OPEN ACCESS International Journal of Applied Biology

ISSN : 2580-2410
eISSN : 2580-2119

The Potential of Seaweed *Gracilaria* sp. as An Organic Waste Bioremediation Agent.

Khusnul Khatimah[1*], Andi Sompa[2], Haerunnisa[2], St. Zaenab[3]

[1]Department of Aquaculture Technology, Pangkajene Islands State Agricultural Polytechnic, Pangkep, Indonesia.
[2]Department of Water Resources Management, Puangrimaggalatung University, Wajo, Indonesia.
[3]Department of Aquaculture, Cokroaminoto University, Makassar, Indonesia.

Abstract

Increased fishing and agricultural activities trigger eutrophication events in marine waters. Excessive availability of nutrients can cause changes in the composition of community structures in marine ecosystems. Therefore an effort is needed to mitigate the eutrophication process in coastal and marine areas by increasing the nutrient partition coefficient in compartments that can absorb or consume these nutrients such as macroalgae or seaweed. The aim of this study was to determine the absorption capacity of nutrients (N and P) from *Gracilaria sp*. Seaweed as a mitigating agent for eutrophication in waters. This research was conducted in October–November 2018 in a controlled manner at the PUI-P2RL Wet Laboratory, Hasanuddin University. Seaweed maintenance media is intensive pond wastewater that has gone through a filtering and sterilization process. The results showed that *Gracilaria sp.* able to absorb nutrients in the form of NH_3, NO_2 , NO_3 and PO_4. *Gracilaria* sp. able to reduce NO_3 by 0.840 ± 0.065 µg/l/day, NO_2 by 2.100 ± 0.609 µg/l/day, NH_3 by 1.506 ± 0.204 µg/l/day and PO_4 by 8.756 ± 2.785 µg/l/day.

Article History
Received February 22, 2023
Accepted June 14, 2023

Keyword
Gracilaria, Bioremediation, Eutrophication, Nutrients, Organic Waste

Introduction

The health of ecosystems and their use in coastal areas is an indicator of water quality, especially in the field of marine conservation and various activities in other fisheries sectors. Aquaculture and agricultural activities are a major challenge in maintaining water quality in coastal areas, where increasing concentrations of nutrients (Nitrogen, Phosphorus and Silicon) from these activities can cause water eutrophication which has a negative impact on organisms. This condition has been found in the coastal waters of the west coast of South Sulawesi where nutrient enrichment occurs quite large (Lukman et al., 2014; Nasir et al., 2015) and symptoms of eutrophication have been identified (Nurfadillah, 2016).

CONTACT : KHUSNUL KHATIMAH khusnul.khatimah@polipangkep.ac.id

Disposal of aquaculture waste, especially shrimp and fish ponds, and agriculture is the main source of nutrients that cause eutrophication which has an impact on the ecosystem of the west coast of South Sulawesi (Nasir et al., 2016; Hopkins et al., 1995). Excessive availability of nutrients can cause changes in the composition of community structures in marine ecosystems such as microorganisms (Kegler et al., 2017), plankton (Nasir et al., 2015) and coral reef ecosystems (Teichberg et al., 2018; Edinger et al., 1998). Therefore efforts to overcome eutrophication need serious attention. The increase in nutrients in waters, especially in coastal areas, needs to be mitigated by increasing the nutrient partition coefficient in compartments that can absorb or consume these nutrients (Carpenter, 2008). On the other hand, seaweed is known to live in waters that have a fairly high concentration of nutrients (Kasim, 2016). Seaweed utilizes nutrients (N and P) as a food source for its growth and development. Nitrogen is needed as a constituent of amino acids, while phosphorus is used as energy in photosynthesis (Graham & Wilcox, 2000). Therefore, seaweed has the potential to become a phytoremediation agent because it is able to adapt to the aquatic environment with extreme nutrient conditions around aquaculture ponds (Komarawidjaja, 2005). This ability to absorb natural nutrients can then be an alternative to mitigating the effects of eutrophication in waters.

Furthermore, from an economic standpoint, the Indonesian government is pushing for increased seaweed cultivation and industrialization in response to the increasing demand for seaweed commodities both on a national and international scale (Radiarta et al. 2016). The potential and good quality of Indonesian seaweed has made it in demand by various countries in the world. There are more than 550 types of seaweed in Indonesia and most of the products from seaweed have been exported as dried seaweed or in processed form. The various opportunities that are owned by one of the biological resources in Indonesia make seaweed a superior export product (Directorate General of National Export Development/MJL/ 004/9/2013).

Rhodophyta and Chlorophyta are seaweeds that are widely distributed in Sulawesi waters and are found to be quite abundant (Admadja et al., 1996). In addition to its relatively easy cultivation techniques and high tolerance for environmental factors, this group of seaweed is known to have good potential in absorbing nutrients that enter the waters. *Gracilaria* sp. is a seaweed that can be used as a phytoremediation agent for organic matter. This type of seaweed has the ability to accumulate organic matter in cells (Komarawidjaja, 2005). In addition, *Gracilaria* also has good growth at sufficient nitrogen concentrations. However, comprehensive research on the absorption capacity of seaweed in the context of eutrophication mitigation efforts has not been widely carried out, especially in the South Sulawesi region.

Based on this, research on the absorption capacity of seaweed is important. The capacity of various types of seaweed, which are used economically by the community, in absorbing nutrients as well as in reducing eutrophication in coastal and marine waters needs to be calculated as a reference in developing integrated coastal management based on a balance between environmental and economic levels. This research is useful in developing the application of ecosystem-based seaweed cultivation, a sustainable aquaculture system which is becoming a world trend (FAO, 2007).

This study aims to determine the ability of *Gracilaria* sp seaweed to absorb NO3, NO2, NH3 and PO4 and see the potential of *Gracilaria* sp. in mitigating significantly the excess of nutrients (eutrophication) in the waters.

Materials and Methods

This research was carried out at the PUI-P2RL Wet Laboratory at Hasanuddin University in October - November 2018 which included literature studies, laboratory tests, data collection, sample analysis, data processing, and data analysis. Analysis of water samples was carried out at the Productivity and Water Quality Laboratory, Faculty of Marine and Fisheries Sciences and analysis of seaweed samples was carried out at the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University.

The tools and materials used in this study were seaweed and intensive pond wastewater as materials to be tested, chemicals to analyze nutrient parameters (NO3, NO2, NH3 and PO4), as well as instruments to measure water quality (salinity, pH and DO).

Sampling Stage

Gracillaria sp seaweed was taken from the waters of Takalar Regency. Seawater to be used as a medium in a controlled container is taken from the intensive pond disposal area of Takalar Regency.

Measurement of Water Quality Parameters

Measurement of nutrient parameters (NO2, NO3, PO4, NH3) and water quality (salinity, temperature, pH and DO) in the test media was carried out every 10 days (for 1 period/30 days). The methods used in measuring the parameters of water quality and seaweed during rearing and sampling are as follows:

Table 1. Water Quality Parameters

No	Parameter	Sample	Methods/Tools
1	P - Total	seaweed	$HNO_3^-HClO_4^-$ Spectrophotometry
2	N-Total	seaweed	Kjeldahl
4	Growth	seaweed	Analytical balance
6	Phosphate (PO4)	sea water	Spectrophotometry
7	Nitrates (NO3)	sea water	Spectrophotometry
8	Nitrite (NO2)	sea water	Spectrophotometry
9	Ammonia (NH3)	sea water	Spectrophotometry
10	Light intensity	sea water	Lux-meter
11	Temperature	sea water	Thermometer
12	Salinity	sea water	Hand Refractometer
13	DO	sea water	DO meters
14	pH	sea water	PH meter

Measurement of Nutrient Parameters (NO2,NH3,NO3 and PO4)

Measurement of nutrient parameters using the spectrophotometric method and the linear standard curve method following the measurement procedure of Grasshoff et al., 1983. Nitrate measurement using the cadmium reduction method, nitrite measurement using the sulfanilamide method, ammonia measurement using the ammonium molybate method, and phosphate measurement using the stony chloride method.

Measurement of The Growth Rate of Seaweed Biomass

The growth rate of biomass is obtained from the total final biomass growth minus the initial biomass divided by the time required. The formula for calculating the growth rate of biomass (Harianto et al., 2012):

$$\text{LPB} == \frac{\text{Bt} - \text{B0}}{\text{t}}$$

Where:

LPB : Biomass growth rate (gram/day)

Bt : Final biomass (gram)

B0 : Initial biomass (grams)

t : Time (days)

The Rate of Reduction of Nutrients in Water

$$\text{Decline rate} = = \frac{\textbf{concentration of nutrient T1} - \textbf{concentration of nutrient T2}}{\textbf{t}}$$

Where:

T1 : Initial nutrient concentration

T2 : Final nutrient concentration

t : Observation period (days)

Total N Analysis

Measurement of total N in seaweed was carried out at the beginning and end of rearing. Standard measurement of total N in seaweed using the Kjeldahl method (AOAC, 1980). To calculate the total N, the following formula is used:

$$\text{Total N (\%)} \frac{[\{0,0007*\times(\text{Vb}-\text{Vs})\times\text{F}\times 20}{\text{S}} \times 100$$

Where:

Vs = ml 0.05 N NaOH titrant for sample

Vb = ml of NaOH titrant for blank

F = correction factor of 0.05 NaOH solution

S = sample weight

* = per ml 0.05 NaOH equivalent to 0.0007 g N

Data Analysis

Research data were processed and analyzed in the form of tables and graphs using Microsoft Excel 2013 Software and the Prism Application.

Results and Discussion

Nutrient Concentrations (Ammonia, Nitrate, Nitrite, and Phosphate) in *Gracillaria* Sp.

Figure 1 shows that there was a decrease in the concentration of nutrients in the form of nitrate, nitrite, ammonia and phosphate in the seaweed rearing medium in each measurement period (per 10 days). However, on the last day of the study (30th day) there was an increase in the concentration of nitrite and nitrate in the rearing medium.

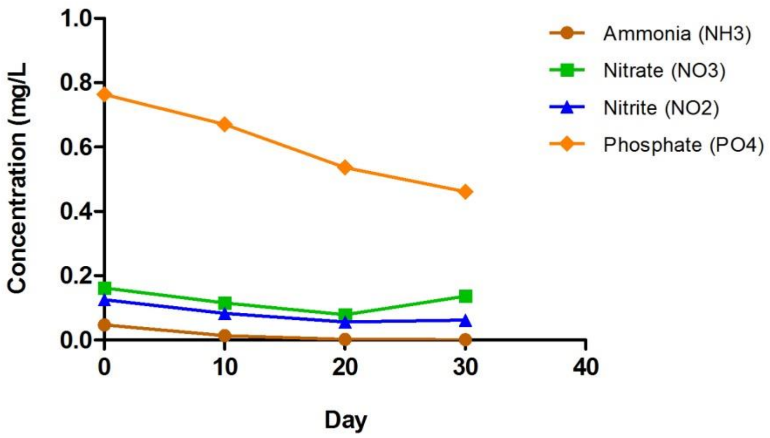

Figure 1. Graph of nutrient concentrations in *Gracillaria* sp.

Nutrient concentrations (ammonia, nitrate, nitrite, and phosphate) in the *Gracilaria* sp. on the 0th, 10th, 20th and 30th day respectively for Ammonia (0.047 mg/l ± 0.003 mg/l; 0.014 mg/l ± 0.003 mg/l; 0.002 mg/l ± 0.001 mg/l; 0.001 mg/l ± 0.002 mg/l), Nitrate (0.162 mg/l ± 0.004 mg/l; 0.115 mg/l ± 0.009 mg/l; 0.079 mg/l ± 0.019 mg/l; 0.136 mg /l ± 0.001 mg/l), Nitrite (0.125 mg/l ± 0.006 mg/l; 0.083 mg/l ± 0.006 mg/l; 0.056 mg/l ± 0.005 mg/l; 0.062 mg/l ± 0.005 mg/l), and Phosphate (0.764 mg/l ± 0.013 mg/l; 0.670 mg/l ± 0.024 mg/l; 0.536 mg/l ± 0.053 mg/l; 0.461 mg/l ± 0.046 mg/l).

The absorption of nutrients by seaweed can be calculated from the value of the rate of decrease in nutrient concentrations found in the seaweed rearing medium. This can occur if the decrease in nutrient concentration is not caused by nitrogen transformation factors (nitrification and ammonification).

The decrease in N concentration in the rearing water medium was not necessarily caused by the absorption of seaweed. The decrease in nutrient concentration can be caused by the process of ammonia oxidation or nitrification, where in this condition there is a decrease in the concentration of ammonia and an increase in the concentration of nitrite, which then nitrite will also undergo oxidation to become nitrate.

Based on Figure 1, there was a decrease in ammonia concentration in the *Gracilaria* sp. caused by absorption by seaweed that occurs in each measurement period (day 0 to day 30). The same pattern of decrease in concentration also occurred in nitrate and nitrite content until the 20th day, but showed the opposite pattern on the 30th day or the last day of the study, namely an increase in concentration in the *Gracillaria* seaweed rearing medium. Figure 1 shows the symptoms of nitrification in the water media for seaweed *Gracillaria* sp. at the end of the study (30th day), where there was a decrease in ammonia and an increase in nitrite and nitrate concentrations at the same time. This is thought to be a symptom of nutrient oxidation in the seaweed rearing medium at the end of the study, but this symptom still needs further investigation because the O_2 indication did not show a significant change during the trial period.

In *Gracilaria* sp. the rate of decrease in the concentration of phosphate in the rearing medium occurred at the beginning of the study until the end of the study (Figure 1). In the *Gracilaria* seaweed rearing medium, the decrease in phosphate concentration was due to absorption by the seaweed which occurred on day 10 to day 30. Phosphate has a role as forming cell membranes and energy transfer within cells (Pramesti, 2013). Phosphate at the end of the study is thought to inhibit metabolic processes because no energy is used for the process so that the growth of seaweed decreases.

Nutrient Depletion Rate (Ammonia, Nitrate, Nitrite, and Phosphate)

Table 1. The Rate of Nutrient Reduction (Mean ± Standard Deviation) in Each Treatment During Observation.

Nutrient Type	Average Nutrient Depletion Rate (µg/L/day)		
	10th Day		
	1	2	3
Ammonia	**3.300 ± 0.265**	**1.133 ± 0.208**	0.085 ± 0.139
Nitrate	4.633 ± 1.274	3.667 ± 2.434	-5.779 ± 2.487
Nitrite	4.167 ± 1.002	2.667 ± 0.709	-0.533 ± 0.115
Phosphate	9.367 ± 1.815	13.367 ± 5.008	3.533 ± 1.531

The value of the daily reduction rate of ammonia, nitrate, nitrite, and phosphate concentrations on *Gracillaria* sp. can be seen in Table 2. On the maintenance media of *Gracilaria* sp. there was a decrease in nutrient concentrations in the form of ammonia and phosphate until the end of the study, while a decrease in nutrient concentrations in the form of nitrate and nitrite only occurred until the 20th day, after the 30th day it was seen that there was an increase in the concentration of nitrate and nitrite in the rearing medium. The highest rate of decrease in ammonia, nitrate and nitrite concentrations occurred in the first 10 days (0-10 days) and the lowest occurred at the end of the study (second 10 days), in contrast to phosphate concentrations which showed the opposite condition, namely a decrease in phosphate concentrations in the second 10 days. observation is greater than the first 10 days.

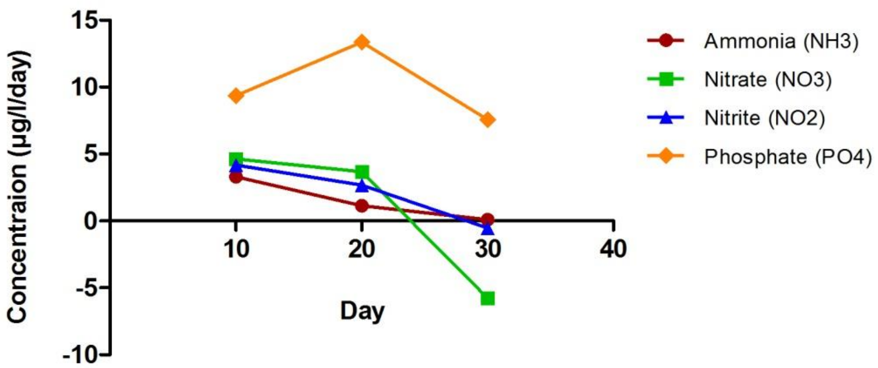

Figure 2. Graph of Nutrient Depletion Rate Per 10 Days

Value of the average amount of reduction of ammonia, nitrate, nitrite, and phosphate in the media maintenance of *Gracillaria* sp. respectively 1.506 ± 0.204 μg/l/day; 0.840 ± 0.065 μg/l/day; 2.100 ± 0.609 μg/l/day; and 8,756 ± 2,785 μg/l/day. The minus value in the figure above shows that there is no rate of decrease in nitrite and nitrate concentrations on the 30th day. This means that there was an increase in nitrite and nitrate indicating the occurrence of nitrification process at that time.

Total N And Total P Values of *Gracillaria* Sp.

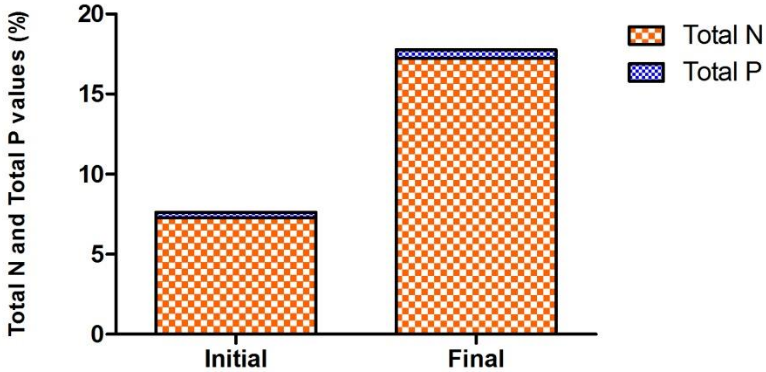

Figure 3. Total N and P Total Values of *Gracillaria* sp. at The Beginning and End of The Study

In Figure 3, the highest Ntotal and Ptotal values were found at the end of the study (30th day), namely 17.23 ± 0.557% and 0.547 ± 0.039%, while the Ntotal and PTtotal values for *Gracillaria* sp. at the beginning of the study that is equal to 7.280 ± 0.355% and 0.337 ± 0.014%. The mean difference between the initial (0th day) and final (30th day) Ptotal values of maintenance on *Gracilaria* sp was 0.21 ± 0.050% and the Ntotal was 9,950 ± 0,911%. This shows that *Gracilaria* sp. has the ability to absorb more nitrogen elements compared to phosphorus elements contained in the rearing medium.

Based on the results of NTotal analysis on *Gracilaria* sp., there was an increase in NTotal and PTotal at the end of the rearing (Figure 3). The results of the analysis showed that the NTotal content was higher than the Ptotal in seaweed. This shows that the uptake of nitrogen by grass is higher than the consumption of phosphorus. Excess nitrogen available in the waters can stimulate seaweed to consume nitrogen in large quantities, which results in high concentrations of N tissue (Gordon et al., 1981).

The process of absorption of nutrients in seaweed is carried out by diffusion through all parts of its body (Supriyantini et al, 2018). The cell membrane which is the outermost part of the cell after the cell wall acts as a protector of the contents of the cells in the body will regulate nutrients that go out and enter the cell. The amount of nutrients that diffuse into the cell depends on the concentration of nutrients inside and outside the cell. This statement was confirmed by Lobban and Harrison (1994) that nutrients will increase cell metabolic activity by entering the cell gradually and then developing vacuoles in the cell. Where vacuoles play a very important role in life because the survival mechanism of seaweed depends on the ability of vacuoles to maintain the concentration of dissolved substances in its thallus.

Seaweed Growth

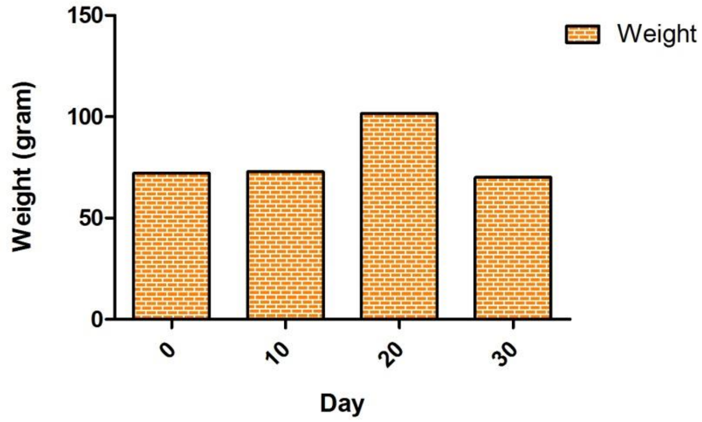

Figure 4. The Average Weight Value of Seaweed During The Study

Seaweed growth can be seen from the weight added value of the seaweed. The average weight value of *Gracillaria* sp. from the beginning to the end of the study respectively 72.013 ± 0.023 gr, 72.830 ± 0.235 gr, 101.513 ± 1.825, and 20.007 ± 0.076 gr. Figure 3 shows that there is an increase in the weight of *Gracilaria* sp. on day 10 to day 20 and then decreased in weight at the end of the study (day 30). The highest weight gain of *Gracilaria* sp. occurred on the 20th day, which was 98.51 gr, then on the 30th day there was a decrease of 67.01 gr.

In Figure 4, there is an increase in the weight of seaweed *Gracilaria* sp. on day 10 to day 20. The increase in seaweed weight until the 20th day was due to the absorption of nutrients (N, P) at that time. According to Gordillo et al., (2002) that the rate of absorption of phosphate and nitrate accordingly has a positive correlation with increasing the rate of growth of seaweed.

On the 30th day there was a decrease in the weight of the seaweed which indicated that the ability of the seaweed to absorb nutrients decreased. On the 30th day, some of the seaweed thallus died and rotted, resulting in decomposition and an increase in nutrients. In addition, light penetration in the seaweed rearing medium was less than optimal which caused some of the seaweed thallus to turn white and die. According to Atmadja (1996) that the growth of seaweed is closely related to the penetration of light and nutrients found in the environment where the seaweed lives.

Water Quality Parameters

The results of measuring water quality parameters on *Gracillaria* sp. presented in Table 3.

Table 3. Water Quality Parameter Values During The Study (Mean ± Standard Deviation)

Parameters	Days			
	0	10	20	30
Salinity (ppt)	31 ± 0.58	30 ± 0.24	29 ± 0.38	30 ± 0.15
pH	7.37 ± 0.13	7.77 ± 0.22	8.10 ± 0.06	8.16 ± 0.06
DO (ppm)	5.80 ± 0.40	6.67 ± 0.33	6.72 ± 0.19	6.91 ± 0.11
Temperature (°C)	29 ± 0.58	29 ± 0.64	30 ± 0.37	30 ± 0.18

Table 3 shows that the salinity values in the *Gracillaria* sp seaweed rearing media ranged from 29-31 ppt, pH ranged from 7.37-8.16, DO ranged from 5.80-6.91 ppm, and temperature ranged from 29-30°C.

During the research process, the water quality in the experimental pond unit was in a relatively stable condition. The stability of the water quality conditions from the beginning to the end of the experiment can explain that the absorption of nutrients by seaweed is not affected by water quality factors so that the ability to absorb nutrients from seaweed can be identified.

Conclusion

Gracilaria sp seaweed is able to absorb nutrients in the form of NH3 and PO4 up to 30 days and in the form of NO2 and NO3 up to 20 days, with the ability to reduce NO3 concentrations by 0.840 ± 0.065 µg/l/day, NO2 by 2.100 ± 0.609 µg/day l/day, NH3 of 1,506 ± 0,204 µg/l/day and PO4 of 8,756 ± 2,785 µg/l/day.

References

Association of Official Analytical Chemists (AOAC). 1980. Official Methods of Analysis Association of Official Analytical Chemist. 13th Edition

Atmadja WS, Kadi A, Sulistijo, Rachmaniar. 1996. Introduction to the Types of Indonesian Seaweed. Oceanology Research and Development Center. LIPI. Jakarta.

Carpenter, S. R. (2008). Phosphorus Control is Critical to Mitigating Eutrophication. Proceedings of the National Academy of Sciences, 105(32), 11039–11040. https://doi.org/10.1073/pnas.0806112105.

Directorate General of National Export Development/MJL/004/9/2013 September. In http://djpen.kemendag.go.id/app_frontend/admin/docs/publication/6201390367517.pdf accessed in March 2019

Edinger, E.N., J. Jompa, G.V. Limmon, W. Widjatmoko, M.J. Risk (1998). Reef Degradation and Coral Biodiversity in Indonesia: Effects of Land-Based Pollution, Destructive Fishing Practices and Changes Over Time. Marine Pollution Bulletin 36(8), 617-630.

FAO. 2011. The Cassava Transformation in Africa. The Food and Agriculture Organization of The United Nations (FAO).

Gordillo, F. J., Dring, M. J., & Savidge, G. 2002. Nitrate and Phosphate Uptake Characteristics of Three Species of Brown Algae Cultured at Low Salinity. Marine Ecology Progress Series, 234, 111-118.

Gordon, D. M., Birch, P. B., & McComb, A. J. 1981. Effects of Inorganic Phosphorus and Nitrogen on The Growth of An Estuarine Cladophora in Culture.

Graham, L.E., L. E. Wilcox, 2000. Algae (Chapter 23 – Macroalgal and Perihyton Ecology). Prentice-Hall, Inc. p.s. 619.

Grasshoff, K., Enrhardt, M., Kremling, K. 1983. Method of Seawater Analysis. Second, Revised and Extended Edition. Verlag Chemie. Germany

Harianto, E., Budiardi, T., & Sudrajat, A. O. 2014. Growth Performance of 7-g Anguilla bicolor bicolor at Different Densities. Journal of Indonesian Aquaculture, 13(2), 120-131.

Hopkins, J. S., M. R. De Voe, and A. F. Holland. 1995. Environmental Impacts of Shrimp Farming Effluent in The Continental United Stated.

Kasim, M. 2016. Ecological, Biological Studies, Utilization and Cultivation of Macroalgae. Self-help Spreader. Jakarta.

Kegler HF, Lukman M, Teichberg M, Plass-Johnson J, Hassenrück C, Wild C and Gärdes A., 2017. Bacterial Community Composition and Potential Driving Factors in Different Reef Habitats of the Spermonde Archipelago, Indonesia. Front. Microbiol. 8:662. DOI: 10.3389/fmicb.2017.00662.

Komarawidjaja, W. 2005. Gracilaria sp. Seaweed. as Phytoremedian Organic Matter in Aquaculture Ponds. J.Tek. Ling. P3TL-BPPT, 6 (2): 410 – 415.

Lobban, C. S., & Harrison, P. J. 1994. Seaweed Ecology and Physiology. Cambridge UniversityPress.

Lukman, M., A. Nasir, K. Amri, R. Tambaru, M. Hatta, Nurfadilah, R.J. Noer, 2014. Dissolved Silicates in Coastal Waters of South Sulawesi. Journal of Tropical Marine Science and Technology, 6(2): 461-478.

Nasir A, Lukman M, Tuwo A, Hatta M, Tambaru R and Nurfadilah, 2016. The Use of C/N Ratio in Assessing the Influence of Land-Based Material in Coastal Water of South Sulawesi and Spermonde Archipelago, Indonesia. Front. Mar. sci. 3:266. DOI: 10.3389/fmars.2016.00266.

Nasir, A., M. Lukman, A. Tuwo, and Nurfadilah, 2015b. Ratio of Nutrients to Diatom-Dinoflagellate Communities in Spermonde Waters, South Sulawesi. Journal of Tropical Marine Science and Technology, Vol. 7, No. 2: 587-601.

Nasir, A., Tuwo, A., Lukman, M., Usman, H., 2015a. Impact of increased nutrient on the variability of chlorophyll-a in the west coast of south Sulawesi, Indonesia. International Journal of Scientific & Engineering Research, 6(5): 821-826.

Nurfadilah. 2016. Comprehensive Assessment of Eutrophication Conditions with Primary and Secondary Symptom Approaches in Muara Sungai Pangkep Waters. Hasanuddin University Masters Degree, Makassar.

Pramesti, R. 2013. Enriched Seawater Media on the Growth Rate of Seaweed *Gracilaria* lichenoides (L) Harvey. Marina Oceanographic Bulletin, 2(1), 66-73.

Radiarta IN, Erlania, Haryadi J, Rosdiana A. 2016. Analysis of The Development of Seaweed Cultivation on Sebatik Island, Nunukan Regency, North Kalimantan. Journal of Indonesian Fisheries Policy. 8(1) : 29-40.

Supriyantini, E., Santosa, G. W., & Alamanda, L. N. 2018. Growth of *Gracilaria* sp. Seaweed. in Media Containing Copper (Cu) with Different Concentrations. Marina Oceanographic Bulletin, 7(1), 15.

Teichberg, M., Wild, C., Bednarz, V. N., Kegler, H. F., Lukman, M., Gärdes, A. A., ... & Plass-Johnson, J. G. 2018. Spatio-Temporal Patterns in Coral Reef Communities of The Spermonde Archipelago , 2012–2014, I: Comprehensive Reef Monitoring of Water and Benthic Indicators Reflect Changes in Reef Health. Frontiers in Marine Science, 5, 33.