The effectiveness of *Sargassum polycystum* C.Agardh (1824) density to reduce nitrate and phosphate in vannamei shrimp aquaculture

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Abstract. The current development of aquaculture raises problems in the form of pollution of liquid waste generated during the production process. Aquaculture wastewater contains very high concentrations of inorganic nutrient. This study aims to determine the effectiveness of *Sargassum polycystum* density as a biofilter to reduce nitrate and phosphate in whiteleg shrimp pond. The study was conducted experimentally at indoor tank culture using a Completely Randomized Design (CRD) consisting of 3 density treatments with 3 replications, which were 1 gL\(^{-1}\), 2 gL\(^{-1}\), and 3 gL\(^{-1}\). The parameters observed were water quality such as nitrate, phosphate, temperature, salinity, pH, DO (Dissolved Oxygen), TSS (Total Suspended Solid), and TDS (Total Dissolved Solid); and also *Saragassum’s* growth. The results showed that the density of 3 gL\(^{-1}\) able to reduce nitrate 80% and phosphate 86.30%. The growth of *S. polycystum* for the three treatments decreased due to several factors, such as low nitrogen availability, environmental conditions, predators, and differences in stocking density. The average water quality measured during the study was temperature 27.84 °C; salinity 31.31 ppt; DO 4.72 mgL\(^{-1}\); pH 7.69; TDS 34.94 mgL\(^{-1}\); and TSS 4807.63 mgL\(^{-1}\). *S. polycystum* has the ability as biofilter for white shrimp culture because able to reduce nitrate and phosphate concentration. *S. polycystum* has potential benefits as alternative commodity candidate for polyculture and Integrated Multi Throphic Aquaculture (IMTA).

1. Introduction
The need for global fish consumption continues to increase along with the increasing human population growth. FAO reports that there has been an increase in fish production, including shrimp, globally from 2015 to 2018 by 27.28% [1]. The increase in shrimp production will also lead to an increase in the use of pellets in their aquaculture. However, unfortunately, 20-30% of the total N and P content in the pellet is wasted to form aquaculture waste [2]. Residues of undigested feed and shrimp metabolism will increase nutrient waste in aquaculture wastewater. High organic nutrient content in aquaculture waste when it is discharged in open water body system will cause eutrophication. Eutrophication can cause the death of organisms that live around aquaculture, threaten the ecological and economic integrity of coastal waters [3],
as well as the degradation of marine ecosystems [4]. So that aquaculture water management needs to be done to improve the quality of aquaculture wastewater so that it can be recirculated and safe before being discharged into open waters system.

The utilization of seaweed as an alternative commodity is considered to reduce nutrient waste in aquaculture waste. In addition to functioning as a biofilter, seaweed can also increase the economic value in a cultivation cycle. Sargassum is one type of brown algae that is easy to find and has a high abundance on the south coast of Yogyakarta. In addition, Sargassum also has excellent potential to be used as a biofertilizer, fodder, as well as various bioactive ingredients used for drugs and nutraceutical [5; 6; 7; 8; 9; 10].

Sargassum spp. reported to be able to act as a biosorption remediation process from aquaculture wastewater so as to reduce the impact of pollution in the sea [10]. Sargassum's ability to carry out bioremediation and bioaccumulation of nutrient waste and heavy metal waste, as well as its bioactive content for various benefits, makes Sargassum has great potential and high economic value as a biofilter to be combined with polyculture systems and Integrated Multi Trophic Aquaculture [10; 11; 12]. Mai et al. [13] using Sargassum sp. combined with western king prawn cultivation shows that Sargassum is able to optimize water quality and reduce the negative impact of waste on the environment. Utilization of macroalgae for bioremediation also needs to pay attention to the optimal macroalgae density factor to obtain effective results [14]. So that the optimal density of Sargassum to determine its effectiveness in reducing nutrient waste also needs to be investigated. So this study aims to determine the density of S. polycystum which is used as a biofilter for vanname shrimp (whiteleg shrimp) cultivation to determine its effectiveness in reducing nutrient waste.

2. Material and method
This research was conducted at the Seawater Cultivation Unit of Sundak Village, Tepus District, Gunung Kidul Regency, Special Region of Yogyakarta. The study was conducted for 25 days with sampling time every 5 days for 25 days. The culture wastewater used is the intensive system of vanname shrimp culture with a density of 150 fish m\(^{-2}\) and 63 days old with an average size of 15 grams of shrimp per head. The stages of research implementation can be described as follows.

2.1 Acclimation
Before use, S. polycystum was cleaned of impurities and existing organisms. In addition, before the preliminary.

2.2 Preliminary test
Preliminary tests were carried out to determine the appropriate dilution for cultured water in the study. Several combinations of seawater and aquaculture water ratios were tried in this preliminary test, including 3 : 1; 1 : 3; and 1 : 1 of seawater : wastewater aquaculture ratios; and the seawater only and cultivated only as controls. The preliminary test was carried out for seven days by maintaining the seaweed used in the primary test. Preliminary test results showed that S. polycystum was the best in the combination of 1 to 1 ratios of seawater and aquaculture water. Therefore, the primary test was carried out using a mixture of 15 L of cultured shrimp water and 15 L of seawater.

2.3 Main test
The main test was carried out for 25 days. The main test was carried out using a completely randomized design method with 3 replications. The treatment carried out was the treatment of different S. polycystum densities, which included:

\[
P 1: \text{S. polycystum with density } 1 \text{ gL}^{-1}.
\]
P 2: *S. polyctum* with density 2 gL\(^{-1}\).

P 3: *S. polyctum* with density 3 gL\(^{-1}\).

2.4. Determination of degree of hydrolysis
Measurement of water quality parameters was carried out every five days from the beginning to the end of the study. Water quality parameters measured include Dissolved Oxygen (Lutron-5519); salinity, Total Dissolved Solid, pH, Temperature (EZ-9909 5 in 1). Measurement of Nitrate (NO\(_3\)-) was carried out using the 2017 APHA test method, Section 4500 - NO\(_3\)B. Phosphate (PO\(_4\)) was measured by the Phosphormolybdenum blue method.

2.5. Thallus absorption
Analysis of seaweed absorption was obtained from measurements of total N and total P content in the seaweed thallus at the beginning and end of the study. The method used to measure the N content is AOAC 976.05 20th Ed. 2016, while the method of measuring P content follows SNI 2803-2012. Measurement of the total absorption of N and P in the thallus of *S. polycystum* seaweed using the following formula:

\[
\text{Percentage of absorption} = \frac{C_f - C_i}{C_f} \times 100\%
\]

where :

C\(_f\): N or P concentration at the end of research; C\(_i\): N or P concentration at the beginning of research.

2.6 The growth of *S. polycystum*
Growth is calculated using the formula:

\[
\text{Seaweed Growth} = \frac{W_t - W_0}{W_0} \times 100\%
\]

where W\(_t\): wet weight at the end of research; W\(_0\): wet weight at the beginning of research.

2.7. Data Analysis
Data of concentration of nitrate and phosphate in wastewater, also seaweed’s growth were tested with normality and homogeneity tests. If normal and homogeneous then proceed with analysis of variance (ANOVA). If the results of the analysis show a significant difference, then further analysis is carried out, namely the Tukey Test with a 95% confidence level. Meanwhile, the analysis of seaweed absorption of nutrients was carried out by paired t-test to find out whether there was a significant difference between before and after being given treatment. Statistical analysis was performed using SPSS version 20.0. Supporting parameter data such as temperature, salinity, pH, Dissolved O\(_2\), Total Dissolved Solid, and Total Suspended Solid were analyzed descriptively.

3 Result and discussion

3.1. Nitrate concentration in wastewater of Vannamei aquaculture during research

The vannamei shrimp wastewater used in this study had an initial nitrate content of 0.55 mgL\(^{-1}\). The concentration of nitrate in aquaculture wastewater used during the study can be seen in Table 1.
Table 1. Nitrate concentration

| Density treatment | Day | Percentage reduction for 25 days (%) |
|-------------------|-----|-------------------------------------|
|                   | 0   | 5        | 10      | 15      | 20      | 25      |
| 1 gL⁻¹            | 0,56| 0,48     | 0,44    | 0,41    | 0,33    | 0,27    | 51,79a  |
| 2 gL⁻¹            | 0,55| 0,48     | 0,39    | 0,29    | 0,31    | 0,21    | 61,82b  |
| 3 gL⁻¹            | 0,55| 0,47     | 0,41    | 0,36    | 0,24    | 0,11    | 80c     |

Description:
*) Values in the column followed by the same letter show no significant difference at the 95% confidence level.

Treatment 3 (3 gL⁻¹) could reduce the highest nitrate concentration by 80% (with a significance level of 0.05), compared to the percentage reduction in other treatments. This decrease was due to the optimal uptake of Nitrate by S. polycystum. It proves that S. polycystum reared in vaname shrimp culture water can improve the quality of these waters by absorbing existing nutrients. Ginting et al. [15] stated that the decrease in nitrate and phosphate in vaname shrimp culture water was due to the seaweed carrying out the process of absorbing nitrate and phosphate to support seaweed growth. Nitrate (NO₃⁻) and ammonium (NH₄⁺) are the main forms of nitrogen in water as the main nutrients for algae growth. The presence of these compounds in large quantities will spur the explosion of the phytoplankton population, which can negatively affect the quality of aquaculture water and the growth of white shrimp (Litopenaeus vannamei).

3.2 Phosphate concentration in wastewater of Vannamei aquaculture during research

Phosphorus content is the second nutrient limiting factor for thallus growth after nitrogen [16]. The concentration of water phosphate from vaname shrimp culture during the study can be seen in Table 2.

Table 2. Phosphate concentration (mgL⁻¹) in wastewater of Vannamei aquaculture

| Density Treatment | Day | Percentage of reduction for 25 days (%) |
|-------------------|-----|----------------------------------------|
|                   | 0   | 5        | 10      | 15      | 20      | 25      |
| 1 gL⁻¹            | 1,3 | 1,36a    | 1,2²    | 1,33¹   | 1,1²    | 1,06²   | 18,46ab |
| 2 gL⁻¹            | 1,43| 1,26ab   | 1,03b   | 0,9b    | 0,73b   | 0,73b   | 48,95bc |
| 3 gL⁻¹            | 1,46| 1,13b    | 0,63b   | 0,53c   | 0,3c    | 0,2c    | 86,30cd |

Description:
*) Values in the column followed by the same letter show no significant difference at the 95% confidence level.

The results showed that S. polycystum as a biofilter could reduce the concentration of phosphate in vaname shrimp culture water. Tsagkamilis et al. [17] state that the use of seaweed as a biofilter is effective in reducing phosphate levels in water. The highest decrease in phosphate concentration in aquaculture wastewater occurred in treatment 3 (3 gL⁻¹) of 86.30%, which was significantly different from the decrease in phosphate concentration in treatment 1 of 18.46%. So it can be said that the higher the density of seaweed used, the higher the rate of nutrient absorption. This is in accordance with the statement of Fouroughifard et al. [18] that the density of algae has a significant effect on the total concentration of ammonia, nitrite,
nitrate, and phosphate in water, where an increase in the density of algae causes a higher decrease in these compounds. The phosphate biosorption process consists of extracellular and intracellular transfer. The extracellular transfer includes the uptake of phosphate by the active site on the surface of the biosorbent and chemical bonds. In contrast, the intracellular transfer consists of biotransformation and the accumulation of intracellular phosphate [19].

3.3 The growth of *S. polycystum* during research

The results of the growth of *S. polycystum* during the study period are presented in Figure 1.

The results of observations of the wet weight of *S. polycystum* during the study period are presented in Figure 1. *S. polycystum*, used as a biofilter for vaname shrimp culture, showed a decrease in biomass until the end of the study for all treatments. There was a reasonably low decrease in biomass in the first week, except in treatment 3, because the seaweed was still adapting to its new environment. Aquilino et al. [20] stated that the adaptation process of seaweed inhibits its growth rate because some of the energy is used to survive due to a reduction in incoming energy and increased energy output. The decrease in weight of Sargassum as a biofilter also occurs due to differences in environmental physics parameters between natural and different research conditions [13]. In addition, it is suspected that the nitrate content is getting thinner and depleted so that it cannot support the growth of *S. polycystum* optimally so that it becomes a limiting factor for its growth. According to Roleda and Hurd [16] if the nitrate and phosphate needed by the thallus is greater than its availability, the nitrate and phosphate will be a limiting factor for its growth. Seaweed growth is influenced by extrinsic factors in the form of the aquatic environment, as well as internal factors in the form of genetics from the type of seaweed itself. Biological characteristics of each seaweed also need to be considered to determine its adaptability and effectiveness as a biofilter. Research conducted by Izzati [21] reported that Sargassum has a relatively lower growth rate than Gracilaria.

The stocking density used affected the growth of *S. polycystum* reared as a biofilter in Vaname shrimp culture water. The specific growth of seaweed was significantly higher at lower stocking densities. It is related to the availability of nutrients in the waters and self-shading [22]. In addition, the lower stocking density of seaweed will provide faster growth and reduce seaweed competition for nutrients. With less stocking density, seaweed can absorb more nutrients. Meanwhile, the high stocking density will inhibit the entry of light due to the shadow cover of the seaweed population itself and cause the photosynthesis process to be less than optimal, causing reduced nutrient absorption and insufficient nutrient needs for seaweed to grow appropriately [23].
3.4. Water quality parameters

Water quality parameters were analyzed to determine the effect of physical water parameters that affect the life of S. polycystum as a biofilter and whether the presence of S. polycystum can improve the quality of the cultured shrimp water. The results of observing environmental parameters during the study are presented in Table 3.

**Table 3. Water quality parameters during research**

| Parameters                      | 0    | 5    | 10   | 15   | 20   | 25   |
|---------------------------------|------|------|------|------|------|------|
| Water temp. (°C)                | 27.6 | 27.8 | 29.1 | 28.1 | 25.8 | 26.6 |
| Dissolved Oxygen (mgL⁻¹)        | 5.9  | 5.6  | 7.4  | 5.2  | 6.3  | 4.9  |
| pH                              | 7.5  | 7.6  | 7.5  | 6.7  | 6.8  | 6.9  |
| Total Dissolved Solid (mgL⁻¹)   | 31.4 | 32.3 | 32.9 | 33.6 | 35   | 35.9 |
| Salinity (ppt)                  | 28.8 | 29.1 | 29.7 | 30.3 | 31.6 | 32.3 |
| Total Suspended Solid (mgL⁻¹)   | 336  | 357  | 273  | 447  | 625  | 489  |

Water temperature during the study ranged from 25.8-29.1 °C (Table 3). Temperature affects the adaptability and absorption of nutrients by seaweed [24]. Therefore, temperature and nutrient availability are significant factors for the growth of the Sargassum [25]. The range of salinity measured during the study was 28.8 -32.3 ppt, with a mean of 30.3 ppt. Salinity for the three treatments during the study tended to be due to the occurrence of water evaporation. The pH of the water ranges from 6.7 to 7.6. Fluctuations in pH will affect the ability and capacity of seaweed as a bio sorbent [11]. The results of the dissolved O2 measurement during the study ranged from 4.9 to 7.4 mgL-1. On the 15th and 25th days of treatment, dissolved O2 content was decreased conceivably due to the nitrification process by the Nitrosomonas bacteria. However, the dissolved O2 value in this study was at the optimum threshold for seaweed growth. The high value of Total Suspended Solid during the study was thought to be due to the dead and decomposed pieces of the *Sargassum polycystum* thallus, so that the TSS content was very high. According to Mai et al. [13] the dead and decomposed thallus can reduce the condition of cultured water quality.

4. Conclusion

*S. polycystum* reduced Nitrate and phosphate concentration in whiteleg shrimp culture water, particularly with the three-gram per liter (gL⁻¹) density as the most effective treatment. Thus, S. Polycystum can improve water quality and be a candidate in polyculture systems and Integrated Multi Trophic Aquaculture (IMTA).

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References

[1] FAO. 2020. The state of World Fisheries and Aquaculture 2020. Sustainability in Action. Rome. https://doi.org/10.4060/ca9229en.

[2] Kawasaki N, Kushairi MRM, Nagao N, Yusoff F, Imai A, Kohzu A 2016 Release of nitrogen and phosphorus from aquaculture farms to Selangor River, Malaysia. Internat. J. Envir. Sci & Dev. 7(2): 113-116.

[3] Williamson SC, Rheuban JE, Costa JE, Glover DM and Doney SC. 2017. Assessing the impact of local and regional influences on nitrogen loads to Buzzards Bay, MA. Front. Mar. Sci. 3: 1-17.

[4] Tirkaso W and Gren IM. 2016. Habitat quality and fish population: impacts of nutrient enrichment on populations of European perch off the east coast of Sweden. Working Paper Series 3:1-21.

[5] Yende SR, Harle UN, Chaugule BB. 2014. Therapeutic potential and health benefits of Sargassum species. Pharmacog. Rev. 8(15):1-7.

[6] Liu J, Luthuli S., Yang Y, Cheng Y, Zhang Y, Wu M, Choi J-i, Tong H. 2020. Therapeutic and nutraceutical potentials of a brown seaweed Sargassum fusiforme. Food Sci. & Nut. 8: 5195-5205. DOI: 10.1002/fsn3.1835.

[7] Puspita M, Setyawidati NAR, Stiger-Pouvreau V, Vandanjon L, Widowati I, Radjasa OK, Bedoux G, Bourgougnon N. Chapter Five Indonesia Sargassum species bioprospecting: potential applications of bioactive compounds and challenge for sustainable development. Advanc. in Bot. Res. 95: 113-161. https://doi.org/10.1016/bs.abr.2019.12.002.

[8] Kusumawati R, Nurhayati, Pangestu HE, Basmal J. 2021. Effect of Trichoderma addition on Sargassum organic fertilizer. IOP Conf Series. Earth & Env. Series 175: 012059. doi:10.1088/1755-1315/715/1/012059

[9] Fatimah S, Alimon H, Daud N 2018 The effect of seaweed extract (Sargassum sp.) used as fertilizer on plant growth of Capsium annum (Chilli) and Lycopersicon esculentum (tomato). Indonesian J. Sci. & Tech. 2:115-123.

[10] Yu Z, Zhu X, Jiang Y, Luo P, Hu C. 2014. Bioremediation and fodder potentials of two Sargassum spp. in coastal waters of Shenzhen, South China. Mar. Poll. Bull. 85(2):797-802. https://doi.org/10.1016/j.marpolbul.2013.11.018.

[11] Saldarriaga-Hernandez S, Hernandez-Vargas G, Iqbal HMN, Barceló D, Parra-Saldivar R. 2020. Bioremediation potential of Sargassum sp. biomass to tackle pollution in coastal ecosystems: circular economy approach. Review. Sci. Total Envir. 715: 136978. https://doi.org/10.1016/j.scitotenv.2020.136978.

[12] Garcia-Poza S, Leandro A, Cotas C, Cotas J, Marques JC, Pereira L, Gonçalves AMM 2020 The evolution road of seaweed aquaculture: cultivation technologies and the industry 4.0 Inter. J. Envir. Res. & Pub. Health 17: 6528. doi:10.3390/ijerph17186528.

[13] Mai H, Fotedar R, Fewtrrel J. 2010. Evaluation of Sargassum sp. as a nutrient-sink in an integrated seaweed-prawn (ISP) culture system. Aquaculture 310: 91-98.

[14] Bambaránda BVASM, Sasaki N, Chirapat A, Salin KR and Tsusaka TW 2019 Optimization of macroalgal density and salinity for nutrient removal by Caulerpa lentillifera from aquaculture effluent. Processess 7(5):303. https://doi.org/10.3390/pr7050303.

[15] Ginting ES, Rejeki S, Susilowati T 2015 Pengaruh peredaman pupuk organik cair dengan dosis yang berbeda terhadap pertumbuhan rumput laut (Caulerpa lentillifera). J. Aquac. Manag. & Tech. 4 (4) : 82-87.

[16] Roleda MY and Hurd CL. 2019. Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. Phycologia 58(5): 552-562.

[17] Tsagkamilis P, Danielidis D, Dring MJ and Katsaros C. 2010. Removal of phosphate by the green
seaweed *Ulva lactuca*, in a small-scale sewage treatment plant (ios island, aegan sea, Greece). *J. Appl. Phycol*, 22 (3): 331–339.

[18] Fourooghifard, H., A. Matinifar, M.S. Mortazavi, G. K. Roohani, dan M. Mirbakhsh. 2018. Nitrogen and phosphorous budgets for integrated culture of whiteleg shrimp *Litopenaeus vannamei* with red seaweed *Gracilaria corticata* in zero water exchange system. *Iranian J. Fish. Sci.* 17(3): 471-486.

[19] Arumugam N, Chelliapan S, Kamyab H, Thirugnana S, Othman N and Nasri NS. 2018 Treatment of wastewater using seaweed: a review. *Int. J. Envir. Res. Pub. Health* 15 (2851): 1-17.

[20] Aquilino KM, Bracken MES, Faubel MN and Stachowicz JJ 2009 Local-scale nutrient regeneration facilitates seaweed growth on wave-exposed rocky shores in an upwelling system *Limnol. & Oceano*. 54(1): 309-317.

[21] Izzati M 2011 The role of seaweeds *Sargassum polycistum* and *Gracilaria verrucosa* on growth performance and biomass production of tiger shrimp (*Penaeous monodon fabr*). *J. Coast. Dev.* 14(3) : 235-241.

[22] Martins AM, daSilva VF, Tarapuez PR, Hayashi L, Vieira FN. 2020. Cultivation of the seaweed *Ulva* spp. with effluent from a shrimp biofloc rearing system: different species and stocking density. *Boletim Do Instituto De Pesca* 46(3): 1-6.

[23] Wandira AW, Sunaryo, Sedjati S. 2018. Rumput laut *Gracilaria* sp. sebagai bioremedian dalam sistem budidaya polikultur dengan kepiting bakau. *J. Mar. Res.* 7(2): 113-124.

[24] Wu H, Huo Y, Hu M, Wei Z, He P. 2015. Eutrophication assessment and bioremediation strategy using seaweeds co-cultured with aquatic animals in an encloses bay in China. *Mar. Poll. Bull.* 95:342-349. http://dx.doi.org/10.1016/j.marpolbul.2015.03.016

[25] Yu Z, Sun H, Huang W, Hu C, Zhou Y. 2019. *Sargassum henslowianum* as a potential biofilter in mariculture farms of a subtropical eutrophic bay. *Mar. Poll. Bull.* 149: 110615. https://doi.org/10.1016/j.marpolbul.2019.110615.