WITHDRAWN: Nutrient removal effectivity of seaweeds as biofilter and water pollution control in hybrid grouper aquaculture wastewater at low salinity

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Research

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Abstract

This research aims to determine the performance and nutrient removal effectivity of seaweeds species, namely *Ulva fasciata., Sargassum illicifolium, Gelidium sp.,* and *Dictyota sp.* as a biofilter in hybrid grouper aquaculture wastewater at low salinity (14-17 ppt). This study used a Completely Randomized Design with 3 replications. The statistical analysis was carried out by Analysis of Variance continued with the Least Significance Difference test, and the Kruskal-Walis test with the Mann-Whitney analysis, and T-test with a confidence level of 95%. The concentrations of ammonia, nitrate, phosphate, and water quality parameters were observed every 4 days. The seaweeds uptake of N and P in water, and Specific Growth Rate were also observed. The results showed that *U. fasciata* was able to reduce ammonia by 75.95% and nitrate by 79.53%, which were the highest compared to other treatments, while *Dictyota* sp. was able to reduce the highest phosphate by 87.5% for 20 days. The highest SGR was achieved by *U. fasciata* at 1.91 %day^{-1}. The highest N content uptake by seaweeds was 104.4%, and the total P of 182.3% occurred in *U. fasciata*. Overall, *U. fasciata* has the highest performance and effectiveness as a biofilter that is able to reduce nutrient waste in low-salinity from grouper aquaculture, for re-circulation or before being discharged into the environment to reduce eutrophication and harmful Algal Blooms (HABs) in aquatic environment. The highest growth rate of *U. fasciata* can be used as food with highly nutritional and economical value.

Background

The need for animal protein derived from fish is increasing, FAO reported that the world fish consumption was 156 million tonnes in 2018 [1]. Therefore, in order to meet these needs, cultivation efforts are carried out including the groupers' species. The Indonesian government increases grouper cultivation to meet the domestic and export demand, as well as reduce natural catchment to maintain its sustainability. The Indonesian Ministry of Maritime Affairs and Fisheries reported that, there has been an increase of this species in 2012 by 11,950 tons to 70,294 tons in 2017. Grouper is a hybrid fish (*Epinephellus fuscoguttatus-lanceolatus*), widely cultivated because of its fast growth, high tolerance for salinity [2;3], and relatively resistant to disease [4]. Furthermore, it can be cultivated in the highlands (393 m above sea level) far from the coast, at a relatively low salinity of around 14-17 ppt.

However, the increasing grouper culture without proper waste management before being discharged into open water cause eutrophication, due to the accumulation of leftover feed and faeces. The use of "pellet" feed in intensive modern cultivation has created new problems in the waters [5], such as triggering eutrophication, due to the input of excessive nitogen and phosphorus into the water, which leads to harmful algal blooms (HABs). HABs threaten biodiversity, habitat loss, and increase the harmful algal blooms' frequency [6], as well as having impact on the social, economic, and health of coastal communities. The ingrated multi-trophic aquaculture (IMTA) is an effort to overcome this problem through the use of seaweeds as an inorganic extractive species [7;8]. Seaweeds biofilter is the most preferred wastewater treatment technology in marine fish aquaculture, because it is eco-friendly, abundantly available, and cheap [9]. Therefore, it is important to sellect appropriate seaweeds as inorganic extractive species for reducing nutrient wastewater, increasing water quality and fish growth.

Seaweed utilization in biofilter and bioremediator have been widely studied, such as *Ulva, Palmaria palmata, Gracilaria, Kappaphycus, and Hypnea aspera* [8;10;11;12;13;14;15;16;17;18;19;20]. Seaweeds are reported to have the ability to reduce nutrients in water. However, their activity in IMTA as an inorganic extractive species must be considered its suitability between the main commodity (*finfish*), and the environmental conditions of the aquaculture. Besides, the effectivity as biofilter of each species is different capability [19], dependent on volume and circulation of water, initial biomass, water quality and the physiology of each seaweeds [20]. This is because each seaweeds has different tolerance for life suitability, therefore, it affecting its ability to carry out nutrient removal, life survival, and growth process. Therefore, the hybrid grouper aquaculture which cultivated in the highlands at a salinity of 14-17 ppt, also requires appropriate seaweeds as a biofilter that able to tolerate these water conditions, and works efficiently. This research aims to determine the most effective species of *Ulva fasciata, Sargassum illicifolium, Gelidium sp.,* and *Dictyota sp.* as biofilter in hybrid grouper cultivation at low salinity. The result of this research is expected as a method to reduce nutrient wastewater, so it can be used for recirculating water, and reducing eutrophication in open water system.

Materials And Methods

Collection and acclimation

Seaweed species of *U. fasciata., Chaetomorpha* sp.*, Caulerpa* sp.*, S. illicifolium, Padina* sp.*, Gracilaria* sp.*, Gelidium* sp.*,* and *Dictyota* sp. were collected from the intertidal area of Tenggole Beach, Gunungkidul, Yogyakarta. Then cleaned of any attached animals and epiphytes. The acclimation process was carried out in stages, from the salinity of 30 ppt, continued with 25 ppt, then 20 and 15 ppt for 3 days, respectively. This process was performed because the grouper culture wastewater has a salinity of 14-17 ppt, then the water quality was observed and aerated in the closed system. After the acclimation process, only *U. fasciata, S. illicifolium, Gelidium* sp.*,* and *Dictyota* sp. survived, therefore, these four species were used for the main test.

Main Test

Wastewater from grouper culture was obtained from hybrid grouper farm which is cultivated in mountainous areas (393m height above sea level), and then soaked in 15 aquariums which each volume was 15 L. Every treatments used 3 g L^{-1} of each seaweeds that has been acclimated before was tested as biofilter in grouper culture wastewater, in a closed system with aeration to determine its performance and effectiveness to remove nutrients wastewater for 20 days. The treatments consisted of P0 (Control), P1 (*U. fasciata*), P2 (*S. illicifolium*), P3 (*Gelidium* sp.), and P4 (*Dictyota* sp.). Water quality parameters were observed using water quality checker including temperature, pH, salinity, Total Dissolved Solid (5 in 1 meter, PH-9909) and Dissolved Oxygen (DO Meter Lutron-5519). Total Suspended Solids (TSS) was calculated by filtration method using Whatman GF / C filter paper, and then compared between initial and final weight. Ammonia, Nitrate, and Phosphate contents were tested using the Visicolour ECO test kit method and a Compact photometer PF-12. Nutrient uptake efficiency was calculated using the following formula :

$$\text{Nutrient removal effectivity in water (\%)} = \frac{Water\ N0 - Water\ Nt}{Water\ N0}\ x\ 100$$

Total N content in seaweed was tested using the Kjeldahl method, while for total P with the Spectrophotometry method.

The absorption of N and P by seaweed is assumed by the percentage of N and P added in the seaweed thallus with the following formula:

$$\text{Addition of N or P in thallus (\%)} = \frac{Cf - Ci}{Ci}\ x\ 100$$

Where Cf and Ci =final and initial concentration

Specific growth rate (SGR) seaweeds was calculated using the formula :

$$SGR\ (\%day^{-1}) = [(\ln Wf - \ln Wi)/t] \times 100\%$$

Where Wf and Wi =final and initial weight, t is period of time.

Statistical Analysis

The data collected were analyzed using SPSS (Statistical Package for the Social Sciences) version 18.0. The statistical analysis was conducted by performing the normality and homogeneity test to determine the parametric or non-parametric statistical assessment. Furthermore, the ANOVA test was used to observe the significant difference between the treatments in each water quality parameter, for the data that was normally distributed and homogeneous was tested using the LSD (*Least Significant Difference*) analysis. While, the data that were not normally distributed and not homogeneous was tested using the Kruskal- Walis analysis with further T-test or Mann-Whitney test. A confidence level of 95% (α = 0.05) was used for all statistical tests.

Result

3.1. Water Quality Parameter

The water quality parameter during the experiments with four different species as biofilter of hybrid grouper aquaculture wastewater was shown in table 1. Water temperature, salinity, Total Dissolved Solid (TDS), and Total Suspended Solid (TSS) showed no significant difference. Water temperature ranges from 27-29 ºC during the experiment. The overall salinity gradually increased from 14 to 17 ppt, due to the evaporation, also with TDS. The TSS showed the lowest decrease (0.38-0.66 mgL^{-1}) on day 8th from 2.53 ± 0.11 mgL^{-1} on day-0.

Table 1. Water quality parameter during experiments with four different species as biofilter of hybrid grouper aquaculture wastewater

Treatment	Parameter					
	Temp. (°C)	Salinity (ppt)	pH	DO (mgL^{-1})	TSS (ppm)	TDS
P0 (Control)	27-29	14,4-16,4	7,2-8,2	3,2-9,8	0,23-2,53	16,43-18,13
P1 (*U. fasciata*)	27-29	14,3-14,4	7,3-8,2	3,3-11,6	0,20-2,37	16,23-18,57
P2 (*S. ilicifolium*)	27-29	14,4-16,2	7,1-8,1	2,6-10,5	0,32-2,57	16,30-18,17
P3 (*Gelidium* sp.)	27-29	14,5-16,6	7,2-7,9	2,9-10,1	0,17-2,67	16,00-18,50
P4 (*Dictyota* sp.)	27-29	14,5-16,6	7,3-8,3	3,1-11	0,36-2,59	16,13-18,43

The dissolved oxygen and pH showed significant differences between treatments. The dissolved oxygen in all treatments after day 4th showed decreasing to 3 mgL^{-1}, this was caused by the activity of bacteria to decompose wastewater that needed oxygen. This DO concentration then increased after the 4th day. The highest DO occurred on the 12th day of treatment with *U. fasciata* until it reached 11.37 mgL^{-1} that indicating a significant difference from others. After increasing on days 12th and 16th, the DO decreased to an average of 6.1 mgL^{-1} on day 20th (Fig. 1a.).

3.2 Nutrient Removal

The concentration of ammonia in wastewater with *U. fasciata* treatment on day 4th was successfully reduced to 0.57 ± 0.04 mgL^{-1} (63.92%), which was the lowest compared to others. In all treatments, there was an increase in ammonia on the day 16th, and then decreasing on the day 20th until 73.88% (Fig. 2a).

The concentration of nitrate in water in the *U. fasciata* treatment increased until day 4th, and then consistently decreased until it reached 1.47 ± 0.3 mgL^{-1}, or reduced the nitrate by 79.49% on day 20th. Meanwhile, other treatments showed that the increase in nitrate concentration occurred up to day 8th (Fig.

2b). This increase occurred because, ammonia at that time was still experiencing a change to become ammonium, nitrite, and nitrate which took a certain time because it was influenced by environmental factors, such as temperature, pH, and dissolved oxygen.

Phosphate concentration during treatment showed a consistent decreasing until the 20th day. However, *Dictyota* sp*.* showed the fastest decreasing in phosphate concentration on day 12th that reached 0.73 ± 0.09 mgL^{-1} (81.75%), and the lowest was 0.50 ± 0.06 mgL^{-1} (87.50%). Meanwhile, *U. fasciata, Gelidium* sp., and *S. illicifolium* were able to reduce phosphate concentrations by 83.05%, 75.61%, and 70.27% on the 20th day, respectively (Fig. 2c).

The largest percentage of ammonia reduction in water was shown by the treatment of *U. fasciata*, followed by *S. illicifolium, Gelidium* sp*.* and *Dictyota* sp. However, the percentage reduction of ammonia in the water showed no significant difference in all the treatments. The highest percentage of nitrate reduction in water was shown in the treatment of *U. fasciata* that can reduce nitrate 79.53%, although it was not significantly different with *S. illicifolium* and *Gelidium* sp*.*. Furthermore, the largest percentage reduction in phosphate was shown by *Dictyota sp.* That can reduce phosphate until 87.50%. Eventhough it was not significantly different with *U. fasciata* (83.05%), however it was significantly different from the control, *S. illicifolium*, and *Dictyota* sp (Figure 3.)

3.3. Content of N total and P total in thallus

Figure 4 showed that the percentage of the total P content addition in thallus seaweed tends to be higher than that of total N content. Overall, *U. fasciata* has the most effective absorption of N and P in wastewater because it has the highest addition of total N and P contents in thallus. Meanwhile, *Gelidium* sp. has the lowest N and P absorption compared to *U. fasciata*, *Dictyota* sp., and *S. illicifolium.*

3.3 Specific growth rate (SGR)

U. fasciata has the best growth rate at 1.91% day^{-1}, followed by *Dictyota* sp at 0.36% day^{-1}, and *Gelidium* sp at 0.25% day^{-1}. While *S. illicifolium* has the lowest SGR -1.71% day^{-1} (Figure 5) because some thalli were fragmented, therefore effect on the final weight of seaweed .

Discussion

Treatments that used *U. fasciata* and *Dictyota* sp. has a relatively higher DO than the control, because the these species have a better metabolism, survival, and photosynthesis process, which then increased DO and reduced the dissolved carbon dioxide in aquaculture wastewater. Meanwhile, the DO concentration with *S. illicifolium* and *Gelidium* sp. Treatments were lower than the control, because the metabolic process was disrupted to adapt to the conditions of wastewater quality, therefore the photosynthesis process was not optimal. *U. fasciata* and *Dictyota* sp., which were able to increase DO than other treatments, also showed a tendency to have higher in pH (Fig. 1b). This was happened because these seaweed was able to produce DO so that increase water pH, while concentration of dissoved CO_2 which causes acidity tend to decrease in water [21]. Therefore, *U. fasciata* and *Dictyota* sp. have capability to balance the pH in acidic water conditions, which are less favorable for aquatic organisms.

The increasing of ammonia occured until day 16th , it might be caused that on the 16th day the process of converting ammonia to ammonium and nitrate was disrupted due to a decrease in water temperature which reduces the ability of nitrifying bacteria to decompose ammonia. This was reinforced by [22] that nitrogen uptake is efficient and reaches its maximum in summer when air and water temperatures increase. Also, the ability of nutrient uptake by the biofilter was influenced by temperature and water exchange rate at various environmental conditions [23]. Biofilter treatment using *U. fasciata* showed significantly different results compared to other treatments (control; *S. illicifolium*; *Gelidium* sp.; and *Dictyota* sp.). Meanwhile, *U. fasciata* showed the most efficient performance to reduce nitrate from day 4th, while other seaweeds treatments indicated a decrease after day 8th . The same result was also shown by [24], which stated that *Ulva* spp was able to reduce nutrients in the form of nitrogen more efficiently than *Gracilaria birdiae*. This decrease in nitrate concentration indicated that the nitrate in the water has been absorbed by seaweeds, which role as nutrient removal. *Ulva* absorbs dissolved inorganic nitrogen for less than 1 hour and stores nitrate as an internal reserve in the thallus [15].

The addition of the total N and P content in seaweed thallus was assumed as its ability to absorb or remove nutrients in the water. Therefore, the greater percentation of the total N and P addition, the more effective the seaweed in absorbing and utilizing these nutrients for metabolism and growth. The highest percentage of the total N addition was shown by *U. fasciata* (104.4%), while the lowest was indicated by *Gelidium* sp. (2,6%). The high concentrations of ammonium and nitrate in water trigger an increase in the accumulation of total dissolved protein [18]. Therefore the nitrogen content in the thallus of *Hypnea Aspera* also increased, even though not all absorbed nutrients were assimilated and stored. This was because all nitrates and ammonium returned to the water in other forms. The percentage of the total P content addition, tends to be higher than that of total N content addition to thallus seaweed. This is occurred because the concentration of phosphate in water was higher than that of ammonia. Therefore, the phosphate absorbtion was higher than the nitrate. The highest percentage of total P content addition of the thallus was found in *U. fasciata* (182.3%), while the lowest was in the *Gelidium* sp. (9.3%). However, not all absorbed inorganic phosphate was used for growth, also, it increase the concentration of pigments, protein, and phosphorus in the thallus [18].

1. *fasciata* has the highest Specific Growth Rate because it has the best ability to deal with unfavorable wastewater conditions. This was in accordance with the statement that *Ulva* has a high tolerance and good environmental adaptability, to fluctuations in irradiance, temperature, salinity, and drying [24;25]. This was also confirmed by [26] which stated that *Ulva* survives in low salinity conditions. Furthermore, during this study *U. fasciata* also has the highest ability to absorb nutrients compared to other seaweeds, and is able to utilize these absorbed nutrients for metabolic processes properly, therefore it has the highest of Specific Growth Rate (SGR) than other treatments. *S. illicifolium* has the lowest SGR because some parts of the thallus had died, which was characterized by the thalus being easily fragmented and flabby that affect on the final weight and SGR. Temperature and salinity

are environmental limiting factors that affect the growth, carbon, and nitrogen content of the thallus [27]. *S. illicifolium* during this study was unable to withstand with the conditions of low salinity, consequently became non efficient in absorbing nutrients, and resulted death in some parts of thallus. Some tips of Gelidium thalli also became green, while some tips of *Dictyota* sp. thalli also became pale and fragile. This change in colour to a pale whitish is often referred to as ice-ice disease, which is triggered by stress in aquatic environmental conditions that are not in accordance with the tolerance for its life. Therefore, in this case, some tips of *Dictyota* sp. thalli were stricken with ice-ice disease, and fragmented because they were unable to tolerate the condition of hybrid grouper wastewater. The salinity of hybrid grouper wastewater tended to be low (14-17 ppt), while naturally *Gelidium*, *Dictyota*, and *Sargassum* inhabit in salinity waters above 25 ppt. This condition impact in the life survival, therefore, it was easily infected by viruses and bacteria which further the thallus health became worse. Some parts of the thallus that experienced the ice-ice disease are usually followed by the attachment of epiphytes, which are green moss that sticks to the dead white surface of the thallus. The same incident also occurred in *Gracilaria chilencis* which infected with filamentous algae when tested in the IMTA system with red abalone [28]. Meanwhile, *U. fasciata* did not show any epiphytic infection. Therefore, *U. fasciata* was able to grow optimally and did more efficient in absorbing nutrients than *S. illicifolium*, *Gelidium* sp., and *Dictyota* sp..

Conclusion

Ulva fasciata is the most effective biofilter for the nutrient removal from wastewater grouper aquaculture compared to *S. illicifolium, Gelidium* sp, and *Dictyota* sp.. *U. fasciata* shows the best and most efficient performance as a biofilter, besides easily to obtain, more tolerate with various conditions, resistant to epiphytic attack, and also has a high growth rate. Therefore, *U. fasciata* become a promise candidate species that can be integrated in IMTA system to realize sustainable environmentally friendly fisheries, and as a water pollution control in the open water system to reduce eutrophication.

Declarations

Availability of data and materials

The authors declared that the data and materials in this manuscripts are not publicly available due to originality reasons but are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no competing interests

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Authors' Contributions

For authors contribution : RIA conducted and coordinated all the research and publication; M and NP arranged the research flow and conducted the research; TBS and RYS analyzed and interpreted data

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Figures

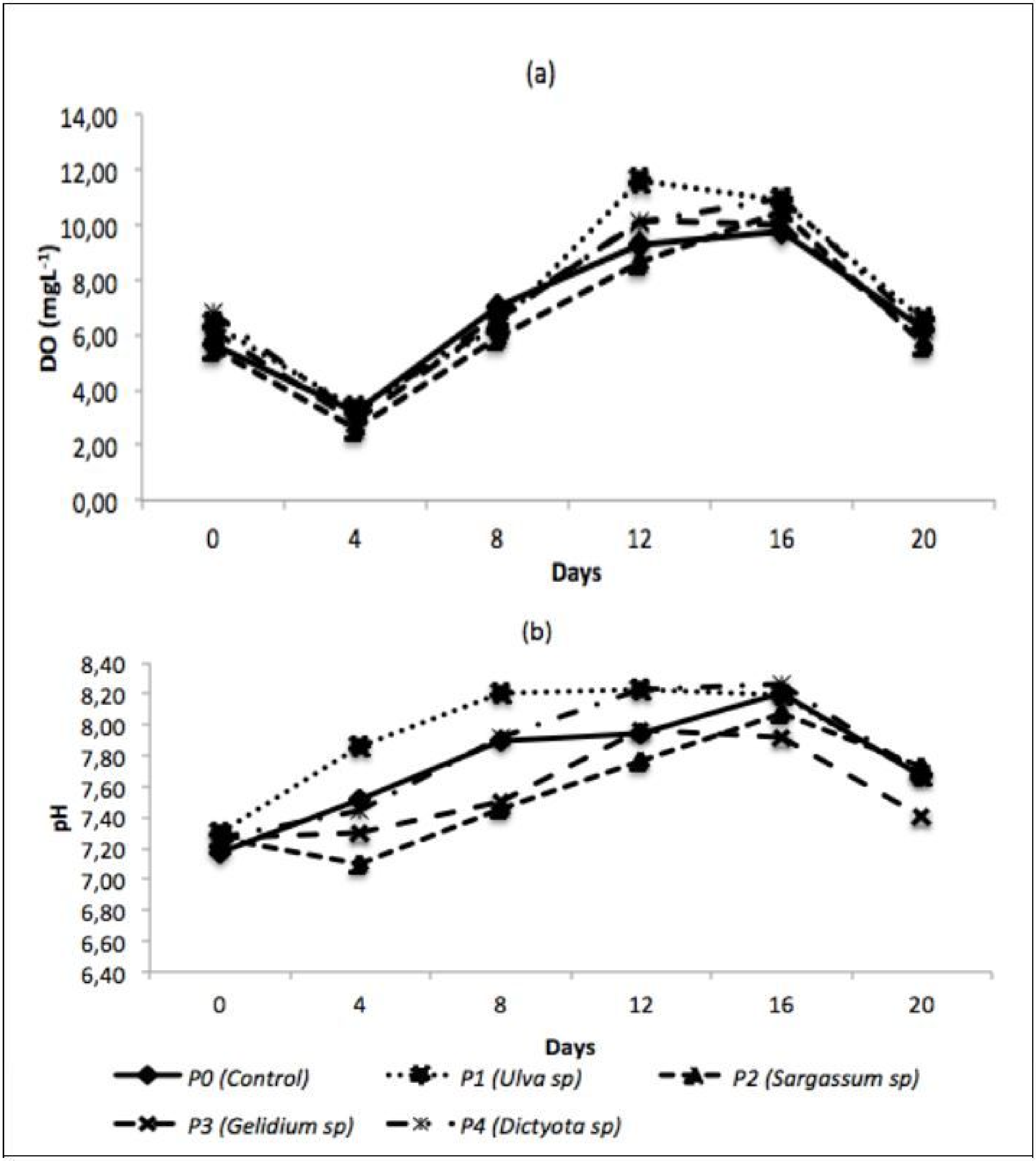

Figure 1

Dynamic of (a) pH and (b) Dissolved Oxygen during experiments with four different seaweeds as biofilter of hybrid grouper aquaculture wastewater in low salinity

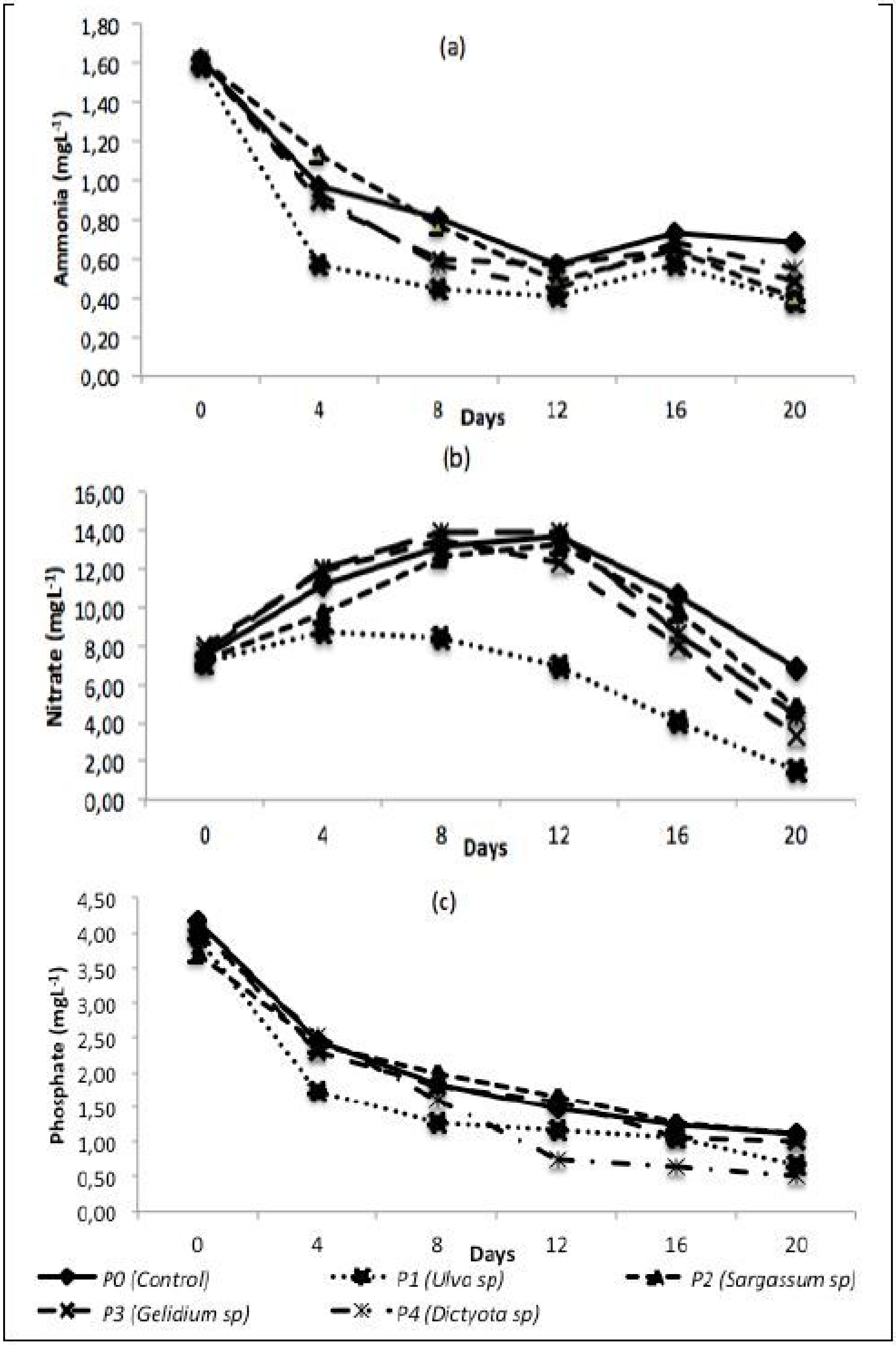

Figure 2

The dynamical of (a) Amonia (NH3), (b) Nitrate (NO3-), and (c) Phosphate (PO43-) concentration during experiment with four different seaweeds as biofilter of hybrid grouper aquaculture wastewater in low salinity.

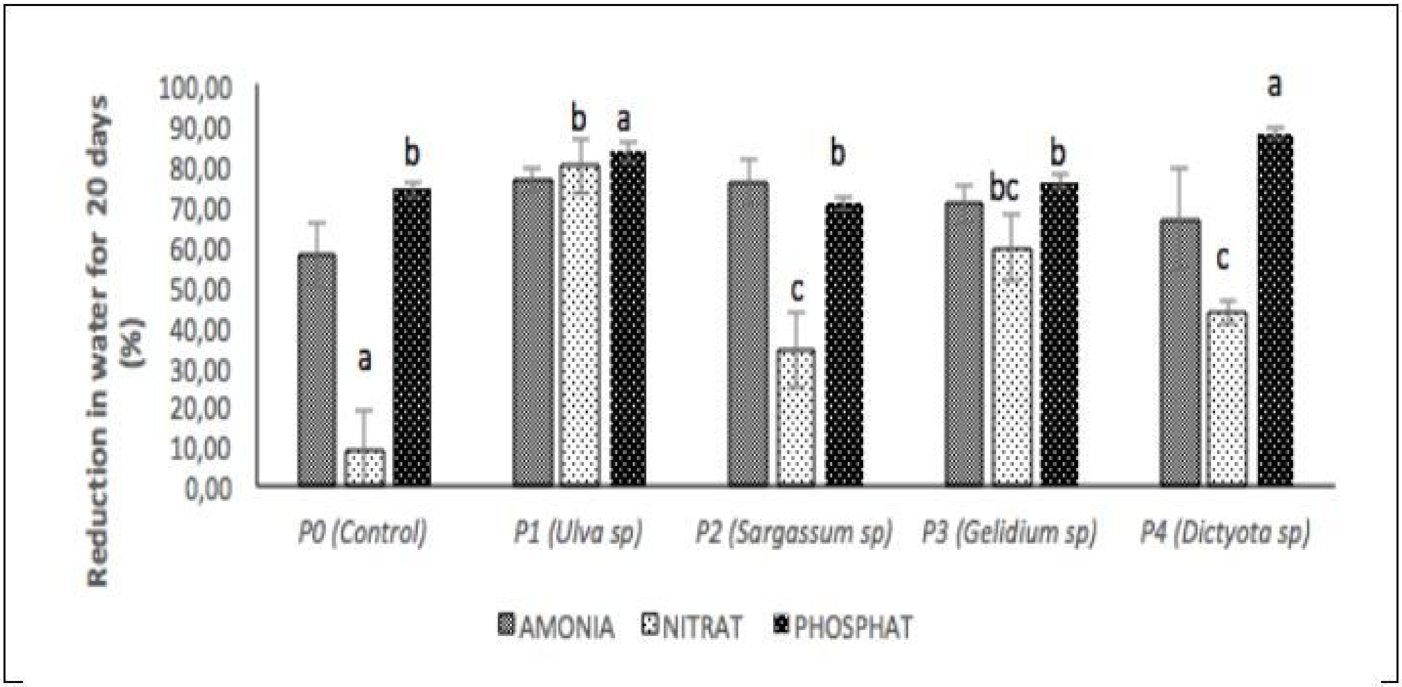

Figure 3

Reduction of Amonia (NH3); Nitrate (NO3-); Phosphate (PO43-) in water for twenty days with four different species as biofilter for hybrid grouper aquaculture wastewater in low salinity

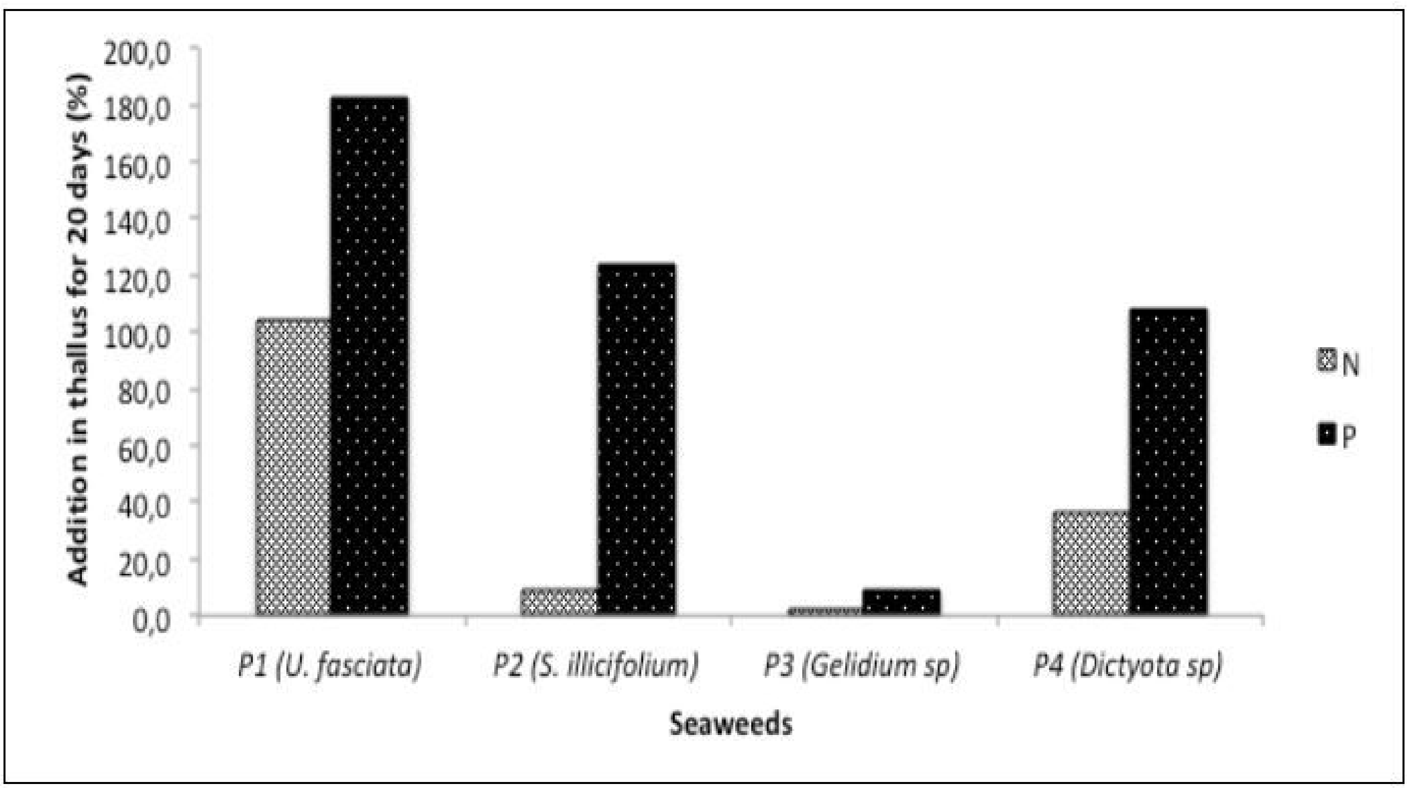

Figure 4

Percent addition of total N and P contents in different thallus of seaweed, for twenty days as biofilter for hybrid grouper aquaculture wastewater in low salinity.

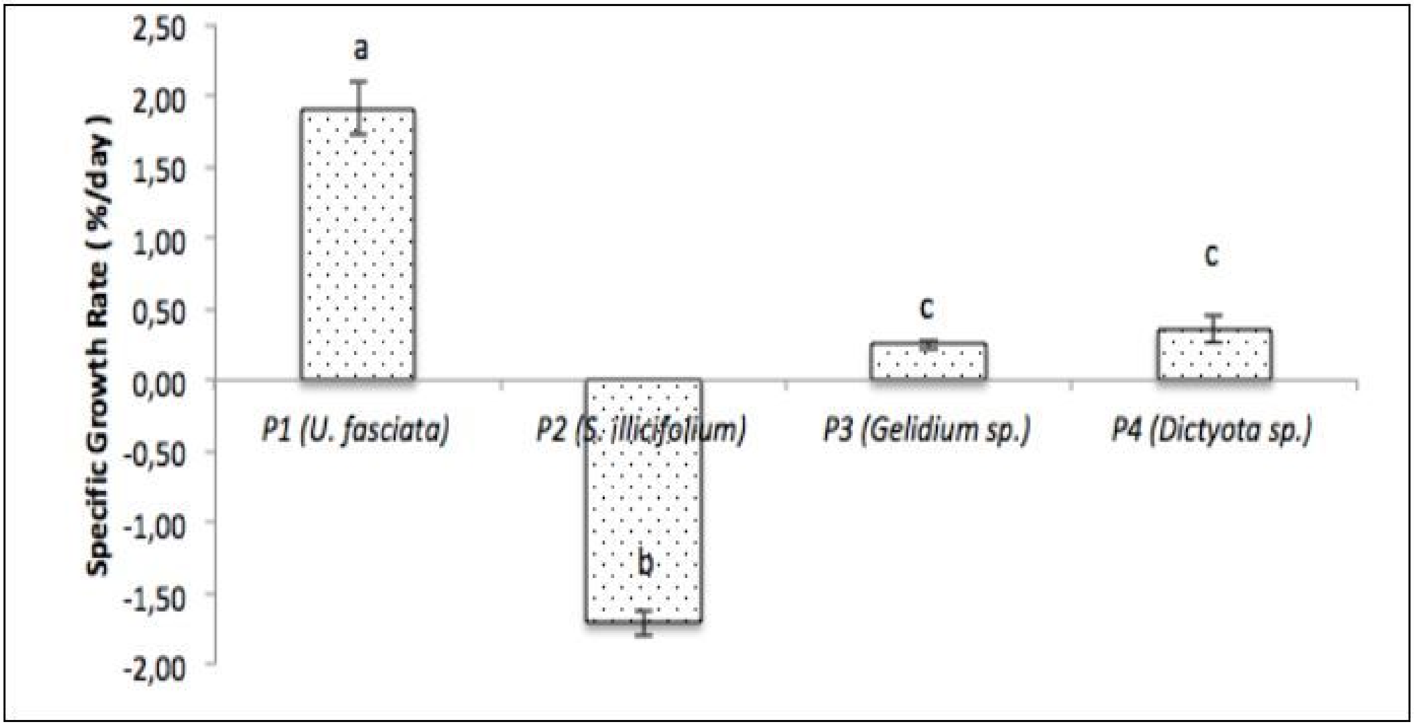

Figure 5

Specific Growth Rate (SGR) of different seaweeds in twenty days as biofilter of hybrid grouper aquaculture wastewater in low salinity.