Seaweed *Gracilaria changii* as a bioremediator agent for ammonia, nitrite and nitrate in controlled tanks of Whiteleg Shrimp *Litopenaeus vannamei*

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**Abstract.** Intensive shrimp cultures are characterized by high stocking densities and use large quantities of artificial feed. The artificial feed was consumed only a small part by shrimp, while the rest decomposes in ponds and produces inorganic nitrogen compounds such as ammonia, nitrite, and nitrate, which are poisonous to shrimp and decrease water quality. One alternative that can be applied to improve water quality in the shrimp pond is the use of bioremediation agents. One of the bioremediation agents is seaweed *Gracilaria changii*. The study was designed using a Completely Randomized Design consisting of three treatments, namely (A) shrimp culture without seaweed as bioremediator; (B) shrimp culture with seaweed as bioremediator in the middle of the treatment (starting from fifth week), and (C) shrimp culture with seaweed as bioremediator since the beginning of the treatment. Each treatment was repeated three times each. During the study, ammonia, nitrite, and nitrate were measured every week. *t*-test is used to compare the average values of ammonia, nitrate, and nitrite. This study aims to analyze the potential of *G. changii* as bioremediator agents in the cultivation of whiteleg shrimp *Litopenaeus vannamei* in controlled tanks. This study indicated that seaweed *G. changii* was an effective bioremediator for shrimp *P. vannamei* culture because seaweed could reduce ammonia that is toxic for shrimp. Seaweed was effective as bioremediator until the ninth week, and after the effectiveness decrease indicated by increase the nitrate concentration. Seaweed could be a shelter for shrimp during molting that indicated by higher survival rate at the treatments with seaweed. It is recommended to harvest seaweed at the ninth week, then proceed with the second seaweed culture cycle, so there will two cycles of seaweed culture every one shrimp culture cycle.

1. **Introduction**

Sustainable aquaculture is an important issue in increasing food production without reducing the quality of the aquatic environment. The various cultivation efforts, which were also an effort to mitigate the environment, continue to be developed, both with monostrophic cultivation patterns [1], and with multitrophic cultivation patterns [2-4]. Currently, there are two mainstay aquaculture
commodities, that are shrimps and seaweed; shrimp is to increase the ability to meet the needs of protein [5-9], and seaweed is to increase the production of the raw materials for food, medicines, cosmetics, renewable energy, and environmental mitigation [10-13]. The development of shrimp and seaweed farming simultaneously through multitrophic cultivation patterns can be the right choice to meet market demand and environmental mitigation.

The development of shrimp farming was triggered by an increase in market demand. The increase in market demand was followed by the intensification of shrimp culture, which was characterized by an increase in stocking density. This intensive cultivation uses artificial feed in very large quantities; this feed was not all consumed by shrimp and become organic waste; this organic waste has an impact on water quality.

The organic waste pollution of intensive shrimp cultivation was enormous [14, 15]; if the food conversion rate was between 1.2 and 1.5, with 40% protein feed content, the potential organic waste from shrimp cultivation would reach 48 to 70 kg nitrogen each one ton of shrimp production. Decomposition of this organic waste that has high protein content will produce inorganic nitrogen compounds which are the toxic compounds for shrimp [16, 17].

Increased organic matter content in shrimp ponds as a result of using artificial feed increases the risk of water quality degradation. Therefore, efforts are needed to overcome them. One of the countermeasures that can be done is the use of bioremediation agents. Bioremediation is the environmental biotechnology development by utilizing biological processes in controlling pollution. Bioremediation has the potential to become one of the clean, natural, and inexpensive environmental technologies. One of the potential bioremediator agents to be developed is seaweed Gracilaria changii. As a primary producer, G. changii can use ammonia, nitrite, and nitrate that produced from the decomposition of leftover feed.

G. changii is a macroalga that has a high tolerance ability for changes in environmental factors, such as salinity and turbidity. G. changii is also known as macroalgae, which has the ability to absorb nitrates in water; this ability can be taken into consideration to cultivate G. changii together with shrimp with a multitrophic system. This cultivation system has two advantages. The first is to increase production. The second is to improve water quality by absorbing nitrate. The amount of nitrate produced from the feed waste and the shrimp metabolism waste can be utilized by G. changii.

The previous studies indicate that some seaweed species, such as Kappaphycus alvarezii and E. denticulatum can be used as bioremediator in the aquaculture waste treatment with a water circulation system. K. alvarezii can reduce the concentration of nitrates in the waters around 40.50±13.29%; and E. denticulatum can reduce nitrate concentrations by 47.40±11.37% [18]. This study aims to analyze the potential of G. changii as a bioremediator agent in the cultivation of whiteleg shrimp Litopenaeus vannamei in controlled tanks.

2. Materials and methods
The study was done at the Hatchery of Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia. The organism used in this study was seaweed G. changii [19] (Figure 1a), with a density of 100 g m⁻²; and postlarvae (PL-30 days) of shrimp vannamei L. vannamei, with a density of 160 shrimps m⁻³. The PL-30 used to have an average weight of 0.2315±0.0034 g and an average length of 2.86±0.42 cm (Figure 1b).

This research is an experimental study on a laboratory scale. This study was designed using a Completely Randomized Design [20] consisting of three treatments, namely (A) shrimp culture without seaweed as bioremediator; (B) shrimp culture with seaweed as bioremediator in the middle of the treatment (starting from the fifth week), and (C) shrimp culture with seaweed as bioremediator since the beginning of the treatment. Each treatment was repeated three times each so that there were nine experimental units. Water quality parameters measured were salinity, temperature, light intensity, pH, ammonia, nitrite, and nitrate.

The experimental tank is measuring 90 x 75 cm (Figure 2a), which was filled as high as 80 cm water. Tanks are filled with seawater with 20 ppt salinity that has been sterilized with chlorine.
During the sterilization process with chlorine (Figure 2b), water was aerated for two days then neutralized with thiosulfate. Before being used, the seawater was analyzed for its chlorine content to ensure that the water was chlorine-free.

![Figure 1. Seaweed *Gracilaria changii* (a) and Whiteleg Shrimp *Litopenaeus vannamei* (b).](image1)

Before treatment began, shrimp and seaweed were acclimatized for 12 days. The treatment lasted for ten weeks. During the treatment of shrimps were fed using pellets with complete nutritional composition. Feed dose was 3% of body weight, with the frequency of feeding six times in 24 hours. The shrimps were counted and weighed at the end of the study.

![Figure 2. Treatment tanks (a) and sea water sterilization (b).](image2)

During the study, ammonia, nitrite, and nitrate were measured every week. The concentrations of the ammonia, nitrite, and nitrate were measured using a spectrophotometer by the method APHA 2012

![Figure 3. Seaweed *Gracilaria changii* (a) and shrimp *Litopenaeus vannamei* acclimatization (b).](image3)
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[21]. Shrimp survival rate and growth rate were used as supporting data. The data were analyzed statistically and descriptively. T-test is used to compare the average values of ammonia, nitrate, and nitrite [22].

3. Results
3.1. Ammonia
Ammonia concentration during treatment varies greatly (Figure 4). Ammonia concentration range in treatment A or shrimp culture without seaweed as bioremediator was 0.0010-0.0350 ppm, with an average value of 0.181±0.0116 ppm; Ammonia concentration range in treatment B or shrimp culture with seaweed as bioremediator in the middle of the treatment (starting from the fifth week) was 0.0010-0.0420 ppm, with an average value of 0.0187±0.0129 ppm, and range of ammonia concentration in treatment C or shrimp culture with seaweed as bioremediator since the beginning of the treatment was 0.0010-0.0280 ppm, with an average value of 0.0158±0.0081 ppm. Treatments A and B were not significantly different (p <0.05), treatments A and C were significantly different (p> 0.05), and treatments B and C were significantly different (p> 0.05).

Figure 4. Ammonia concentration during the treatment.

3.2. Nitrite
Nitrite concentration during treatment varies greatly (Figure 5). The range of nitrite concentration in the treatment A or shrimp culture without seaweed as bioremediator was 0.0020-1.1050 ppm, with an average value of 0.44625±0.3763 ppm; the range of nitrite concentration in treatment B or shrimp culture with seaweed as bioremediator in the middle of the treatment (starting from fifth week) was 0.1520-1.1870 ppm, with an average value of 0.6366±0.4032 ppm, and the range of nitrate concentration in treatment C or shrimp culture with seaweed as bioremediator since the beginning of the treatment was 1920-1.8890 ppm, with an average value of 0.6825±0.5490 ppm. All treatment pairs (A and B, A and C, and B and C) were significantly different (p> 0.05).

3.3. Nitrate
Nitrate concentration during treatment varies greatly (Figure 6). The range of nitrate concentrations in the treatment A or shrimp culture without seaweed as bioremediation was 0.1930-6.1160 ppm, with an average value of 1.9626±1.6933 ppm; the range of nitrate concentrations in the treatment B or shrimp culture with seaweed as bioremediator in the middle of the treatment (starting from the fifth week) was 0.7750-5.3040 ppm, with an average value of 2.3494±1.3870 ppm, and the range of nitrate...
concentrations in the treatment C or shrimp culture with seaweed as bioremediator since the beginning of the treatment was 0.3020-4.8750 ppm, with an average value of 1.7151±1.4112 ppm. All treatment pairs (A and B, A and C, and B and C) were significantly different (p > 0.05).

3.4. Survival rate and growth of shrimp
Survival rates were higher in both treatments with seaweed compared to controls (without seaweed as bioremediator) (Figure 7a). Absolute growth was higher in treatment with seaweed as bioremediator in the middle of the treatment (starting from the fifth week) compared to two other treatments (without seaweed as bioremediator and with seaweed as bioremediator since the beginning of the treatment) (Figure 7b).

4. Discussion
The use of seaweed *K. alvarezii* as a biofilter for aquaculture waste has been proven to reduce the eutrophication process [23]. However, the use of *K. alvarezii* as a bioremediator in shrimp ponds
cannot be used because the physical and chemical conditions of the ponds do not support the growth of *K. alvarezi*, so the alternative bioremediator need to be sought. *Gracilarias* sp., which has long been cultivated in ponds can be an option [24-26].

**Figure 7.** Survival rate (a) and absolute growth (b) of shrimp *vannamei* *Litopenaeus vannamei*.

4.1. Ammonia
Seaweed can utilize inorganic nitrogen in various forms of compounds, such as ammonia, nitrates, and nitrates [27, 28]. Ammonia, nitrates, and nitrates are three of the four compounds in the nitrogen cycle (Figure 8) [29].

**Figure 8.** Schematic representation of nitrogen cycle [29]. 1 is the uptake of ammonium or nitrate by microorganisms; 2 is released ammonium by decomposition; 3 is microbial oxidation of ammonium to nitrite; 4 is microbial oxidation of ammonia to nitrate; 5 is microbial denitrification in anaerobic condition; 6 is nitrogen fixation, and 7 is nitrate leaching from the soil.

During the study, the lowest ammonia concentration was found in treatment C, and the highest in treatment A, this was thought to occur because in treatment C there was a seaweed that in the process of photosynthesis produced O$_2$. The presence of O$_2$ is used by aerobic bacteria in the nitrification process using ammonia and producing nitrates. Nitrate is used by seaweed in its growth process. Ammonia can be toxic to shrimp and reduce water quality. Nitrite is a compound that is unstable, where if there is no O$_2$ it will turn into NH$_3$, but if the O$_2$ concentration is high, it will turn into nitrate.
Nitrate is an important nutrient for seaweed growth. Increased organic matter content in shrimp ponds due to the use of artificial feed can increase the risk of water quality degradation. Therefore, efforts are needed to overcome them. One of the best alternatives that can be applied to improve water quality in shrimp ponds is the use of bioremediation agents. One of the bioremediation agents is seaweed *G. changii*. The same trend increase and decrease in ammonia concentration between treatment with and without seaweed can be due to the too low of ammonia concentration (<0.045) compared to the natural concentration for seaweed cultivation brackish water ponds (0.125-0.2141 ppm) [30]. Greater ammonia concentration may be needed to able to indicate the effectiveness of *G. changii* seaweed as a bioremediator.

4.2. Nitrite

Although the results of all treatments were statistically significantly different, the same trend of nitrite concentration in all treatments, probably, due to the accumulation of nitrites in the tank (± 1,200 ppm). The natural concentration of nitrite in seaweed cultivation brackish water ponds was 0.0033-0.0406 ppm [30]. This accumulation may have relation with the presence of microbes or bacteria in the tank. Nitrite is a compound that is unstable, and in high concentrations is toxic. The presence of nitrite depends on the presence of O₂ and decomposing bacteria in the waters. Water sterilization removes microbes or bacteria so that there are no microbes or bacteria that oxidize nitrites to nitrates [29]. This was one of the weaknesses in shrimp cultivation using sterile sea water; on one side, sterilization can eliminate the disease, but on the other side, sterilization can eliminate microbes or bacteria that functions as a nitrogen recycler. The nitrogen cycle requires several bacteria of Proteobacteria, such as *Nitrobacter* sp, *Nitrosomonas* sp, *Nitrosococcus* sp, and *Nitrospina* sp. [31]. This study shows the importance of using probiotics the shrimp cultivation [32].

4.3. Nitrate

Nitrate is one of the main sources of nitrogen for autotroph [29]; therefore, under normal conditions, nitrate concentration in the brackish water pond was not too big (0.0379-1.4892 ppm). Nitrate content that continues to increase up 5.0 ppm at the end of treatment in all treatments indicated there was accumulation of nitrate in the tanks. Accumulation is thought to occur because the nitrate produced in the tanks is more than what is needed by seaweed.

4.4. Survival rate and absolute growth

Survival rate was higher in treatment with seaweed, treatment with seaweed as bioremediator since the beginning showed a higher survival rate. Since water quality parameters have the same trend, the difference in survival rate was thought to be related to the presence of seaweed. Seaweed is one of the epiphytic hosts which can be a natural food for shrimps, especially during the juvenile phase, so that shrimp cultivate in the seaweed treatment as bioremediator since the beginning, have a higher survival rate. Another inference that can explain the higher survival rate of the seaweed treatment as bioremediator since the beginning was the presence of seaweed can be a shelter for shrimps, especially when molting so that the shrimps were protected from the cannibalism.

Growth did not indicate the consistent correlation with the presence of seaweed because the treatment with seaweed as bioremediator since the beginning has the lowest growth rate. However, if the growth rate was related to survival rate, then it appears that the higher survival rate has a lower growth rate. This was thought to be related to feeding availability, at the treatments where higher survival rates were found, greater competition for feed resources, resulting in lower growth.

5. Conclusion

This study indicated that seaweed *G. cangii* was an effective bioremediator for shrimp *P. vannamei* culture because seaweed could reduce ammonia that is toxic for shrimp. Seaweed was effective as bioremediator until the ninth week, and after the effectiveness decrease indicated by increase the nitrate concentration. Seaweed could be a shelter for shrimp during molting that indicated by higher
survival rate at the treatments with seaweed. It is recommended to harvest seaweed at the ninth week, then proceed with the second seaweed culture cycle, so there will two cycles of seaweed culture every one shrimp culture cycle.

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