EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2022, 23(5), 137–144
https://doi.org/10.12912/27197050/151880
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Received: 2022.06.27
Accepted: 2022.07.18
Published: 2022.08.01

Reducing Nitrate and Phosphate Concentrations in Shrimp Wastewater Aquaculture Using *Gelidium corneum*

Nabila Indraswari[1], Ratih Ida Adharini[1*]

[1] Laboratory of Aquatic Resource Management, Department of Fisheries Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Caturtunggal, Kec. Depok, Kabupaten Sleman, Daerah Istimewa Yogyakarta 55281, Indonesia

* Corresponding author's e-mail: ratih.adharini@ugm.ac.id

ABSTRACT

Shrimp culture wastewater contains residual feed and shrimp metabolism. Shrimp wastewater if not managed properly will be harmful to the long term viability of the farming itself and threatens environmental sustainability. This study aims to determine the potential and effectiveness of *Gelidium corneum* with different densities as a biofilter for vaname shrimp culture waste. This study used a completely randomized design method consisting of 3 treatments and 3 replications using different density treatments, namely T1 (1 gL^{-1}); T2 (2 gL^{-1}); and T3 (3 gL^{-1}) in an aquarium volume of 30 L. The parameters observed included the concentration of nitrate, phosphate, temperature, TDS, TSS, salinity, DO, pH. Absorption effectiveness and growth of *G. corneum* were measured at the beginning and end of the study. *G. corneum* is able to reduce the concentration of phosphate in water is higher than lowering the content of nitrate. *G. corneum* with a density of 1 gL^{-1} showed the best performance in absorbing nitrate by 8% on day 15. While the 2 gL^{-1} treatment was able to reduce the phosphate concentration by 92% in 25 days. Absorption of Total Nitrogen content in the thallus obtained in the treatment of 2 gL^{-1} was 32% and total phosphate was 58% for 25 days. Therefore *G. corneum* can be used as a candidate for commodity in Integrated Multi Trophic Aquaculture (IMTA).

Keywords: nutrient, wastewater, shrimp, removal, growth rate, *Gelidium corneum*.

INTRODUCTION

The need for food consumption globally continues to increase, including the need for shrimp commodity. The increasing global demand for shrimp has been translated into an increase the global aquaculture production of vannamei shrimp. FAO noted that there had been an increase in shrimp farming production by 2648.5 thousand tons in 2010 to 4966.2 thousand tons in 2018 (FAO 2020). Meanwhile, the national shrimp production volume in the last five years (2013–2018) also showed a positive growth trend with an average annual growth of 15.7% (Indonesian Ministry of Marine and Fisheries, 2018).

Shrimp culture which is managed semi-intensively or intensively has serious problems related to water quality degradation. Dissolved nitrogen and phosphate produced by intensive shrimp farming comes from the excretion of shrimp and decomposition of feed residues, where about 60–90% of nitrogen waste is found in dissolved form (van Rijn 2013). From the feed given to shrimp, about 80–60% nitrogen and 90–85% phosphorus become feed waste in the culture water (Kawasaki et al. 2016).

High nutrient waste in aquaculture systems has a major impact on cultured shrimp due to the presence of toxic nitrogen elements (ammonia and nitrite), the growth of harmful microorganisms caused by increased nutrient concentrations, or an imbalance in the N:P ratio (da Silva 2013). Other impacts that often occur are an increase in suspended solids sedimentation, increased turbidity, eutrophication, increased algae and microbial growth, and a higher need for biological and chemical oxygen (Santhi et al. 2017; Rabiei et al. 2016). So that if shrimp culture waste is disposed of directly into open waters, it has the potential to cause pollution and degradation of coastal and marine ecosystems (Williamson et al. 2017; Tirkaso & Gren 2016) which in turn will have an impact on decreasing biodiversity. For this reason, a good cultivation technique is needed to improve the quality of aquaculture water to support sustainable shrimp production levels as well as to reduce the impact of pollution on aquatic ecosystems.

Gelidium is an edible red seaweed as a functional food containing agar which is used for nutraceutical and pharmaceutical (Lee et al. 2018; Rajeshkumar et al. 2018). *Gelidium* has also been widely used for biofilters and bioremediation of heavy metals (El-Naggar et al. 2018), as well as for the pulp industry (Seo et al. 2010; Kustantiny 2011). Red algae as a biofilter is claimed to be able to reduce nutrient waste because it is able to absorb nitrogen compounds (NH3, NO3, and NO2) and phosphorus from cultivation activities (Marinho-Soriano et al. 2009). The use of red algae, especially *Gelidium* as a biofilter to reduce aquaculture waste, has been carried out by several researchers. Research by Rabiei et al. (2016) which uses *G. elegans* as a biofilter can grow in shrimp broodstock waste which functions to reduce nitrogen compounds. Liu et al. (2004) used *G. amansii* as a biofilter to reduce nitrogen levels in culture water. *G. corneum* is easy to find and very abundant on the southern coast of Yogyakarta. The optimal density of seaweed used needs to be investigated to determine its effectiveness and growth (Bambaranda et al. 2019). Therefore, research is needed to determine the ability of *G. corneum* with different densities to reduce nitrate and phosphate concentrations in water, as well as the effectiveness of their absorption ability. This research is expected to reveal the potential of *G. corneum* as a biofilter of shrimp culture water which can be used as a candidate for a combination of commodities in profitable and sustainable aquaculture.

MATERIALS AND METHODS

The research was conducted at the Sundak Sea Water Cultivation Unit, Gunungkidul, Yogyakarta. Aquaculture water was obtained from vanname shrimp culture water at the Sundak Seawater Aquaculture Work Unit, Gunungkidul, Yogyakarta. *G. corneum* was obtained in the intertidal area of Trenggole Beach, Gunungkidul, Yogyakarta.

Preliminary research

Preliminary tests were carried out to determine the appropriate dilution for the main test. The preliminary test phase was carried out by testing dilution 1:1 shrimp cultured water and seawater, 1:3 shrimp cultured water and seawater, and shrimp cultured water and water. 3:1 sea in 5 different aquariums for 7 days. *G. corneum* was reared in the aquarium with the addition of aeration. The best dilution was obtained at 1:1 seawater and vaname shrimp culture water.

Acclimation

The acclimatization step was carried out by maintaining *G. corneum* for 3 days with the basic stocking method with the addition of 7 mL of fertilizer. The purpose of this acclimatization step is to ensure that the seaweed can adapt to the new environment.

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 used was seaweed density treatment, which consisted of P1 = 1 gL^{-1}, P2 = 2 gL^{-1}, and P3 = 3 gL^{-1}. The main test was carried out by maintaining *G. corneum* in a mixture of 15 L of shrimp wastewater aquaculture and 15 L of seawater (1:1).

Water quality measurements

The physical parameters measured were temperature, pH, and salinity. Chemical parameters measured were dissolved oxygen (DO), nitrate (NO_3) and phosphate (PO_4). Measurement of phosphate (PO_4) and nitrate (NO_3) was performed every 5 days. Measurements of DO, temperature, salinity, and pH parameters were carried out at the *G. corneum* rearing location in the Sundak Seawater Aquaculture Unit. DO, temperature, salinity and pH measurements were carried out every 5 days. Growth of *G. corneum* was measured every 7 days.

Absorption measurement

The absorption ability of *G. corneum* to absorb the N and P content in the vannamei shrimp culture water, an analysis of the absorption capacity obtained from the measurement of the total N and total P content in the seaweed thallus was carried out 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. The absorption of N and P by *G. corneum* in the thallus is assumed using the percentage with the formula (1) as follows:

$$(\%) = \frac{Cf - Ci}{Ci} \times 100 \quad (1)$$

where: Cf is the initial concentration and Ci is the final concentration.

Data analysis

This research was conducted using a completely randomized design. The results of water quality measurement data including nitrate, phosphate, biomass, nitrogen and phosphorus content were analyzed by analysis of variance (ANOVA). Analysis of variance (ANOVA) was used to determine whether there was a significant difference between treatments and time. If the results obtained during the study were significantly different, further analysis was carried out using the Tukey test. Analysis of variance (ANOVA) and Tukey's test were performed with the SPSS version 20 application using One Way ANOVA analysis.

RESULTS AND DISCUSSION

The reduction of nitrate and phosphate concentration in vannamei wastewater aquaculture

The concentration of nitrate during the treatment fluctuated. The highest value of nitrate concentration was 0.71 mgL^{-1} on the 25th day of 2 gL^{-1} treatment. The lowest nitrate concentration value was 0.53 mgL^{-1} on the 15th day of 1 gL^{-1} treatment with a decrease of 8%. Based on the results of statistical analysis, there was no significant difference ($p>0.05$) from treatment 1 gL^{-1}, treatment 2 gL^{-1}, and treatment 3 gL^{-1} so that it did not have a significant effect on nitrate levels in shrimp cultured water (Table 1).

The dynamics of nitrate concentration in the three treatments had the same pattern. Although on the 2^{nd} day there was a slight decrease, then

Table 1. Average of nitrate concentration of shrimp wastewater aquaculture during treatment

Treatment	Nitrate (mgL^{-1})					
	0 day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	25^{th} day
1 (1 gL^{-1})	0.58 ± 0^a	0.53 ± 0.01^a	0.58 ± 0.01^a	0.53 ± 0.01^a	0.61 ± 0.01^a	0.65 ± 0.01^a
2 (2 gL^{-1})	0.58 ± 0^a	0.58 ± 0.03^a	0.62 ± 0.02^a	0.55 ± 0.01^a	0.65 ± 0.02^a	0.71 ± 0.04^a
3 (3 gL^{-1})	0.58 ± 0^a	0.55 ± 0.02^a	0.64 ± 0.02^a	0.57 ± 0.03^a	0.68 ± 0.03^a	0.66 ± 0.03^a

Note: *values in the column followed by the same letter show no significant difference at the 95% confidence level.

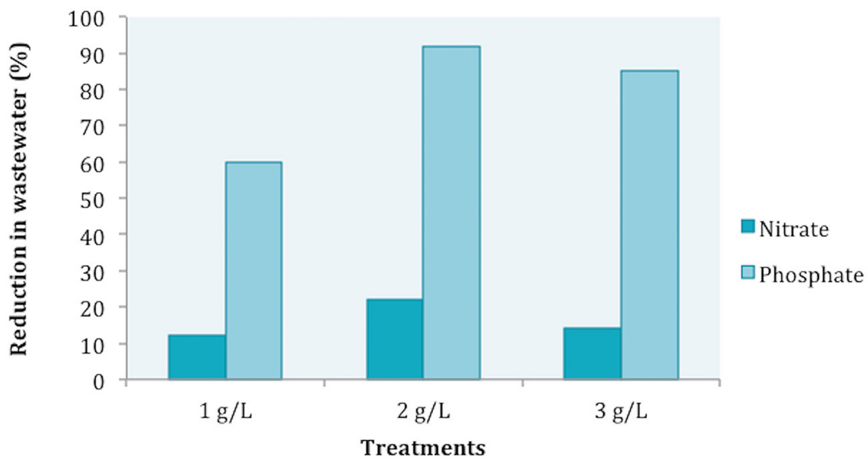

Figure 1. Percentage reduction of Nitrate and Phosphate concentration for 25 days

the nitrate concentration in the three treatments increased until the 10th day. It is suspected that the nitrification process is still ongoing until the 10th day. Furthermore, on the 15th day the nitrate concentration decreased because nitrate had begun to be absorbed by *G. corneum*. However, after being absorbed, NO_{3-} still undergoes a reduction process by nitrate reductase enzyme (Carneiro et al. 2021) before being stored in the thallus so that the nitrate absorption process is relatively slower. Then on the 20th day, nitrate levels increased again, presumably because the nitrification process continued. On the 25th day there was an increase in nitrate again in the 1 gL^{-1} treatment and the 2 gL^{-1} treatment, presumably due to the decreased condition of *G. corneum*. According to Carneiro et al. (2021) nitrate is the nitrogenous form with the highest concentration in water compared to ammonium and nitrite. However, ammonium is the form that is most easily absorbed by seaweed than other forms (Liu et al. 2004; Rabiei et al. 2016). The condition of *G. corneum* on the 25th day from the treatment of 1 gL^{-1}, 2 gL^{-1}, and 3 gL^{-1} experienced a decrease in quality such as decreased biomass, slightly faded color, and weakened thallus. Rabiei et al. (2016) stated that the limited nitrogen concentration in water will affect the ability of pigment formation so that the thallus looks pale. The condition of *G. corneum* has decreased causing less than optimal *G. corneum* in absorbing nitrate causing the nitrate in the water to increase.

Phosphate concentration in wastewater aquaculture in the all treatments decreased from 1.6 mgL^{-1} on day 0 to 0.33 mgL^{-1} on day 25. The lowest decrease in phosphate concentration occurred in the 2 gL^{-1} and 3 gL^{-1} treatments which showed a significant difference with the 1 gL^{-1} treatment (Table 2).

The concentration of phosphate in the vannamei wastewater aquaculture continued to decrease until the 25th day. On the 25th study day, the phosphate concentration experienced the highest decrease with an average decrease of 79%. The lowest phosphate concentration value was 0.13 mgL^{-1} on the 25th day of 2 gL^{-1} treatment with a decrease of 92%. The results showed that the phosphate levels ranged from 0.13 to 1.67 mgL^{-1}.

The use of *Gelidium* as a biofiter in this study showed that *Gelidium* was able to reduce the concentration of phosphate more than reducing nitrate in vanname shrimp culture water. This is different from the research of Rabiei et al. (2016) who reported that *G. elegans* was more efficient in reducing nitrate (48%) than reducing phosphate (20%). Seaweed's ability to absorb different nitrogen is because it is influenced by the species of seaweed (Liu et al. 2004). *Gelidium* density treatment of 2 gL^{-1} showed the highest efficiency as a biofilter because it was able to reduce the highest nitrate concentration by 22% and phosphate by 92%.

Absorbability of *G. corneum* to total inorganic nitrogen and total phosphate in aquaculture wastewater

Red algae are considered efficient in nutrient absorption because red algae have a great ability to store nutrient reserves. The concentration of nitrate and phosphate in the culture water which tends to decrease in the three treatments indicates that there has been absorption of total inorganic nitrogen (TIN) and phosphate in the thallus of *G. corneum*. The absorption of TIN and water phosphate into the thallus was indicated by the addition of total kjeldahl nitrogen (TKN) and phosphate content in the thallus after treatment. The increase in TKN and phosphate in the thallus in each treatment is shown in Figure 2.

Based on the results of the analysis using the One Way ANOVA test, the three treatments did not have a significant effect on the absorption of TIN by *G. corneum*. Although on the 25th day, the TKN content in the thallus increased. The highest increase in total TKN in the thallus occurred in the 2 gL^{-1} treatment with an increase of 32% (Table 3). According to Rabiei et al. (2016) that the nitrogen content absorbed by the seaweed is stored in the thallus and converted into protein.

Table 2. Average of phosphate concentration of shrimp wastewater aquaculture during treatment

Treatment	Phosphate concentration (mgL^{-1})					
	0 day	5th day	10th day	15th day	20th day	25th day
1 (1 gL^{-1})	1.60 ± 0 ^{a}	1.30 ± 0.06^{a}	1.23 ± 0.03^{a}	1.07 ± 0.09^{a}	0.90 ± 0.15^{a}	0.63 ± 0.09^{a}
2 (2 gL^{-1})	1.60 ± 0 ^{a}	1.17 ± 0.03^{b}	0.90 ± 0.00^{b}	0.67 ± 0.12^{b}	0.43 ± 0.03^{b}	0.13 ± 0.03^{b}
3 (3 gL^{-1})	1.60 ± 0 ^{a}	1.00 ± 0.06^{b}	0.73 ± 0.09^{b}	0.33 ± 0.03^{b}	0.30 ± 0.06^{b}	0.23 ± 0.07^{b}

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

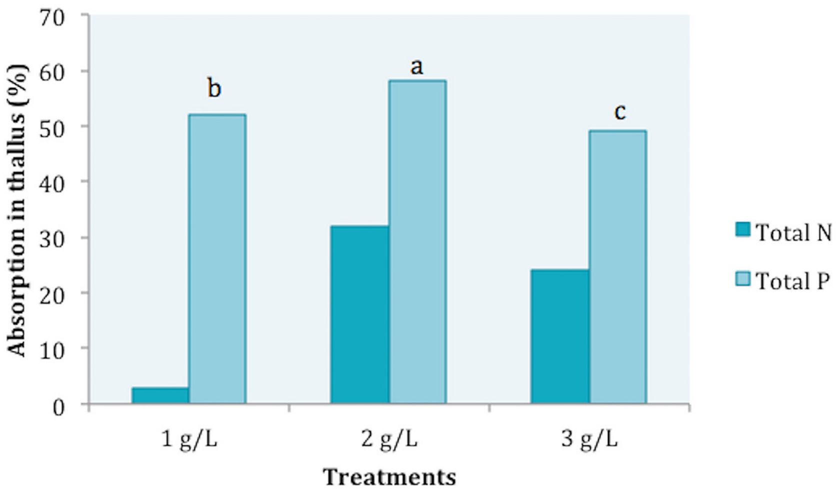

Figure 2. Absorbtion of total inorganic nitrogen and total phosphate in thallus

There was a significant difference ($p<0.05$) on absorption in the thallus until the 25th day in the three treatments. The highest total phosphate absorption in the thallus occurred in the 2 gL^{-1} treatment with an increase of 58%. *G. corneum* is able to absorb well the phosphate content contained in the vaname shrimp culture waste used. According to Ben-Ari et al. (2014), the effectiveness of seaweed as a biofilter is influenced by several controllable factors such as the type and density of seaweed, pond design, nutrient input and aeration regime.

The results obtained during the research period showed that the concentration of nitrogen (TIN) and phosphate from vaname shrimp culture waste decreased because it was absorbed by *G. corneum* and then stored in the thallus so that the content of TKN and total phosphate in the thallus increased in all treatments. With the absorption of nitrogen (TIN) and phosphate by *G. corneum*, it is evident that there has been a decrease in nutrient waste in aquaculture water so that it can improve the quality of vaname shrimp aquaculture wastewater. In addition, when the demand for nutrients from seaweed is greater than the supply, then nutrients become a limiting factor which means that it can limit the growth of seaweed (Roleda & Hurd, 2019).

The growth rate of *G. corneum*

The average wet weight increased on the 7th day in the 1 gL^{-1} and 2 gL^{-1} treatments. While in the 3 gL^{-1} treatment, it decreased on the 7th day. Then on the following days, the wet weight of *G. corneum* decreased both in the 1 gL^{-1}, 2 gL^{-1}, and 3 gL^{-1} treatments (Figure 3).

The decrease in wet weight in all treatments after the 7th day was thought to be because the nutrients in the culture water had been completely

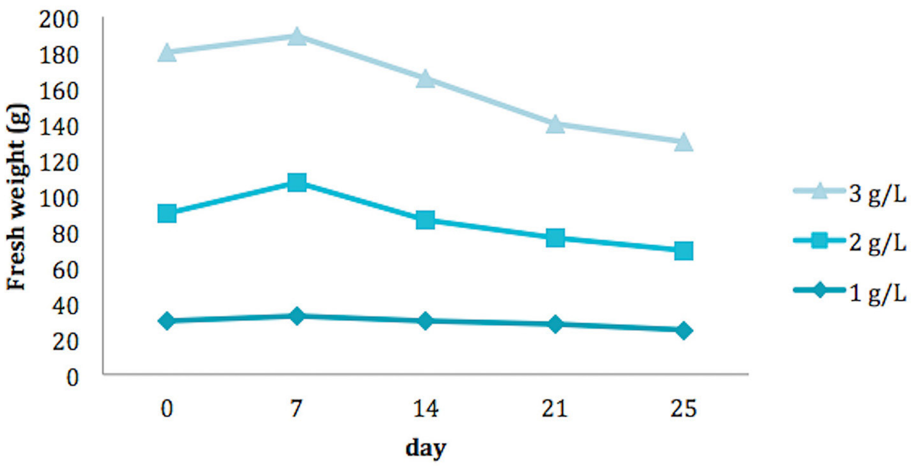

Figure 3. The dynamical of fresh weight of *G. corneum* during experiment

absorbed by the thallus of *G. corneum* so that as the days went on, the nutrient concentration was getting depleted, thus affecting the growth of *G. corneum* until the end of the study. Rabiei et al. (2016) also stated that the decreased growth of *Gelidium* as a biofilter was due to increasingly limited nutrients. In addition, wastewater quality conditions such as pH, salinity, turbidity, and light also affect the survival of *G. corneum*.

Significant weight loss was obtained in the 3 gL^{-1} treatment, where *G. corneum* on the 25^{th} day experienced a decrease in weight with an average of 33% (Figure 4).

Treatment density of 1 gL^{-1} was also decreased but not significantly. So that the weight loss of *G. corneum* during the study period showed that the treatment of lower seaweed density as biofilter experienced a less significant decrease. This happens because the use of a lower density of seaweed as a biofilter will reduce competition in the utilization of nutrients to be absorbed. On the other hand, the use of seaweed with a higher density as a biofilter in the same media volume will accelerate the absorption of nutrients in the absorbed waste so that the concentration of nutrient waste in aquaculture water will decrease and run out, but then there will be competition for seaweed to get nutrients back if it is in a closed system. This is reinforced by Roleda & Hurd (2019) that when the demand for nutrients from seaweed is greater than the supply, then nutrients become a limiting factor which means it can limit the growth of seaweed.

Water quality parameters

The survival and ability of seaweed as a biofilter is influenced by the quality of the aquaculture wastewater itself. This is because each seaweed has different adaptability and tolerance when it is in aquaculture waste. So that when the seaweed survives in aquaculture wastewater, the seaweed will be able to work effectively in absorbing nutrients and improving the quality of aquaculture water. The quality parameters of aquaculture wastewater during the study are presented in Table 3.

The temperature obtained from the cultured water during the study ranged from 26.2 °C – 29.3 °C. The increase in temperature also affected the condition of *G. corneum* on the 25^{th}

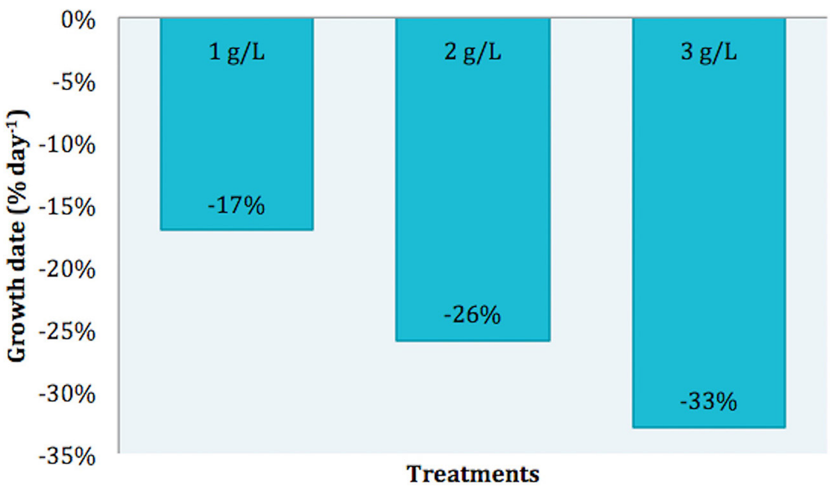

Figure 4. The growth rate of *G. corneum* for twenty five days

Table 3. Water quality parameter during experiment

Parameter	On day						Range
	0	5	10	15	20	25	
Water temp. (°C)	27.3	26.2	29.3	28.4	28.9	27.90	26.2–29.3
pH	7.55	7.71	7.64	8.20	8.20	7.77	7.55–8.20
DO (ppm)	6.9	5.8	5.9	6.4	6.9	8.5	5.8–8.5
Salinity (ppt)	29.1	29.3	30.2	31.5	32.9	33.8	29.1–33.8
TDS (mgL^{-1})	32.0	32.7	33.8	35.1	36.5	37.7	32–37.7
TSS (mgL^{-1})	3793.3	7074.9	2780.7	5258.0	6344.2	5314.0	2780.7–7074.9

day which began to experience a decrease in quality such as the seaweed thallus becoming wilted or pale so it could not grow and develop properly. The degree of acidity (pH) during the study ranged from 7.55 to 8.20. so that the pH parameters obtained during the research period are still in a good range for *G. corneum* to improve water quality by stabilizing the pH of aquaculture wastewater.

DO on days 5 to 10 tend to decrease. This is presumably because the activity of nitrobacteria and nitrosomonas in carrying out nutrient reforms requires oxygen. From the 15th day, the DO concentration gradually increased, presumably because the condition of *G. corneum* had begun to stabilize and oxygen production was carried out through the process of photosynthesis. So that the utilization of *G. corneum* is able to improve the quality of wastewater by increasing the DO concentration in the water. This is of course very profitable for fish/shrimp farming if it is co-cultured with seaweed. Salinity obtained during the study ranged from 29.1 ppt to 33.8 ppt at the end of the study. As the days go by, the salinity obtained also increases. The increase in salinity must be controlled properly because it will affect the seaweed's ability to absorb nutrients and affect the life of the shrimp if the water is recirculated. Ching & Redzwan (2017) stated that salinity affects the activity of nitrosomonas and nitrobacter bacteria in the nitrification and denitrification processes.

The condition of the seaweed also gradually experienced a slight decrease in quality such as the color starting to fade, branches that break easily and decreased biomass. Based on this, the salinity on day 0 to day 20 shows that it is still in the optimal range. Then on the 25'h day there was an increase in salinity which slightly exceeded the optimal salinity range for seaweed growth. In addition, at a salinity of 33.8 ppt the biomass of *G. corneum* also decreased. TSS and TDS values increased until the end of the study. High concentrations of TSS and TDS can increase turbidity thereby inhibiting the penetration of light needed by *Gelidium* to carry out photosynthesis. The inhibition of the photosynthesis process results in a decrease in the metabolic ability of seaweed in its growth and its ability to absorb nutrients. This is different from the research results of Carneiro et al. (2021) where the use of seaweed can significantly reduce TSS in shrimp culture water.

CONCLUSION

G. corneum was able to reduce phosphate concentrations higher than TAN in vaname shrimp cultured water. The treatment of 2 gL^{-1} and 3 gL^{-1} was able to significantly reduce the concentration of phosphate in water. The 2 gL^{-1} treatment was able to absorb 32% total N and 58% total P in the thallus for 25 days. Therefore, *G. corneum* can be used as a candidate for commodity in Integrated Multi Trophic Aquaculture (IMTA).

Acknowledgements

This research was funded through the RTA grant from Universitas Gadjah Mada in 2021.

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