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Scientific Article

Use of the macroalga *Ulva lactuca* for the treatment of effluent from the cultivation of shrimp *Penaeus vannamei* and tilapia *Oreochromis niloticus* in a biofloc system with different fertilization strategies

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ABSTRACT

The objective of this study was to evaluate the growth and nutrient absorption of the macroalga *Ulva lactuca* in effluents from integrated shrimp (*Penaeus vannamei*) and tilapia (*Oreochromis niloticus*) cultivation in a biofloc system dominated by heterotrophic or chemoautotrophic bacteria. The experiment lasted 15 days and was conducted in six aerated, 3.5 m^3 circular tanks decoupled from the integrated multi-trophic aquaculture system. Depending on the fertilization strategy, treatments were dominated by either heterotrophic or chemoautotrophic bacteria. Macroalgae were stocked at 1 $g \cdot L^{-1}$, with three tanks per treatment. Initial nitrate and total suspended solids concentrations differed significantly between treatments. During cultivation, the chemoautotrophic system exhibited higher alkalinity (290.00 ± 36.33 mg *$CaCO_3 \cdot L^{-1}$) and pH (8.40 ± 0.04). The heterotrophic system showed higher total suspended solids concentrations (303.33 ± 68.25 $mg \cdot L^{-1}$) and a nitrate removal rate of 28%, twice that observed in the chemoautotrophic system. The nitrogen to phosphate ratio in the heterotrophic treatment favored nutrient absorption by the macroalgae. These findings suggest that *U. lactuca* can improve nutrient utilization in heterotrophic systems, contributing to effluent treatment, with positive implications for sustainable integrated aquaculture.

Keywords: *Ulva lactuca*; Biofloc; Biomass; Input; Water quality; Sustainability.

Uso da macroalga *Ulva lactuca* para o tratamento de efluente do cultivo de camarão *Penaeus vannamei* e da tilápia *Oreochromis niloticus* em sistema de bioflocos com diferentes estratégias de fertilização

RESUMO

O objetivo deste estudo foi avaliar o crescimento e a absorção de nutrientes da macroalga *Ulva lactuca* em efluentes do cultivo integrado de camarão (*Penaeus vannamei*) e tilápia (*Oreochromis niloticus*) em um sistema de bioflocos dominado por bactérias heterotróficas ou quimioautotróficas. O experimento durou 15 dias e foi realizado em seis tanques circulares de 3,5 m^3, com aeração constante, desacoplados do sistema de aquicultura multitrófica integrada. Dependendo da estratégia de fertilização, os tratamentos foram dominados por bactérias heterotróficas ou quimioautotróficas. As macroalgas foram estocadas na densidade de 1 $g \cdot L^{-1}$, com três tanques por tratamento. As concentrações iniciais de nitrato e sólidos suspensos totais diferiram significativamente entre os tratamentos. Durante o cultivo, o sistema quimioautotrófico apresentou maior alcalinidade (290,00 ± 36,33 mg $CaCO_3 \cdot L^{-1}$) e pH (8,40 ± 0,04). O sistema heterotrófico apresentou maiores concentrações de sólidos suspensos totais (303,33 ± 68,25 $mg \cdot L^{-1}$) e taxa de remoção de nitrato de 28%, duas vezes superior à observada no sistema quimioautotrófico. A relação nitrogênio:fósforo no tratamento heterotrófico favoreceu a absorção de nutrientes pelas macroalgas. Os resultados indicam que *U. lactuca* pode melhorar o aproveitamento de nutrientes em sistemas heterotróficos, contribuindo para o tratamento de efluentes e a sustentabilidade da aquicultura integrada.

Palavras-chave: *Ulva lactuca*; Biofloco; Biomassa; Insumos; Qualidade de água; Sustentabilidade.

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INTRODUCTION

Investment in the production of macroalgae of the genus *Ulva* has evolved over the years due to advances in laboratory reproduction, large-scale cultivation, and the application of the biomass produced. Its reproduction differs from other groups of macroalgae, occurring through the formation and release of zoospores or gametes, and can be induced through various abiotic factors such as light intensity, temperature, and fragmentation of the thallus (Balar & Mantri, 2020). Also, in their adult stage they exhibit a dynamic morphology in response to environmental variables (Schiavon et al., 2012), demonstrating their adaptability to cultivation conditions.

Macroalgae production in open areas can be subject to nutrient availability, herbivory, and current dynamics (FAO, 2024). Multi-scale models that take into account light, temperature, and nutrients at different times of the year can be used to obtain greater growth and nutrient sequestration, an important tool for maximizing sustainable macroalgae production (Zollmann et al., 2021). Production on land has also shown promise, as demonstrated by the work carried out by Mata et al. (2016), which showed that the production of *Ulva ohnoi* in six fiberglass tanks with a useful volume of 10 m^3 produced 138 tons of dry weight·ha^{-1}·$year^{-1}$, with high variations in biomass yield during the year. This production is directly linked to the availability of limiting nutrients in the aquatic environment, such as nitrate and phosphate (Hadley et al., 2015; Lundberg et al., 1989). According to Shakouri and Balouch (2020), testing different proportions of nitrate and phosphate to maximize macroalgae biomass production found the best results at concentrations of 30 mg·L^{-1} of nitrate and 15 mg·L^{-1} of phosphate.

The use of chemical fertilizers for nutrient input can represent a high cost in land-based production (Mansilla et al., 2014). For this purpose, as a way of reusing inorganic waste produced through aquaculture activities, macroalgae have been inserted into the system through integrated cultivations or effluent treatments. The use of macroalgae as a bioremediation agent in fish and shrimp cultivation has been increasing as a way of mitigating impacts on production and nutrient release. Martins et al. (2020) used the macroalga *U. ohnoi* to treat shrimp effluent, obtaining a high yield with a specific growth rate of 3%·day^{-1}. Therefore, the use of macroalgae as an inorganic consumer becomes viable due to the need to absorb nitrogen and phosphorus as an energy source, carrying out physiological and biochemical processes that are essential for growth and reproduction (Duke et al., 1989).

Therefore, the treatment of effluent generated in aquaculture aims to improve sustainability by diversifying the products generated in cultivation. Among intensive shrimp and tilapia production systems, the biofloc system (BFT) is particularly important, since it allows the use of high stocking densities without compromising water quality or the use of renewals (Krummenauer et al., 2011; Wasielesky et al., 2013). In the biofloc system, nitrogen is managed by inducing the growth of microorganisms in the water that will cycle nitrogen compounds in the system and convert them into bacterial biomass (Wasielesky et al., 2013). Different initial fertilizations in the BFT will provide the growth of several bacteria, which can be heterotrophic bacteria, chemoautotrophic bacteria, or a mixed system with both bacteria present in the system (Brandão et al., 2021). The use of organic carbon will favor the growth of heterotrophic bacteria, which will use ammonia to grow bacterial biomass, resulting in higher production of total suspended solids (bioflocs) in the system (Gaona et al., 2017). The use of inorganic carbon, on the other hand, will result in the predominance of nitrifying bacteria, which will be responsible for oxidizing ammonia to nitrite and then to nitrate, with this compound accumulating in high concentrations in the cultivation (Brandão et al., 2021).

In both fertilization strategies, nutrients and organic matter will accumulate and, if discharged into the environment without prior treatment, can lead to human health problems and environmental eutrophication (Macedo & Sipaúba-Tavares, 2010). Inserting macroalgae into the cultivation effluent can therefore be an option for treating water for disposal or reuse it for a new cycle.

Aquaculture sustainability is defined as the production of aquatic organisms to meet the required demand without damaging the ecosystem around it or exhausting the natural resources necessary to maintain the activity (Boyd et al., 2020). However, little is known about maintaining the water quality of the BFT when active species are not inserted into the system to move the floc. In addition, high concentrations of nutrients can be a stress factor for the macroalgae, becoming a trigger for reproduction and causing a loss of biomass (Copertino et al., 2009), as well as high concentrations of solids may interfere with the absorption of light and photosynthesis, causing the macroalgae to grow at a low rate (Carvalho et al., 2023b). For this reason, the aim of this study was to evaluate the growth and absorption of nutrients by the macroalga *U. lactuca* in an effluent from an integrated system with shrimp and tilapia in biofloc obtained from organic and inorganic fertilization.

MATERIALS AND METHODS

Location and origin of the macroalgae

The experiment was carried out in an agricultural greenhouse, with constant aeration promoted by a blower that injected air into micro-perforated hoses (aerotubes). The greenhouse was located at the Marine Aquaculture Station, Institute of Oceanography, at the Universidade Federal do Rio Grande, located on Cassino beach, Rio Grande, Rio Grande do Sul, Brazil.

The macroalgae were obtained from a maintenance cultivation at the Marine Aquaculture Station, produced in a 1 m^3 circular tank, with constant aeration and 5% inoculum from a biofloc shrimp cultivation system, maintaining a concentration of 35.1 ± 2.74 and 2.24 ± 1.2 $mg \cdot L^{-1}$ of nitrate and phosphate, respectively.

Characterizing the biofloc system

The experiment used two effluents from an integrated cultivation with *Penaeus vannamei* shrimp, *Oreochromis niloticus* fish, and the macroalga *Ulva lactuca* in different BFT.

The first effluent came from integrated cultivation with a chemoautotrophic system. In this system, chemical fertilization occured prior to stocking the animals, lasted 35 days, and used ammonium chloride and sodium nitrite at a concentration of 1 $mg \cdot L^{-1}$ in the water and an artificial substrate to attach the bacteria. After 35 days of formation and stabilization of the bacteria in the chemoautotrophic system, a 30% inoculum was added to the three tanks and the animals were stocked. During the 55-day experimental period, only inorganic fertilization with sodium bicarbonate was carried out to correct the alkalinity for the bacteria to be more effective (Furtado et al., 2011), the average temperature was maintained at 22.82 ± 0.30°C, and nutrients averaged 0.95 ± 0.07 $mg \cdot L^{-1}$ for ammonia, 7.96 ± 1.16 $mg \cdot L^{-1}$ for nitrite, 26.41 ± 2.72 $mg \cdot L^{-1}$ for nitrate, 1.04 ± 0.25 $mg \cdot L^{-1}$ for phosphate, and total suspended solids averaged 189.22 ± 26.02 $mg \cdot L^{-1}$. Due to the increase in nitrite concentration at the start of this treatment cultivation, it was necessary to renew the water as a way of managing and controlling nitrite.

The second effluent was obtained from an integrated cultivation in a heterotrophic system, using organic fertilizers. It started with the use of 40% of a mature biofloc inoculum from a 68-day cultivation of *P. vannamei* shrimp at a density of 184 shrimp $\cdot m^{-2}$ and 60% water at a salinity of 20 ppt. Fertilization with organic carbon and molasses was carried out when ammonia exceeded 1 $mg \cdot L^{-1}$ (Ebeling et al., 2006), and sodium bicarbonate was used to maintain alkalinity. In this system, during the 55 days of cultivation in an integrated system, the average temperature was maintained at 22.67 ± 0.38°C, and nutrients averaged 0.18 ± 0.08 $mg \cdot L^{-1}$ for ammonia, 0.86 ± 0.60 $mg \cdot L^{-1}$ for nitrite, 26.04 ± 4.03 $mg \cdot L^{-1}$ for nitrate, 1.19 ± 0.21 $mg \cdot L^{-1}$ for phosphate, and total suspended solids averaged 335.38 ± 47.92 $mg \cdot L^{-1}$. No water was renewed in this system.

Experimental design

At the end of the integrated cultivation of both systems, the animals were harvested, and the water was kept in the tanks for the experiment, which was carried out over 15 days. There were two treatments with three replicates each:

- Chemoautotrophic: use of inorganic fertilizers to maintain the system's water quality;
- Heterotrophic: use of organic fertilizers with molasses to maintain the system's water quality.

The experiment was carried out in circular tanks with a useful volume of 3.5 m^3 and constant aeration by micro-perforated hoses (aerotubes). A density of 1 $g \cdot L^{-1}$ of macroalgae was adopted, with an average initial weight of 3.52 ± 0.10 g. The macroalgae was arranged in a single floating structure in each cultivation tank, with a water column depth of 0.10 m to keep the macroalgae close to the surface, and dimensions of 1 m × 1 m × 0.30 m (length × width × height). The initial nutrient and solids parameters for each treatment are described in Table 1.

Table 1. Initial parameters (mean ± standard deviation) of nutrients and solids in the chemoautotrophic and heterotrophic treatments.

Parameters	Treatments	
	Chemoautotrophic	Heterotrophic
Total ammoniacal nitrogen ($mg \cdot L^{-1}$)	0.07 ± 0.03	0.07 ± 0.03
Nitrite ($mg \cdot L^{-1}$)	0.11 ± 0.03	0.15 ± 0.03
Nitrate ($mg \cdot L^{-1}$)	25.00 ± 0.00 a	32.33 ± 2.08 b
Phosphate ($mg \cdot L^{-1}$)	2.43 ± 0.21	2.17 ± 0.35
Total suspended solids ($mg \cdot L^{-1}$)	165.00 ± 77.78 a	346.67 ± 115.58 b

*Different letters on the same line indicate a significant difference between treatments after Student's t-test.

Water quality

Temperature (°C), dissolved oxygen ($mg \cdot L^{-1}$), and pH were measured daily using a Pro-20 model multiparameter probe (YSI Inc., Yellow Springs, OH, United States of America) and a bench pH meter (Seven2Go S7 Basic, Mettler Toledo, São Paulo, SP, Brazil). Salinity (ppt) and alkalinity (mg $CaCO_3 \cdot L^{-1}$) were measured once a week using a Pro-20 model probe (YSI Inc., Yellow Springs, OH, United States of America) and the methodology proposed by the American Public Health Association (APHA,

2005), respectively. Total ammoniacal nitrogen ($mg \cdot L^{-1}$) and nitrite ($mg \cdot L^{-1}$) were measured daily according to the methodology proposed by the United Nations Educational, Scientific and Cultural Organization (Unesco, 1983). Nitrate ($mg \cdot L^{-1}$) and phosphate ($mg \cdot L^{-1}$) were measured four times a week according to the methodology proposed by Aminot and Chaussepied (1983). Total suspended solids ($mg \cdot L^{-1}$) were measured at the beginning and at the end of the experiment according to the methodology proposed by Baumgarten et al. (1996).

Macroalgae performance

The macroalgae were weighed at the beginning and at the end of the experiment. The weighing procedure consisted of removing the floating structure from the water, leaving it in the fresh air for 10 minutes to remove excess water and then weighing it on a digital scale (BL3200H, MARTE®, Santa Rita do Sapucaí, Minas Gerais, Brazil). Equations 1 and 2 were used to measure the relative growth rate (RGR) and nutrient removal rate (NRR) by the macroalgae.

$$RGR\ (\%d^{-1}) = 100 \times [\ln(\text{final weight (g)}/\text{initial weight (g)})/(\text{final time} - \text{initial time})] \quad (1)$$

$$NNR\ (\%) = 100 \times [(\text{nutrient concentration at the initial time } (mg \cdot L^{-1}) - \text{nutrient concentration at the end } (mg \cdot L^{-1})/\text{nutrient concentration at the initial time } (mg \cdot L^{-1})] \quad (2)$$

Statistical analysis

Data is represented as mean ± standard deviation. The normality and homoscedasticity of the data were assessed using the Shapiro-Wilk's and Levene's tests, respectively. When the assumptions were satisfied, a Student's t-test was performed to assess differences between treatments. In cases where the Student's t-test assumptions were not met, the non-parametric Kruskal-Wallis' test was used. A significance level of 5% ($p \leq 0.05$) was applied to all analyses.

RESULTS

Water quality

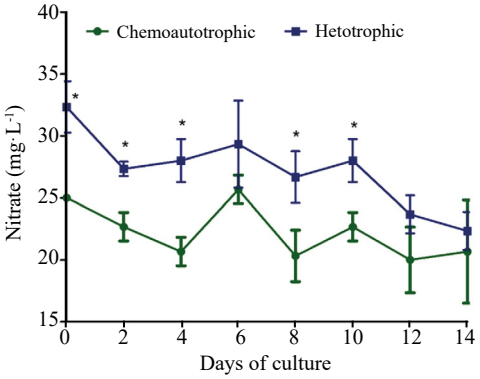
The results showed a higher concentration of alkalinity and pH in the chemoautotrophic treatment compared to the heterotrophic treatment. Regarding nutrients, total ammoniacal nitrogen and nitrate showed lower concentrations in the chemoautotrophic treatment, as did the concentration of total suspended solids, which showed a significant difference ($p \leq 0.05$) with the heterotrophic treatment (Table 2).

Table 2. Average water quality parameters (mean ± standard deviation) during the 15 days of cultivation in the chemoautotrophic and heterotrophic treatments*.

Parameters	Treatments	
	Chemoautotrophic	Heterotrophic
Temperature (°C)	17.76 ± 1.19	17.21 ± 1.34
Dissolved oxygen ($mg \cdot L^{-1}$)	6.88 ± 0.32	6.99 ± 0.35
pH	8.40 ± 0.04 a	8.29 ± 0.04 b
Salinity	20.08 ± 0.50	21.74 ±1.33
Alkalinity ($mg\ CaCO_3 \cdot L^{-1}$)	290.00 ± 36.33 a	218.33 ± 34.74 b
Total ammoniacal nitrogen ($mg \cdot L^{-1}$)	0.12 ± 0.02 a	0.19 ± 0.05 b
Nitrite ($mg \cdot L^{-1}$)	0.11 ± 0.02	0.10 ± 0.04
Nitrate ($mg \cdot L^{-1}$)	22.36 ± 1.93 a	27.33 ± 3.13 b
Phosphate ($mg \cdot L^{-1}$)	3.54 ± 0.72	3.27 ± 0.70
Total suspended solids ($mg \cdot L^{-1}$)	157.50 ± 81.32 a	303.33 ± 68.25 b

*Different letters on the same line indicate a significant difference between treatments after Student's t-test.

The experiment showed a decrease in nitrate concentrations during the trial in both treatments. The heterotrophic treatment had a higher initial nitrate concentration, showing significant differences ($p \leq 0.05$) with the chemoautotrophic treatment in the days of cultivation. However, at the end of the experiment there was no significant difference ($p > 0.05$) between the treatments, showing similar nitrate concentrations (Fig. 1). The phosphate concentration did not differ between the treatments ($p > 0.05$), but it showed an increase over the course of the experiment (Fig. 2).



*A significant difference between treatments on the same day.

Figure 1. Average nitrate concentration ($mg \cdot L^{-1}$) during the days of cultivation in the chemoautotrophic and heterotrophic treatments.

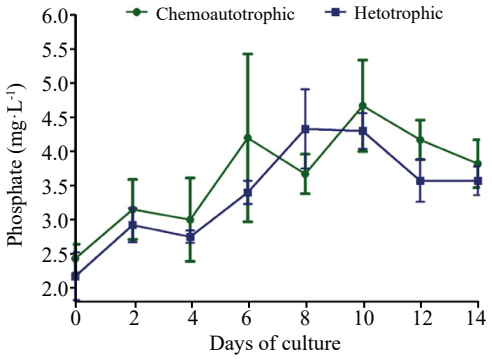


Figure 2. Average phosphate concentration (mg·L^{-1}) during the days of cultivation in the chemoautotrophic and heterotrophic treatments.

Macroalgae performance

There was no significant difference ($p > 0.05$) between the average final weight of the macroalgae between the treatments, showing positive biomass gain values in both treatments. However, a higher nitrate removal rate was found in the heterotrophic treatment compared to the chemoautotrophic one (Table 3).

Table 3. Macroalgae performance (mean ± standard deviation) during 15 days of cultivation in the chemoautotrophic and heterotrophic treatments*.

Parameters	Treatments	
	Chemoautotrophic	Heterotrophic
Average final weight (kg)	5.98 ± 0.13	6.47 ± 1.03
RGR (% day^{-1})	3.53 ± 0.15	3.99 ± 1.12
Biomass gain (kg)	2.46 ± 0.13	2.95 ± 1.03
NNR – nitrate (%)	14.67 ± 7.25 b	28.02 ± 10.71 a

NNR: nutrient removal rate; *different letters on the same line indicate a significant difference between treatments after Student's t-test.

DISCUSSION

The different types of fertilization in the BFT can affect the concentration of nutrients and solids in the system, as well as interfering with the organisms' performance. The maintenance of the chemoautotrophic system occurs with the insertion of inorganic carbon, such as sodium bicarbonate and calcium carbonate, with the aim of increasing the alkalinity of the system for better establishment and activity of the nitrifying bacteria (Ebeling et al., 2006). According to Furtado et al. (2011), alkalinity should be maintained above 150 mg $CaCO_3 \cdot L^{-1}$, however, for chemoautotrophic systems, alkalinity can be maintained at 300 mg $CaCO_3 \cdot L^{-1}$. As a result, the higher alkalinity and pH values found in chemoautotrophic treatment reflect the need to maintain the system at higher concentrations to ensure the efficient performance of the bacteria.

However, during the experimental period, it was not necessary to make corrections with inorganic carbon to raise alkalinity. It is known that nitrifying bacteria consume 7.14 g of alkalinity, resulting in 1.69 g of inorganic carbon, for each gram of ammonia converted into nitrate in the chemoautotrophic system, compared to the heterotrophic system, which operates with the consumption of 3.57 g of alkalinity, resulting in 0.86 g of inorganic carbon for the conversion of 1 g of ammonia into bacterial biomass (Ebeling et al., 2006). This maintenance of alkalinity in both treatments may have resulted from the consumption of carbon dioxide by the macroalgae. According to Chopin (2015), macroalgae contribute to combating ocean acidification and are important in marine ecology. The cultivation of macroalgae could therefore reduce the amount of inputs used in production. Carvalho, Braga et al. (2024) and Legarda et al. (2021) used 0.38 and 0.07 g·L^{-1} of inorganic carbon in 35 and 45 days of integrated cultivation with shrimp and macroalgae, showing·Lower inorganic carbon utilization values compared to monoculture systems.

In addition to maintaining alkalinity, the insertion of macroalgae for effluent treatment is aimed at bioremediation with the absorption of nutrients accumulated during the cultivation period. According to del Río et al. (1996), the use of 2.5 g·L^{-1} of fresh weight of the macroalga *Ulva rigida* was able to absorb an average of 1.7 g of dissolved inorganic nitrogen·m^{-2}·d^{-1} in the treatment of effluent from a cultivation of *Sparus aurata*. Compared to conventional systems with water renewal, the BFT operates as a closed system, resulting in the accumulation of nitrate and phosphate (Da Silva et al., 2013; Luo et al., 2020). In this study, the average nitrate concentration in the chemoautotrophic treatment was lower than in the heterotrophic treatment. This result was due to the lower nitrate concentrations in the chemoautotrophic treatment at the start of the experiment. However, when the entire experimental period was evaluated, the heterotrophic treatment showed twice the nitrate removal rate compared to the chemoautotrophic system, and it was measured that at the end of cultivation both treatments had similar nitrate values.

This result may be associated with better physical and chemical conditions in the heterotrophic treatment, which resulted in maximized nitrate absorption. For macroalgae, balancing the nitrogen and phosphorus ratio is essential for better removal rates. According to Wheeler and Björnsäter (1992), the N:P ratio

in the macroalgae tissue is higher than 10, which is different to that found for phytoplankton, probably due to a greater demand for nitrogen by the macroalgae. Therefore, the greater availability of nitrogen present in the heterotrophic treatment ensured that this nutrient was not limiting, compared to the chemoautotrophic treatment. In addition to the availability of nitrogen, the N:P balance in the 30:1 ratio (N:P) could help maximize growth and the rate of nutrient removal by the macroalgae (Harrison & Hurd, 2001). In addition, the heterotrophic treatment also had an N:P ratio closer to the ideal, compared to the chemoautotrophic treatment, which initially had a ratio below 30:1, which may have resulted in nitrogen limitation.

The removal rate results were lower than those found by Carvalho, Brandão et al. (2024) with the same fertilization system in bioflocs in an integrated system with shrimp and fish, obtaining a nitrate removal rate of 57% and a phosphate one of 47%. The constant production of nutrients in the integrated system becomes an available food source for the macroalgae, with minimal nitrogen or phosphorus limitation, unlike effluent treatment, which, with the reduction of any of the nutrients, will cause limitation and unbalance the ideal ratio. Nitrogen production comes from animal excretion and leaching from the feed (Da Silva et al., 2013), factors that are not present when working only with the effluent from aquaculture production. Using the macroalga *U. ohnoi* to treatment shrimp effluent, Martins et al. (2020) resulted in the absorption of 58% of the nitrate in three weeks of experimentation using twice as much biomass as this experiment.

Phosphate is an important nutrient for photosynthesis and tissue formation, and when it is limited in the medium, it can interfere with the growth of the macroalgae (Zirino et al., 2016). In this experiment, phosphate values increased over the course of the culturing days in both treatments, which may be associated with a higher production of phosphate in the tanks in parallel with a lower consumption of phosphate by the macroalgae. Despite being an important nutrient for the growth of macroalgae, the requirement for phosphate in relation to nitrogen is lower. According to Shakouri and Balouch (2020), for optimal growth of the macroalga *U. rigida*, 30 $mg \cdot L^{-1}$ of nitrate and 15 $mg \cdot L^{-1}$ of phosphate are required, which means that more nitrogen available in the medium is used than phosphate, and so the macroalga was unable to perform a NNR.

According to Da Silva et al. (2013), despite the fact that phosphorus does not have toxic concentrations, high accumulated concentrations can trigger phytoplankton blooms and eutrophication, as this phosphorus is produced through leaching from the feed and the death of organisms. In this experiment, as no feed was given, the phosphorus produced may have come from the decomposition of the biofloc. The microbial floc is made up of a cluster of bacteria, phytoplankton, and zooplankton (Reis et al., 2019), and the death of these organisms releases the nitrogen and phosphate accumulated in the tissue.

Unlike water recirculation and renewal systems, the BFT operates with a high organic load formed by microbial aggregates (Gaona et al., 2017). In the chemoautotrophic system, the concentration of solids was lower than in the heterotrophic system, result found by Ferreira et al. (2021). This is due to the use of organic carbon in the heterotrophic system, which has the function of maintaining the Carbon:Nitrogen ratio to favor bacteria that will consume the ammonia and transform it into bacterial biomass and thus increase the concentration of total suspended solids in the system (Hostins et al., 2019). However, despite the intense aeration, the effluent treatment system had no animals such as shrimp or fish to move the water and suspend the solids, so at some locations in the tank, decantation zones may have formed and the floc decomposed, which led to an increase in phosphate in the water. The insertion of a sessile organism such as macroalgae also helps in the settling of solids, forming a barrier to the movement of water and its deposition on the macroalgae lamina (Carvalho et al., 2023a).

This solids retention on the surface of the macroalgae can also affect its growth due to the low incidence of light and photosynthesis. However, unlike the work carried out by Carvalho et al. (2023a), Legarda et al. (2021) and Morais et al. (2023) with macroalgae cultivation in an integrated system with bioflocs, which showed a loss of biomass or relative growth rate of $0.14\% \cdot day^{-1}$, in this study the rates were higher, with an overall average of $3.76 \pm 0.76\% \cdot day^{-1}$. This high growth rate may be related to the stabilization of solids production when operating with effluent treatment, in addition to the possible settling of solids at the bottom of the tank, making the water clearer. In an integrated system, the production of solids is continuous due to the presence of organisms (Gaona et al., 2017), which can be a stressful factor for macroalgae and which is not evident when working with effluent treatment. In addition to the concentration of solids, temperature can also be an important factor for higher macroalgae growth rates. According to Hiraoka and Oka (2008), the highest growth rate for *Ulva fasciata* using deep seawater was 22°C, but growth was still observed in water with a temperature of 13°C and a decrease in biomass at temperatures above 22°C. Therefore, the average temperature values in our work may have contributed to a better growth rate.

The results were similar to those found by Resende et al. (2022) with a specific growth rate of 3.82%·day^{-1} in the first week of cultivation of the macroalga *Ulva* in the fish farm effluent, noting that the experiment was carried out under controlled laboratory conditions and using mesh to filter the effluent beforehand, unlike the conditions used in this experiment. The results obtained in this experiment were higher than those found by Ramos and Gallardo (2021) using *U. lactuca* to treat *Seriola lalandi* effluent, obtaining only 0.78% day^{-1}, probably due to the low concentration of nutrients found in the effluent. Therefore, due to the high availability of nitrogen and phosphorus in the BFT resulting from the action of bacteria and zero water renewal, the effluent from the BFT becomes a viable alternative for the growth and bioremediation of macroalgae.

CONCLUSION

The use of effluent with organic fertilization for cultivation of the macroalga *U. lactuca* proved to be viable, with a nitrate removal rate of 28% and a RGR of 3.99%. The use of macroalgae as a bioremediator, in addition to nitrogen removal, also acted on the controlling of the pH and alkalinity of the system, reducing the use of inputs. However, better forms of management should be adopted to maintain total suspended solids so that biofloc decomposition does not occur in the system.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be provided upon request.

AUTHORS' CONTRIBUTION

Conceptualization: Carvalho, A., Cardozo, A.P., Poersch, L.H.; **Investigation:** Carvalho, A., Rungo, N.; **Methodology:** Carvalho, A., Rungo, N., Cardozo, A.P.; **Formal Analysis:** Carvalho, A., Rungo, N.; **Data Curation:** Carvalho, A., Rungo, N., Cardozo, A.P., Turan, G.; **Writing – original draft:** Carvalho, A., Cardozo, A.P.; **Writing – Review & Editing:** Rungo, N., Cardozo, A.P., Turan, G., Poersch, L.H.; **Validation:** Cardozo, A.P., Turan, G., Poersch, L.H.; **Supervision:** Cardozo, A.P., Turan, G., Poersch, L.H.; **Project Administration:** Turan, G., Poersch, L.H.; **Resources:** Turan, G., Poersch, L.H.; **Final approval:** Poersch, L.H.

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DECLARATION ON THE USE OF ARTIFICIAL INTELLIGENCE TOOLS

No artificial intelligence tools were used in the preparation of this manuscript.

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