobacteria growth (JOURDAN, 2012).

The selection of the cyanobacteria or microalgae species for the culture should take into account its capacity not only to grow rapidly in an organic environment without losing its nutritional characteristics, as well as adapting to large scale external cultivation. It should also be easily separated from water by filtering, tolerate variations in salinity and temperature and possess a commercial value (CHUNTAPA; POWTONGSOOK; MENASVETA, 2003).

The *S. platensis*, aside from these characteristics, has the capacity to adapt to different quantities of salt in the water and to quickly grow in alkaline environments, facilitating its cultivation in selective production systems (LAVENS; SORGELOOS, 1996).

CONCLUSIONS

1. The results demonstrate the efficiency of *S. platensis* in the treatment of effluents from tilapia production by reducing the concentrations of the major dissolved nutrients that can cause aquatic environments eutrophication to values really close to zero, making the water suitable for reuse in the fish production or for correct disposal of the effluent without causing negative impacts to the environment. Since the 9th day it was possible to verify the reduction of the levels of total ammonia, nitrite, nitrate and total phosphate contents accumulated in the fish production waters to acceptable levels for effluent disposal according to the Brazilian legislation;
2. The technique presented several concomitant benefits, such as the production of a high protein and commercial biomass, the reduction of aquaculture impacts, the removal of nutrients from the effluents and the reuse of water according to the recirculation system used;
3. The entire experiment was employed on a pilot scale with a total volume of 4,500 L, however, it is necessary to carry out tests on industrial scales to analyze the economic viability of the methodology. As a main result it is possible to carry out the development of *S. platensis* consortium with other organisms, such as shrimp or other species of fish, in polyculture systems, or integrated farming, with or without water recirculation. Also, the algal biomass produced can be used in the formulation of animal feed as well as human food supplement due to its high nutritional value.

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