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Phytorremediation of urban and fish farming wastewater and growth performance responses of *Litopenaeus vannamei* using microalgae *Chlorella vulgaris*

Fitorremediação de efluentes urbanos e piscícolas e respostas do desempenho de crescimento de *Litopenaeus vannamei* utilizando a microalga *Chlorella vulgaris*

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ABSTRACT

Phytoremediation, as the implementation of microalgae in the treatment of effluents, is used to remove nitrogen compounds and phosphorus that can cause eutrophication in receiving water bodies. The present study evaluated the phytoremediation of urban and fish farming wastewater and growth performance of *Litopenaeus vannamei* using microalgae *Chlorella vulgaris*. Microalgae were cultivated stationary with three treatments: T1 sewage treatment station effluent (STS), T2 fish farming effluent (FF) and T3 with standard medium (SM) Guillard f/2), conducted in quintuplicate for each culture medium, using a 20 L carboy for eight days. 5.0 L inoculum (25%) plus 15 L culture medium, T1 STS, T2 FF and T3 SM (75% of each) were used to start the experiment. The shrimp experiment was realized with three treatments in quintuplicates, completely randomized design, during 15 days. The animals were maintained in tanks of 100 L under constantly aerated, resulting in a concentration above 5.0 mg L^{-1} of dissolved oxygen, in a density of 70 animals m^{-3}, fed four times per day (8:00, 11:00, 14:00 and 16:00 h) offering to T1 commercial feed (C-Feed) with 35% crude protein, T2 and T3 with commercial feed plus addition of 5.0% dry biomass of *C. vulgaris*. The best result for algal perfomance, lipid biomass and nutrient removal was with Fish farm wastewater. The additive with the highest performance was FF-Feed**.** *C. vulgaris* has a high potential for removing nutrients from wastewater, producing biomass and lipids, in addition to increasing shrimp productivity.

Index terms: Water quality; nutrients removal; marine shrimp.

RESUMO

Fitorremediação, como implementação de microalgas no tratamento de efluentes, é utilizada para remover compostos nitrogenados e fósforo que podem causar eutrofização. O presente estudo avaliou a fitorremediação de águas residuárias urbanas e piscicultura e o desempenho zootécnico do *Litopenaeus vannamei,* usando *Chlorella vulgaris*. As microalgas foram cultivadas em três tratamentos (efluente da estação de tratamento de esgoto T1 STS, efluente aquícola T2 FF e T3 meio padrão (SM) Guillard f/2), conduzidos em quintuplicata para cada meio de cultura, utilizando garrafão de 20 L por 8 dias. 5 L de inóculo (25%) mais 15 L de meio de cultura, T1 STS, T2 FF e T3 SM (75% de cada) foram usados para iniciar o estudo. O experimento com camarões foi realizado com três tratamentos em quintuplicata, em delineamento inteiramente casualizado, durante 15 dias. Os animais foram mantidos em tanques de 100 L sob aeração constante, resultando em concentração acima de 5,0 mg L^{-1} de oxigênio dissolvido, densidade de 70 animais m^{-3}, alimentados quatro vezes ao dia (8:00, 11 : 00, 14:00 e 16:00 h) ofertando ração comercial T1 (C-Feed) com 35% de proteína bruta, T2 e T3 com ração comercial mais adição de 5% de biomassa seca de *C. vulgaris*. O melhor resultado para o desempenho de algas, biomassa lipídica e remoção de nutrientes foi o FF. O aditivo com melhor desempenho foi FF-Feed. *C. vulgaris* possui alto potencial para remoção de nutrientes de águas residuárias, produção de biomassa e lipídios, além de aumentar a produtividade do camarão.

Termos para indexação: Qualidade da água; remoção de nutrientes; camarão marinho.

INTRODUCTION

The generation of urban and aquaculture effluents, rich mainly in nitrogen and phosphorus, are challenges faced by society, a large part of aquaculture projects and cities in Brazil do not have an adequate disposal of their effluents before discarding them, which can contaminate and eutrophicate receiving water bodies with excessive growth of macrophytes, cyanophyceae, low specimen diversity, anaerobic conditions and mortality of aquatic organisms (Evans et al., 2017). Most studies on effluent treatment are based on physical processes such as stabilization ponds and decanters, chemicals with use flocculation or flotation compounds and biological processes such as water recirculation system that use aggregated biofilters with decantation tanks and processes with and without the presence of oxygen (Arun et al., 2017). These processes are mainly aimed at removing nitrogenous compounds and phosphorus, resulting in steps with disadvantages due to high infrastructure and professional costs, high operational complexity, demanding large areas, in addition to producing large amounts of sludge (Ajayan; Harilal; Selvaraju, 2018).

Some bioremoval studies work with plants, such as wetlands that use superior plants and macroalgae (Sujuan et al., 2021; Saberi et al., 2020), both processes achieve a lower removal of nitrogen compounds and phosphorus when compared to the use of microalgae, in addition to requiring a longer period for the development of plants and macroalgae. These characteristics make these processes more environmentally and financially costly (Yajing et al., 2021). Phytoremediation using microalgae is an excellent alternative for wastewater treatments, as it is more environmentally efficient by absorbing high levels of nutrients, economically with use of biomass and bioactive compounds with high commercial value in various applications in pharmaceutical, cosmetics, biogas, biofuels, biofertilizers, human and animal nutrition industries (Mujtaba; Rizwa; Lee, 2017; Mujtaba; Lee, 2017). Currently, several studies demonstrate the advances in the efficiency of microalgae in the treatment of urban (Pacheco et al., 2021), industrial (Rajak; Jacob; Kim, 2020) and agricultural effluents (Wen et al., 2017), indicating the possible applications of the algal biomass produced, but without using it.

Various effluents can be an excellent alternative for algal cultivation and biomass recovery (Ortenzio et al., 2015), as they are culture media of reduced financial value and with sufficient concentration of nutrients for an efficient growth performance (Wenhai et al., 2019). The removal of nutrients from the culture medium occurs because the microalgae incorporates these to its biomass, effecting a high removal rate (Cao et al., 2018) and not causing damage to the ecosystem if chosen correctly, according to environmental factors (Lam et al., 2017).

Phytoremediation, as the implementation of microalgae in the treatment of effluents, is used to remove nitrogen compounds and phosphorus that can cause eutrophication in receiving water bodies (Mohd-Sahib et al., 2017). Microalgae can remove more than 95% of nitrogen compounds and 90% of phosphorus in effluents (Praveen; Loh, 2016). The use of native algae, such as *Chlorella vulgaris*, in the phytoremediation of wastewater is very important because the species presents rapid cell growth, CO_2 removal, oxygen production, resistance to contamination, excellent biochemical composition and efficient nutrient absorption (Rodrigues-Sousa et al., 2021; Miao et al., 2016; Silva et al., 2015). *C. vulgaris* was used by Wang et al. (2017), to evaluate the capacity to remove nitrogen in the form of ammonia or ammonium ion, being able to drastically remove nitrogen compounds in just 48 h, showing its high potential for wastewater treatment. Liu et al. (2017), which used an urban effluent with carbon dioxide injection and observed a removal of 99% of phosphorus and 87% of ammonia, while Mujtaba and Lee (2017), used urban treatment wastewater, achieving a removal of 95% ammonia and 90% phosphorus, and Mujtaba, Rizwan and Lee (2017), found that *C. vulgaris* removed 70% ammonia and 66% phosphorus when domestic sewage effluent was used.

C. vulgaris is also used as additives in the diet of aquatic organisms, due to its excellent biochemical composition with a high content of proteins, vitamins, minerals and polyunsaturated fatty acids. *C. vulgaris* biomass improves feed palatability and digestibility, reducing nitrogen and phosphorus content in excreta and optimizing zootechnical performance, achieving environmental and economic gains, promotes good nutrition, elevates individuals immunity and resistance to pathogens and management, allowing for greater cultivation density and greater survival, achieving excellent results in terms of productivity, profitability and sustainability (Alagawany et al., 2021). The production of *Litopenaeus vannamei* is growing rapidly, with a world production of approximately 4.1 million tons (Food and Agriculture Organization of the United Nations - FAO, 2020), demanding a food with better nutritional quality, environmentally correct and economically viable. The feed must have a high content of proteins, lipids, vitamins and minerals (Gao et al., 2019), important substances for the development of the shrimp and that are part of the composition of *C. vulgaris* in the necessary amounts.

Silva et al. (2020b), added 0.5% *C. vulgaris* dry biomass to the feed and compared it with the commercial diet of *L. vannamei*. The authors found greater final biomass and productivity when adding algal biomass, with an increase of 1.05 times compared to the commercial diet. Pakravan et al. (2017), used different experimental diets with *C. vulgaris* added to *L. vannamei* feed and confirmed that their biomass can partially or completely replace fishmeal in the feed formulation without causing adverse effects in the zootechnical performance of shrimp, demonstrating environmental feasibility and economic gain when using algae, reducing environmental impacts on fish stocks and feed manufacturing costs due to the high value of fishmeal.

The differential of present study was aggregate environmental and economic value to phytoremediation by using algal biomass as an additive in the *L. vannamei* feed and enabling the reuse of aquaculture effluent after phytoremediation in the production of fish and shrimp by achieving quality parameters required by cultivated aquatic organisms. This factor demonstrates the importance of multidisciplinarity in the pursuit of sustainable food production and water use. The present work evaluated the *C. vulgaris* as a phytoremediation of urban and fish farming wastewater and comparing their kinetic yield and water quality, and as an nutritional additive in the feeding of *L. vannamei* and determining the zootechnical parameters.

MATERIAL AND METHODS

C. vulgaris, Shrimp and Effluents

The microalgae *C. vulgaris* strain was obtained from the algal culture collection of the Aquaculture Technology Laboratory of the Federal Institute of Ceará (LTA - IFCE) - Aracati Campus, where it is kept in a Guillard f/2 medium (Guillard, 1975) under controlled temperature conditions (20.0±1.0 ºC) and light intensity maintained at 54 µmol m^{-2} s^{-1} using a fluorescent light with a controlled photoperiod (16 h dark:08 h light).

Shrimp were obtained from the shrimp farm CEAQUA, located in the municipality of Beberibe, CE. and transported in a tank of 1,000 L, containing water from the cultivation nursery with salinity 15 and oxygen supply provided by cylinder, distributed by aerotubes with a flux of 6.0 ± 1.0 L air min^{-1}. In LTA - IFCE, shrimp were acclimated and populated in experimental tanks of 100 L with average initial weight of 5.53±0.68 g. The effluents used as a culture medium, urban sewage and fish farming were collected in sewage treatment station (STS) and fish farming (FF) of IFCE - Aracati Campus, respectively. They were transported in two 100 L tanks to the Aquaculture Technology Laboratory (LTA of IFCE – Aracati), during collection, the effluents were filtered in a 25 µm mesh to remove plankton and suspended material, then autoclaved at 120 °C for 15 minutes to eliminate microorganisms that can interfere with algal development and nutrient dynamics.

Experimental design

The inoculum for the beginning of the experiment was produced and acclimated by adding 1,250 mL of *C. vulgaris* (25%) to 3750 mL of effluents (75%) used as a culture medium, using 5.0 L erlemeyers under the same conditions of temperature, luminosity and aeration of the experiment for three days.

Microalgae were cultivated stationary (Ohse et al., 2009) with three treatments (T1 effluent from STS, T2 effluent from FF and T3 with standard medium (SM) Guillard f/2), conducted in quintuplicate for each culture medium, using a 20 L carboy for eight days. 5.0 L inoculum (25%) plus 15 L culture medium, T1 STS, T2 FF and T3 SM (75% of each) were used to start the experiment. The microalgae had an initial dry weight of 0.194, 0.196 and 0.191 g L^{-1} for STS, FF and SM, respectively. After eight days of cultivation, the algal biomass was recovered by chemical flocculation using 2N NaOH, washed to remove the fluctuant and dried in oven with air renewal at 60 °C for 24 h (Silva et al., 2015).

All material used and culture media were sterilized in an autoclave for 15 minutes at 120 °C to avoid any type of contamination. The growing conditions remained constant, with a temperature of 28 ± 1.0 °C, light intensity 108 µmol m^{-2} s^{-1}, having as light source two 40 W fluorescent lamps, aeration provided by an electromagnetic blower with a flux of 3.0 ± 1.0 L air min^{-1}, pH 7.8 and salinity 0.

The shrimp experiment was realized with three treatments in quintuplicates, completely randomized design, during 15 days. The animals were maintained in tanks of 100 L under constantly aerated by radial blower and aerotubes, resulting in a concentration above 5.0 mg L^{-1} of dissolved oxygen, in a density of 70 shrimps m^{-3}, fed four times per day (8:00, 11:00, 14:00 and 16:00 h) offering to T1 commercial feed (C-Feed) with 35% crude protein, T2 and T3 with commercial feed plus addition of 5.0% dry biomass of *C. vulgaris*, after pretreatment with sonication to break the cell wall, cultivated in effluents of FF-Feed and STS-Feed, respectively (Table 1). The T2 and T3 feed formulation followed the method described by Silva et al. (2020a).

Table 1: Nutritional characteristics of shrimp feed.

Ingredient composition (%)	Diets		
	C-Feed	FF-Feed	STS-Feed
Crude protein	35	35	35
Humidity	13	13	13
Crude fat	5.5	5.5	5.5
Fibrous matter	5.0	5.0	5.0
Mineral matter	12.5	12.5	12.5
Calcium	2.5	2.5	2.5
Phosphor	0.8	0.8	0.8
Dry biomass *C. vulgaris*	-	5.0	5.0

C-Feed – Commercial feed. FF-Feed – Commercial feed plus 5.0% dry biomass of *C. vulgaris* cultivated in Fish Farming effluent. STS-Feed – Commercial feed plus 5.0% of dry biomass of *C. vulgaris* cultivated in Sewage Treatment Station effluent.

The amount of feed started at 5.0% biomass. Animal weight was determined weekly per tank to monitor growth and adjust the amount of feed. 100% of the water in the tanks was renewed daily to maintain the water quality parameters at acceptable levels for the species.

C. vulgaris and shrimp growth performance

Daily, determined the dry biomass weight (DBW) (Lopes et al., 2020), to monitor the development of cultures. At the end of the experiment, the kinetic indices were determined: dry biomass yield (DBY) (g), biomass yield (g L^{-1}), biomass productivity (g L^{-1} day^{-1}), lipid content (%), lipid yield (mg L^{-1}) and lipid productivity (mg L^{-1} day^{-1}) The weight of the shrimp was monitored weekly to determine the growth of the animals and adjust the amount of feed offered.

The specific growth rate (K) was calculated by the Equation (1) (Ohse et al., 2009).

$$K = \frac{lnN_2 - lnN_2}{t_2 - t_1} \tag{1}$$

where N_2 and N_1 are the DBW at time t1 and t2, respectively.

At the end of experiment were calculated, mean final weight (g), daily weight gain (mg day^{-1}), feed conversion rate (FCR), survival (%), final biomass (g), productivity (kg m^{-3}) and bioremediation potential (%).

Determination of lipid content and fatty acid composition in *C. Vulgaris*

Lipid extraction and quantification of total concentration were evaluated by a modified Bligh and Dyer (1959) method and the characterization of the lipids extracted from *C. vulgaris* was done by gas chromatography (GC), using the Ce 2-66 method of the AOCS (Association of Official Analytical Chemists - AOCS, 1997) at the Laboratory of Analysis and Process Development of the Department of Chemical Engineering of the Federal University of Ceará (Fortaleza, Ceará, Brazil).

Water quality

Dissolved oxygen, salinity, pH and temperature were determined (HANNA HI 9829) twice a day (8:00 and 16:00 h) for shrimp cultures. Alkalinity, ammonia, nitrite, nitrate and orthophosphate (American Public Health Association - APHA 2012) were determined at the beginning and end for algal cultures and weekly for shrimp cultivation. After phytoremediation of urban and aquaculture effluents, nutrient concentrations were compared to CONAMA Resolution n°. 357/2005 (Brasil, 2005) and Boyd and Tucker (2014).

Statistical analysis

Analysis of variance (ANOVA) ($P \leq 0.05$) was used for analyzed water quality, *C. vulgaris* and shrimp performance. When significant differences were found between treatments ($P \leq 0.05$) used Tukey's test. All statistical data were performed using Assistat 7.6.

RESULTS AND DISCUSSION

Microalgae growth

The three tests showed similar behavior and satisfactory growth for the different culture medium, but with different productivity. In the first two days, the cells adapting to the new culture conditions. After acclimatization, the cells showed exponential growth due to the favorable conditions of light and nutrient, with highlighted to FF that got better development, followed by SM and STS, respectively, until the final in the stationary phase of cultivation with reduced growth rates (Figure 1).

According to Silva et al. (2020a), after inoculation of a microalgae in a nutrient-enriched culture medium, population grows over time, generally presents a curve with five distinct phases. This nutrient absorption capacity demonstrates the efficiency of *C. vulgaris* in the treatment of effluents, being important in the management of water resources, as the effluent discarded will respect environmental standards, reducing costs and the load of toxic and pathogenic nutrients in the environment.

In all tests, the growth rate had an initial tendency to increase until the third day of cultivation, with a tendency to decrease until the end of the experiment on the eighth day (Figure 1). Similar to the present experiment, studies such as of Katiyar et al. (2021), Zhang, Ren and Jiang (2021), Wu et al. (2020), report, in *Chlorella* sp. cultures, the daily deceleration of growth rate as the nutrients are absorbed, mainly nitrogen and phosphorus, which are essential nutrients for algae growth.

The highest specific growth rates of *C. vulgaris*, during the experiment, were achieved in FF, followed by SM and STS. There was a significant difference during the growing period between FF, STS and SM, with FF with an increase in growth rate 1.23 and 1.85 times greater than STS and SM, respectively. The growth of STS compared to SM was significantly slower, with the SM culture 1.13 times fastest than STS (Table 2). Lower concentrations of nitrogen compounds, possibly, influenced a higher growth rate, as high absorbed concentrations can be toxic to *C. vulgaris*.

Kong et al. (2021), used simulated wastewater aiming to produce biomass of *C. vulgaris*, their results presented that maximal specific growth rate of *C. vulgaris* was 0.21–0.35 d^{-1} and 0.33–0.43 d^{-1} at 90 mg L^{-1} and 60 mg L^{-1} of nitrogen source, respectively. The authors indicate in this study that a higher concentration of nitrogen source reduces the specific growth rate, especially in the first days, as algal cells are adapting to new culture conditions and possible toxicity due to excess nutrient.

Biomass and lipid yield of *C. Vulgaris*

Table 3 demonstrates that the FF and SM treatments were similar to each other and different with STS, showed higher dry biomass, biomass yield and biomass productivity, possibly, because FF and SM absorb phosphorus more efficiently, with 100% removal on both, than STS (69.7%). As phosphorus was fully absorbed from the culture environment in FF and SM, the cells did not accumulate this nutrient, reducing storage and increasing utilization, resulting in greater vegetative growth in 8 days of culture. Dry biomass, biomass yield and biomass productivity of FF and SM grew up 1.35 e 1.22 times more, respectively, than STS.

High concentrations of nitrogen and phosphorus reduce lipid synthesis, because they offer favorable cultivation conditions, not inducing stress on the cells and energy reserve in the form of lipids, which reduces the production of bioactive compounds with high economic and nutraceutical value.

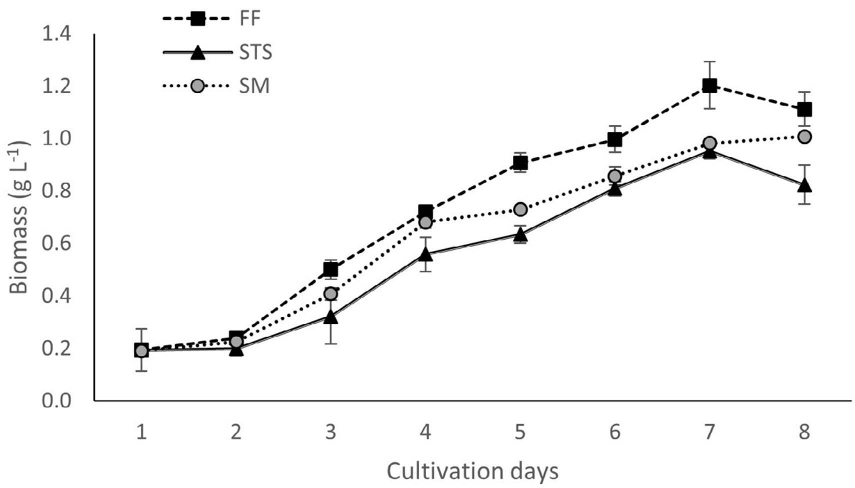

Figure 1: Growth kinetics (g L^{-1}) of three tested *C. vulgaris* under different culture medium. (FF) Fish farming effluent, (STS) Sewage treatment station and (SM) Standard medium.

Table 2: Means and standard deviation of specific growth rate (K d^{-1}) of *C. vulgaris* in different culture medium.

Time (d)	FF	STS	SM
1	0.291 ± 0.05[a]	0.044 ± 0.01[b]	0.243 ± 0.04[a]
2	0.679 ± 0.02[a]	0.363 ± 0.02[c]	0.546 ± 0.05[b]
3	0.627 ± 0.02[a]	0.508 ± 0.03[b]	0.611 ± 0.03[a]
4	0.554 ± 0.03[a]	0.427 ± 0.05[b]	0.483 ± 0.01[b]
5	0.470 ± 0.01[a]	0.412 ± 0.03[b]	0.433 ± 0.02[b]
6	0.437 ± 0.03[a]	0.382 ± 0.01[b]	0.394 ± 0.02[b]
7	0.358 ± 0.02[a]	0.298 ± 0.01[b]	0.343 ± 0.03[a]

Different letters in the same line represent statistical difference between treatments (p<0.05). FF – Fish Farming Effluent. STS – Sewage Treatment Station Effluent. SM – Standard Medium.

Carneiro et al. (2021), evaluated the growth of *Chlorella* using municipal wastewater as a nutrient source. Their results showed that the co-cultures grew well in the centrate, achieving the maximum biomass densities 1.3 g DW L^{-1} by the end of semi-continuous regime in thin-layer cascade, which increases the algal photosynthetic area, resulting in more chemical energy and cell division, on the other hand, raises production costs. In the present work, the economic costs are reduced, as it did not use laminar culture mechanisms or bioreactors, but achieving good algal and lipid productivity results. Molazadeh et al. (2019) cultivated *C. vulgaris* in urban sewage treatment plant effluent, generating maximum biomass concentration, biomass productivity of 0.7900 g L^{-1} and 0.0850 g L^{-1} d^{-1}, respectively. Even using CO_2, the authors found inferior result of biomass concentration and biomass productivity when compared to the three tests of the cultivation of *C. vulgaris* in the present study, possibly due to the inadequate N: P ratio for biomass production.

The FF treatment had the best lipid results along with SM in the lipid content and lipid productivity. The STS got the lowest lipid yield of the three treatments, followed by SM second minor lipid yield and FF showing the best result (Table 2). The cultures FF and SM accumulated more lipid content, they were improved by 1.59 and 1.35 times in comparison with SM. The lipid productivity of FF and SM were 2.16 and 1.65 times higher, respectively, than STS, possibly, because of phosphorus depletion in cultivation and energy accumulation in the form of lipids.

Katiyar et al. (2021), produced some species of *Chlorella*. Their lipid productivity of *Chlorella minutissima, C. sorokiniana,* was 36.66 and 48.33 mg L^{-1} day^{-1}, respectively, in municipal wastewater. Amini, Wang and Shahbazi (2016) evaluated the lipid productivity of *C. vulgaris* cultivated in swine wastewater, obtaining the highest lipid productivity 40.41 mg L^{-1} day^{-1}. Gao et al. (2019), used secondary effluent from municipal wastewater treatment plant to cultived *C. vulgaris*, their lipid content and lipid productivity were 13% and 25.76 mg L^{-1} day^{-1}, respectively. The three studies cited obtained superior results to the present study, possibly due to the stress of nutrient reduction, stimulating lipid biosynthesis in the cells of the *C. vulgaris* in their respective studies. Future studies are needed with the dilution of fish farm effluents to reduce the concentration of nitrogen compounds and induce lipid increase.

Characterization of the fatty acids present in *C. vulgaris*

As show in Table 4, the characterization of fatty acids from *C. vulgaris* identified a significant difference between the effluents used (FF and STS), but with similar

Table 3: Means and standard deviation of kinetic algal and lipid yield in *C. vulgaris* cultures with fish farm (FF), sewage treatment station (STS) effluents and standard medium (SM), during eight days in 20 L of cultivation.

Parameter	Effluent		
	FF	STS	SM
Dry biomass (g)	22.24 ± 0.205[a]	16.46 ± 0.188[b]	20.14 ± 0.197[a]
Biomass yield (g L^{-1})	1.112 ± 0.082[a]	0.823 ± 0.055[b]	1.007 ± 0.026[a]
Biomass productivity (g L^{-1} day^{-1})	0.139 ± 0.005[a]	0.102 ± 0.012[b]	0.125 ± 0.008[a]
Lipid content (%)	13.250 ± 0.731[a]	8.308 ± 0.317[b]	11.224 ± 0.696[a]
Lipid yield (mg L^{-1})	147.340 ± 0.015[a]	68.375 ± 0.022[c]	113.025 ± 0.018[b]
Lipid productivity (mg L^{-1} day^{-1})	18.417 ± 0.097[a]	8.547 ± 0.085[b]	14.128 ± 0.076[a]

Different letters represent statistical difference between treatments and equal letters do not differ statistically, in the same line (*p*<0.05). FF – Fish Farming Effluent. STS – Sewage Treatment Station Effluent. SM – Standard Medium.

sums of SFA and MUFA. The MUFAs showed proportions greater than 63% of the total fatty acids methyl ester (FAME) for FF and STS, with a significant increase in C16:1 and C22:1 and a reduction in C18:1 when using FF as the culture medium.

The SM obtained a considerably higher C16:0 concentration (5.04-3.51 times), resulting in a higher SFA concentration than the other treatments. Concentrations of C18:2 were similar for FF and SM, while the proportion for STS significantly increased by 27.1%. There was an increase in C18:0 of 57.4% in FF and 31.16% in STS compared to SM. The use of FF and STS effluents increased the biosynthesis of C18:0, C16:1, C18:1 and C22:1 fatty acids, while significantly reducing the synthesis of C16:0, when compared to SM. The results demonstrate that *C. vulgaris* cultivated in aquaculture and urban effluents produced a higher concentration of MUFAs and PUFAs, respectively, unsaturated fatty acids related to improved immunity (Guo et al., 2021), increased brain functions (Cisbani et al., 2021) and nervous system (Gu et al., 2021), reduced risk of cardiovascular diseases (Evangelista-Silva et al., 2021; Mezouar et al., 2016). They are also used in the formulation of drugs such as anti-inflammatory and antioxidants (Khaddaj; Morin; Rousseau, 2016; Marrapu et al., 2020), in food industries as a human/animal food supplement (Rojas et al., 2020; Hossain; Peng; Samll, 2021) and cosmetics sector such as moisturizers and lubricants (Yarkent; Gurlek; Oncel, 2020). The SFAs obtained from *C. vulgaris* biomass are important in the manufacture of waxes and oil for burning and energy production (Nirmala; Dawn, 2021; Davoodbasha et al., 2021).

Fazal et al. (2021), evaluated the lipid production of *C. vulgaris* cultivated in textile wastewater, found the SFAs yield in diluted medium higher (11.07 mg g^{-1}) than the undiluted medium (9.12 mg g^{-1}), mainly C16:0 (10.64 mg g^{-1}), C16:0 concentration similar to the present study when using effluents as a culture medium. However, when using undiluted medium, the study obtained the highest concentration of MUFAs, with emphasis on C22:1 (3.49 mg g^{-1}), concentration significantly lower than that of the present study. The best result for PUFAs was achieved when using standard medium, with 31.08 mg g^{-1} to C18:2, indicating that textile wastewater is more efficient in the synthesis of C18:2 than the effluents used in present study. Zheng et al. (2021), used *Pyropia*-processing wastewater as a culture medium to *Chlorella* sp. The *Pyropia*-processing wastewater was more efficient in the biosynthesis of SFAs and PUFAs, mainly C16:0 (22.28%) and C18:2 (17.69%), respectively, however, it synthesized less MUFAs than the effluents used in the present study. The synthesis of fatty acids depends on substrates and enzymes, can produce different SFA, MUFA and PUFA at different concentrations, expanding their use in diverse applications.

Table 4: Percentage of fatty acids in oil obtained from *C. vulgaris* cultures with fish farm (FF), sewage treatment station (STS) effluents and standard medium (SM).

Fatty acids	Effluent			Rincon et al. (2017)	Mohd-Sahib et al. (2017)	Fazal et al. (2021)	Zheng et al. (2021)
	FF	STS	SM				
Palmitic (C16:0)	7.8^c	11.2^b	39.3^a	18.15	20.4	10.64	22.28
Stearic (C18:0)	15.9^a	10.1^b	7.7^c	3.03	3.7	ND	6.22
Palmitoleic (C16:1)	13.9^a	9.5^b	6.2^c	2.71	0.4	0.43	5.34
Oleic (C18:1)	24.3^b	35.1^a	19.5^c	37.68	36.8	ND	19.51
Erucic (C22:1)	26.2^a	19.1^b	15.5^c	ND	1.2	3.49	ND
Linoleic (C18:2)	11.9^b	15.0^a	11.8^b	32.91	0.6	31.08	17.69
ΣSFA	23.7^b	21.3^b	47.0^a	21.18	24.1	10.64	30.03
ΣMUFA	64.4^a	63.7^a	41.2^b	40.39	38.4	3.92	27.58
ΣPUFA	11.9^b	15.0^a	11.8^b	32.91	ND	31.08	42.39

Different letters in the same line represent statistical difference between treatments (p<0.05).
ΣSFA: the total of saturated fatty acids; ΣMUFA: the total of monounsaturated fatty acids; ΣPUFA: the total of polyunsaturated fatty acids. ND: not detected.

Nutrient removal

Table 5 shows that the reductions in inorganic nutrients from effluents and standard culture medium were significant when using *C. vulgaris* as a phytoremediator. The cultivations of *C. vulgaris* in FF and STS were more efficient in reducing the ammonia concentration, with 80.7 and 75.7% removal, respectively. Nitrite decreased in its entirety for the three treatments, however, nitrate had similar removal, with 95.1, 92.0 and 98.6% for FF, STS and SM, respectively. Cultures with FF and SM totally absorbed phosphorus, this efficiency in phosphorus consumption is of great interest for the treatment of aquaculture effluents and the feasibility of water reuse. Unlike STS, which absorbed 69.7%, showing a significantly lower efficiency.

Microalgal growth depends mainly on three factors: light, temperature and amount of nutrients available. When considering a medium that is not nutrient-limited, the factors that interfere with algal growth are light and temperature. And the light will still depend on the photoperiod that directly influences photosynthesis, cell division and growth (Amini; Wang; Shahbazi, 2016). According to Fernández-Linares et al. (2017), even considering the same species of microalga under the same growing conditions, its growth may be variable. With this, the factors that determine the growth were all controlled during the cultivation, revealing an efficient cell multiplication, since it was provided light, temperature, and nutrients necessary for algal development. Znad et al. (2018) used *C. vulgaris* and obtained a result compared to FF, with 79% removal of total nitrogen and 100% removal of total phosphorus from effluents from wastewater treatment plants, and with alternative medium, in this same study, total phosphorus removed was 100% and for alternative medium and 79.4% total nitrogen removal rate was achieved using alternative medium as culture medium. The cultivation of microalgae reduced by up to 90.51% of total nitrogen and 91.54% of total phosphorus, improving the quality of swine wastewater, converting these nutrients into biomass that can be used in the production of biogas or syngas, and reducing possible eutrophication in the receiving bodies of this final effluent (Wen et al., 2017). These studies point to microalgae as an alternative that added value and a high nutrient removal rate, mainly of the *Chlorella* genus, which absorb the compounds under conditions of high nitrogen and phosphorus load, converting them into biomass rich in proteins, fatty acids, minerals and vitamins, in addition to producing biogas as an energy source.

Table 5: Mean and standard deviation of nutrient removal in in *C. vulgaris* cultures with fish farm (FF), sewage treatment station (STS) effluents and standard medium (SM).

Parameters	Effluent								
	FF			STS			SM		
	Initial	Final	% Removal	Initial	Final	% Removal	Initial	Final	% Removal
Ammonia (mg L^{-1})	0.088 ± 0.002	0.017 ± 0.002	80.7^a	1.486 ± 0.002	0.362 ± 0.002	75.7^a	0.220 ± 0.002	0.070 ± 0.001	68.2^b
Nitrite (mg L^{-1})	0.009 ± 0.001	0.0	100.0^a	1.345 ± 0.001	0.0	100.0^a	0.010 ± 0.001	0.0	100.0^a
Nitrate (mg L^{-1})	1.235 ± 0.001	0.061 ± 0.002	$95,1^a$	1.013 ± 0.001	0.081 ± 0.002	92.0^a	7.001 ± 0.004	0.100 ± 0.002	98.6^a
Phosphorus (mg L^{-1})	2.519 ± 0.001	0.0	100.0^a	11.872 ± 0.001	3.601 ± 0.003	69.7^b	0.411 ± 0.003	0.0	100.0^a

Different letters in the same line represent statistical difference between treatments ($p<0.05$).

Ayatollahi, Esmaeilzadeh and Mowla (2021) cultured *C. vulgaris* municipal wastewater integrated with CO_2 capture and obtained ammonia, nitrate and phosphorus removal of 99, 81.05 and 87.95%, respectively, demonstrating that *C. vulgaris* is efficient in removing nitrogen compounds and phosphorus, essential compounds for amino acid biosynthesis, cell membrane formation and cellular energy production. This result was similar to the present study, with capacity total nitrogen removal exceeding 80% and almost 70% phosphorus removal in effluents. Praveen and Loh (2016) used effluent from the tertiary wastewater and reported a high efficiency of *C. vulgaris* in the removal efficiency for ammonia, nitrate and phososrus reached as high as 95%, 53% and 89%, respectively. Different of present study, *C. vulgaris* prefered ammonia as a primary source of nitrogen, revealing that there are several factors that influence the growth and consequently the removal rate, since the microalgae incorporate nitrogen into the biomass for protein biosynthesis.

Shrimp zootechnical parameters

Regarding shrimp growth performance, significant differences was found in final biomass and yield with the following result among treatments: FF-Feed > C-Feed > STS-Feed (Table 6). Final biomass and yield of FF-Feed were 1.05 and 1.13 times greater than C-Feed and STS-Feed, respectively, C-Feed was 1.07 times greater than STS-Feed. Survival, even without significant difference, influenced the differences in final biomass and yield, as it increased these data at the end of the experiment. For the other parameters, no significant differences were found. The zootechnical indices in FF were better, possibly due to the higher lipid concentration of the biomass that influenced the shrimp immunity, higher average weight and final biomass obtaining better productivity and profitability.

Silva et al. (2020b), added 0.5% dry biomass of *C. vulgaris* to the feed and compared it with the commercial diet of *L. vannamei*. Similar to the present study, the authors found greater final biomass and productivity when adding algal biomass, with an increase of 1.05 times compared to the commercial diet. These results indicate that the addition of *C. vulgaris* to *L. vannamei* feed increases the shrimp farming's zootechnical, health and economic indices, in addition to increasing the digestibility of the feed and reducing the concentration of toxic nutrients in the environment. Silva et al. (2021), cultivated marine shrimp in two systems, one with bioflocs and the other with bioflocs plus microalgae *Navicula* sp. The microalgae system obtained final weight and yield 1.23 and 1.22, respectively, superior to the system without microalgae, demonstrating the efficiency of adding microalgae to the marine shrimp diet in terms of production and productivity gains, in addition to increasing shrimp immunity due to bioactive compounds present in the alga.

Ge et al. (2016), evaluated the effects of feed microalgae in *L. vannamei* culture. They compared the zootechnical indices when cultivating the shrimp in tanks without microalgae and tanks with *C. vulgaris*. Due to the absorption of *C. vulgaris* microalgae cells by shrimp in the microalgae tank, final weight and productivity were significantly larger (17.64 g and 4.20 kg m^{-3}, respectively) that the results of the tank without microalgae (14.30 g and 2.41 kg m^{-3}, respectively), corroborating the present study on the efficiency of *C. vulgaris* in increasing biomass and productivity in marine shrimp farming.

Water quality

Table 7 demonstrates the concentration of water quality variables in the treatments were within the ranges recommended for the marine shrimp farming (Boyd and Tucker, 2014).

Table 6: Means and standard deviation of zootechnical parameters of *L. vannamei* marine shrimp after inclusion of *C. vulgaris* in the diet.

Parameters	C-Feed	FF-Feed	STS-Feed
Survival (%)	84.06 ± 0.38^a	86.37 ± 0.21^a	80.31 ± 0.27^a
Mean final weight (g)	6.28 ± 0.18^b	6.43 ± 0.17^a	6.13 ± 0.22^a
Daily weigth gain (mg day^{-1})	0.05 ± 0.02^a	0.06 ± 0.01^a	0.04 ± 0.02^a
Final biomass (g)	369.52 ± 0.05^b	388.75 ± 0.03^a	344.61 ± 0.02^c
Productivity (kg m^{-3})	3.69 ± 0.20^b	3.88 ± 0.31^a	3.44 ± 0.29^c
FCR	1.23 ± 0.02^a	1.24 ± 0.03^a	1.24 ± 0.04^a

Different letters in the same line represent statistical difference between treatments (p<0.05). C-Feed – Commercial feed. FF-Feed – Commercial feed plus 5.0% of dry biomass of *C. vulgaris* cultivated in fish farming effluent. STS-Feed – Commercial feed plus 5.0% of dry biomass of *C. vulgaris* cultivated in sewage treatment station effluent.

Based on Boyd and Tucker (2014) and CONAMA Resolution n°. 357/2005 (Brasil, 2005), after phytoremediation with *C. vulgaris*, FF and SM showed totally efficient removal of water quality variables, enabling the reuse and discharged in accordance with current legislation, nitrogen is a limiting factor for eutrophication for freshwater classes 1 and 2, not exceeding the total nitrogen value of 1.27 mg L^{-1} for lentic environments and 2.18 mg L^{-1} for lotic environments, at the reference flow rate. Phosphorus concentrations for FF and SM, with total removal, are in accordance with current legislation for disposal of lotic, intermediate and lentic environments of class 1 for fresh water, as the maximum requirement is 0.020 mg L^{-1}. In contrast, STS was not efficient in the removal of ammonia and phosphorus, above recommended for the correct disposal of effluents (Brasil, 2005), being indicated a longer phytoremediation time for a possible reduction of these parameters (Table 8). Silva et al. (2020a), Ge et al. (2016) and Maliwat et al. (2021), demonstrate the positive effects on water quality and shrimp performance when using *C. vulgaris*, in water resources management, profitability and public health.

The study demonstrates the importance of advancing the verticalization of water use, with use in the cultivation of aquatic organisms, biotreatment, reuse and production of algal biomass for shrimp feed additive, adding value to the product, reducing environmental impacts and generating employment in the sector. These results demonstrate promising economic, social and environmental benefits of the production of microalgae for the extraction of proteins, lipids, vitamins, carotenoids and polysaccharides of high financial value and used in

Table 7: Means and standard deviations of water quality parameters of the marine shrimp *L. vannamei* culture during the experiment with addition of *C. vulgaris* in the diet.

Variable	Treatment			Boyd and Tucker (2014)
	C-Feed	FF-Feed	STS-Feed	
Dissolved oxygen (mg L^{-1})	5.24 ± 0.001	5.25 ± 0.001	5.28 ± 0.001	5.0 - 15
Salinity	25 ± 0.014	25 ± 0.011	25 ± 0.013	2.0 - 35
pH	7.96 ± 0.001	7.93 ± 0.001	7.95 ± 0.001	7.0 - 9.0
Temperature (°C)	29.69 ± 0.005	29.87 ± 0.007	29.71 ± 0.008	25-30
Alkalinity (mg CaCO3 L^{-1})	131 ± 1.569	130 ± 1.957	129 ± 1.883	> 120
Ammonia (mg L^{-1})	0.18 ± 0.05	0.19 ± 0.04	0.19 ± 0.06	< 0.2
Nitrite (mg L^{-1})	0.26 ± 0.05	0.28 ± 0.06	0.21 ± 0.05	< 0.3
Nitrate (mg L^{-1})	1.19 ± 0.04	1.24 ± 0.05	1.22 ± 0.04	0.2 ≤ 10
Phosphorus (mg L^{-1})	0.45 ± 0.05	0.40 ± 0.06	0.48 ± 0.07	< 0.5

C-Feed – Commercial feed. FF-Feed – Commercial feed plus 5.0% of dry biomass of *C. vulgaris* cultivated in fish farming effluent. STS-Feed – Commercial feed plus 5.0% of dry biomass of *C. vulgaris* cultivated in sewage treatment station effluent.

Table 8: Means and standard deviation of water quality parameters after biorremediation with *C. vulgaris*.

Variable	Treatment			Boyd and Tucker (2014)
	FF	STS	SM	
Dissolved oxygen (mg L^{-1})	6.35 ± 0.001	6.48 ± 0.001	6.41 ± 0.001	5.0 - 15
pH	8.56 ± 0.001	8.49 ± 0.001	8.53 ± 0.001	7.0 - 9.0
Temperature (°C)	29.72 ± 0.005	29.61 ± 0.007	29.77 ± 0.008	25-30
Ammonia (mg L^{-1})	0.017 ± 0.002	0.362 ± 0.002	0.070 ± 0.001	< 0.2
Nitrite (mg L^{-1})	0.0	0.0	0.0	< 0.3
Nitrate (mg L^{-1})	0.061 ± 0.002	0.081 ± 0.002	0.100 ± 0.002	0.2 ≤ 10
Phosphorus (mg L^{-1})	0.0	3.601 ± 0.003	0.0	< 0.5

FF – Fish farming effluent. STS – Sewage treatment station effluent. SM – Standard medium.

the animal and human nutrition, nutraceutical, cosmetic, biofertilizer and aquatic biotreatment industries. More studies should be developed with the addition of bioactive compounds from microalgae in the diet, for a better understanding of the zootechnical parameters and immunity of the shrimp.

CONCLUSIONS

C. vulgaris proved to be an excellent alternative to treatment of farming fish and urban wastewater, efficiently reducing nutrients, increasing water quality and enabling reuse, mitigating environmental problems such as eutrophication, in addition to effective productivity of algal biomass and benefits for the algaculture, aquaculture nutrition, wastewater treatment and water reuse sectors. The addition of *C. vulgaris* to marine shrimp feed, influenced the quantity and lipid quality of diet, with emphasis to FF-feed, which presented better zootechnical parameters, increasing productivity and profitability.

AUTHOR CONTRIBUTION

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