

Nannochloropsis oculata D. microalgae growth in a treated effluent from superintensive shrimp cultivation

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Abstract

The use of microalgae biomass in order to obtain lipids is an important alternative to be studied and it has great potential to be applied in order to produce food and biofuel, for instance. However, there are some processes of its production which need further study, such as the cultivation inputs. A possibility for an alternative raw material is the effluent from superintensive shrimp cultivation with bioflocs (BF). Therefore, the objective of this study was to evaluate the productivity and nutrient removal rate of *Nannochloropsis oculata* cultivation in three systems: (i) f/2 - produced integrally with chemical fertilizers, (ii) BF - using 100% of the effluent for superintensive shrimp cultivation with bioflocs and (iii) 50/50 - using 50% of shrimp cultivation effluents and 50% from f/2 system. The microalgae presented greater biomass growth and productivity in BF system but less lipids and esters accumulation. Concerning nutrient removal, f/2 system showed better performance, which may indicate that the cultivation in BF systems takes longer to reach the stationary growth phase.

Keywords: Microalgae cultivation. *Nannochloropsis oculata* D. Superintensive shrimp cultivation. Bioflocs.

Introduction

Due to the increasing demand for alternative power sources, the possibility of using microalgae for this purpose has become frequent in discussions concerning fuel. These microorganisms have been cultivated for commercial application in various areas such as food, chemical and pharmaceutical, and now present themselves as a potential source of raw material for biofuels production, especially biodiesel and bioethanol (ANTONI; ZVERLOV; SCHWARZ, 2007).

According to Clarens et al. (2010), algae have several more favorable characteristics that differ themselves from other biomass sources. Algae tend to produce more biomass than terrestrial plants per unit area. Moreover, unlike these plants, they can be grown in non-arable soils, using fresh or salt water and thus not competing directly with food crops (CHISTI, 2007). Another point is that algae

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can be cultivated in systems developed for CO₂ absorption and removal of certain pollutants due to its high growth rate and aquatic habitat (POWELL et al., 2008). Among the microalgae species evaluated in order to identify potential biodiesel producers stand the species from *Chlorella* and *Nannochloropsis* genus due to its high rate of lipid content (SINGH; GU, 2010).

However, the conventional microalgae cultivation uses chemical fertilizers rich in nitrogen and phosphorus as main sources of nutrients, which can be considered a restriction of this production process due to the large environmental burden generated (LARDON et al., 2009). Clarens et al. (2010) declare that if the fertilizers used in the process were replaced by partially treated effluent, there would be reduction in the environmental impacts of this process.

McGinn et al. (2011) evaluated the integration of microalgae cultivation and industrial wastewater remediation, using it as the culture medium. The paper states that microalgae present great potential for nitrogen, phosphorous and metal pulls removal from industrial and domestic effluents. It is noticed that although there are studies that mention wastewater use as an alternative to reduce the impacts of the microalgae production, technical feasibility on performing cultivation using different culture media still needs further studies.

Among the effluents possibilities to be studied as potential raw materials is the effluent from bioflocs (BFT, Biofloc Technology) production (AVNIMELECH, 2006). This technology is used in superintensive marine shrimp cultivation and is generated from the fertilization of the media with carbon sources and strong oxygenation. In the current situation, at the end of shrimp farming, the effluent is intended for treatment and subsequent release to the environment. Since this organic material has high environmental burden, especially nitrogen and phosphorus, there is a possibility of integrating marine shrimp superintensive cultivation with microalgae growth using effluent in algae cultivation in order to reduce organic matter and increase the production of these organisms. The aim of this study is to evaluate the feasibility of microalgae cultivation in a treated effluent from marine shrimp superintensive cultivation.

Material and methods

Biological material

The microalgae species chosen for this study was *Nannochloropsis oculata* D., which is a unicellular green algae present in marine environment and widely used as food source for shrimp larvae. This microalgal species has great potential for accumulation of lipids and has been studied as a potential source of fatty acids for biodiesel production (CHIU et al., 2009).

Culture media

The used culture media were: Guillard's f/2 (GUILLARD, 1975) and the liquid fraction from marine shrimp cultivation effluent, pretreated with decantation process and surface filtration. The treatments had the following configuration:

- f/2: 100% f/2 medium. The volume of each experimental unit was composed entirely of Guillard's f/2 medium.

- 50/50 medium: 50% f/2 and 50% pretreated effluent. The volume of each experimental unit was composed of equal parts of Guillard's f/2 medium and the pretreated effluent previously mentioned.

- BF medium: 100% pretreated effluent from shrimp superintensive cultivation with bioflocs. The volume of each experimental unit was composed entirely by the pretreated effluent previously mentioned.

Experimental design

The volume of each experimental unit was stored in fiberglass cylinders, with 120 L capacity. These units were filled with the corresponding culture medium (f/2, 50/50 or BF) and *N. oculata* inoculum was added so that the initial biomass was 0.1 g/L in a final 100 L volume. There were three replicates of each treatment and the experiment cylinders were kept indoors with no temperature and lighting control, with constant aeration generated by an air compressor. The growth experiment was continued until the cultures reach the stationary phase of growth (12 days).

At the end of the experiment, the algae biomass was collected from the cultivation through flocculation method, the lipid fraction was extracted and fatty acid methyl esters (FAME) contents were determined according to Zhu et al. (2002) methodology. Based on these data, the cultivations volumetric productivity was calculated, in grams per liter per day (dry weight) and the productivity per area, in grams per square meter per day (dry weight), considering a relation of 200 liters per square meter of illuminated area (surface).

Representative samples were also collected (200 ml) from each experimental unit at the beginning and at the end of the experiment and the dissolved nutrients in the experimental units were quantified. The physico-chemical parameters analyzed were Biological Oxygen Demand (BOD), nitrogen as ammonia (N-NH₃), nitrogen as nitrite (N-NO₂), nitrogen as nitrate (N-NO₃) and orthophosphate (PO₄). All procedures follow APHA/AWWA/WEF (2005).

Results and discussion

Growth and productivity

After 12 days of cultivation, it was observed that from the initial biomass (0.1 g/L), a higher biomass increase was obtained in BF medium, that showed a final concentration of 0.4 g/L. F/2 and 50/50 media showed less biomass gain, having both 0.33 g/L final biomass concentration (Figure 1).

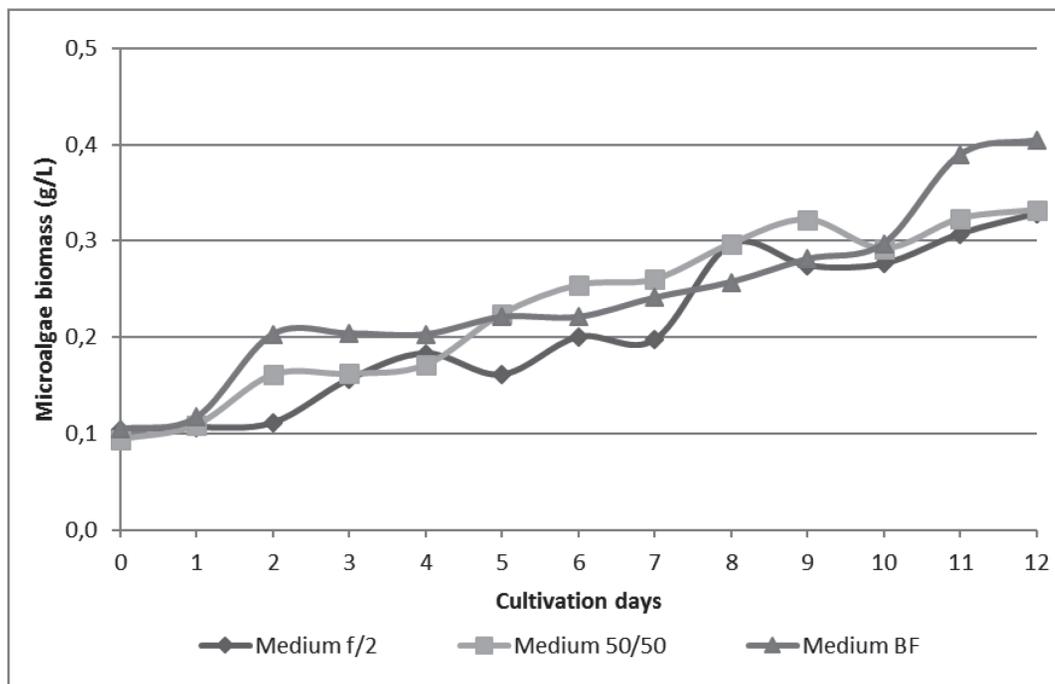


Figure 1. Biomass growth of *N. oculata* using f/2, 50/50 and BF media.

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The cultures performed in this study showed 0.4 g/L maximum biomass concentrations when the effluents from superintensive shrimp cultivation were used as medium. However, this value is lower than those described in the literature for *N. oculata*, that range from 0.5 to 1.0 g/L (Richmond, 2004).

Table 1 shows that BF medium represented the highest average volumetric productivity (0.03 g/L/d) and higher average productivity per area (5.0 g/m²/d). On the other hand, f/2 and 50/50 media showed 33.3% lower average volumetric productivity (0.02 g/L/d) and 24.4% lower average productivity per area (3.83 g/m²/d).

Table 1. Average productivity of *N. oculata* in different culture media.

Culture media	Bi ¹ (g/L)	MaxB ² (g/L)	AcB ³ (g/L)	Time ⁴ (days)	Vol prod ⁵ (g/L/d)	Prod area ⁶ (g/m ² /d)	Prod area ⁷ (t/ha/y)
f/2	0.10	0.33	0.23	12	0.02	3.83	13.99
50/50	0.10	0.33	0.23	12	0.02	3.83	13.99
BF	0.10	0.40	0.30	12	0.03	5.00	18.25

1- Biomass; 2- Maximum biomass achieved; 3- Accumulated biomass (MaxB -Bi); 4- Time in which MaxB was achieved; 5- Volumetric Productivity (AcB/Time); 6- Productivity per area m²/day (Vol prod x 200 = 200 L/m²); 7- Productivity per area in hectares/year (Prod area in m²/day x 365 days).

Source: created by the authors

It can be noticed that all the treatments from this study showed low volumetric productivity as well as low productivity per area. While the maximum volumetric productivity obtained in this study was 0.03 g/L/d, there are productivity records ranging from 0.025 to 0.125 g/L/d (FULKS; MAIN, 1991). In a similar situation to this study, cultivating the same microalgae species in fiberglass cylinders, James and Al-Khars (1990) obtained average 0.05 g/L/d volumetric productivity.

These findings are associated with the fact that in the present study artificial lighting and temperature control were not used. Therefore, the algae were submitted to natural lighting photoperiod and to changes in ambient temperature, which may have reduced growth, biomass production and therefore productivity.

Comparing the present study with others that also use natural light, it still shows lower productivity per area. In these studies, productivity greater than 10 g/m²/d (RICHMOND, 2004) was observed. However, it is important to emphasize that James and Al-Khars (1990), Fulks and Main (1991) and Richmond (2004) studies were developed in suitable structures for microalgae cultivation, both open ponds and photobioreactors, are designed in order to increase the crops illuminated surface, optimizing the photosynthesis and growth rate. The fiberglass cylinders used in this study are more suitable for crops that use artificial lighting.

Total lipids and FAME

The analysis of total lipid content in biomass presented similar average values for the three culture media. 50/50 medium showed the highest average lipid accumulation, 15.36%; the f/2 medium obtained 14.93% and BF presented 12.99% lipid content in biomass.

Several conditions are important to assess whether a raw material can be used for the biodiesel production. For microalgae, we evaluate the yield of direct transesterification, as FAME content in biomass. The 50/50 medium samples presented the highest esters levels (94.6 milligrams of esters

per gram of biomass). The f/2 medium showed 90 mg of esters per gram of biomass. In BF culture medium, the lower accumulation of this material was found, 88.8 milligrams of esters per gram of biomass.

Considering the amount of esters relatively to the total lipid and the yield of biomass per area, it is possible to observe that although there is a minor lipids and esters microalgae accumulation in BF medium, the esters productivity per area is still higher (1.62 t/ha/year) than the values found in f/2 media (1.26 t/ha/year) and 50/50 (1.32 t/ha/year). This is directly related to the increased productivity of biomass per area located between BF, as shown in Table 2.

Table 2. Productivity per area related to total lipids and FAME.

Culture Media	Prod Area (t/ha/y)	Total Lipids (% average)	FAME (% average)	Lipid Prod per Área (t/ha/y)	Ester Prod Per Area (t/ha/y)
f/2	13.99	14.93	9.00	2.09	1.26
50/50	13.99	15.36	9.46	2.15	1.32
BF	18.25	12.99	8.88	2.37	1.62

Source: created by the authors

Regarding lipid and FAME in biomass, the maximum value obtained in this study was 15.36% in 50/50 culture medium. Chisti (2007) states that, for *Nannochloropsis* sp., the percentage of accumulated lipid in biomass can vary from 31 to 68%, depending on cultivation type used. It is perceived that improvements are needed in cultures concerning productivity per area, such as adjustment of culture structures, so that they become feasible for commercial production of biodiesel.

Nutrient consumption.

After 12 days, significant reduction in nitrate (N-NO₃) and orthophosphate (PO₄) of the culture media in the three treatments was observed. BOD rates showed little variation, with an increase in 50/50 medium and decrease in f/2 and BF media. The concentrations of nitrite (N-NO₂) presented total consumption in f/2 and BF; in some samples, they were not detected.

Regarding ammonia (N-NH₃) concentration in the cultures, there was a reduction of its concentration in the three crops: 26% in f/2 (from 1.6 to 1.2 mg/l), 22.7% in 50/50 (from 1.9 to 1.5 mg/l) and 35.6% BF (from 2.3 to 1.5 mg/l). The f/2 medium presented the lowest concentrations, both initial and final, for this parameter.

BOD was reduced to 58% in f/2 culture media (from 7.7 to 3.2 mg/l) and 43% in BF (from 7.1 to 4.0 mg/l). However, the 50/50 medium had an increase of 26% in BOD (from 7.4 to 10.0 mg/l) after algae cultivation (Figure 2).

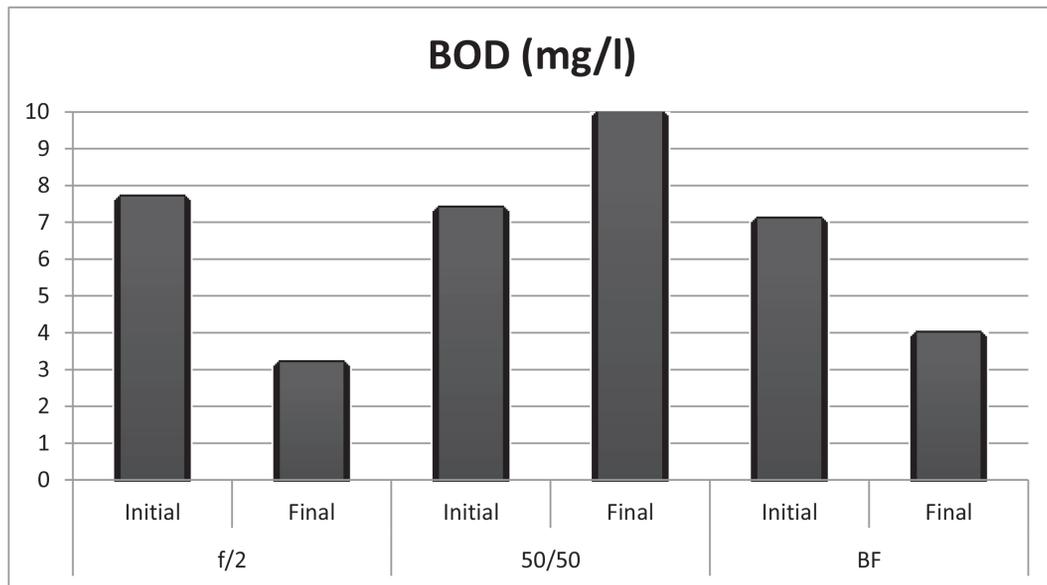


Figure 2. Biological Oxygen Demand (BOD) of cultivation systems (mg/l).
Source: created by the authors

Regarding nitrate concentration, it is possible to realize that there was nearly complete consumption of nitrate dissolved in f/2 medium (from 12.5 to 0.01 mg/l). Moreover, 50/50 and BF media showed higher final values of nutrient concentration, which means less nitrate consumption (53.7 and 22.7%, respectively). Figure 3 shows that the highest concentrations were obtained in BF system (15.32 mg/l initial and 11.9 mg/l final) and 50/50 system presented 11.1 mg/l initial and 5.2 mg/l final concentrations.

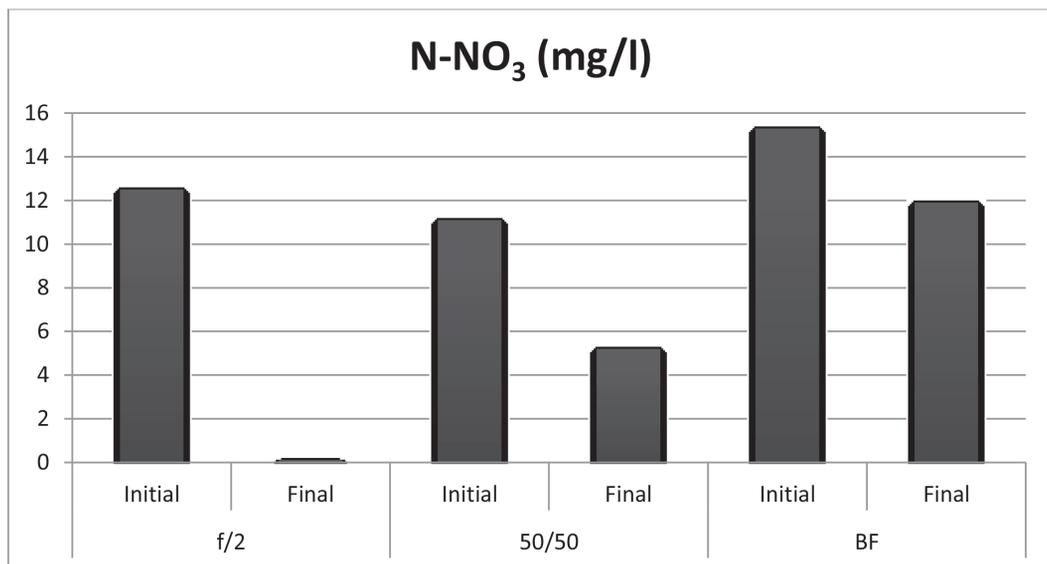


Figure 3. Nitrogen as nitrate (N-NO₃) of cultivation systems (mg/l).
Source: created by the authors

The concentrations of orthophosphate presented the most significant reduction in all three culture media, almost being completely consumed in all of them (Figure 4). The reduction in concentration was 99.2% in f/2 (from 12.5 to 0.1 mg/l), 91.7% in 50/50 (from 14.5 to 1.2 mg/l) and 94% in BF (from 16.1 to 1.0 mg/l).

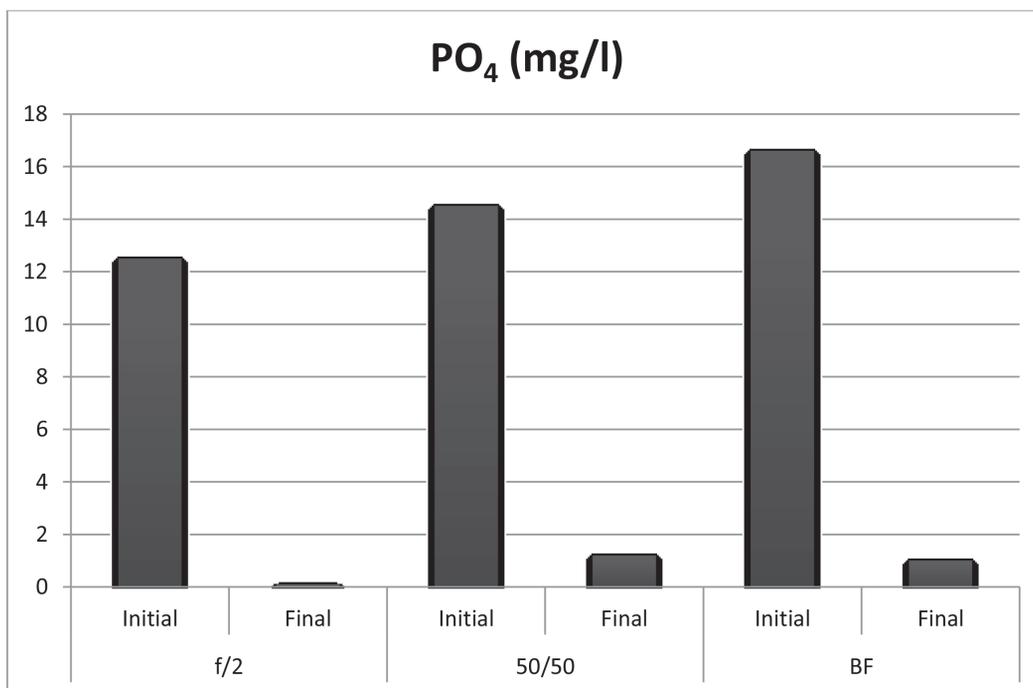


Figure 4. Orthophosphate (PO₄) of cultivation systems (mg/l).

Source: created by the authors

Observing the growth curves on Figure 1, it is possible to infer that, apparently, 50/50 and BF microalgae crops were still in the exponential growth phase when the experiment was finished. This can be further corroborated by the observation of chemical parameters, which can show that there was nutrients availability in these media at the end of the experiment, especially nitrate and phosphate. If the growth of microalgae is extended for a longer time, it is possible that higher productivity could be generated in these treatments. As for f/2 medium, we can infer that its productivity was lower than other crops because all the available phosphate was assimilated and, therefore, this nutrient may have limited the production of biomass.

Regarding to nutrient removal from the culture media, the microalgae cultivated in f/2 media presented the highest removal efficiency for all the analysed parameters, except ammonia, in which f/2 was the most efficient one. We also observed that microalgae cultivation may represent a potential nutrient remover from superintensive shrimp cultivation with bioflocs effluent, with a removal rate varying from 25 to 50%, depending on the analysed parameter.

Chisti (2007) states that the phosphate availability in the medium is one of the main limiting growth factor for microalgae. In the present study, the phosphate was totally consumed in all the cultures, but the presence of a small amount of phosphate in 50/50 and BF may indicate that the algae have not reached the stationary growth phase yet.

For all other parameters, McGinn et al. (2011) state that the removal of the dissolved nutrients in microalgae cultivation using wastewater is approximately 80%. This study obtained similar values removal in f/2 and less on 50/50 and BF. However, it is important to point out that the conditions in which treatments of this study were submitted were different from those described in the literature, especially regarding to lighting. This may have contributed to reducing the growth of microalgae and the removal of dissolved nutrients in 50/50 and BF culture medium.

Conclusions

Through this study, it was established that *N oculata* microalgae species have potential to grow in culture media that use the effluent of superintensive shrimp farming. However, the low productivity in relation to lipid content indicates that this form of cultivation is probably not feasible on a large-scale production. The results also seem to indicate that microalgae can be used to treat effluents from superintensive shrimp farming.

Regarding the gain of biomass, algae showed superior growth in BF system compared to the traditional f/2 medium growth, which uses chemical fertilizers. The option to combine these two culture media, using 50% of each in the final composition, showed similar growth and biomass gain to BF system.

The results indicate that the concentration of phosphate, nitrate, nitrite, and ammonia and BOD have been reduced in the environment after using effluent as a growth medium for microalgae. Further studies are needed to assess other physical and chemical effluent parameters and the removal of nutrients through cultivation.

Acknowledgments

This research Project would not have been possible without the support of *Universidade Federal de Santa Catarina*, especially the researchers from *Laboratório de Cultivo de Algas* and *Laboratório de Camarões Marinhos*. Special thanks to Carlos Manoel do Espírito Santo, Caio Magnotti and Rafael Arantes.

Crescimento da microalga *Nannochloropsis oculata* D. em um efluente oriundo de cultivo superintensivo de camarões

Resumo

A utilização de microalgas para a obtenção de lipídios é uma alternativa importante a ser estudada e tem demonstrado grande potencial de aplicação para a produção de alimentos e biocombustíveis, por exemplo. No entanto, há poucos estudos relacionados ao seu processo de produção, especialmente quando se refere a insumos para cultivo. Nesse sentido, uma alternativa viável para o cultivo das algas pode ser a utilização de efluentes oriundos de tanques de cultivo superintensivo de camarões. O objetivo deste estudo foi avaliar a produtividade e a taxa de remoção de nutrientes do cultivo da microalga *Nannochloropsis oculata* em três sistemas: (i) f/2 – utilizando apenas fertilizantes químicos, (ii) BF – utilizando 100% do efluente do cultivo **superintensivo** de camarões com bioflocos e (iii) 50/50 – utilizando 50% de efluentes de cultivo de camarões e 50% de meio f/2. A microalga apresentou maior crescimento em biomassa e produtividade no sistema BF, porém, menor acumulação de lipídios e ésteres. No que se refere à remoção de nutrientes, o sistema f/2 apresentou melhor desempenho, o que pode indicar que o cultivo em meio BF leva mais tempo para atingir a fase estacionária de crescimento.

Palavras-chave: Cultivo de microalgas. *Nannochloropsis oculata*. Cultivo superintensivo de camarões. Bioflocos.

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Histórico editorial

Submetido em: 04/10/2014

Aceito em: 12/02/2015

