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Phycoremediation of fish farm wastewater by *Chlorella sorokiniana* and autochthonous microalgae

Ficorremediação da água residual da piscicultura por *Chlorella sorokiniana* e microalgas autóctones

Ficorremediación de aguas residuales de piscifactorías por *Chlorella sorokiniana* y microalgas autóctonas

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Emerson Machado de Carvalho
ORCID: https://orcid.org/0000-0002-4865-6784
Universidade Federal do Sul da Bahia, Brazil
E-mail: carvalho.em@gmail.com
Carolina Ramos dos Santos
ORCID: https://orcid.org/0000-0003-0677-7745
Federal University of Grande Dourados, Brazil
E-mail: crsbiotec@hotmail.com
Mônica Ansilago
ORCID: https://orcid.org/0000-0002-7866-3619
Federal University of Grande Dourados, Brazil
E-mail: monica_ansilago@hotmail.com
Mariana Lara Menegazzo
ORCID: https://orcid.org/0000-0001-8029-0279
Federal University of Grande Dourados, Brazil
E-mail: marianamenegazzo@ufgd.edu.br
Nathaskia Silva Pereira Nunes
ORCID: https://orcid.org/0000-0002-0433-6412
Federal University of Grande Dourados, Brazil
E-mail: nathaskia.spn@gmail.com

Abstract
With the disorderly increase in global environmental problems, the cultivation of aquatic organisms is a promising path for sustainable food production. The quality of water, both at the entrance and exit of the production of aquatic animals, needs to be maintained following the parameters specified by local legislation. This study aimed to investigate the removal of contaminants from fish farming wastewater associated with the production of freshwater microalgae biomass. Six completely randomized treatments were used in triplicate: with the addition of microalgae *C. sorokiniana* in fish farm wastewater (W+Cs), the addition of *C. sorokiniana* in wastewater enriched with NPK fertilizing (W+F+Cs) or sugarcane vinasse (W+V+Cs), only wastewater (W), wastewater supplemented with fertilizer (W+F) or vinasse (W+V). The wastewater was used *in natura* to allow the development of autochthonous microalgae. The microalgae *C. sorokiniana* grew rapidly in effluents enriched with NPK and vinasse. After 28 days of bioassay, the concentrations of several contaminants in the water were reduced: zinc (20 to 88%), lead (5 to 83%), aluminum (56 to 75%), manganese (56 to 72%), cadmium (9 to 52%), calcium (16 to 24%) and magnesium (12 to 33%). Our results indicated that the production of microalgae biomass can be integrated with the treatment of fish farming effluents to reduce the environmental burden and increase the economic bonus for adopting a sustainable production method. However, our results also indicated the importance of introducing a microalgae strain with high productive performance and supplementing the wastewater to obtain rapid biomass.
Keywords: Aquaculture; Bioassay; Environmental biotechnology; Chlorophyceae; Kinetics.

Resumo
Com a escalada dos problemas ambientais globais, o cultivo de organismos aquáticos é um caminho promissor para a produção sustentável de alimentos. A qualidade da água, tanto na entrada como na saída da produção de animais aquáticos precisa ser mantida de acordo com os parâmetros especificados pela legislação local. Este estudo teve como objetivo investigar a remoção de contaminantes da água residual da piscicultura consorciado à produção de biomassa de microalgas dulcícolas. Foram utilizados seis tratamentos inteiramente casualizados em triplicata: com adição da microalga *C. sorokiniana* em água residual da piscicultura (W+*Cs*), adição de *C. sorokiniana* em água residual enriquecida com fertilizando NPK (W+F+*Cs*) ou vinhaça de cana-de-açúcar (W+V+*Cs*), somente água residual (W), água residual suplementada com fertilizante (W+F) ou vinhaça (W+V). A água residual foi utilizada *in natura* para permitir o desenvolvimento de microalgas autóctones. A microalga *C. sorokiniana* cresceu rapidamente em efluentes enriquecidos com NPK e vinhaça. Após 28 dias de bioensaio as concentrações de vários contaminantes na água foram

reduzidas: zinco (20 a 88%), chumbo (5 a 83%), alumínio (56 a 75%), manganês (56 a 72%), cádmio (9 a 52%), cálcio (16 a 24%) e magnésio (12 a 33%). Nossos resultados indicaram que a produção de biomassa microalgácea pode ser integrada ao tratamento de efluentes da piscicultura de forma a diminuir o ônus ambiental e aumentar o bônus econômico por adotar um método de produção sustentável. Porém, nossos resultados também indicaram a importância de introduzir uma cepa de microalga com alto desempenho produtivo e suplementar a água residual para obter biomassa rápida.
Palavras-chave: Aquicultura; Bioensaio; Biotecnologia ambiental; Chlorophyceae; Cinética.

Resumen
Con el aumento desordenado de los problemas ambientales globales, el cultivo de organismos acuáticos es un camino prometedor para la producción sostenible de alimentos. La calidad del agua, tanto a la entrada como a la salida de la producción de animales acuáticos, debe mantenerse de acuerdo con los parámetros especificados por la legislación local. Este estudio tuvo como objetivo investigar la eliminación de contaminantes de las aguas residuales de la piscicultura asociados con la producción de biomasa de microalgas de agua dulce. Se utilizaron seis tratamientos completamente aleatorizados por triplicado: con adición de microalgas *C. sorokiniana* en aguas residuales de piscifactoría (W+ Cs), adición de *C. sorokiniana* en aguas residuales enriquecidas con fertilizante NPK (W+F+Cs) o vinaza de caña de azúcar -de- azúcar (W+V +Cs), solo aguas residuales (W), aguas residuales suplementadas con fertilizante (W+F) o vinaza (W+V). El agua residual se utilizó *in natura* para permitir el desarrollo de microalgas autóctonas. La microalga *C. sorokiniana* creció rápidamente en efluentes enriquecidos con NPK y vinaza. Después de 28 días de bioensayo, las concentraciones de varios contaminantes en el agua se redujeron: zinc (20-88%), plomo (5-83%), aluminio (56-75%), manganeso (56-72%), cadmio (9-52%), calcio (16-24%) y magnesio (12-33%). Nuestros resultados indicaron que la producción de biomasa de microalgas se puede integrar con el tratamiento de los efluentes de la piscicultura para reducir la carga ambiental y aumentar la bonificación económica por adoptar un método de producción sostenible. Sin embargo, nuestros resultados también indicaron la importancia de introducir una cepa de microalgas con alto rendimiento productivo y complementar las aguas residuales para obtener biomasa rápida.
Palabras clave: Acuicultura, Bioensayo, Biotecnología ambiental, Chlorophyceae, Cinética.

1. Introduction

Fish farming is economically important and has been growing at a notably higher rate than other rural industries. According to the SOFIA report (The State of World Fisheries and Aquaculture) of the FAO (Food and Agriculture Organization of the United Nations), fish accounts for 20% of the total animal protein consumed worldwide (triennial report 2013-2015), and in 2016 global fish production was 171 million tonnes (FAO, 2018).

With the escalation of global environmental issues, the farming of aquatic organisms is a promising avenue for sustainable food production. However, fish farming, like any other activity in the productive sector, needs to be sustainable, which requires complete overall knowledge of associated processes and adopting practices to remedy and/or minimize the potential negative impacts of production on the environment (Ballester-Moltó, Sanchez-Jerez, Cerezo-Valverde & Aguado-Giménez, 2017).

Water quality is vital for fish health and that can be influenced by a variety of factors, including pH, dissolved oxygen, organic matter, mineral content, and presence of pathogens (Banerjee & Ray, 2017). Therefore, the quality of water used in the production of aquatic animals needs to be maintained according to parameters specified by the local legislation. It is also necessary that the quality of the effluent generated by productive systems is high to minimize harmfully affecting receiving water bodies.

Several tools are needed to ensure proper food quality and safety in the production of aquaculture products, and for this purpose, the FAO has developed numerous documents which outline how to achieve these objectives, such as the Code of Conduct for Responsible Fisheries and the Technical Guidelines for Aquaculture Certification. The Environmental Protection Agency of The United States (USEPA, 1986) has provided guidelines concerning water quality that details the permitted concentrations of harmful compounds in aquatic environments intending to protect aquatic life after both short and long-term exposure.

Low-cost alternative measures can be incorporated into the productive processes of aquacultures to mitigate the negative effects of fish farming effluent discharge. Among these technologies, microalgae are particularly promising as they require a

large number of nutrients and are resilient to metals and other contaminants found in effluents (Jung et al., 2017). These microorganisms allow the independent maintenance of water in their respective fish tanks, decreasing the volume of effluent needing to be released into water bodies.

Microalgae are unicellular organisms, often exhibiting little or no cellular differentiation and being capable of converting solar energy into chemical energy via CO_2 fixation more efficiently than higher plants (Sathasivam, Radhakrishnan, Hashem & AbdAllahd, 2019). They are predominantly aquatic and microscopic and are considered a very heterogeneous group of microorganisms. They have the potential to produce various biomolecules such as lipids, carbohydrates, and proteins and are used in the pharmaceutical and food industries (Mostafa, 2012).

Algae are also of significant use in the performance of bioassays, in the mitigation of environmental damage, and wastewater treatment systems such as biofloc technologies (Jung et al., 2017). Microalgae are capable of assimilating inorganic compounds, heavy metals, and nutrients present in aquatic environments (Mcginn et al., 2012; Wuang, Khin, Chua & Luo, 2016) and have been increasingly employed in laboratory cultivation tests due to their high productivity and the ease at which they are maintained (Carvalho et al., 2012; Ansilago, Otonelli & Carvalho, 2016).

Among the microalgae that are commonly used in industry, the genus *Chlorella* includes green microalgae which are used as supplements in human food and animal feed (Sathasivam et al., 2019). The species *Chlorella sorokiniana* is small-sized algae (4.5 μm) that grow rapidly, exhibits high biomass production, has a competitive advantage over other species, and can be grown in mixotrophic environments. Therefore it is ideal for cultivation in wastewater (Lizzul et al., 2014).

The use of algal biomass requires its separation from the liquid medium in which it is contained. One of the most common techniques used to recover algal biomass is centrifugation, which, while highly efficient and relatively fast, often causes cell damage through cell disruption and has high energy- and equipment-related costs, and requires considerable maintenance (Barros, Gonçalves, Simões & Pires, 2015). Flocculation with different organic and inorganic agents is also frequently used, being a low-cost method with high efficiency and the capacity to process large volumes of liquid media.

Singh, Singh and Taggar, (2017) compared the separation of biomass by centrifugation and the use of chitosan as a flocculant and found that the two methods were 98.4% and 97.23% efficient, respectively. Kim et al. (2017) assessed the use of ferric sulfate in the recovery of *Chlorella* sp. biomass via chemical flocculation and reported efficiencies of up to 98% using a concentration of 0.9 g L^{-1}. Lal and Das (2016) tested the efficiency of ferric chloride and alum (potassium aluminum sulfate) in the flocculation of *Chlorella* sp. and also reported efficiencies of up to 98% using 1 g L^{-1} chemical compounds.

Given the above, the objective of this study was to evaluate the removal of residual water contaminants in fish farms using freshwater microalgae in a controlled environment bioassay. To define the experimental design, we raised two hypotheses: 1. Supplementation of fish farming wastewater with vinasse or NPK chemical fertilizer could induce the development of autochthonous microalgae and, therefore, allow phycoremediation of pollutants in the environment; 2. The introduction of the microalgae *Chlorella sorokiniana* strain in fish farm wastewater supplemented with vinasse or NPK chemical fertilizer may increase the efficiency of the phytoremediation of pollutants in the environment.

2. Methodology

2.1 Bioassays

Chlorella sorokiniana (Trebouxiophyceae) - André Tosello Foundation reference number 211-32) - was used in bioassays. Wastewater was collected from fish farms in tarpaulin tanks at properties in the municipality of Glória de Dourados, MS. The bioassay design was completely randomized, containing six treatments in triplicate, totaling 18 experimental units: treatments with the addition of microalgae *C. sorokiniana* only in wastewater (W+*Cs*), the addition of microalgae *C. sorokiniana* in fish farm wastewater supplemented with NPK chemical fertilizer (W+F+*Cs*) or vinasse (W+V+*Cs*), only autochthonous

microalgae grown in fish farm wastewater (W), only autochthonous microalgae grown in fish farm wastewater supplemented with NPK chemical fertilizer (W+F) or vinasse (W+V).

To supplement the wastewater 10 mL per liter of NPK stock solution or 1 mL per liter of crude sugarcane vinasse were added. Wastewater was not autoclaved to preserve autochthonous microorganisms. The stock solution NPK was prepared with 0.70 g L^{-1} of N:P:K chemical fertilizer (20-5-20 g L^{-1}) according to the methods of Carvalho et al. (2012). The composition of the crude vinasse was: Zinc (Zn) 1.60, Lead (Pb) 0.4, Cadmium (Cd) 0.05, Nickel (Ni) 0.20, Iron (Fe) 42.50, Manganese (Mn) 5.50, Copper (Cu) 0.95, Chrome (Cr) 0.07, Cobalt (Co) 0.22, Aluminum (Al) 43.80, Calcium (Ca) 1.13, Magnesium (Mg) 234, Sodium (Na) 25.30, Potassium (K) 2.45, Boron (B) 27.3, Nitrogen (N) 0.06, Phosphorus (P) 0.06 (mg L^{-1}).

The bioassays were packaged in suspended plastic bags (1000 mL) for 28 days using a non-axenic static culture system, constant aeration, a controlled room temperature, and a photoperiod of 2500 lux provided by white fluorescent lamps (12 h light / 12 h dark). Samples were collected from 18 experimental units every 7 days apart to measure the cell duplication rates and monitor the pH and electrical conductivity of the water. Microalgae were identified and grouped into *Chlorella* sp. and others. For the *Chlorell*a sp. it was not possible to distinguish between the species of the pure strain introduced in the treatments (*Chlorella sorokiniana*) and the native species. The duplication rate was calculated by counting cells in a Neubauer chamber and calculated by dividing the difference between the algal density values from the last and first day of the experiment by the time elapsed in days.

After 28 days of bioassay, the 18 experimental units were flocculated with 0.75 g of ferric chloride ($FeCl_3$) per liter and filtered on qualitative filter paper (Whatman® Grade 40 Circles) to separate the microalgal biomass. The samples were acidified with 1% nitric acid and conditioned in a refrigerator for analysis of their physical and chemical composition.

The chemical analysis of samples was performed at the Laboratory of Applied Mass Spectrometry and Chromatography (LECA - Laboratório de Espectrometria e Cromatografia Aplicada) of the Faculty of Exact Sciences and Technology (FACET - Faculdade de Ciências Exatas e Tecnologia) of UFGD. The analyses were performed using flame atomic absorption spectrometry (FAAS). The chemical elements measured in the analysis were: Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), Calcium (Ca), Magnesium (Mg), Chromium (Cr), Lead (Pb), Molybdenum (Mo), Aluminium (Al), Cadmium (Cd), and Nickel (Ni). The procedures for the collection, storage, and analysis of the samples followed the methods detailed in the *Standard Methods for Examination of Water and Wastewater* (APHA, 2005).

2.2 Data Analysis

To verify statistical differences in the data of cell duplication rate, pH, and electrical conductivity of the culture medium, an analysis of variance was used to compare the six treatments (ANOVA $p < 0.05$), followed by the Tukey test. Tests were also carried out to verify significant differences in the percentage of contaminants removed for each treatment. The original data were transformed to suit the analysis used.

To evaluate the nutrient reduction potential as analyzed using FAAS, the concentrations of each nutrient present in whole samples were subtracted from those of the supernatant both before and after chemical flocculation. The amount of each nutrient removed throughout the experiment was expressed as a percentage and calculated using the following equation (1).

$$R\% = \left(\frac{(C_0 - C_e)}{C_0}\right) \times 100$$

Equation (1)

Where C_0 and C_e are the concentrations of the nutrient in the liquid phase (mg L^{-1}) before and after chemical flocculation, respectively.

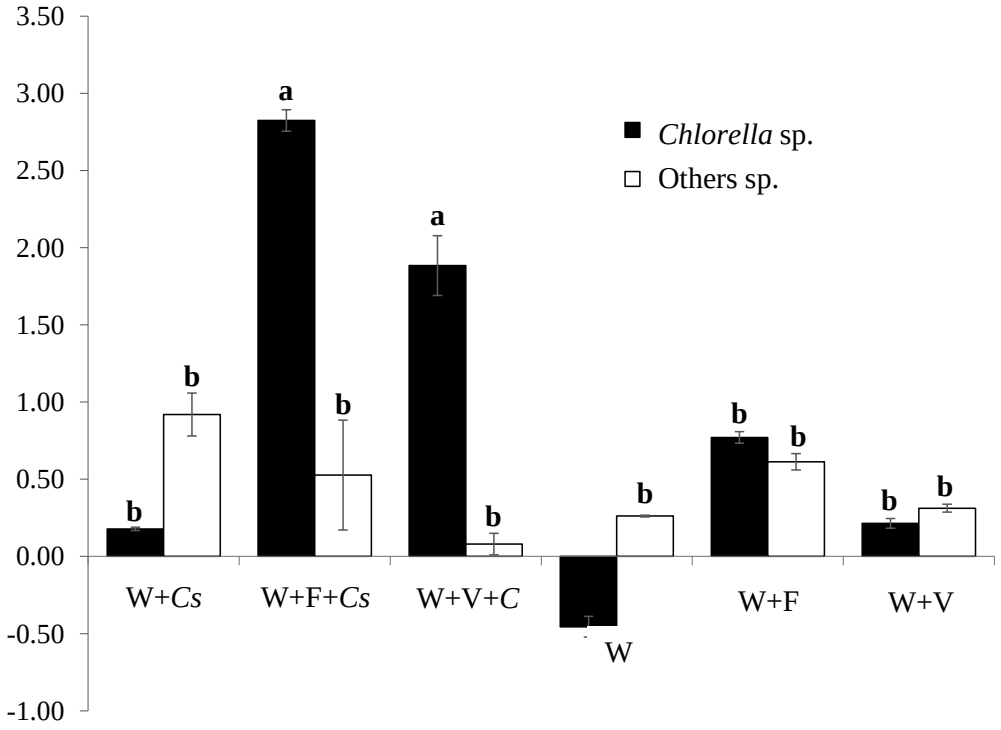
3. Results

The cell duplication rate of microalgae of the group composed of *Chlorella* species was significantly higher in treatments with fish farm wastewater supplemented with chemical fertilizer NPK and sugarcane vinasse ($F_{5,16}$= 343,12 p<0.001). Treatment with *C. sorokiniana* cultivated only in fish farm wastewater (without supplementation) or treatments with only high-altitude microalgae showed a low duplication rate (Figure 1). These values demonstrate that the microalgae *C. sorokiniana* supplemented with fertilizer or vinasse had the highest product performance.

The hydrogenic potential of the culture medium measured on the 28th day showed a trend towards more acidic values for the treatments supplemented with the chemical fertilizer NPK (Figure 2a). The electrical conductivity of water also showed a trend for treatments supplemented with fertilizer with higher values (Figure 2b).

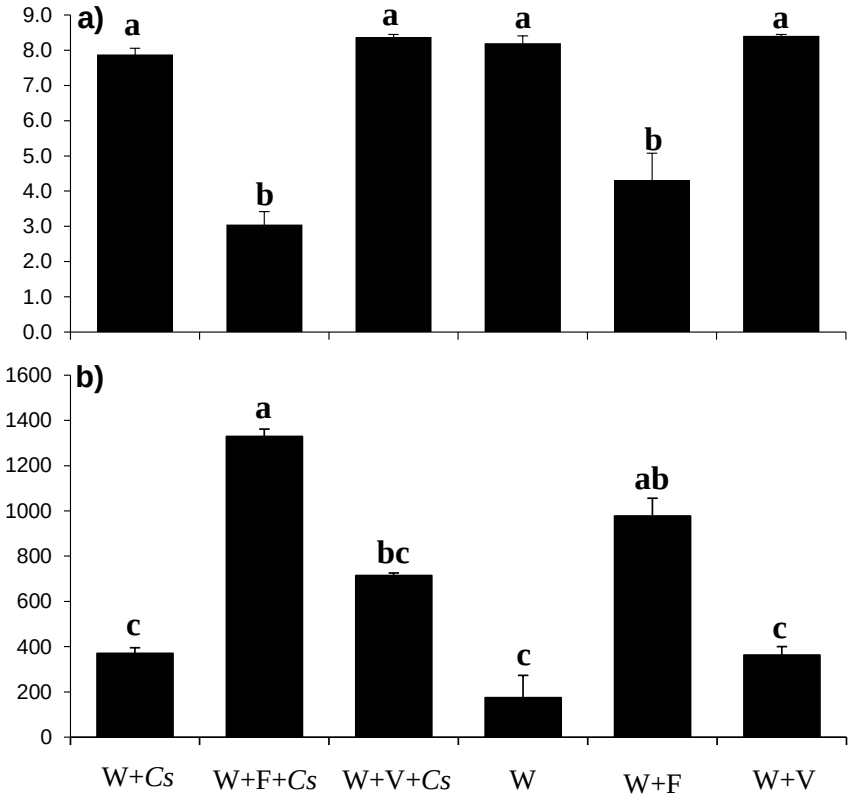
The results of the percentage reduction in a load of pollutants after the period of microalgae cultivation were: (1) a reduction in the contaminants aluminum, cadmium, magnesium, lead and zinc was observed in all treatments; (2) the contaminants calcium and manganese showed reduction in some treatments; (3) the contaminants chromium, copper, nickel, cobalt, and molybdenum were below the limit of detection (LD); (4) the iron contaminant increased the concentration by about 99% (Table 1). Despite these results, a pattern of increase in the removal rate was not observed as a function of supplementation of the culture medium or the addition of the *C. sorokiniana* strain to the culture medium.

Figure 1. Duplication rates of microalgae in each treatment as measured on the 28th day of the experiment (mean ± standard error): treatments with the addition of *C. sorokiniana* only in wastewater (W+*Cs*), the addition of *C. sorokiniana* in wastewater supplemented with fertilizer (W+F+*Cs*) or vinasse (W+V+*Cs*), only autochthonous microalgae grown in wastewater (W), autochthonous microalgae grown in wastewater supplemented with fertilizer (W+F) or vinasse (W+V). Analysis of variance was performed ($p < 0.05$) followed by the Tukey test when comparing the rows, where equal letters indicate statistically equal means and different letters indicate statistically different means.



Source: Authors.

Figure 2. pH monitoring (a) and conductivity (b) of treatments (mean ± standard error) on the 28th day of culture: subtitles see Figure 1.



Source: Authors.

Table 1. Rate of reduction in the concentration of pollutants present in the culture medium after 28 days of bioassay (mean ± standard error): subtitles see Figure 1.

	W+*Cs*	**W+F+*Cs***	**W+V+*Cs***	**W**	**W+ F**	**W+V**	**F**	***P***
Fe	-99.24 ^{NS} ± 0.01	-99.98 ^{NS} ± 0.06	-99.52 ^{NS} ± 0.01	-99.79 ^{NS} ± 0.02	-99.87 ^{NS} ± 0.01	-99.61 ^{NS} ± 0.04	748.30	0.64
Al	55.54 ^{B} ± 0.65	60.37 ^{B} ± 0.54	58.97 ^{B} ± 5.31	62.06 ^{AB} ± 1.74	51.59 ^{B} ± 1	74.94 ^{A} ± 0	11.54	<0.05
Cd	50.82 ^{A} ± 6.93	45.85 ^{AB} ± 0.86	8.91 ^{B} ± 1.78	52.07 ^{A} ± 2.71	20.17 ^{AB} ± 9.77	12.06 ^{AB} ± 12.38	7.86	<0.05
Mn	< LD	72.48 ^{NS} ± 7.24	55.13 ^{NS} ± 7.99	< LD	< LD	< LD	9.36	0.06
Mo	< LD	< LD	< LD	< LD	< LD	< LD	-	-
Mg	21.71 ^{BC} ± 0.49	23.81 ^{B} ± 0.68	12.19 ^{D} ± 2.66	16.92 ^{CD} ± 0.11	19.86 ^{BC} ± 0.19	33.13 ^{A} ± 0,13	38.50	<0.05
Pb	82.61 ^{A} ± 0.03	34.93 ^{B} ± 1.47	75.84 ^{A} ± 2.62	71.62 ^{A} ± 1.21	5.50 ^{C} ± 4.58	44.55 ^{B} ± 1.54	156.11	<0.05
Zn	84.06 ^{AB} ± 0.28	65.65 ^{C} ± 0.3	79.81 ^{B} ± 2.82	88.36 ^{A} ± 0.33	66.50 ^{C} ± 0.35	19.67 ^{D} ± 1.27	378.64	<0.05
Cr	< LD	< LD	< LD	< LD	< LD	< LD	-	-
Cu	< LD	< LD	<LD	< LD	< LD	< LD	-	-
Ni	< LD	< LD	<LD	< LD	< LD	< LD	-	-
Ca	24.05 ^{NS} ± 1.89	16.29 ^{NS} ± 2.32	< LD	20.44 ^{NS} ± 0.11	14.33 ^{NS} ± 0.61	11.04 ^{NS} ± 3.83	1.42	0.33
Co	< LD	< LD	<LD	< LD	< LD	< LD	-	-

Source: Authors.

Analysis of variance was performed ($p < 0.05$) followed by the Tukey test when comparing the rows, where equal letters indicate statistically equal means and different letters indicate statistically different means. ^{NS} is not significant. < LD: below the limit of detection. Subtitles see Figure 1.

4. Discussion

In the present work we used alternative means - NPK chemical fertilizer and sugarcane vinasse - to supplement fish farm wastewater and produce biomass of the microalgae *C. sorokiniana* in a controlled laboratory bioassay. When cultivated in effluent enriched with either fertilizer or vinasse, the duplication rate of the microalgae *Chlorella sorokiniana* was significantly increased. We also adopted the same supplementation procedure to stimulate the production of autochthonous microalgae biomass from fish farm wastewater. We inferred the great potential of bioremediation of contaminants in the culture medium in association with the production of microalgae biomass. The results of this study indicated that supplementation with alternative media was essential for the production of microalgae biomass and that the removal of contaminants (phycoremediation) was highly efficient during the period of microalgae cultivation.

These results may also subsidize the production of microalgae biomass on an industrial or semi-industrial scale in partnership with effluent treatment plants. The process of phycoremediation can be more efficient and less costly than conventional wastewater treatment (Lugo et al., 2020). Furthermore, the alternative use of agro-industrial residues to replace synthetic nutrients may further reduce the costs of microalgae biomass production and, consequently, expand its

application. Many kinds of researches are being carried out to determine possible applications for the biomass of microalgae, many of them returning promising results, managing to add value to the generated compounds and/or insertion in existing processes (see review by Dias et al., 2019).

As an alternative source of nutrients, sugarcane vinasse was highly efficient in the enrichment of the algal culture. It is an acidic liquid, dark brown, and rich in organic compounds such as glycerol, lactic acid, sugars, nitrogen, and phosphorus (Ortegón, Arboleda, Candela, Tamoh & Valdes-Abellan, 2016). Some authors have already evaluated the cultivation of microalgae in media enriched with vinasse; *Chlorella vulgaris* showed higher specific growth rates and lipid production when cultivated in anaerobically treated vinasse than when grown in a synthetic medium (Marques, Nascimento, Almeida & Chinalia, 2013), and exhibited growth rates of up to 1.2 cells day^{-1} when grown in 60% conventional filtered vinasse and 80% biodigested vinasse (Candido & Lombardi, 2017). The enrichment of the medium with the chemical fertilizer NPK also provided the micronutrients needed for the development of *C. sorokiniana*. These nutrients were then supplied in a sufficient quantity to potentiate the development of microalgae.

The study of microalgal growth kinetics in an alternative culture medium is important for the use of formulations that allow faster and more efficient low-cost production strategies. The use of wastewater as a culture medium to support the production of microalgae improves the sustainability of the process and reduces the environmental burden generated when the effluent is improperly discharged into the soil or water resources. Nunes et al. (2021) presented results for the production of *Chlorella vulgaris* in wastewater from the dairy industry similar to those obtained in control culture (Bold Basal Medium). Andreotti et al. (2017) evaluated the potential use of *Tetraselmis suecica*, *Isochrysis galbana*, and *Dunaliella tertiolecta* in a multitrophic integrated aquaculture system, resulting in high *T. suecica* biomass yield (603 ± 34 mg) and the removal of more than 90% of dissolved inorganic nitrogen and inorganic diphosphorus.

Moreover, several studies have reported the highly efficient removal of inorganic nutrients, organic material, and heavy metals from effluent by microalgae, and some have reported improvements in effluent color following treatment with microalgae (Carvalho et al., 2012; Pires, Alvim-Ferraz, Martins, & Simões, 2013; Satpal & Khambete, 2016). This process, combined with the production of algal biomass and the decontamination of wastewater, reinforces the idea that microalgae have great potential for use in sustainable aquaculture.

Some metals present in the aquatic environment are of importance due to their high inherent toxicity, such as lead, cadmium, chromium, nickel, mercury, and, to a lesser extent, copper and zinc. Copper, zinc, and iron, among others, are micronutrients, and ideally should be present in trace concentrations in all aquatic environments. A trace element is a chemical element whose average concentration is very low (a "trace amount") and, therefore, does not cause any risk to human and animal health (Soto-Jiménez, 2011). The presence of these heavy metals at high concentrations and their toxicity to the environment and humans is a major challenge when considering the treatment of wastewater effluent before its discharge into water bodies (Gautam, Sharma, Mahiya, & Chattopadhyaya, 2014).

It was also observed a high rate of reduction of contaminants present in the culture medium in the 28 days of bioassay with microalgae. However, the adopted methodology limited us to certifying whether the microalgae promoted better the adsorption, biosorption, and/or transport of highly xenobiotic chemical elements in isolation. The ph monitoring and electrical conductivity of the water indicated that there is a constant biotransformation process during the 28 days of cultivation and intrinsic for each treatment. Encouraging the development of autochthonous microalgae in fish farm wastewater also played a key role in removing contaminants from wastewater. The elements that presented the greatest reductions in their concentrations, in decreasing order, were: Zinc, Lead, Aluminum, Manganese, Cadmium, Calcium, and Magnesium.

The reduction of high levels of pollutants in water can be observed in the literature that tested different sources of these pollutants through the use of different species of microalgae. Gani et al. (2017) reported the high efficiency of the

algae *Botryococcus* sp. in the removal of Cd, Zn, Fe, and Mn compared to a control group (wastewater without algae); in household wastewater, Zn, Fe, and Mn were removed by up to 71.5%, 51.2%, 83.5%, and 97.2%, respectively; and in food processing wastewater, the concentration of these metals was reduced by up to 64.4%, 53.3%, 52.9%, and 26.7%, respectively. Liu et al. (2018), Lugo et al. (2020), Shivagangaiah, Sanyal, Dasgupta and Banik (2021) also showed that microalga cultivation in wastewater has great potential to reduce contamination while generating economic benefits.

The production of *Chlorella vulgaris* in aquaculture wastewater was responsible for the reduction of high concentrations of phosphorus and total nitrogen (Blanco-Carvajal, González-Delgado, García-Martínez, Sánchez-Galvis & Barajas-Solano, 2017). Barnharst, Rajendran and Hu, (2018) developed a synthetic lichen-type biofilm using the fungus *Mucor indicus* and the microalgae *C. vulgaris*, and simulated the contamination of an aquacultural system. They found that the incorporation of this biofilm reduced the concentrations of several chemicals, including phosphorus and nitrogen, converting them into proteins and other cellular products and purifying the water. The system also rescued total ammonia levels in the water by 69%.

Despite the promising results in the phycoremediation from wastewater, it is important to emphasize the methodological limitation in microalgal biomass flocculation. After chemical flocculation, there was a significant increase in iron concentration in the culture media, which required the addition of 0.75g of ferric chloride ($FeCl_3$) per liter of medium. Thus, the use of chemical flocculation with ferric chloride to separate algal biomass should be performed with caution. During an evaluation of different flocculation techniques, Lal and Das (2016) determined that electro-flocculation was the most adequate and promising technique for the recovery of algal biomass in *Chlorella* sp. and *Synechocystis* due to its low cost and ease of use relative to chemical flocculation ($FeCl_3$; $KAl(SO_4)_2$) and the use of chitosan. However, they did not investigate whether the chemical agents used during flocculation left residues in the water. In an investigation of non-chemical agents with the potential to be used in algal biomass separation, Abdul Hamid et al. (2014) concluded that using derivatives of *Moringa oleifera* as bio-coagulants provided several advantages, including a reduced impact on the environment, lower associated harvest costs, and is chemical-free. Singh et al (2017), however, reported that centrifugation is more efficient and results in higher biomass yields in *C. sorokiniana*.

Based on the results we obtained, further studies are needed to identify flocculants that are less cumulative in the water. Studies are also required to better understand the synergistic and antagonistic processes involved in contaminant removal in residual fish farm wastewater by microalgae. However, the cultivation of microalgae using wastewater from artificial biosystems (aquaculture, fish farming, industrial and domestic effluent treatment) is a promising concept for the integration of biomass generation and chemical contaminant removal into wastewater.

5. Conclusions

Our results indicated that the production of microalgae biomass can be integrated with the treatment of fish farming effluents to reduce the environmental burden and increase the economic bonus for adopting a sustainable production method.

However, our results also indicated the importance of introducing a microalgae strain with high productive performance and supplementing the wastewater to obtain rapid biomass. The introduction of the *Chlorella sorokiniana* strain in fish farming wastewater and its enrichment with the chemical fertilizer NPK and sugarcane vinasse was essential for the high productive performance of microalgae biomass.

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