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Pemanfaatan *Gracillaria* sp. sebagai Agen Biofilter pada Udang Vaname (*Litopenaeus vannamei*) yang Dibudidayakan di Laboratorium

Use of *Gracillaria* sp. as a biofiltering agent in laboratory cultured Vaname shrimp *(Litopenaeus vannamei*)

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ABSTRAK

Pakan udang mengandung nitrogen dan fosfor, yang merupakan nutrisi penting untuk pertumbuhan udang. Namun, tidak semua pakan dikonsumsi oleh udang, dan beberapa di antaranya menjadi limbah yang terakumulasi di dalam air. Ada berbagai metode untuk mengurangi amonia dan fosfat, salah satunya dengan bioremediasi. Salah satu bahan biofilter yang menjanjikan untuk budidaya udang adalah *Gracillaria* sp yang dapat menyerap amonia dan fosfat dari air. *Gracillaria* sp. juga dapat menghasilkan agar-agar yang merupakan bahan yang sangat berharga untuk berbagai industri. Penelitian ini menggunakan *Gracillaria* sp dengan berat yang berbeda untuk bioremediasi limbah cair budidaya udang vannamei. Metode yang digunakan adalah eksperimental di Laboratorium dengan 4 perlakuan, yaitu K: *Gracillaria* sp dengan berat 100 gram (kontrol tanpa udang), A: *Gracillaria* sp dengan berat 300 gram, B: *Gracillaria* sp dengan berat 450 gram, dan C: *Gracillaria* sp dengan berat 600 gram. Hasil penelitian menunjukkan bahwa *Gracillaria* sp. dapat secara efektif menurunkan kadar amonia dan fosfat dalam air, dengan efisiensi biofilter mencapai 92% untuk fosfat dan 65% untuk amonia. Semakin tinggi berat *Gracillaria* sp. yang digunakan dalam biofilter, maka semakin efisien dalam menyisihkan amonia dan fosfat. Selain itu, *Gracillaria* sp. juga dapat menghasilkan agar, zat yang penting untuk berbagai industry.

Kata kunci: Amonia-N; biofilter; fosfat; *Gracillaria* sp.; *L. vannam*ei;

ABSTRACT

Shrimp feed contains nitrogen and phosphorus, which are essential nutrients for shrimp growth. However, not all of the feed is consumed by the shrimp, and some of it becomes waste that accumulates in the water. There are various methods to reduce ammonia and phosphate, one of it is by bioremediation. One promising biofilter material for shrimp farming is *Gracillaria* sp that can absorb ammonia and phosphate from the water. *Gracillaria* sp. can also produce agar, a valuable substance for various industries. This research uses *Gracillaria* sp with different weights for bioremediation of vannamei shrimp farming effluent. Method used was experimental in Laboratory with 4 treatments, there are K: *Gracillaria* sp weight of 100 grams (control without shrimp), A : *Gracillaria* sp weight of 1 300 grams, B: *Gracillaria* sp. weight of of 450 grams and C: *Gracillaria* sp weight of 600 grams. The results showed that *Gracillaria* sp. can effectively reduce the levels of ammonia and phosphate in water, with biofilter efficiency reaching up to 92% for phosphate and 64% for ammonia. The higher the weight of *Gracillaria* sp. used in the

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biofilter, the more efficient it was at removing ammonia and phosphate. Additionally, *Gracillaria* sp. can also produce agar, a valuable substance for various industries.

Keywords: Ammonia-N; biofilter; *Gracillaria* sp.; *L. vannamei*; phosphate;

INTRODUCTION

Shrimp feed can increase the levels of ammonia and phosphate in water due to the nitrogen and phosphorus content in the feed, which is essential for shrimp growth. However, not all of the feed is consumed by the shrimp, and some of it becomes waste that accumulates in the water. The decomposition of the feed and the shrimp excretion by bacteria produces ammonia, which is toxic to the shrimp and can cause stress, reduced growth, and mortality. The feed and the shrimp excretion also release phosphate into the water, which can stimulate algal growth and cause eutrophication. Therefore, it is important to monitor and control the ammonia and phosphate levels in the water to ensure optimal shrimp health and production (Septory et al., 2021).

Effects of excessive feed might increase levels of ammonia dan organic phosphate. Shrimp feces and the results of microorganisms in the decay of organic matter rich in nitrogen (protein) are the cause of high ammonia levels in water (Iber & Kasan, 2021). Ammonia is toxic to shrimp and can cause stress, reduced growth, and mortality. High ammonia levels can also stimulate the growth of plankton and benthic algae, which consume oxygen and contribute organic matter to ponds (Zhao et al., 2020) .

Phosphorus has lower toxicity than ammonia or nitrite, but the indirect consequences of eutrophication are hazardous to aquatic life 3. Shrimp feed and feces decompose in water, releasing phosphate into the water, which can stimulate algal growth and cause eutrophication 4 . Algae have a lifespan of a few days, so they die continually and contribute organic matter to ponds 5.

It is important to monitor and control the ammonia and phosphate levels in the water to ensure optimal shrimp health and production. There are various methods to reduce ammonia and phosphate in water, such as water exchange, water circulation, aeration, biofiltration, nitrification, denitrification, and bioremediation. Some of these methods involve using living organisms, such as seaweed, bacteria, or algae, to absorb or transform ammonia and phosphate into less harmful forms.

A particular species of seaweed called *Gracillaria* sp. can function as a biofilter for the cultivation of Litopenaeus vannamei shrimp. A biofilter is a system that uses living organisms to remove pollutants from water. *Gracillaria* sp. can absorb nutrients such as nitrate and phosphate from the shrimp waste, and also produce agar, a valuable substance for various industries. According to some studies, *Gracillaria* sp. can increase the production and quality of vannamei shrimp by improving the water quality and providing natural food for the shrimp. Some of the benefits of using *Gracillaria* sp. as a biofilter are could reduce the cost of water exchange and artificial feed; to enhance the immune system and growth rate of the shrimp; to prevent the occurrence of diseases and parasites in the shrimp; and to generate additional income from harvesting the seaweed (Yudiati et al., 2020; Andayani et al., 2016).

In aquaculture systems, ammonia can build up and be toxic, affecting the wellbeing and development of aquatic organisms. Because it uses ammonia for photosynthesis and the creation of agar, an important material for many industries, *Gracillaria* can lower the ammonia concentration in water. Depending on the quantity of seaweed and zeolite employed as a biofilter, *Gracillaria* sp can remove up to 100% of the ammonia in water within 7 days, according to some research (Royan et al., 2019).

The intensive shrimp farming makes high feed inputs, where uneaten feed will be able to accumulate in the

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water, which can also lead to increased phosphate levels in the water. Phosphate sources apart from feed, can also come from water sources, fertilizers and the use of other materials such as supplements. Phosphate is a harmful substance that can cause eutrophication and algal blooms in aquatic ecosystems.

Water quality in ponds used for raising Vannamei shrimp is typically impacted by the usage of *Gracillaria* sp. as biofilters. However, it is not yet clear how much seaweed is the appropriate dosage to use or how well it can reduce phosphate and ammonia levels in pond water. In order to determine how much *Gracillaria* sp is present and how successful it is at enhancing water quality, this study will evaluate these factors.

RESEARCH METHODS

The research was conducted in the Laboratory of Aquaculture (Division of Reproduction) and Freshwater Aquaculture Unit Sumberpasir, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya.

Gracillaria sp weighed according to treatments. In aquariums, vannamei shrimp of the size PL 15 were acclimatized. Fed 3 times a day, with commercial feed. Water quality such as temperature, dissolved oxygen and pH were measured daily, while ammonia and phosphate were measured 3 times during research using spectrophotometer.

The research used experimental methods in Laboratory. The treatment used in this study is using *Gracillaria* sp. seaweed as a biofilter material in the cultivation of vannamei shrimp (*P. vannamei*). Every treatment was replicated three times and placed randomly in this study. The treatments in this study were K: *without Gracillaria* A : *Gracillaria* sp weight of 1 300 grams, B: *Gracillaria* sp weight of of 450 grams and C: *Gracillaria* sp weight of 600 grams. Seaweed weight treatment was determined by preliminary research, while the control treatment used the method suggested by Widiastuti, (2011). Shrimp density 90 vannamei shrimp for each aquarium using the method of Supriyono et al.(2006).

Preparing containers and research equipment for use in research. Start by preparing 24 aquariums, each measuring 30x30x30 cm3 for *Gracillaria* sp aquariums and 60x30x30 cm3 for shrimp aquariums. Aquariums were cleaned by washing with soap and letting them dry in the sun. The aquarium was then set up in the designated location, and the *Gracillaria* aquarium and the vannamei shrimp aquarium were each filled with water, the *Gracillaria* aquarium to a height of 25 cm, the shrimp aquarium to a volume of 45 liters, and the vannamei shrimp aquarium to a density of 90 shrimp per aquarium, respectively. A conduit to drain water into the shrimp tank was added to each treatment A, B, and C of *Gracillaria* sp. seaweed tank. The water also drained to the tank's base where the shrimp were kept.

Shrimp acclimatization in the aquarium for 15 days. During acclimatization shrimp were given commercial feed 3 times a day (at 07.00 am, 12.00 pm and 17.00 pm). At the time of research vannamei shrimp fasting for one day to empty the stomach. With the following adjustments, the filter efficiency formula from (Lekang, 2013) is used to calculate the reduction of nitrogen in the form of ammonia and phosphate in the form of orthophosphate.

$$Cc = \left(\frac{Cstart - Cend}{Cstart}\right) x\ 100 \qquad (1)$$

Description:
Cc = Filter efficiency (%)
Cstart = Concentration of substance at the beginning of the study
Cend = Concentration of substance at the end of the study

Agar content test was conducted in accordance with the procedure proposed by Rosyida and Nasmia (2015), to determine the development of the utilization of N and P by *Gracillaria* sp. Agar was extracted by using 10 grams of

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dried seaweed soaked in 0.25% chlorine solution for 3x24 hours. After rinsed and cleaned, then soaked in fresh water for 3 hours. The seaweed then soaked in 0.1% H_2SO_4 for 15 minutes and washed thoroughly, following soaked in fresh water again for 15 minutes. Seaweed was cooked with 500 ml of distilled water, filtered and poured into trays and dried. The dried agar was then weighed. The content of agar in dried seaweed (yield) was calculated after extraction using a formula to determine the percentage of agar content in seaweed.

$$Agar\ content = \frac{agar\ weight\ (g)}{sampel\ weight\ (g)} x\ 100 \quad (2)$$

Water quality data displayed descriptively while filter efficiency and agar content were analyzed using ANOVA with 95% confidence interval. In addition, a correlation test was conducted to determine whether there was a relationship between the efficiency of the *Gracillaria* sp biofilter and the agar content.

RESULTS AND DISCUSSION

The nutrient phosphorus and nitrogen in pond water can be absorbed by *Gracillaria* sp. Problems with pollution and an excessive amount of algae development might result from too many nutrients in pond water. *Gracillaria* sp. aids in lowering the concentration of nutrients that could be detrimental by absorbing nutrients.

A decrease in ammonia-N and phosphate content in water occurred in all treatments at the end of the study (Figure 1). The ammonia-N reduction was lowest in the Control with an efficiency of 51%, but not in the other three treatments. The highest reduction arose in treatment C, shown by an efficiency of 64%, followed by treatments B and A at 63% and 49%, respectively. There were significant differences (Tabel 1) in the efficiency of the *Gracillaria* sp. filter, appears that the greater weight of seaweed used in the filter, the more efficient the filter works (Figure 2). The equation of the line on the graph is y = 0.0292x + 46.167, where the weight in grams and the filter efficiency in percent. The R-squared value of 0.6052 indicates that there is a moderately strong correlation between the granular particle weight and the filter efficiency, which suggests that *Gracillaria* sp. can be used as an effective filter for aquaculture.

The biofilter efficiency for phosphate was greatest in treatment C (92%), followed by treatments B and A at 90% and 86%. The lowest efficiency was in the control treatment at 61%. However, no differences amongst the treatments as shown in Table 1.

The biofilter efficiency can vary depending on the type and characteristics of the biofilter media, the operating conditions, and the pollutant properties. There is no universal standard for defining low, medium, and high efficiency for biofilters, but some general ranges are Low efficiency (less than 50% removal of pollutants), Medium efficiency (50% to 80% removal of pollutants) and High efficiency (more than 80% removal of pollutants). The volumetric flow rate of air or water, the pollutant concentration and loading rate, the contact time, the moisture content, the temperature, and the pH of the biofilter must all be taken into account when designing a biofilter to achieve the desired efficiency. These findings support the inclusion of *Gracillaria* sp as having high P uptake efficiency and medium N uptake efficiency.

Gracillaria sp can absorb nutrients such as nitrogen (N) and phosphorus (P) from the water through its surface cells, which have specialized transporters for different forms of N and P. *Gracillaria* can use both inorganic and organic sources of N and P, such as nitrate (NO_3^-), ammonium (NH_4^+), urea, phosphate (PO_4^{3-}), and dissolved organic matter (Pedersen & Johnsen, 2017) and (Smit, 2002). Moreover, *Gracillaria* sp. has different strategies to adapt to the availability and fluctuations of nutrients in the water. For example, *Gracillaria* sp. can store excess N and P in its tissues when the nutrient concentrations are high, and use them later when the nutrient

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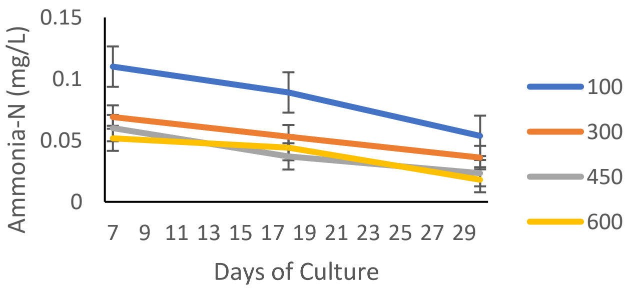
concentrations are low. *Gracillaria* sp. can also adjust its uptake rates and preferences for different forms of N and P depending on the environmental conditions. For instance, *Gracillaria* sp. can preferentially take up NH_4^+ over NO_3^- when both are present, because NH_4^+ is more readily assimilated and requires less energy than NO_3^- (Liu et al., 2016). It can also increase its affinity and capacity for NO_3^- uptake when the water temperature is low or when it is N-limited. Hence, *Gracillaria* sp. can enhance its nutrient uptake by reducing the diffusion boundary layer around its surface cells with the help of water motion (Smit, 2002) and (Wedchaparn et al., 2015).

The agar content was not significantly different in all treatments. However, there was a decrease in agar content with increasing weight of seaweed used (Table 1). Agar may be varies depending on the species, the cultivation conditions, and the extraction methods. According to some studies, the agar content in *Gracillaria* sp. can range from 15% to 46% of the dry weight (Lee et al., 2022).

Table 1. Value of N and P in water, Agar Content and Filter Efficiency (average$\pm$ stdev)

Treatments *Gracillaria* sp. (g)	Ammonia (mg/L)	Phosphate (mg/L)	Agar Content (%)	Filter Efficiency for NH_3 (%)	Filter Efficiency for PO_4^{3-} (%)
0	0,08 ± 0,02 ^{a}	0,068 ± 0,03^{a}	0,65 ± 0,03 ^{a}	51 ± 0,04^{a}	61 ± 0,04^{a}
300	0,05 ± 0,01^{a}	0,11 ± 0,10 ^{a}	0,75 ± 0,03 ^{a}	49 ± 0,10^{b}	86 ± 0,10^{a}
450	0,04 ± 0,01^{a}	0,068 ± 0,08 ^{a}	0,567 ± 0,03 ^{a}	64 ± 0,08^{c}	90 ± 0,08^{a}
600	0,04 ± 0,01^{a}	0,076 ± 0,05 ^{a}	0,427 ± 0,03 ^{a}	63 ± 0,05^{c}	92 ± 0,06^{a}

*Values with different superscripts in the columns indicate very significant differences (P< 0,05)



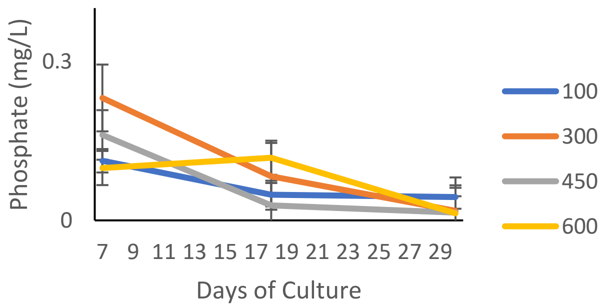


Figure 1. Concentration of Ammonia-N and Phosphate in water (average+SE) of different treatments

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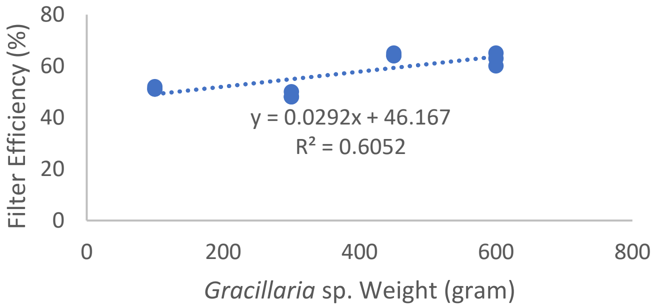

Figure 2. Linear Regression of Filter Efficiency for Ammonia

The agar content in *Gracillaria* sp. from Indonesia was between 20.7% and 28.7%. The agar content can also be influenced by factors such as light, temperature, salinity, nutrients, and harvesting time (Lee, WK., Lim, YY., Ho, 2022) and (Skriptsova & Nabivailo, 2009). Therefore, the agar content in *Gracillaria* sp. is not a fixed value, but rather a variable that depends on various conditions such as light, temperature, salinity, nutrient and harvesting time.

Light is essential for photosynthesis and growth of *Gracillaria* sp. However, too much light can cause photoinhibition and reduce the agar content. The optimum light intensity for *Gracillaria* sp. may vary depending on the species and the culture system. Temperatur affects the metabolism and enzim activity of *Gracillaria* sp. High temperatures can increase the water loss and decrease the agar content. Low temperatures can slow down the growth and reduce the agar yield. The optimum temperature for *Gracillaria* sp. may range from 20°C to 30°C. Salinity affects the osmotic balance and ion exchange of *Gracillaria* sp. High salinity can cause dehydration and decrease the agar content. Low salinity can cause swelling and increase the water content. The optimum salinity for *Gracillaria* sp. also may vary depending on the species and the culture system. Nutrients are essential for the growth and development of *Gracillaria* sp. However, too much nutrients can cause eutrophication and reduce the agar quality. The optimum nutrient level for *Gracillaria* sp. may depend on the type and concentration of the nutrient, as well as the interaction with other factors. Harvesting at different stages of the life cycle or seasons can result in different agar yields and qualities. Generally, harvesting at the peak of growth or before sporulation can produce higher agar content.

Salinity, temperature, and light conditions can all affect *Gracillaria*'s preferred oxygen level (Israel et al., 1999). Warm water, typically between 20 and 30°C, is preferred by *Gracillaria* sp. Although it can survive in temperatures between 10 and 35°C, if it becomes too hot or too cold, it will either stop growing or perish. It can endure a wide pH range, from 6.5 to 9.0 or higher. However, the optimal pH value is at around 8.0. If the pH falls below 7, its fronds may be lost. *Gracillaria* sp. can flourish in a eutrophic environment, which is one with lots of nutrients but little oxygen. However, oxygen is still required for respiration and photosynthesis (Figure 3).

Water quality in this research is still suitable for the maintenance of shrimp and seaweed. The Indonesian National Standard (SNI 8037.2:2014) for water quality shrimp rearing has been established to regulate the water quality requirements needed in the shrimp rearing process, especially the vaname shrimp species. This standard document details the physical, chemical, and biological parameters that must be maintained in shrimp rearing media, including variables such as temperature, salinity, pH, dissolved oxygen content, ammonia, nitrite, nitrate, phosphate, silicate,

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alkalinity, carbon dioxide, iron content, sulfide, chlorine, and bacteria levels. The guidelines also provide guidance on appropriate measurement and examination methods for monitoring such water quality. Water quality parameter values proposed by the shrimp rearing water quality SNI include temperature ranging from 28°C to 32°C, salinity of about 10 to 40 ppt, a recommended pH range of 7 to 8.5, optimal dissolved oxygen above 4 mg/L, and ammonia limits that should be less than 0.1 mg/L. Additional values include values for nitrite, nitrate, phosphate, silicate, alkalinity, carbon dioxide, iron, sulfide, chlorine, and Vibrio sp. bacteria, all of which have limit values to ensure suitable environmental conditions for shrimp growth and welfare during the culture process.

Based on the results of statistical analysis, there is no positive correlation between the efficiency of *Gracillaria* sp biofilter and agar content. This is indicated by the correlation coefficient of 0.189 which is smaller than the R table of 0.0576. A correlation coefficient value close to 0 indicates that the relationship between the two variables is very weak.

In addition, the significance value of 0.556 is greater than 0.05. A significance value greater than 0.05 indicates that there is no significant relationship between the two variables.

In simple terms, it can be said that agar content does not affect the efficiency of the *Gracillaria* sp. biofilter. The two variables do not have a strong or significant relationship.

There may be other factors affecting biofilter efficiency, such as the size and shape of the filtered particles, or the environmental conditions in which the biofilter operates. Further research needs to be done to examine the relationship between these two variables.

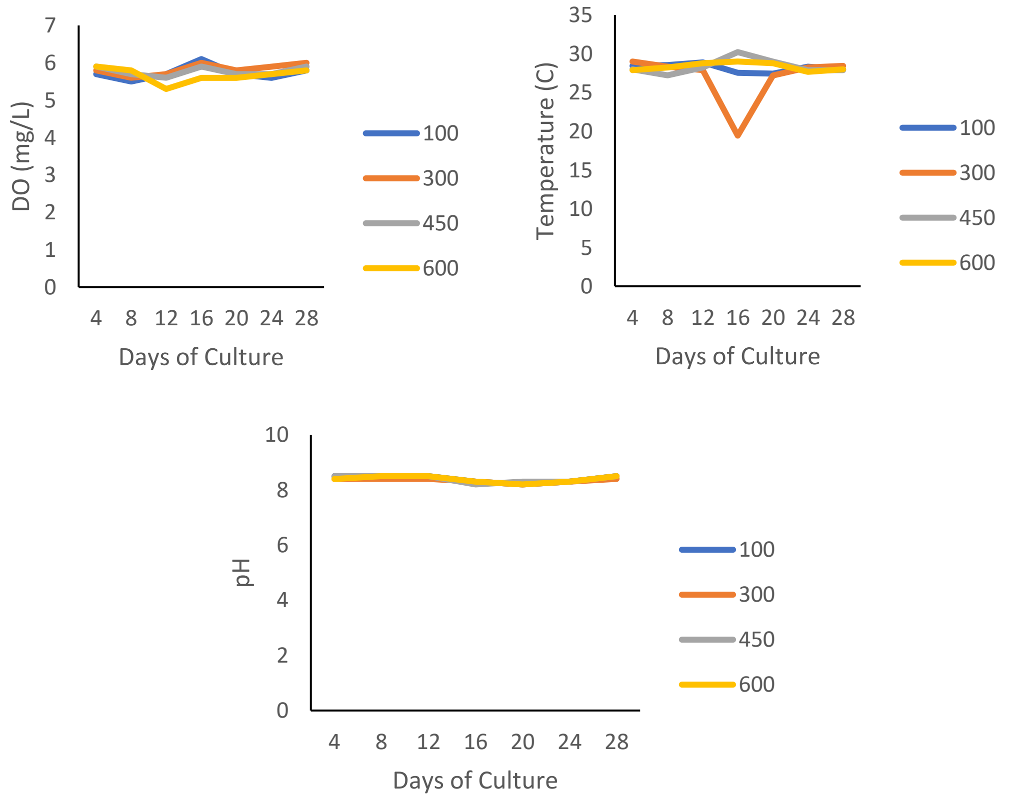

Figure 3. Water quality parameter (average+SE)

CONCLUSION

The efficiency of the biofilter exhibited no positive correlation with the weight of the seaweed used, with treatment C (600 g) demonstrating the highest efficiency. These findings underscore the potential of *Gracillaria* sp. as an efficient nutrient uptake tool, particularly for phosphorus, while also revealing the significance of factors like seaweed weight in influencing its performance. While there's no universal standard, typical ranges include low, medium, and high efficiency categories. The study emphasizes that parameters like flow rate, pollutant concentration, contact time, temperature, and pH must be considered when designing biofilters. The study provides insights into the factors affecting *Gracillaria*'s growth, agar content, and preferred oxygen levels, emphasizing the importance of optimal conditions for its cultivation.

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