Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Composition Characterization of Ulva Ohnoi Cultivated in a Biofloc System

Jamilly Sousa Rocha
Universidade Federal de Santa Catarina
Daniele Santos
Universidade Federal de Santa Catarina
Efrayn Wilker de Souza Candia de Souza Candia
Universidade Federal de Santa Catarina
Leila Hayashi
Universidade Federal de Santa Catarina
Claúdia Marlene Bauer
Universidade Federal de Santa Catarina
Marcelo Maraschin
Universidade Federal de Santa Catarina
Felipe do Nascimento Vieira (felipe.vieira@ufsc.br)
Universidade Federal de Santa Catarina

Research Article

Keywords: ulvan, protein, shrimp effluent, macroalgae

Posted Date: June 30th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1778076/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

This work evaluated the cultivation of the macroalgae *Ulva ohnoi* on the performance of algal biomass and changes in its proximate and biochemical composition after cultivation in a biofloc system. *U. ohnoi* was cultivated in three experimental units with aeration, heating, initial density of 6 g L^{-1} for 28 days. The water used for cultivation was collected from a biofloc matrix tank and exchanged 90% once a day throughout the experiment. Temperature, salinity and illuminance were measured twice a day. To evaluate the growth of the macroalgae, weekly weighing was performed, and the daily growth rate was calculated. The density of each experimental unit was weekly adjusted to the initial density (6 g L^{-1}), and the remain was saved for the composition analyses. Results showed an average plant growth of 1.15% day^{-1}, totaling 49.5 g of weekly biomass increase. A significant difference was observed when comparing the initial 2.64±0.3% and final 4.62±0.2% ulvan concentration, and an increase of 30.2% in protein concentration at the end of the cultivation. No statistical differences were found regarding the concentration of phenolic compounds and chlorophylls (a and b) during the weeks. An increase in flavonoid concentration was observed on days 7 and 14 (0.41±0.04; and 0.41±0.07 µg g-1 of dry weight), as well as a decrease in carotenoid concentration of approximately 41.3 %. In conclusion, an increase in protein and ulvan concentrations were observed after the macroalgae *U. ohnoi* was cultivated in the biofloc system.

Introduction

Ulva Linnaeus (1753) is one of the four oldest genera of macroalgae and with the highest number of species. This genus belongs to the Ulvaceae family, popularly known as “sea lettuce” (Guiry and Guiry 2020). Species of the genus *Ulva* are cosmopolitan, opportunistic, have a short life cycle and are predominantly marine. They occur in coastal zones and subtidal habitats, and can be found at 10 m depth, free, floating or attached to substrates (Chapman 1956; Hoffman et al. 2010; Masakiyo and Shimada 2014). The wide distribution of this macroalgae is related to its high tolerance and adaptation to the main environmental factors (light, temperature, salinity, etc.), and specimens have already been found in environments with salinity ranging from ≥ 0.5 to 49 ‰ (Rybak 2015).

Within this genus is the species *Ulva ohnoi*, considered exotic, invasive and opportunistic in several parts of the world. Its composition contains proteins, vitamins, minerals, amino acids and polyunsaturated fatty acids (Angell et al. 2015), which means that it can be used as a natural source of high value-added compounds (Bikker et al. 2016) and low-cost organic molecules such as polysaccharides (Alves et al. 2013). Therefore, it has attracted great interest from the industry for the extraction of specific compounds, which can be used in the pharmaceutical, cosmetic, biofertilizer, medicinal and animal feed areas (Plaza et al. 2009).

Ulvan is a type of sulfated polysaccharide (Lahaye and Axelos 1993) that is considered a functional ingredient as it has shown positive effects on the immune system of aquatic organisms (Peso-Eharri et al. 2012). The main pigment present in *Ulva* sp. is chlorophyll, which is involved in the photosynthesis

prosses. In addition, this genus contains accessory pigments called carotenoids, which are synthesized only by algae, plants and microorganisms (Goodwin 1962). These compounds have biological functions in these organisms acting as antioxidants, membrane stabilizers and accessory pigments in photosynthetic organisms (Guaratini et al. 2009). Aquatic organisms such as shrimp and fish do not synthesize these carotenoids, so dietary supplementation is necessary (Lorenz and Cysewski 2004). Thus, the macroalgae *Ulva* sp. can improve the nutritional value of feed in aquaculture (Calheiros et al. 2019), in addition to often acting as a nutraceutical feed ingredient (Shpigel et al. 2017; Wells et al. 2017).

Macroalgae can be cultivated in continental areas, open lagoons, ponds and laboratories (Sebök et al. 2017), presenting ideal characteristics for cultivation in aquaculture, including in the Integrated Multitrophic Aquaculture (AMTI) model. AMTI provides nutrient bioremediation capability (Bolton et al. 2009; Hoang et al. 2016), benefits to co-cultivated organisms, and economic diversification through the production of other value-added aquaculture products (Chopin et al. 2001). In fact, species of the genus *Ulva* are promising in this system, since they present considerable rates of assimilation of nutrients that are used for their growth, such as nitrogen in different forms (ammonia, nitrite, nitrate), for example (Troell et al. 2003).

IMTA and biofloc system (Biofloc Technology - BFT) has been pointed out by researchers as a viable alternative to traditional systems. Characterized by being an intensive or super-intensive cultivation system and with low water exchange (Avnimelech 2015), the biofloc system is composed of detritus, bacteria, microalgae, zooplankton, fungi, feces and exoskeletons of dead animals. The bioflocs are rich in protein and have significant amounts of micronutrients, which contributes to shrimp nutrition and increases their yield, survival, and decreases their feed conversion rate (Avnimelech and Kochba 2009; Taw 2010, Ray et al. 2012).

In BFT, ammonia is controlled within the system in two ways. One of them is the heterotrophic pathway, which is stimulated by the addition of an external source of organic carbon, in which heterotrophic bacteria assimilate inorganic nitrogen and orthophosphate, transforming them into cellular biomass (Avnimelech 2015). The other pathway is called the chemoautotrophic pathway, which oxidizes ammonia and as a result produces nitrate by nitrifying bacteria (Ebeling et al. 2006). However, despite the positive points presented by the biofloc system, there is an increase in the accumulation of feed residues, excreta and inorganic compounds in the system, since shrimp retain only a small percentage of nutrients (25% total nitrogen and 11% phosphorus). The disposal of this effluent rich in inorganic and organic nutrients is still a challenge, as it can negatively impact the environment (Crab et al. 2007; Avnimelech 2015; Avnimelech and Ritvo 2003).

Several studies have been carried out to minimize these negative effects caused by the intensification of the cultivation of aquatic organisms. In this sense, the use of macroalgae has shown great potential, since these organisms take advantage of the residual nutrients made available by animal production (Xu et al. 2008; Xu et al. 2008). Martins et al. (2020) cultivated two species of macroalgae (*U. ohnoi and U.*

fasciata) using biofloc effluent from shrimp farming as fertilizer and obtained positive results in growth and nutrient uptake in both species. The integration of *U. fasciata* in the cultivation of shrimp and mullet in a biofloc system retained phosphorus and nitrogen, in addition to increasing the concentration of chlorophyll and carotenoids without affecting the productivity of the ulvan extract (Legarda et al. 2021). According to Khoi and Fotedar (2011), the macroalgae *U. lactuca* increased the rate of conversion of nutrients into total biomass in the integrated systems (shrimps and algae) and removed part of the inorganic nitrogen dissolved in the closed aquaculture recirculation system. This removal is the result of photosynthetic activities that facilitate the absorption of nutrients from effluents, improving the water quality of cropping systems (Nelson et al. 2001).

Green algae, besides acting as agents to remove organic and inorganic nitrogen compounds from the aquatic environment, also have a high capacity for absorbing compounds such as ammonia (Cohen et al. 1991) and nitrate (Lapointe and Tenore, 1981; Naldi and Viaroli 2002). In general, macroalgae have shown promising results and aroused economic interest in several sectors, whether in the form of fresh biomass or algal extracts. For example, in a work carried out by Cruz-Suárez et al. (2010), *U. clathrata* supplementation in shrimp feed provided a positive effect on the growth rate of 60% and decreased the feed conversion rate by up to 45%. In addition, green macroalgae supplementation also improves shrimp immunity (Valente et al. 2006). In view of all the benefits mentioned above, new discoveries and functionalities in the use of macroalgae have emerged, showing the importance of knowing their biochemical and nutritional characteristics. Still, it is worth mentioning that no work was carried out to analyze possible differences in the characteristics of macroalgae cultivated in bioflocs. Therefore, this work evaluated the cultivation of the macroalgae *Ulva ohnoi* on the performance of algal biomass and changes in its proximate and biochemical composition after cultivation in a biofloc system.

Material And Methods

Macroalgae

Macroalgae were collected in the sedimentation pond of the Laboratory of Marine Molluscs of the Federal University of Santa Catarina (LMM/UFSC) in September 2021. All biomass was stored in plastic containers (20L) with water from the pond and transported to the Macroalgae Section of the Marine Shrimp Laboratory from the Federal University of Santa Catarina (LCM/UFSC). The epiphytes and any material encrusted in the macroalgae were manually removed, which were then washed with salt water. After this procedure, the algae were placed in a circular polyethylene tank with a capacity of 500 liters equipped with an aeration system with three porous stones. The temperature was gradually increased until reaching the temperature indicated in the experiment, 28°C.

Experimental design and conditions

Three experimental units were used, consisting of rectangular white polypropylene boxes with a capacity of 60 L (55.5x37.7x31 cm) and a useful volume of 40 L, containing constant aeration and a HT-1900

100W heater. The initial density of macroalgae cultivation was 6 g L^{-1} and the cultivation time was 28 days. The water used for the experiment was collected from the LCM biofloc matrix tank (capacity of 40,000 L), where 5,000 shrimp of approximately 15 g were being cultivated in a biofloc system. Shrimp were fed 4 times a day with commercial feed (35% crude protein) at a rate of 3% biomass. From this effluent, the water quality parameters were evaluated: total ammoniacal nitrogen, nitrite, pH, dissolved orthophosphate, total suspended solids, alkalinity and nitrate **(Table 1).**

The water exchange of the experimental units was performed once a day (morning), throughout the experiment, at a rate of 90% of the useful volume of the box. The biofloc from the matrix tank was taken to the experimental units through a water recirculation system coupled to an aquarium pump and a 25 µm polyester bag filter to remove solids. The pump was turned on only when the experimental units were renewed. The temperature (YSI multiparameter, Professional Plus), salinity (with a refractometer (Instrutherm®) were measured twice a day. The values of illuminance (digital luxmeter) were then converted to quantum irradiance (µmol photons m-2 s-1) by multiplying them by 0.018 (Gensler, 1986). Lighting was provided naturally inside an agricultural greenhouse with 70% shading. To assess the growth of macroalgae, weekly weighing was performed with the aid of a digital scale, with excess water removed before the procedure. The daily growth rate was calculated according to the equation GR (% day $^{-1}$) = [(Fb / Ib) $^{1/t}$ -1] x 100, where Fb (final biomass), Ib (initial biomass) and t (time), proposed by YONG et al. (2013). The density of each experimental unit was then adjusted to an initial density of 6 g L^{-1} and the remainder of the sample was saved for proximate and biochemical analyses. The proximate composition of the algae was performed according to the methods described by AOAC (1999).

(Fig. 1)

Extraction and quantification of chlorophylls and total carotenoids design

Samples of *U. ohnoi* (3g fresh weight, n = 3) were macerated in a crucible with liquid N2 and 5 ml of 80% methanol (v/v) were added to them. The material was incubated for 1 h in the dark and then centrifuged (12000 g, 10 min). Finally, the supernatant was recovered by vacuum filtration. The total contents of phenolic compounds were determined by the Folin-Ciocalteu colorimetric method (λ = 750 ηm), as described by Rhandir et al. (2002). The gallic acid standard curve was used to calculate the analyte levels (Sigma-Aldrich, St. Louis, MO, USA – 100 - 1250 µg.mL^{-1}, y = 0.0108x, r2 = 0.999). The results, in triplicate, were expressed in mg of gallic acid equivalent (C7H6O5) per g of dry mass.

Extracion and quantification of total phenolic compounds

Throughout the experiment, the following water quality parameters were monitored: dissolved oxygen and temperature twice a day (YSI 55, YSI Inc., Yellow Springs, OH, USA); salinity (YSI 55, YSI Inc., Yellow Springs, OH, USA), pH (YSI 55, YSI Inc., Yellow Springs, OH, USA), TSS, alkalinity, total ammonia and nitrite (Strickland and Parsons 1984; APHA 1995) twice a week; nitrate (HACH method 8039, cadmium reduction) and phosphate (Strickland and Parsons 1984; APHA 1995) at days 0, 25 and 49 (beginning, middle and end) of the experiment.

Extraction and quantification of total flavonoids

The total flavonoid content was determined by the colorimetric method described by Chang et al. (2002), with adaptations to algal extract carried out by the research group of the Plant Morphogenesis and Biochemistry Laboratory (LMBV). An aliquot of 0.5 mL of extract (as described in item 2.4) of each sample was used to determine the total flavonoid content in the presence of 0.5 mL of methanolic aluminum chloride solution (2%) and 2, 5 ml of ethanol P.A. The material was then vortexed and the sample remained protected from light for 1 h. Then, the absorbance reading was performed at 420 nm in a UV-Visible spectrophotometer (UV-2000A, Instrutherm). The calculation of the total flavonoid content was based on the quercetin standard curve (10 to 200 $\mu g.mL^{-1}$ – r2 = 0.99; y = 0.010x). The analyzes were performed in triplicate and the results were expressed as mg of quercetin equivalent per g of dry mass of the extract.

Ulvan extraction

The ulvan polysaccharide extract was prepared according to the methodology described by Paulert et al. (2009) with modifications. Fresh macroalgae were autoclaved following the proportion of 50 g of fresh mass to 300 mL of distilled water for two hours at 110°C. Then, the supernatant was incubated with three volumes of P.A ethanol for 48 h at -20°C, and then filtered and lyophilized for 24 h at -54°C and 0.160 mBar. Finally, the ulvan yield was calculated using the following formula: ulvan yield = [[dry ulvana (g)/fresh macroalgae (g)] x 100]/percent dry matter. For this calculation, the dry ulvan obtained from the extraction and the fresh mass used in the process were considered, and the result was divided by the dry matter of the macroalgae.

Protein quantification

The physical-chemical characterization analyzes were performed in triplicate according to the methodologies described by AOAC (1999): protein (LECO) Dumas method 990.03, conversion factor 6.25 and ether extract by Soxhlet using the 920.39C method.

Statistical analysis

All results were calculated with mean and standard deviation. The data were submitted to Shapiro-Wilk and Brown-Forsythe test to prove the prerequisites of normality and homogeneity of variances. Then, analysis of variance (one-way ANOVA) was used, followed by Dunnet's test of multiple comparisons. For the initial and final quantification of ulvan, Student's t test was used. All statistical tests were evaluated with a significance level of 5% and were performed using the GraphPad Prism version 9.2 program.

Results

Water quality

The temperature, salinity, dissolved oxygen and illuminance of the laboratory cultivation period are presented in **Table 2**.

Algal performance

(Tabela 3)

In relation to the initial condition, the macroalgae showed changes in their characteristics over the weeks, such as an increase in weight, firmer consistency, and color change, from light green to darker green. In addition to these aspects, a weekly biomass gain was observed.

(Fig. 2)

Phenolic compounds, flavonoids, Chlorophyll a and b, carotenoids from *U. ohnoi*

(Tabela 4)

No statistical differences were observed in relation to the concentration of phenolic compounds and chlorophylls a and b. Regarding flavonoids, a statistical difference was observed on days 7 and 14. It was also observed that carotenoids decreased approximately 41.3% in macroalgae cultivated after 28 days in bioflocs.

Ulvana quantification

(Fig. 3)

A significant difference was observed when comparing the initial 2.64±0.3% and final 4.62±0.2% ulvan concentration.

Protein concentration

(Fig. 4)

Statistical differences were observed in protein concentration throughout the cultivation period, with values of 14.52±0.1% at the beginning and 18.91±0.17% at the end, representing an increase of 30.2%.

Discussion

The importance of vegetative and biochemical characteristics related to the cultivation of macroalgae using water from a biofloc system was evaluated in this work. In order to obtain optimal growth, it is important to establish a positive balance between the experimental conditions of the cultivated organism (Gustavs et al. 2009) and, in the same way, the parameters of water quality are indispensable to maintain the adequate environment (Boyd and Tuker 2014). In the present work, it was possible to observe that all water quality parameters were in accordance with the references found for the species *U. ohnoi* (Notoya 1999; Ohno 1988; Angell et al. 2015). Still, it is a fact that all organisms have levels of tolerance to certain

conditions, and regarding the species *U. ohnoi*, it is known that the ideal range for the growth of the species varies between 25 and 40 ‰ (Angell et al. 2015). In this experiment, this parameter was in the limit (39-40‰), and the macroalgae was able to demonstrate tolerance and adaptive capacity.

In addition to water quality parameters, nutrient availability in crops is another important factor for maintaining growth in *Ulva* species. Imchen (2012) states that the excess of nutrients in the cultivation water of *Ulva* sp. is not harmful, but on the contrary, that its growth is correlated with their availability. The algal performance results were consistent with this author's statement, since we obtained an increase in biomass and growth during the experimental period, showing that the biofloc provides nutrients for algal development. Martins et al. (2020) cultivated two species of *Ulva* (*U. ohnoi* and *U. fasciata*) using the biofloc at a concentration of 25% as a fertilizer for algae achieved positive results for growth rate and nutrient absorption (total ammonium nitrogen, nitrate and orthophosphate) for both species.

Antioxidant compounds (chlorophylls, carotenoids, phenolic compounds and flavonoids) depend on the macroalgae lineage and the impact of physical, chemical or physical-chemical aspects of the environment. These compounds are important because of their anti-inflammatory properties, in addition the possibility of being used as alternative natural antibiotics (Peso-Echarri et al. 2012). Most biochemical studies focusing on green algae are restricted to nutritional value, and in the literature, most values of antioxidant compounds were found expressed in different units.

Eismann et al. (2020) reported a wide range of carotenoids, from 0.005 to 900 mg g^{-1} of fresh weight in *Ulva* sp, while Hong et al. (2007) found contents of 62.7 and 97.3 µg g^{-1} in *U. reticulata* and *Caulerpa racemosa*. Similarly, Legarda et al. (2020) evaluated the carotenoid concentrations of *U. fasciata* cultivated in an integrated mode with shrimp and mullet in a biofloc system and found values of 4.99±0.73 µg g^{-1} (initial) and 16.46±3.41 µg g^{-1} (final) of the fresh weight. Chakraborty and Santra (2008) also quantified the total carotenoids of four species of green algae, *Lola capillaries* (20.19±0.67 µg g^{-1}), *U. lactuca* (19.55 µg g^{-1}), *Rhizoclonium riparium* (19 .16±1.58 µg g^{-1}) and *Enteromorpha intestinalis* (18.85±0.40 µg g^{-1}), with the results being expressed in dry weight. According to Chakraborty and Santa (2008), the profile of carotenoids (vitamins) in macroalgae depend on endogenous and exogenous factor, which explains the fact that variations in values were found in the literature and in this work.

Regarding the concentration of chlorophyll, Legarda et al. (2020) found values of 72.15±12.07 µg g-1 (initial) and 294.66±16.46 µg g-1 (final) of the fresh weight of *U. fasciata* cultivated in salinity 31.6±1.15 g L^{-1} and in biofloc. Raymundo et al. (2004) found the following values for chlorophyll-a, 151.48 and 411.51 mg kg^{-1} of fresh weight, in ulvacean macroalgae collected on the coast of Santa Catarina. On the contrary, the values found in the present work were lower, and a possible explanation is that the content and presence of pigments may vary according to factors such as reproduction, growth phase, environmental changes, seasons and salinity, the latter being a limiting factor for the species in this work (Chakbraborty et al. 2010; Kakinuma et al. 2006; Kakinuma et al. 2004). Most macroalgae utilize a complex set of physiological and biochemical changes to adapt to hypersaline conditions (Kirst GO

1990). Corroborating this, Kakinuma et al. (2004) reported that the macroalgae *U. pertusa* showed changes in these aspects when cultivated under salinity fluctuations. The authors also reported that, in response to high salinity, a decrease in the total pigment content (carotenoids and chlorophyll a and b) was observed, which caused irreversible damage to the algae's photosynthetic activity.

Phenolic compounds occur naturally in terrestrial and aquatic plants, being the main responsible for antioxidant activity (Hayase and Kato 1984; Ramarathnam et al. 1986). Although this class of compounds includes the effective antioxidants, little is known about this activity in algal extracts. Fujimoto and Kaneda (1980) studied 21 species of macroalgae, and 60% of the species showed antioxidant activity to some degree. Phenol extraction yield depends on the variety of active compounds with different properties and polarities, which can be affected according to the solubility of the solvent. For the extraction of polyphenols from a plant matrix, polar solvents such as ethanol, methanol, acetone and ethyl acetate are used (Parekh and Chanda 2007). The species *U. fasciata* collected at Praia de Canasvieiras - SC, presented 635.53 mg/100 g of phenolic compounds, following a protocol using the solvents ethyl ether and methanol, according to Raymundo et al. (2004). Legarda et al. (2020) quantified, using methanol as a solvent, the phenolic compounds of *U. fasciata* grown in bioflocs and obtained values of 0.32±0.05 μg g^{-1} (initial) and 0.19±0.03 μg g^{-1} (final) of dry weight and observed a reduction of 40% at the end of the cultivation. In the present work, methanol was also used as a solvent for analysis of green algae grown in bioflocs, however, no statistical difference was observed. The different concentrations found by the authors may be the result of differences in the chemical composition between the phenolic compounds of the algae, the solvents used in the protocols or the involvement in the antioxidant activity of other compounds, such as chlorophylls and carotenoids (Raymundo et al. 2004).

Flavonoids represent one of the most important groups of phenolic compounds and are widely distributed in the plant kingdom, with more than 4200 varieties already found (Simões et al. 2007). This distribution is associated with several factors according to taxonomic classification, species variation, etc. These factors influence the metabolism and production of these compounds, resulting in different concentrations (Machado et al. 2008). Al-Malki et al. (2018) evaluated the impact of various solvents on the flavonoid yield of *U. lactuca* collected in the Red Sea - Saudi Arabia, and found different values according to each solvent used in the methodology (34.5±4.8 ethanol, 60 ±7.0 ethyl acetate, 31.2±3.3 chloroform, in mg QE/g extract). In the present work, the solvent used in the methodology was ethanol, and we obtained a statistical difference in the second and third week of cultivation. However, the biofloc, in general, did not interfere in the flavonoid yields according to the values found in the literature of algae collected in the natural environment.

Ulvan is the main water-soluble carbohydrate of members of the order Ulvales (Ray and Lahaye 1995b) and has been gaining prominence due to its activities such as anti-carcinogenic, anti-proliferative, antiviral, antioxidant, antihypertensive, anti-inflammatory, anticoagulant, among others (Collén et al. 2011; Costa et al. 2010; Glasson et al. 2017; Karnjanapratum and You 2011; Qi et al. 2012). According to Castelar et al. (2014), no differences were found in ulvan yield (17.7±0.05%) in sea and pond cultures of

the macroalgae *U. flexuosa*. Legarda et al. (2022), when evaluating the productivity of ulvan extract in the macroalgae *U. fasciata* cultivated in bioflocs, found values of 2.74% (initial) and 2.24% (final). In the present work, cultivation of *U. ohnoi* in bioflocs increased the concentration of this polysaccharide in macroalgae by 75%, a very promising result. For aquaculture, ulvan has potential as probiotics for aquatic animal, in addition to functionalities in feed efficiency, immunostimulant action and improvement in the health of fish (Peso-Echarri et al., 2012).

In the biofloc system, nutrients such as nitrogen and phosphorus accumulate, and macroalgae are able to absorb and transform this nitrogen into protein (Duke et al. 1989). Our results clearly show this, since the macroalgae had 30% more protein in their final composition. The same was found by Legarda et al. (2020), who cultivated the alga *U. fasciata* in bioflocs and obtained an increase of 182% in the concentration of nitrogen in its composition at the end of the cultivation. Similarly, Shahar and Guttman (2021) reported that the macroalgae *U. fasciata* absorbed 88% of all final dissolved inorganic nitrogen from an effluent from a mullet (*Mugil cephalus*) pond. This high nitrogen content found in the works occur because macroalgae can store inorganic compounds in their tissues to be used later, for example, for their growth (Fong et al. 2001, 2003; Vidotti and Rollemberg 2004).

Conclusion

The macroalgae *U. ohnoi* showed growth in the effluent of shrimps cultivated in bioflocs as well as alterations in its composition, with an increase in ulvan and protein concentration.

Declarations

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. Leila Hayashi, Marcelo Maraschin and Felipe Vieira received productivity research fellowships from CNPq.

Data availability statement

The authors declare that the data supporting the findings of this study are available within the article and its supplementary information files.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Author contribution

Jamilly Sousa Rocha- Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing,

Daniele Santos - Investigation and data analysis;

Efrayn Wilker de Souza Candia - Investigation;

Leila Hayashi - Conceptualization, Formal analysis;

Claúdia Marlene Bauer - Formal analysis (macroalgae composition);

Marcelo Maraschin - Formal analysis (macroalgae composition);

Felipe do Nascimento Vieira - Conceptualization, Formal analysis, Resources, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

References

1. Al-Malki AL, Barbour EK, Al-Zahrani MH, Moselhy SS (2018) Impact of Various Solvents on Yield and Activity of Phenolics and Flavonoids of Ulva lactuca (Chlorophyta) Algae. J Pharm Res Int 24(3):1–7.
2. Alves RA, Sousa RA, Reis RL (2013) A practical perspective on ulvan extracted from green algae. J Appl Phycol 25: 407–424.
3. Angell AR, Mata L, Nys R, Paul NA (2015) Indirect and direct effects of salinity on the quantity and quality of total amino acids in Ulva ohnoi (Chlorophyta). J Phycol 51(3):536–545.
4. AOAC, Association of Official Analytical Chemists. Official Methods of Analysis. 16th edn. OMA, Arlington, 1999.
5. Avnimelech Y (2015) Biofloc Technology. Baton Rouge, Louisiana: A Practical Guide book, 3th. The World Aquaculture Society.
6. Avnimelech Y, Kochba M (2009) Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using 15N tracing. Aquaculture 287:163–168.
7. Avnimelech, Y, Ritvo G (2003) Shrimp and fish pond soils: Processes and management. Aquaculture 220(1–4):549–567.
8. Bikker P et al (2016) Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. J Appl Phycol 28:3511–3525.
9. Bolton JJ, Robertson-Andersson DV, Shuuluka D, Kandjengo L (2009) Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a swot analysis, J Appl Phycol 21:575–583.
10. Boyd CE, Tucker CS (2014) Handbook for aquaculture water quality. Craftmaster Printers, Auburn, 439.
11. Calheiros AC et al (2019) *Ulva* spp. as a natural source of phenylalanine and tryptophan to be used as anxiolytics in fish farming. Aquaculture 509:171–177.

12. Castelar B, Reis RP, Calheiros ACS (2014). *Ulva lactuca* and *U. flexuosa* (Chlorophyta, Ulvophyceae) cultivation in Brazilian tropical waters: recruitment, growth, and ulvan yield. J Appl Phyc 26:1989–1999.
13. Chakraborty K, Lipton AP, Raj RP, Vijayan KK (2012) Antibacterial labdane diterpenoids of Ulva fasciata Delile from southwestern coast of the Indian Peninsula. Food Chem 119(4):1399–1408.
14. Chakraborty S; Santa SC (2008) Biochemical composition of eight algae colleted from Suberdan. Ind J Mar Scienc 37(3):329–332.
15. Chang CC, Yang MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10(3):178–182.
16. Chapman VJ (1956) Marine Algae of New Zealand. Part I, Myxophyceae and chlorophyceae. J Linn Soc London (Botany) 55(360):333–501.
17. Chopin T et al (2001) Integrating seaweeds into marine aquaculture systems: a key toward sustainability. J Phycol 37(6):975–986.
18. Cohen I; Neori A (1991) *Ulva lactuca* Biofilters for Marine Fishpond Effluent. I. Ammonium Uptake Kinetics and Nitrogen Content. Bot Mar 34:475–482.
19. Collén PN et al (2011) Ulvan Lyases Isolated from the Flavobacterium Persicivirga ulvanivorans Are the first Members of a New Polysaccharide Lyase Family. J Biol Chem 286:42063–42071.
20. Costa L (2010) Biological activities of sulfated polysaccharides from tropical seaweeds. Biomedicine & Pharmacotherapy 64: 21–28.
21. Crab R et al (2007) Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270(1–4):1–14.
22. Cruz-Suárez LE et al (2010) Shrimp: *Ulva* co-culture: A sustainable alternative to diminish the need for artificial feed and improve shrimp quality. Aquaculture 301(1–4):64–68.
23. Duke CS, Litaker W, Ramus J (1989). Effects of temperature, nitrogen supply, and tissue nitrogen on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum*. J Phycol, 25(1):113–120.
24. Ebeling JM, Timmons MB, Bisogni JJ (2006) Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture. 257(1–4):346–358.
25. Eismann AI, Reis RP, Silva AF, Cavalcanti DN (2020) *Ulva* spp. carotenoids: Responses to environmental conditions. Algal Research 48:101916.
26. Fong P, Kamer F, Boyer KE, Boyle KA (2001) Nutrient content of macroalgae with differing morphologies may indicate sources of nutrients to tropical marine systems. Mar Ecol Prog Ser 220:137–152.
27. Fong P, Boyer KE, Kamer F, Boyle KA (2003) Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Mar Ecol Prog Ser 262:111–123.

28. Fujimoto K; Kaneda T (1980) Screening test for antioxigenic compounds from marine algae and fraction from *Eisenia bicyclis* and *Undaria pinnatifida*. Bull Japan Soc Sci Fisheries 46:1125–1130.
29. Gensler WG (1986) Advanced agricultural instrumentation: design and use. Dordrecht: Martinus Nijhoff Publishers. 480.
30. Glassom CRK et al (2017) Cascading biorefinery process targeting sulfated polysaccharides (ulvan) from *Ulva ohnoi*. Algal Research 27(12):83–391.
31. Goodwin TW (1962) Carotenoids, their comparative biochemistry, 3ª ed, Chemical Pub. Co., New York, 378.
32. Guaratini T, Cardozo KHM, Pinto E, Colepicolo P (2009) Comparison of Diode Array and Electrochemical Detection in the C30 Reverse Phase HPLC Analysis of Algae Carotenoids. J Braz Chem Soc 20(9):1609–1616.
33. Guiry MD, Guiry GM (2020) AlgaeBase World-wide electronic publication, National University of Ireland, Galway.
34. Gustavs L, Schumann R, Eggert A, Karsten U (2009) In vivo growth fluorometry: Accuracy and limits of microalgal growth rate measurements in ecophysiological investigations. Aquatic Microbial Ecology 55(1):95–104.
35. Hayase F; Kato H 1984 Antioxidative components of sweet potates. J Nutr Sci Vitaminol (30):37–46.
36. Hiscox, JD; Israelstam GF (1979) A Method for Extraction of Chlorophyll from Leaf Tissue without Maceration. J Bot 57(12):1332–1334.
37. Hoang TH et al (2016) Colour changes of greenlip abalone (*Haliotis laevigata* Donovan) fed fresh macroalgae and dried algal supplement. Aquaculture 456:16–23.
38. Hoffmann LC, Nettleton JC, Neefus CD, Mathieson AC (2010) Cryptic diversity of *Ulva* (Ulvales, Chlorophyta) in the Great Bay Estuarine System (Atlantic USA): introduced and indigenous distromatic species. Eur J of Phycol 45(3):230–239.
39. Hong DD, Hoang MH, Son PN (2007) Seaweeds from Vietnam used for functional food, medicine and biofertilizer. J App Phyc 19(6):817–826.
40. Imchen T (2012) Recruitment Potential of a Green Alga *Ulva flexuosa* Wulfen Dark Preserved Zoospore and Its Development. Plos One 7(3):32651.
41. Kakinuma et al (2006) Physiological and biochemical responses to thermal and salinity stresses in a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta). Mar Biol 149:97–106.
42. Kakinuma M (2004) Salinity stress responses of a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta). Fish Scien 70(6):1177–1179.
43. Karnjanapratum S, You SG (2011) Molecular characteristics of sulfated polysaccharides from Monostroma nitidum and their in vitro anticancer and immunomodulatory activities. Int J Biol Macr 48:311–318.
44. Khoi LV, Fotedar R (2011) Integration of western king prawn (*Penaeus latisulcatus* kishinouye, 1896) and green seaweed (*Ulva lactuca* Linnaeus, 1753) in a closed recirculating aquaculture system.

Aquaculture 322–323:201–209.

45. Kirst GO 1990 Salinity tolerance of eukaryotic marine algae. Annu. Rev. Plant Physiol Plant Mol Biol 40:21–53.
46. Lahaye M, Axelos MAV (1993) Gelling properties of water-soluble polysaccharides fromproliferating marine green seaweed (*Ulva* spp.). Carb Pol 22(4):261–265.
47. Lapoint BE, Tenore KR (1981) Experimental outdoor studies with *Ulva fasciata* Delile I Interacion of light and nitrogen on nutrient uptake, growth, and biochemical composition. J Exp Mar Biol Ecol 53:135–152.
48. Legarda EC et al (2020) Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. Aquaculture 534:736265.
49. Cysewski GR, Lorenz RT (2004) Industrial Production of Microalgal Cell-mass and secondary Products – Species of High Potential: Haematococcus. In: RICHMOND, A. (ed.) Handbook of Microalgal Culture: biotechnology and applied phycology. Blac Sci, UK, 281–288.
50. Machado H et al (2008) Flavonoids and potential therapeutic. Bol Cent Biol da Reprod 27(1/2) 33–39.
51. Martins MA et al (2020) Cultivation of the seaweed *Ulva* spp. with effluent from a shrimp biofloc rearing system: Different species and stocking density. Bol Inst Pesca 46(3):602.
52. Masakyio Y, Shimada S (2014) Species diversity of the genus *Ulva* (Ulvophyceae, Chlorophyta) in Japanese Waters, with special reference to Ulva tépida Masakiyo et S. Shimada sp. Bull Natl Mus Nat Sci Ser B 40:1–13.
53. Naldi M, Viaroli P (2002) Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). J Exp Mar Biol Ecol 269(1):65–83.
54. Nelson SG et al (2001) Cultivation of *Gracilaria parvispora* (Rhodophyta) in shrimp-farm effluent ditches and floating cages in Hawaii: a two-phase polyculture system. Aquaculture 193(3–4):239–248.
55. Neori A (2008) Essential role of seaweed cultivation in integrated multi-trophic aquaculture farms for global expansion of mariculture: An analysis. J Appl Phycol 20:567–570.
56. Notoya M (1999) Utilization of *Ulva* spp. and environmental restoration. Seizandou, Tokyo 137–143.
57. Ohno M (1988) Seasonal Changes of the Growth of Green Algae, *Ulva* sp. in Tosa Bay, Southern Japan. Mar. fouling 7(1–2):13–17.
58. Parekh J; Chanda SV (2007) In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk. J Biol 31:53–58.
59. Paulert R et al (2009) Effects of sulfated polysaccharide and alcoholic extracts from green seaweed *Ulva fasciata* on anthracnose severity and growth of common bean (*Phaseolus vulgaris L.*). J Plan Dis Prot 116:263–270.

60. Peso-Echarri P et al (2012) Polisacáridos de algas como ingredientes funcionales en acuicultura marina: alginato, carragenato y ulvano. J Bio Mar Ocea 47(3):373–381.
61. Plaza M, Herrero M, Cifuentes A, Ilbánêz E (2009) Innovative Natural Functional Ingredients from Microalgae. J Agric Food Chem 57(16):7159–7170.
62. Qi H et al (2012) Synthesis and antihyperlipidemic activity of acetylated derivative of ulvan from *Ulva pertusa*. Int J Biol Macr 50: 270–272.
63. Ramarathnam H, Osawa T, Namiki M, Tashiro T (1986) Studies on the relaionship between antioxidative activity of rice hull and germination ability of rice seeds. J Sci Food Agric 37(8):719–726.
64. Ray AJ et al (2012) Effects of Biofloc Reduction on Microbial Dynamics in Minimal-exchange, Superintensive Shrimp, *Litopenaeus vannamei*, Culture Systems. J World Aquaculture Soc 43(6):790–801.
65. Ray B; Lahaye M (1995b) Cell-wall polysaccharides from the marine green alga *Ulva* "rigida" (Ulvales, Chlorophyta) Chemical structure of ulvan. Carb Res 274:313–318.
66. Raymundo MS, Horta P, Fett R (2004) Antioxidant in vitro activity of extracts of some green seaweed (Chlorophyta) from southern Brazilian coast. Rev Bras Cienc Farm 40(4).
67. Rybak AS (2015) Revision of herbarium specimens of freshwater Enteromorpha-like *Ulva* (Ulvaceae, Chlorophyta) collected from Central Europe during the years 1849–1959. Phytotaxa 218(1):001–029.
68. Sebök S, Herppich WB, Hanelt D (2017) Development of an innovative ring shaped cultivation system for a land-based cultivation of marine macroalgae. Aquacultural Engineering 77:33–41.
69. Shahar B, Guttman L (2021) Integrated biofilters with *Ulva* and periphyton to improve nitrogen removal from mariculture effluent. Aquaculture 532:736011.
70. Shpigel M et al (2018) The sea urchin, Paracentrotus lividus, in an Integrated Multi-Trophic Aquaculture (IMTA) system with fish (Sparus aurata) and seaweed (Ulva lactuca): Nitrogen partitioning and proportional configurations. Aquaculture 490:260–269.
71. Simões CMO et al (2007) (Org.). Farmacognosia: da planta ao medicamento. 6. ed. Porto Alegre: Editora da UFSC e UFRGS.
72. Taw N (2010) Biofloc Technology Expanding At White Shrimp Farms-Biofloc Systems Deliver High productivity with sustainability The advocate:Global Aquaculture Alliance 24–26.
73. Troell M et al (2003) Integrated mariculture: asking the right questions. Aquaculture 226(1–4):69–90.
74. Valente L M P et at (2006) Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 252(1):85–91.
75. Vidotti EA; Rollemberg MCE (2004) Algae: from aquatic environment economy to bioremediation and analytical chemistry. Quím Nova 27(1):139–145.

76. Wellburn AR (1994) The Spectral Determination of Chlorophylls a and b, As Well As Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. J Plant Phys 307–313.
77. Wells et al (2017) Algae as nutritional and functional food sources: revisiting our understanding. J Appl Phycol 29:949–982.
78. Xu Y, Fang J, Tang Q, Lin J, Le G, Liao LV (2008) Improvement of water quality by the macroalga, *Gracilaria lemaneiformis* (Rhodophyta), near aquaculture effluent outlets. J World Aquaculture Soc 39(4).
79. Xu Y, Fang J, Wei W (2008) Application of *Gracilaria lichenoides* (Rhodophyta) for alleviating excess nutrients in aquaculture. J Appl Phyc 20(2):199.
80. Yong YS, Yong WTL, Anton A (2013) Analysis of formulae for determination of seaweed growth rate. J App Phyc 25:1831–1834.

Tables

Table 1. Table 1. Physical-chemical parameters of water quality analyzed weekly from the biofloc matrix tank.

BFT parameters	Day			
	7	14	21	28
Total ammonia nitrogen – N (mg L^{-1})	0.09	0.16	0.10	0.20
Nitrite (mg N-NO2- L^{-1})	0.08	0.10	0.08	0.19
Nitrate (mg N-NO3- L^{-1})	52.4	57.2	62.05	65.65
Dissolved orthophosphate (mg P-PO43- L^{-1})	2.81	3.47	4.12	5.26
Total suspended solids (mg L^{-1})	505.5	520	584.5	566.5
pH	7.74	7.72	7.64	7.69
Alcalinity (mg L^{-1})	181	196	185	184

Table 2. Temperature (°C), salinity (‰), dissolved oxygen (mg L^{-1}) and lux: average, minimum (min) and maximum (max) during 28 days of *U. ohnoi* cultivation in laboratory. Values are represented as mean ± standard deviation (n = 3).

Temperature (°C)			Salinity (‰)		Dissolved oxygen (mg L^{-1})		Photon irradiance (µmol photons m^{-2} s^{-1})	
Day	Mean	Min – Max	Mean	Min – Max	Mean	Min – Max	Mean	Mín – Máx
7	27.5±0.39	26.3-29.4	40±0.00	39-40	7.2±0.21	6.4-7.9	15.3±10.4	12.8-17.1
14	27.9±1.60	26.0-29.8	39±0.52	39-40	7.7±0.43	7.1-8.5	11.4±124.3	5.5-18.4
21	28.0±1.45	26.8-29.9	39±0.00	38-40	7.6±0.16	7.0-8.3	11.7± 168.9	4.5-18
28	26.9±0.64	25.5-28.2	40±0.00	38-41	7.6±0.10	7.0-8.2	8.6±74.4	3.4-16.7

Table 3. Biomass produced and weekly growth % day^{-1} of *U. ohnoi* cultivated for 28 days in biofloc. Data are presented as arithmetic mean ± standard deviation (n=3).

U. ohnoi	Day 0	Day 7	Day 14	Day 21	Day 28
Initial biomass(g)	240±0.0	-	-	-	-
Accumulated final biomass (g)	-	259±9.3	312±43.9	287±24.9	300±37.0
GR (% day^{-1})	-	1.33±0.5	1.49±1.1	1.00±0.4	0.78±0.4

Table 4. Concentration of phenolic compounds, flavonoids, chlorophylls a and b, and carotenoids from *U. ohnoi* cultivated for 28 days using water from a biofloc system. The values represent the mean ± confidence interval (n = 3) and the lower case letters represent the significant differences between the days of cultivation ($p < 0.05$).

	Day 0	Day 7	Day 14	Day 21	Day 28
Phenolic compounds (mg EAG.g^{-1} of dry weight)	0.24±0.01 [a]	0.33±0.2 [a]	0.27±0.1 [a]	0.19±0.03 [a]	0.19±0.01 [a]
Flavonoids (mg EQ.g^{-1} of dry weight)	0.29±0.0 [a]	0.41±0.04 [b]	0.41±0.07 [b]	0.34±0.05 [a]	0.32±0.03 [a]
Chlorophyll a (µg g^{-1} of dry weight)	78.55±4.4 [a]	63.45±13.6 [a]	53.45±36.4 [a]	71.80±47.3 [a]	80.60±28.2 [a]
Chlorophyll b (µg g^{-1} of dry weight)	70.15±3.6 [a]	57.70±14.1 [a]	62.85±49.4 [a]	80±44.4 [a]	72.40±25.5 [a]
Carotenoids (µg g^{-1} of dry weight)	32.20±2.8 [a]	29.25±3.6 [a]	19.55±11.4 [a]	24.50±1.4 [a]	18.90±1.5 [b]

Figures

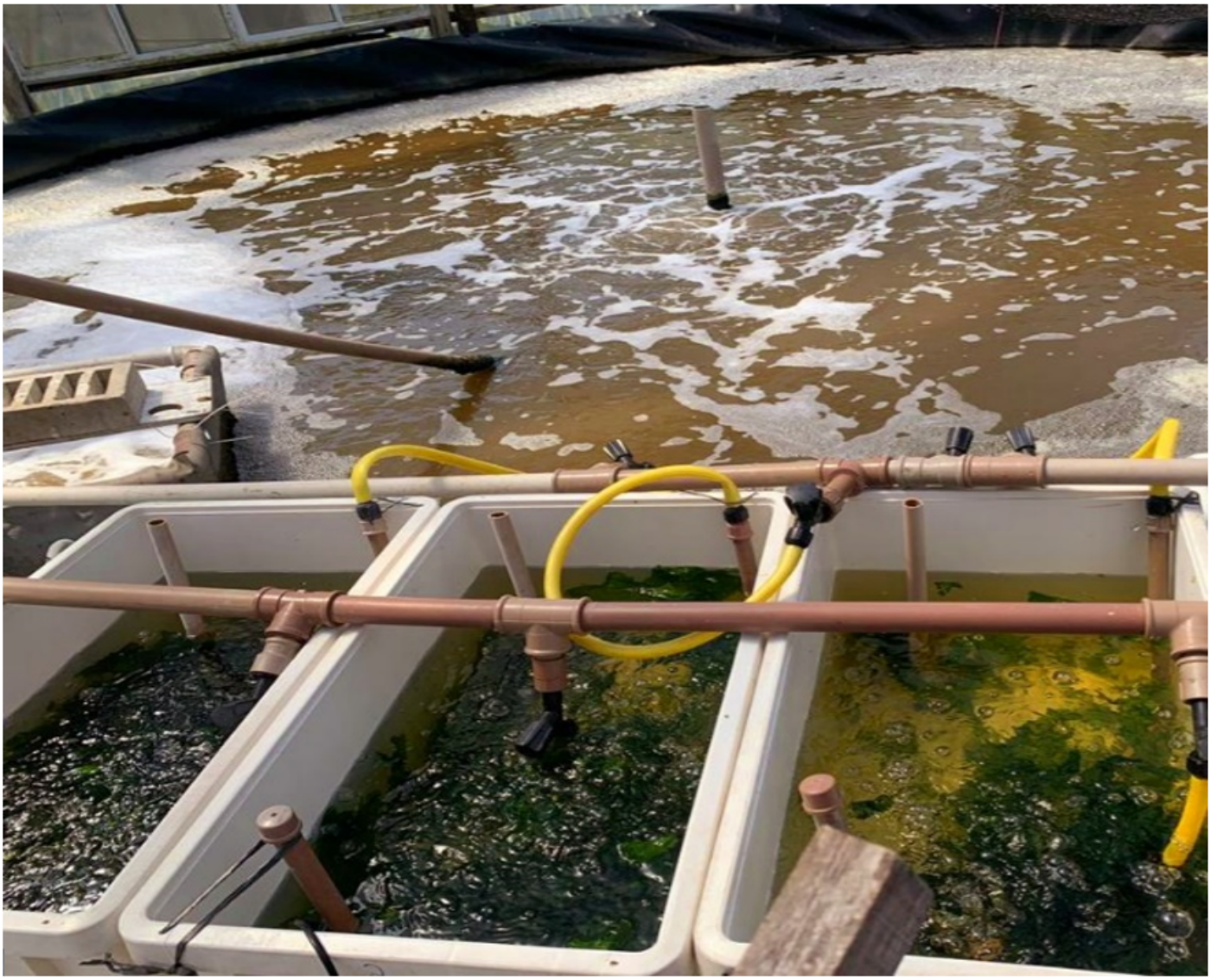


Figure 1

Experimental design used to evaluate the cultivation of *Ulva ohnoi* (6 g L^{-1}) with the effluent from the biofloc matrix tank.

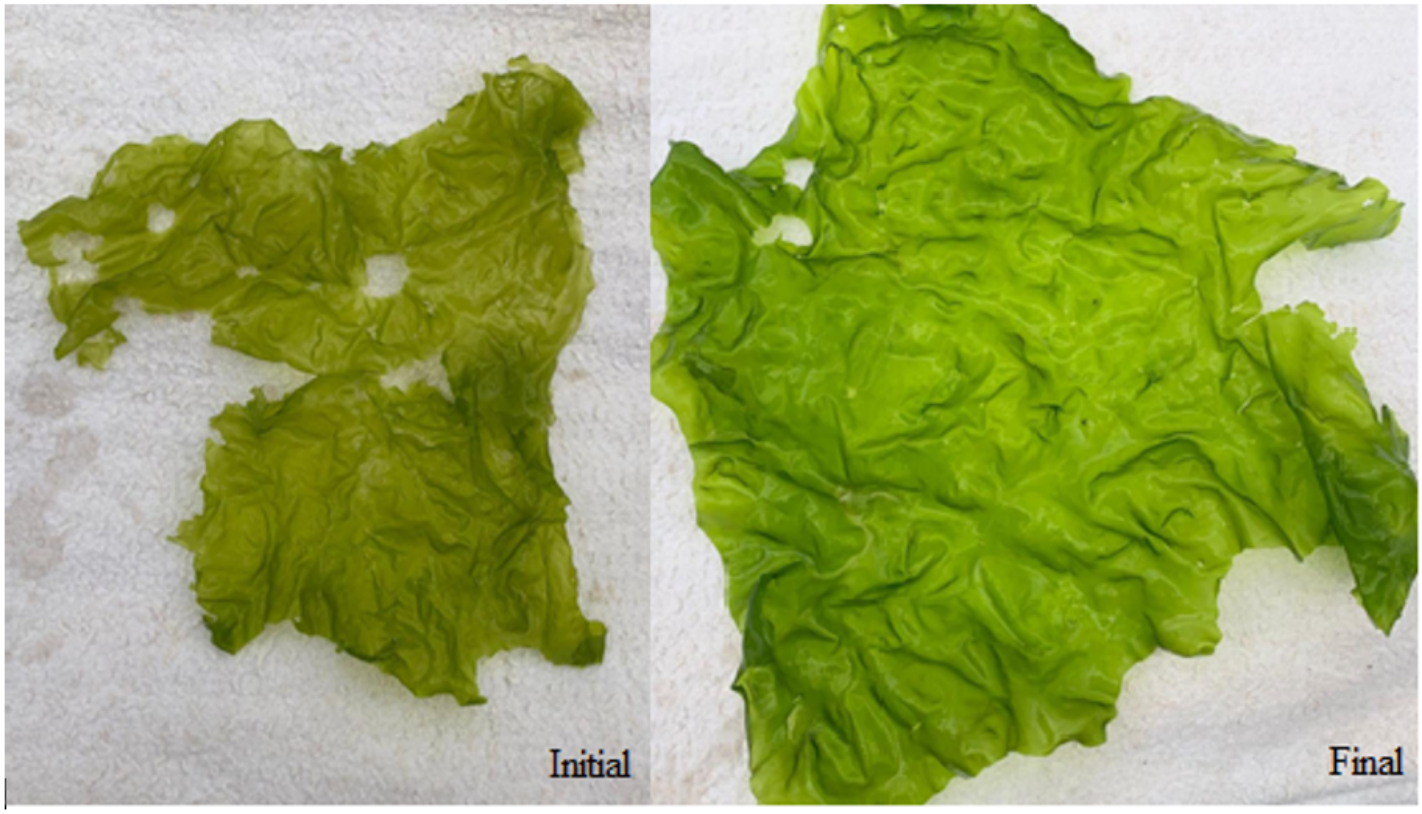


Figure 2

Ulva ohnoi before and after being cultivated during 28 days in effluent from a biofloc system

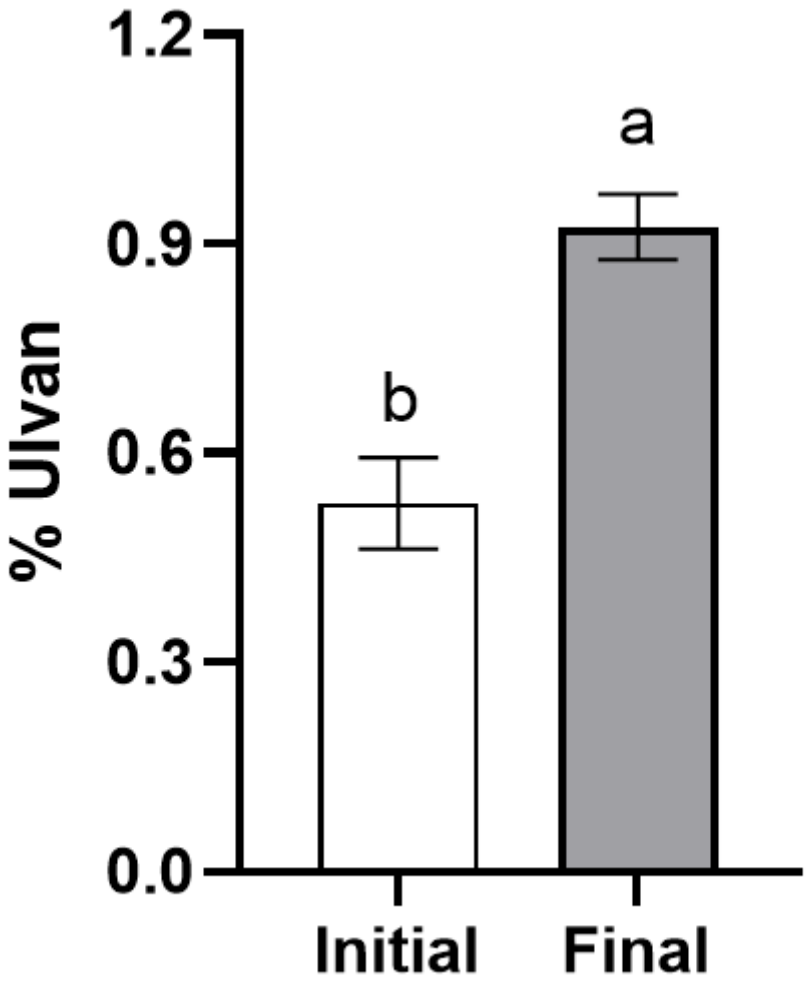


Figure 3

Yield of *U. ohnoi* ulvan at the beginning and end (after 28 days) of the culture period in water from a biofloc system. Values represent the mean ± confidence interval (n = 3) and the lowercase letters represent significant differences on initial and final yield ($p < 0.05$).

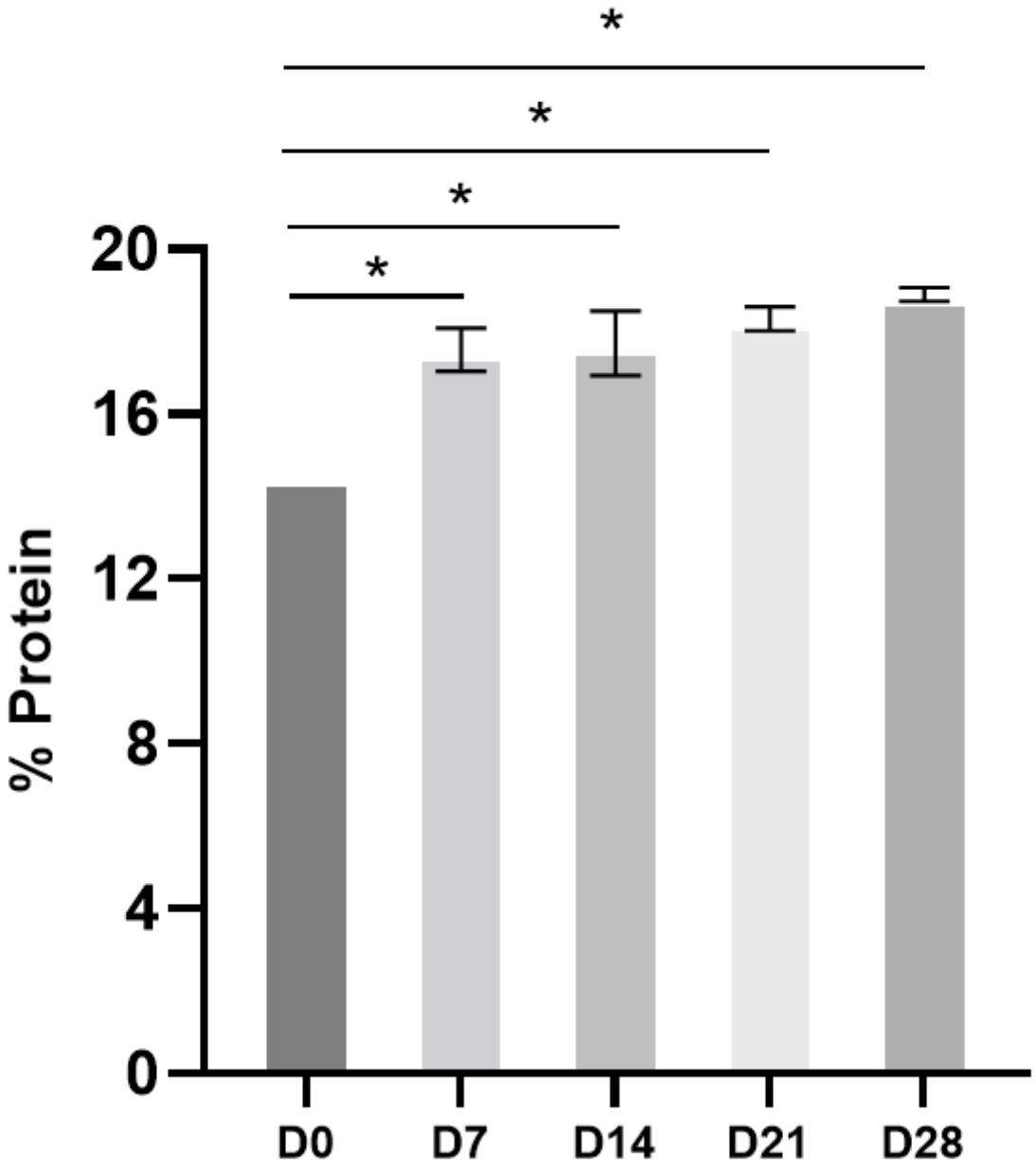


Figure 4

Protein concentration of *U. ohnoi* during the 28-day culture in biofloc. Values represent the mean ± confidence interval (n = 3) and *** represent the significant differences between day 0 compared to later days ($p < 0.05$).