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# Optimal Dosing of Composted Shrimp Pond Solid Waste as Organic Fertilizer for *Gracilaria* sp. Growth and Agar Yield

Hidayat Suryanto Suwoyo[1*], Sri Redjeki Hesti Mulyaningrum[1], Sahabuddin Sahabuddin[1], Rahmi Rahmi[2], Andi Sahrijanna[1], Makmur Makmur[1], Agus Nawang[1], Mat Fahrur[1], Erfan Andi Hendrajat[1], Imam Taukhid[1], Muslimin Muslimin[1]

[1] Research Center for Fishery, National Research and Innovation Agency, Bogor 16911, Indonesia
[2] Aquaculture Study Program, Faculty of Agriculture, University of Muhammadiyah Makassar, Makassar 90234, Indonesia

Corresponding Author Email: hida017@brin.go.id



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**ABSTRACT**

The solid waste from super-intensive shrimp ponds contains substantial nutrients, rendering it appropriate for organic fertilizer production by composting. This study evaluates the impact of varying amounts of organic fertilizer derived from shrimp pond solid waste on the growth and agar yield in seaweed cultivation, *Gracilaria* sp. The trial had a completely randomized design (CRD) featuring four treatments, each replicated three times. The applied doses of organic fertilizer were 2 g/L (A), 4 g/L (B), 6 g/L (C), and a control without organic fertilizer (D). Seaweed was cultivated for over 45 days under 25-45 ppt salinity. The measured variables included water quality, absolute weight, growth rate, agar yield, and characteristic organic fertilizer. The findings indicated that applying organic fertilizer derived from shrimp pond solid waste at several doses did not result in significant differences in the growth rate of seaweed and agar production ($P>0.05$) among the treatments. The ideal dosage of organic fertilizer used was 4g/L, resulting in a total growth of 105.69g, a seaweed growth rate of 1.31% per day, and a yield of 15.58%. The water quality during the research remained sufficient for facilitating seaweed growth. Solid waste from shrimp aquaculture can serve as organic fertilizer for seaweed cultivation.

## 1. INTRODUCTION

In 2022, algae exports surged by 26% relative to the prior year, with a total value of USD 1.6 billion. This growth may be mainly attributed to the notable export increase from Indonesia and Chile to China. In terms of value, Indonesia emerged as the leading exporter of algae in 2022, with the Republic of Korea and Chile following behind. Collectively, these three nations represented 58% of the overall export value of algae in 2022 [1].

*Gracilaria* sp. is a widely cultivated species of algae in Indonesia. Their success derives from their capacity to produce considerable biomass, extensive adaptation to climate factors like temperature and salinity, and the notable commercial worth of their byproducts [2]. *Gracilaria* sp. variety is widely recognized as a primary ingredient for producing agar-agar. This form of seaweed belongs to the class of red algae (Rhodophyta) and is referred to by several names in different regions. The Gracilaria genus encompasses numerous varieties of seaweed, each distinguished by unique morphological and anatomical features and assigned distinct scientific names [3]. Seaweeds are abundant in vitamins A, B1, B12, C, D, and E, as well as riboflavin, niacin, pantothenic acid, and folic acid. They also include minerals such as calcium, phosphorus, sodium, and potassium. Their amino acid composition is equilibrated, supplying all or the majority of essential amino acids necessary for health and well-being [4].

Fertilizers are synthetic compounds utilized to promote plant development. They are generally administered directly to the soil or provided by foliar application. Fertilizers are either organic or inorganic substances that include essential chemicals and minerals. Organic fertilizers are naturally occurring compounds derived from natural processes. Inorganic fertilizers are produced by chemically altering naturally occurring deposits. The proliferation of chemical fertilizers in agriculture enabled numerous developing nations to achieve food self-sufficiency. However, this practice has concurrently resulted in environmental degradation and adverse effects on organisms. The excessive application of chemical fertilizers in agriculture incurs high costs. It has detrimental impacts on soils, including the depletion of water retention capacity and soil fertility, and causes imbalances in soil nutrient levels. Consequently, it is imperative to develop economical, efficient, and eco-friendly fertilizers that operate without disturbing the natural equilibrium. Organic fertilizer is an essential element that improves soil vitality and productivity while facilitating plant growth. Organic nutrients enhance the population of soil organisms by supplying organic matter and micronutrients. Over time, the application of inorganic fertilizers can negatively alter soil microorganisms and fertility [5].

Organic agricultural fertilizer is suitable for cultivating *G. verrucosa*, which produces food for both people and animals. This approach also decreases production costs and may improve the economic feasibility of commercial seaweed cultivation [6]. The application of organic fertilizer significantly enhanced crop quality, elevating the sugar-acid ratio, vitamin C concentration, starch content, and protein levels. The application of organic fertilizer considerably increased plant development metrics, including height, stem diameter, root mass, and leaf area [7]. Organic fertilizer is created from organic materials, such as plant matter or animal excrement, that have undergone an engineering process. Fertilizer is available in both solid and liquid forms to meet the nutritional requirements of plants and improve the physical, chemical, and biological properties of the soil [8]. Composting technology is critical in organic fertilizer production because it facilitates the microbial breakdown of organic materials. Technology now offers a way to reduce municipal waste by converting it into compost. Researchers have documented numerous composting procedures, each with unique processes for fermenting organic matter. In developed countries, compost has attained recognition as a commercially produced and economically significant commodity. Compost can be produced from readily accessible organic resources that are environmentally sustainable and conducive to soil enhancement [9-13].

Pond solid waste has elevated levels of organic matter (C), total nitrogen (N), and phosphorus (P) relative to standard soil. The solid waste from aquaculture ponds comprises 1.92% organic carbon, 0.54% total nitrogen, and 1.70% phosphorus [14]. The nitrogen concentration in pond solid waste is 0.67%, $P_2O_5$ is 4.78%, $K_2O$ is 1%, and organic carbon is 17.84% [15]. The high levels of nitrogen, phosphorus, and organic matter in pond sediments render them an appropriate source of organic fertilizer. Production of organic fertilizers from solid waste for agricultural and fishery applications to reduce waste disposal and environmental degradation, while simultaneously increasing soil productivity [16]. The nutrient profile of organic fertilizer sourced from solid waste in shrimp ponds corresponds with the nutritional needs of seaweed, demonstrating a C/N ratio of 15.30. At the same time, *Gracilaria* sp. necessitates a ratio of 14.8. Brown seaweeds (Ochrophyta) had the highest average carbon to nitrogen (C/N) ratio of 27.5 (range: 7.6-122.5), followed by green seaweeds (Chlorophyta) with a ratio of 17.8 (6.2-54.3), and red seaweeds (Rhodophyta) with a ratio of 14.8 (5.6-77.6). Seaweeds possess, on average, C/N and C/P ratios that are 2.8 and 4.0 times greater than those of phytoplankton, respectively, suggesting that seaweeds may incorporate more carbon into their biomass relative to the available nutritional resources [17]. The majority of macroalgae exhibit a species-specific nutrient ratio, with an average of 92 species demonstrating a ratio of 550C: 30N: 1P. This indicates that seaweeds possess a higher carbon content and necessitate a lower concentration of phosphorus than nitrogen, in contrast to phytoplankton [18]. The C/N and C/P ratios of seaweed tissue exhibit significant fluctuation, determined by various circumstances, including the quantities of inorganic nutrients in saltwater [19], temperature [20], seasonality [21], and water movement [22]. Solid waste from shrimp ponds will serve as fertilizer for the growth of *Caulerpa lentillifera*. Nutrient supply derived from nitrate, ammonium, and phosphate immersion with a concentration of 6 g/L [23].

This study aims to evaluate the effect of the application of organic fertilizer derived from shrimp pond solid waste on the growth and yield of seaweed *Gracilaria* sp.

## 2. MATERIAL AND METHOD

### 2.1 Research location

The study was carried out in the Experimental Pond Installation in Maros Regency. South Sulawesi, Indonesia, which extends from 4°58’0” S to 4°58’15” S and 119°32’5” E to 119°32’10” E, as illustrated in Figure 1.

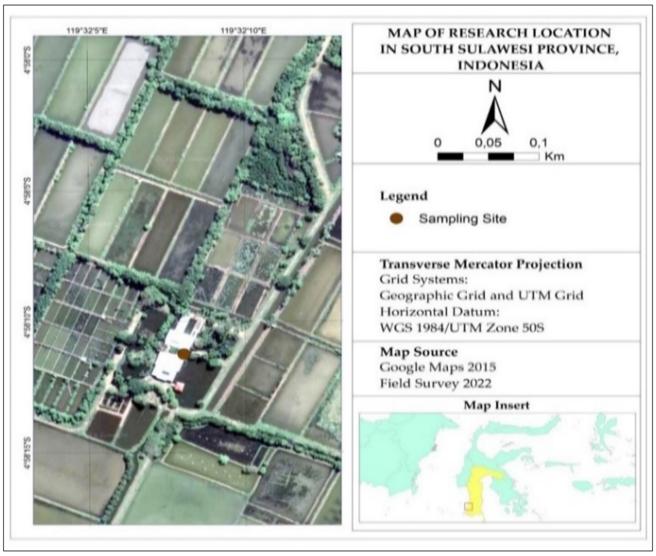



**Figure 1.** Research locations for seaweed cultivation, *Gracilaria* sp.

### 2.2 Production of organic fertilizer utilizing solid waste from shrimp pond aquaculture

Pond solid waste was collected from the Waste Water Treatment Plant (WWTP) located in the super-intensive shrimp pond at Punaga Village, Takalar Regency, South Sulawesi. Solid waste was retrieved from a depth of roughly 30-40 cm beneath the sedimentation pond's surface. Furthermore, the trash underwent drying to eradicate hazardous gases contained in the pond waste, such as hydrogen sulfide ($H_2S$), methane ($CH_4$), and ammonia. The pond's solid debris was initially segregated from other materials, including plastics, gravel, and stones. The sample was subsequently quantified and treated using bioactivators, comprising autochthonous bacteria derived from the pond's solid waste. The bioactivators were administered at a dosage of 1 liter per 0.5 metric tons. The ingredients were fully blended through agitation and thereafter transferred into a container. A black plastic cover was applied to the bucket to sustain moisture within a temperature range of 28-45℃ and humidity levels of 40-60% for 30 days. The solid waste pile was disrupted every week.

### 2.3 Experimental design

The research containers were 12 units, each measuring 67.34 cm³ and capable of containing 50 litres of water. This study utilized a completely randomized design consisting of four treatments and three replications. The organic fertilizer doses applied were 2 g/L (A), 4 g/L (B), 6 g/L (C), and control (D), which did not receive any organic fertilizer. The dose of organic fertilizer applied refers to research [23].

### 2.4 Preparation of research.

Before administering fertilizer treatment, the seaweed maintenance medium is created. Soil media was taken from the pond, well mixed for consistency, and then the soil was put into the research container with a 5cm thickness. Pond soil was desiccated for 7 days until it fissured, after which it was supplemented with 20 cm of brackish water. After that, fertilization is carried out using organic fertilizer according to the treatment dose. The organic fertilizer is first mixed with water and then spread evenly into the experimental container. After fertilizer application, seaweed seeds, as shown in Figure 2, are spread at 130 g/container. During maintenance, mud and dirt attached to the seaweed thallus are cleaned. This research lasted for 45 days.

**Figure 2.** *Gracilaria* seedling used in the study

### 2.5 Observed variables

The evaluated variables were absolute growth, daily growth rate, agar yield, and water quality variables such as temperature, pH, salinity, and dissolved oxygen, employing the YSI Pro Quatro Multi-Parameter instrument. Nitrate ($NO_3$) was analyzed using the sodium reduction method, whereas phosphate ($PO_4$) was assessed using the ascorbic acid method. The evaluations of water quality were performed in the morning. The equipment was calibrated before use.

Absolute growth is determined using the formula [24]:

$$\Delta W = W_t - W_o \quad (1)$$

where, $\Delta W$ represents the absolute growth in grammes, $W_t$ denotes the weight on day $t$ (g), and $W_o$ signifies the initial weight (g).

The daily growth rate (%/day) was calculated as previously advised by [25-27]:

$$DGR(\%/day) = ln(\, Wf/\,Wi)/t \times 100 \quad (2)$$

where, $Wf$ represents the final weight after $t$ days of the culture phase, and $Wi$ denotes the initial weight.

The agar yield was determined using the formula previously proposed by studies [24, 28, 29]:

$$Agar\ yield\ (\%) = \frac{Dry\ weight\ of\ agar\ (g)}{Dry\ weight\ of\ seaweed\ (g)} \times 100 \quad (3)$$

### 2.6 Data analysis

The statistical analysis was conducted using SPSS software, specifically version 25.00. A two-way analysis of variance (ANOVA) was utilized to assess the impact of different organic fertilizer dosages on growth and agar production. Subsequently, Duncan's Multiple Range Test (DMRT) was conducted at a 95% confidence level to further evaluate the data. The water quality data were assessed via descriptive methodologies.

## 3. RESULT

### 3.1 Water quality

The growth rate and agar yield of the seaweed *Gracilaria* sp. are affected by water quality. Table 1 displays the assessed water quality parameters from the experiment.

**Table 1.** Water quality parameters assessed over the 45-day experimental period

| Variables | Treatment | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Temperature (°C) | 22.3-25.2 | 22.5-25.4 | 22.5-26.0 | 22.3-25.6 |
| pH | 7.50-9.00 | 7.50-8.90 | 7.50-8.90 | 7.50-9.00 |
| Salinity (ppt) | 25-40 | 25-42 | 25-45 | 25-43 |
| Dissolved Oxygen, DO (mg/L) | 1.29-4.84 | 1.75-3.67 | 2.03-6.14 | 2.46-4.92 |
| Nitrate, $NO_3$ (mg/L) | 0.1883-0.8457 | 0.0808-0.8757 | 0.0963-0.8915 | 0.0972-0.9725 |
| Phosphate, $PO_4$ (mg/L) | 0.0021-0.0853 | 0.0832-0.1959 | 0.0640-0.2140 | 0.0021-0.1033 |

### 3.2 Absolute growth performance of seaweed *Gracilaria* sp.

The average absolute growth weight of seaweed, *Gracilaria* sp., for 45 days of rearing was observed, and the results varied and improved with longer rearing times for all treatments. Treatment B produced the highest absolute weight gain of seaweed, *Gracilaria* sp. (105.69 g), followed by treatment C (83.06 g), treatment A (80.89 g), and the lowest in treatment D (80.58 g), as illustrated in Figure 3. The analysis of variance (ANOVA) results demonstrated that varying dosages of organic fertilizer sourced from solid shrimp pond waste did not exert a statistically significant effect ($P>0.05$) on the absolute growth of the seaweed, *Gracilaria* sp. The findings indicate that the use of organic fertilizer sourced from solid waste shrimp ponds produced the most substantial development of *Gracilaria* sp., whereas growth was negligible without any fertilizer intervention. Evidence suggests that the utilization of organic fertilizer yields superior results compared to the absence of fertilizer.

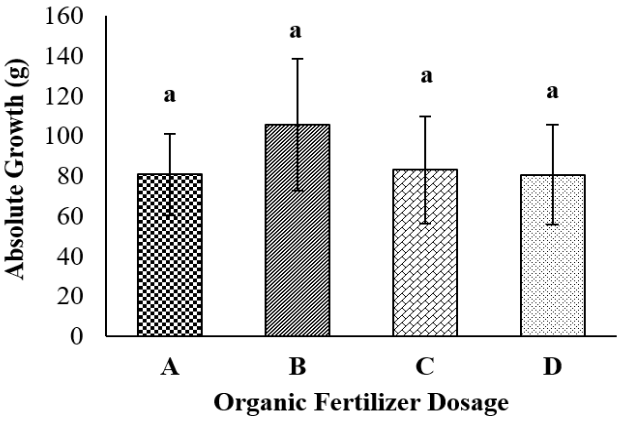



**Figure 3.** Absolute growth of *Gracilaria* sp. seaweed throughout the 45-day cultivation period

### 3.3 Growth rate performance of seaweed, *Gracilaria* sp.

*Gracilaria* sp. exhibited varying growth rates in four different treatments. Treatment B exhibited the highest growth rate at 1.31% per day, followed by treatment C at 1.09% per day. Treatment A exhibited a growth rate of 1.07% per day, whereas treatment D demonstrated the lowest growth rate of 1.06% per day. The data indicate that the utilization of organic fertilizer yielded the highest growth rate of *Gracilaria* sp., while the lack of organic fertilizer led to the lowest growth rate. The research findings demonstrate that the use of organic fertilizer sourced from shrimp pond solid waste at varying doses (2 mg/L, 4 mg/L, 6 mg/L, and a control without fertilizer) did not substantially influence (P>0.05) the growth rate of *Gracilaria* sp., as illustrated in Figure 4.

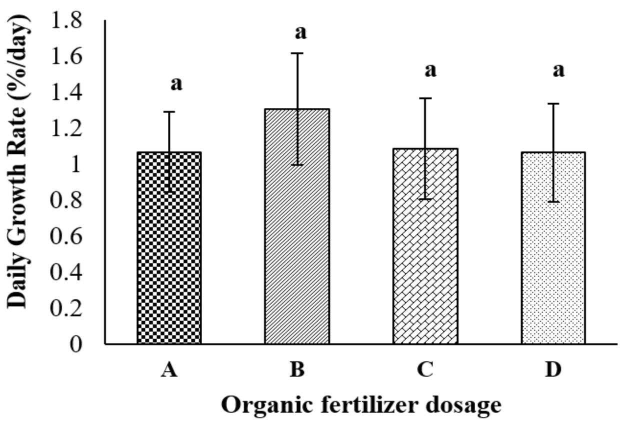



**Figure 4.** Growth rate performance of seaweed, *Gracilaria* sp., during the 45-day cultivation period

### 3.4 Yield of seaweed, *Gracilaria* sp.

The highest average seaweed agar content on day 45 was seen in treatment B, with a value of 15.58±0.52%. Treatment D had the second-highest content at 14.98±0.64%, followed by treatment C at 14.03±0.11%. Treatment A exhibited the lowest amount, recorded at 13.27±0.66%. Figure 5 illustrates the agar production of the seaweed species *Gracilaria* sp. under each experimental setting. The analysis of variance (ANOVA) indicates that differing amounts of organic fertilizer significantly affected (P<0.05) the agar content of the seaweed, *Gracilaria* sp. The subsequent Duncan Test indicated that the agar content of seaweed in treatment A did not demonstrate a significant difference (P>0.05) relative to treatment C. However, it demonstrated a statistically significant change when compared to treatments B and D (P<0.05). Treatment B exhibited a statistically significant difference compared to treatment C (P<0.05), but did not show a statistically significant difference when juxtaposed with treatment D (P>0.05).

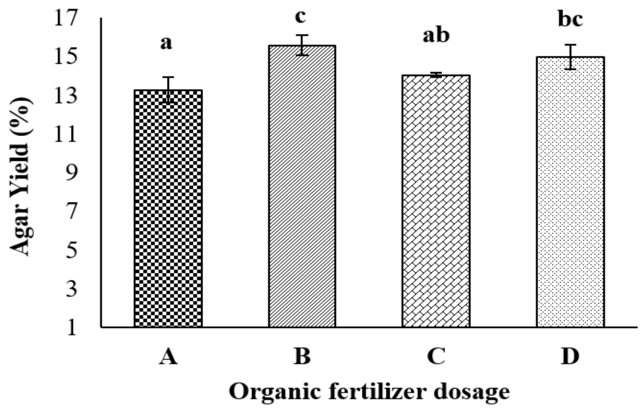



**Figure 5.** Yield of seaweed, *Gracilaria* sp., during the 45-day cultivation period

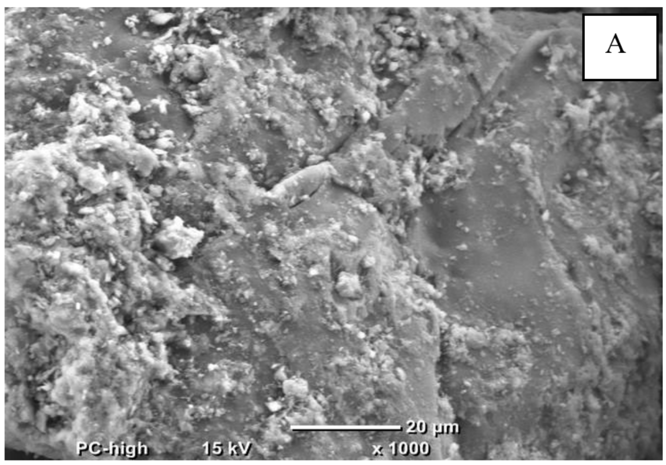
### 3.5 Chemical properties of organic fertilizer

The results of the analysis of the macro and micro nutrient composition of organic fertilizer obtained from shrimp pond solid waste are presented in Table 2.

**Table 2.** Characteristics of organic fertilizer made from shrimp pond solid waste

| Parameters | Value | Quality Standards * |
|---|---|---|
| N Total (%) | 0.70±0.01 | N+P+K maximum 2% |
| $P_2O_5$ (%) | 1.59±0.29 | |
| $K_2O$ (%) | 0.90±0.02 | |
| C Organik (%) | 10.82±2.62 | 9-32% |
| C/N Ratio | 15.30±4.16 | ≤ 25 |
| Fe (ppm) | 8580±11.93 | maximum 15.000 |
| Mn (ppm) | 778±67.88 | maximum 5000 |
| Zn (ppm) | 57.02±3.81 | maximum 5.000 |
| Cu (ppm) | 19.93±0.95 | maximum 5.000 |

*: Source: Minister of Agriculture of the Republic of Indonesia No.261/KPTS/SR.310/M/4/2019.



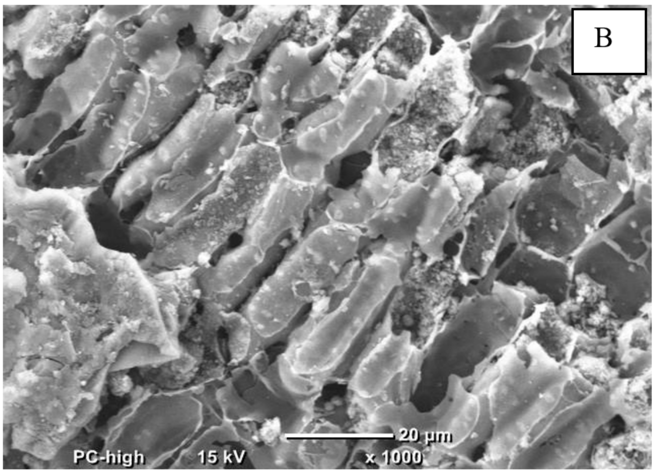



**Figure 6.** Scanning electron microscope (SEM) images of solid waste materials before (A) and after (B) composting

The comparison microstructure between pond solid waste before and after composting using a scanning electron microscope (SEM) is presented in Figure 6.

The SEM technique can be employed to examine the microstructure of the composted material in greater detail. The visual and olfactory evaluation revealed that the decomposed materials released a subtle fragrance during the early stage of composting, marked by grey and black hues. During the final phase of composting, the colouration deepened, the particles became more refined and homogeneous, and the odour dissipated. SEM analysis revealed that the surface and interior of the solid waste particles exhibited increased roughness and the emergence of cracks following composting, compared with those before composting (Figure 6(A)), which showed a compact and smooth surface (Figure 6(B)).

## 4. DISCUSSION

### 4.1 Water quality

The temperature in the cultivation container fluctuates between 22.3 to 26.0℃, which remains conducive to seaweed development. Temperature is essential for the growth and development of seaweed. It directly influences the reproductive cycle, metabolic processes, and photosynthesis in seaweed. The rates of nutrient uptake by active transport are expected to be influenced by temperature due to its impact on the activity of membrane transporters. However, the effect on uptake through passive diffusion may be less significant [30]. Moreover, the principal environmental elements affecting the ability of seaweeds to assimilate nutrients and regulate their growth and productivity are light and temperature. The water temperature of the cultures was recorded at 18.7 to 21.5℃. Salinity levels fluctuated from 33.6 to 40.3 parts per thousand (ppt), whereas pH values spanned from 7.8 to 8.4 [6].

The pH level of water typically ranges from 7.5 to 9.0 during seaweed maintenance. The resultant pH value remains conducive to algae development. Seaweed farming activities typically require an alkaline range of pH. The optimal pH range for seaweed cultivation is 7.8 to 8.2, with an average pH of 8.0 [31]. Various factors, including biological processes like photosynthesis and respiration, temperature, and ion concentration, affect the pH levels of these liquids. The optimal pH range for seaweed cultivation is 6.5 to 8.0 [32]. The water quality parameters were measured during the experimental pH of 8.0±0.1, the temperature of 22±0.1℃, the salinity of 32±0.3 ppt, transparency of 74.5±1.4 cm, nitrate ($NO_3$) concentration of 0.632±0.2 mg/L, and nitrite ($NO_2$) concentration of 0.433±0.11 mg/L [33].

Salinity values obtained during maintenance varied between 25 to 45 ppt. This value is still capable of facilitating the growth of seaweed. The salinity and temperature in the culture substantially impacted the growth of both seaweed species. *Gracilaria chorda* and *G. verrucosa* exhibited robust growth across a broad temperature range of 10-30℃ and an extensive salinity range of 5-35‰. Optimal growth was observed at 17 to 30℃ and salinity of 15-30 ppt [34]. Gracilaria/Gracilariopsis seaweed is a species that has a high tolerance to salinity ranges of 10-40 ppt and grows optimally in the range of 25-33 ppt [35]. The salinity values obtained during the cultivation of seaweed *G.verrucosa* in Karimunjawa waters ranged from 29-33 ppt. The salt level remained within the optimal range for the culture of *G. verrucosa*, promoting the seaweed's growth and development [36]. The cultivation of *G. verrucosa* is substantially influenced by the adequacy of water quality. The growth rate is significantly affected by various factors, including temperature, salinity, light intensity, and drought conditions resulting from tidal influences [37]. The nutritional composition of waterfalls is within the permissible limits for the cultivation of *G. verrucosa* seaweed. Temperature of water in the cultivation media fluctuates between 28 to 30 degrees Celsius, salinity ranges from 14 to 16 ppt, pH is maintained at 7, $PO_4$ concentration varies from 0.27 to 0.61 milligrams per liter (mg/L), $NH_4$ concentration ranges from 0.15 to 0.90 milligrams per liter, and $NO_3$ concentration varies from 0.03 to 0.57 milligrams per liter [38].

The result of dissolved oxygen content readings ranged from 1.29 to 6.14 mg/L. At the start of maintenance, there was a low oxygen level of 1.29 mg/L due to the adjustment period following the application of organic fertilizer. However, from the second week to the end of maintenance, DO levels increased to 3.5-6.5 mg/L. Adequate levels of dissolved oxygen are crucial for the viability of aquatic life. The dissolved oxygen concentration during seaweed maintenance varies between 6.2 to 6.4 milligrams per liter, while the nitrate value ranges from 0.03 to 0.57 milligrams per liter [36]. Dissolved oxygen concentrations at three distinct seaweed sampling sites varied from 5.89 to 7.45 mg/L [29].

The investigation of nitrate concentrations during the research ranged from 0.0808 to 0.9725 mg/L. Seaweed needs $NO_3$ in water to perform its metabolic processes. The optimal nitrate concentration for *Gracilaria* growth ranges from 0.6 to 0.8 mg/L [39]. The range of nitrate concentration for algal growth is 0.1 to 3 mg/L. The bleaching of the red algae thallus signifies inadequate nitrate levels [40].

Phosphate level during the experiment ranged from 0.0021 to 0.2140 mg/L. The phosphate concentration in the medium cultivation can be classified into high fertility rate categories, with a range from 0.051 to 0.2 mg/L [41]. The growth rate of the seaweed was significantly affected by the availability of nutrients, particularly nitrates and phosphates, in the cultivation environment. Nitrate-rich water is a crucial nutrient supply for seaweed, enabling the regulation of primary producer growth.

The increase in $NO_3$ and $PO_4$ levels in the culture medium after fertilization is related to microbial decomposition. Organic fertilizers applied to the culture medium are broken down by microbes into simpler organic compounds through enzymatic processes, producing beneficial nutrients such as nitrate and phosphate. These nutrients are absorbed and utilized by algae as the primary components for protein synthesis and chlorophyll formation for photosynthesis. The higher the chlorophyll pigment content in seaweed, the more optimal the photosynthesis process becomes, enabling the plant to stimulate thallus growth and accelerate the formation of new tissues and shoots, as well as the high carbohydrate content produced. Organic waste from aquaculture is biodegradable in water. This decomposition process involves various biochemical mechanisms, such as hydrolysis, fermentation, and oxidation, which are triggered by extracellular enzymes produced by microbes and convert insoluble organic compounds into water-soluble compounds [42]. Furthermore, bacteria contribute to the mineralisation of organic chemicals into soluble inorganic compounds, yielding nutrients such as phosphate and nitrate [43-45]. The degradation of organic matter in aquatic environments transpires under two principal situations, namely aerobic (with oxygen) and anaerobic (without oxygen), each involving different types of microorganisms.

### 4.2 Absolute growth performance of seaweed *Gracilaria* sp.

Organic fertilizer obtained from the solid waste of shrimp ponds is employed in sustainable agriculture because of its abundant vitamin and macronutrient content. The pond's solid waste comprises essential elements such as nitrogen (N), phosphorus (P), potassium (K), organic carbon (C), zinc (Zn), manganese (Mn), and iron (Fe). These minerals significantly elevate the nutritional content of plants, especially seaweed [15]. The solid waste from shrimp ponds comprises 1.92% organic matter, 0.54% total nitrogen, and 1.70% phosphorus [46]. Seaweed growth requires the presence of essential nutrients, which might be either macro- or micronutrients. Gracilaria sp. requires nutrients, mainly nitrate and phosphate, which are commonly used. The initial weight of seaweed at the onset of planting is negligible, promoting nutrient absorption during its metabolic activities. Nutrient availability profoundly influences seaweed growth. *G. verrucosa* [34, 36]. High-nitrate organic fertilizers have the potential to enhance the growth of *G. verrucosa* [6]. They utilized four distinct organic fertilizers of agricultural origin, namely ammonium sulfate, ammonium nitrate, urea, and sodium nitrate, to cultivate *Chondracanthus squarrulosus*, a species of red algae belonging to the Rhodophyta phylum. The researchers determined that there was no substantial disparity among the nitrogen sources. The study determined that sodium nitrate and urea had the same level of effectiveness in stimulating the growth of this specific seaweed [47].

The seaweed proliferation seen in this study was analogous to that of previous research. The *Gracilaria* sp. exhibited absolute growth of 34.86 g, 22.27 g, and 16.39 g at depths of 30 cm, 45 cm, and 60 cm, respectively. The degree of culture significantly influences the total development and biomass of *Gracilaria* sp. [31]. The absolute growth of *G. verrucosa* was 17.87 g at a spacing of 20cm and 17.31 g at a spacing of 25 cm. A spacing of 10cm demonstrated a growth rate of 16.75 g, whilst the minimal growth rate of 15.62 g occurred at a spacing of 30cm. The optimal distance for the thallus of *G. verrucosa* to ensure sufficient surface area for photosynthesis and the uptake of macronutrients from the water [24]. The mean absolute growth of *G. verrucosa* across several treatments is 71.0 g with liquid fertilizer Ulva sp., 52.4 g with urea fertilizer, and 37.9 g without any fertilizer [48]. The seaweed's development has escalated, indicating that it has reached the cell elongation phase due to its ample supply of nutrients for its growth [3]. The weather conditions during the culture in the pond had a considerable impact on the growth of the seaweed. The meteorological conditions from March to early August were advantageous, characterized by ideal amounts of sunlight and rainfall, which were conducive to the growth of seaweed [40].

### 4.3 Growth rate performance of seaweed, *Gracilaria* sp.

The proliferation of seaweed is intimately correlated with the availability in the aquatic environment. The nutrient absorption rate in seaweed is affected by plant density, leading to increased growth and biomass in the early stages of cultivation, which subsequently declines as cultivation advances. Furthermore, an individual's growth is most favorable during their early years [49]. The daily growth rate was mostly influenced by phosphate, nitrate, salinity, and light penetration levels [40].

The seaweed growth rate in this study was analogous to that recorded in several previous tests. The mean daily growth rate of *G. corticata* over a 90-day rearing period employing three distinct cultivation techniques, Raft method, Polythene method, and Longline method, was 1.52% $day^{-1}$, 1.38% $day^{-1}$, and 1.24% $day^{-1}$, respectively [50]. The square raft method produced a maximum daily growth rate (DGR) of 1.33±0.88% per day throughout the initial harvest period from January to March. Conversely, the DGR value in the second harvest season (May to August) was the lowest, recorded at 0.45±0.35% per day [26]. The growth rates of *G. verrucosa* seaweed cultivated via the longline system over 47 days, with initial weights of 25 g, 50 g, and 75 g, were 1.48%, 0.69%, and 0.29% per day, respectively [36]. The daily growth rates of *Gracilaria* sp. seaweed under varied temperature treatments (20, 25, 30, and 35°C) are 1.7%, 2.3%, 2.5%, and 2.3% $day^{-1}$, respectively. The daily growth rates of *Gracilaria* sp. seaweed are as follows: 2.1%, 3.2%, 3.1%, and 2.7% per day under varied salinity treatments (20, 25, 30, and 35 ppt) [25]. The seaweed growth rate in ponds with acid sulfate soil ranges from 1.52% to 3.63% $day^{-1}$, with an average of 2.88±0.56% $day^{-1}$ [51]. The growth rate of the seaweed *G. verrucosa* in a closed system was 4.03±1.63% per day, while in a greenhouse with a controlled environment, it was 1.21±0.34% per day [52]. The growth rates of *G. verrucosa* seaweed cultivated using the longline system over 47 days, with initial weights of 25g, 50g, and 75g, were 1.48%, 0.69%, and 0.29% per day, respectively. Moreover, an adequate supply of nutrients markedly affects seaweed growth, recorded at 5.03±1.39% $day^{-1}$ and 1.22±0.78% $day^{-1}$, respectively. The weather markedly affected the pace of seaweed development during cultivation, hence influencing the salinity of the pond water [40].

The DGR of *G. verrucosa* during seed propagation by the basic spreading method across two cycles averaged 3.23±0.50% per day for the selected results, surpassing the internal control at 2.12±0.02% per day and the external control at 1.69±0.09% per day [53]. The growth rate of the seaweed *G. verrucosa*, cultivated via the bottom-off method with differing spacing, varied from 2.18% to 2.20% daily. The field experiment revealed that the alga *G. gracilis* displayed satisfactory development, with an average daily growth rate

(DGR) of 0.87% and intermittent peaks of 3.50% [27]. *Gracilaria* demonstrated enhanced growth in low salinity settings, with a daily growth rate that was twice as high as that reported in high salinity conditions. The seaweed species *Gracilaria* sp. benefits from a growth rate that surpasses 3% [54].

The minimal growth performance of seaweed in the control condition (absent organic fertilizer delivery) resulted from insufficient nutrient availability in the culture medium, which impeded growth. The inhibitory effect of nutrient deficiency impacts photosynthesis, cell division, and protein synthesis in the control treatment. Nitrogen limitations in the water cause a decrease in chlorophyll content in seaweed and can lead to growth cessation [55]. Plants experiencing nitrate deficiency may result in suboptimal photosynthesis within their bodies, as the chlorophyll formation process is likely incomplete, while chlorophyll functions as a light absorber and plays a crucial role in photosynthesis [56]. Nutrient deficits frequently diminish photosynthetic capacity, hence resulting in reductions in gross carbon gain and plant output [57, 58]. Nitrogen limitation is known to diminish plant growth rate and development, impair photosynthetic ability, and degrade photosynthetic pigments and proteins [59, 60]. Nutrient availability for plants must be sufficient and balanced according to their needs so that plants can stimulate thallus growth and accelerate the formation of new tissues and shoots.

The growth rate of seaweed is affected by the rate of photosynthesis. The rate of photosynthesis is affected by the age or developmental stage of the photosynthetic organs. The organ's photosynthetic capacity first rises during development but then decreases intermittently before reaching complete maturity. Each plant undergoes an initial phase of increased photosynthesis throughout its growth, followed by a phase of declining photosynthetic rates at a specific age, which varies across different plant species [61].

Algal organisms require nutrients and particular molecules that are dissolved in their water to flourish. Algae necessitate three categories of nutrients: macronutrients (including N, P, and C), micronutrients or trace elements (such as Fe, Zn, Cu, Mn, and Mo), and vitamins (including vitamin B12, thiamine, and biotin). A deficiency of essential nutrients will impede the body's growth rate [27, 62]. The abundance of nitrogen (N), phosphate (P), and carbon (C) in wastewater from aquaculture farms fosters ideal circumstances for rapid algal growth [30].

### 4.4 Yield of seaweed, *Gracilaria* sp.

The quality of agar is influenced by various factors, including the nutritional mix of the algae growth media, the age and reproductive condition of the seaweed, the molecular weight of the polymer, and the concentration of cations such as potassium ($K^+$) and alkali used in the pre-extraction treatment of the seaweed [53, 63].

The agar content of the seaweed obtained in this investigation was comparable to that of various earlier experiments. The seaweeds *G. gigas* and *G. verrucosa* grown in the WWTP equalization pond showed good quality with protein levels between 13.91%-15.32% and agar yield of 14.11% to 17.00% [64]. The agar yield of seaweed *Gracilaria corticata* cultivation in ponds was found to be higher in the summer season (15.65±1.99%), while lower in the winter season (14.24±1.47%). The small quantity of agar may be attributed to the sluggish growth of *G. corticata* [4]. The yield of *G. verrucosa* seaweed in cycle I was higher (17.18±2.76%) than that of the internal control (10.9±1.00%) and the external control (9.48±0.24%). During cycle II, the agar content of the internal control seaweed was found to be greater (29.24±0.86%) compared to the selection results (14.95±0.67%) and the external control (7.09±2.19%) [53]. The effects of species, environment, and their interactions on agar production differed significantly. There is a variation in agar production among different species of *Gracilaria* at both rocky shores and mangrove swamps. The disparity in agar yields among species from the mangrove wetland is significant. The agar yield of *G. salicornia* from the mangrove swamp (13.63%) and rocky beach (13.94%), which exhibited no significant difference in agar content [29]. The agar content of estuarine seaweed *Gracilaria* in inshore tank cultivation is the highest agar yield (20.67%) relative to the control (16.67%), indoor cultivation with brackish water medium (13.8%), and seawater medium (11.64%), suggesting potential for extensive cultivation and agar utilization [65].

The mean agar yield of tissue-cultured seaweed seedlings was 15.50%. In April, the maximum yield reported was 27.84%, and the minimum yield was observed in March at 10.30%. Agar is a carbohydrate produced during photosynthesis, with its synthesis influenced by light intensity [40]. The yield of seaweed, *G. verrucosa,* cultivated using the bottom-off method with varying spacing, ranged from 14.83% to 24.93% [24]. A high agar yield of 28.39% was obtained from *G. edulis*, 8.69% from *G. salicornia,* and 26.2% from *Gracilaria* sp. A, and 27% from *Gracilaria* sp. B [25]. The content of seaweed is closely correlated with 56.2% of the factors that influence seaweed yield, including water quality and nutrients. Other factors influence the remaining 43.8%. The agar yield is significantly impacted by various parameters, including the species, cultivation location, and climate [38]. Seaweed obtained from several geographical regions exhibits variations in agar yield and gel strength. Variations in environmental factors, including nutrient availability, species, localities, and extraction volumes, can affect both the mass production and gel strength of seaweed [28]. Both light intensity and ammonium, individually or in conjunction, significantly influence the growth and agar yield of seaweed [66]. Water quality, including temperature and transparency, significantly influences agar production. The agar production of the seaweed grew in direct correlation with the level of transparency. The impact of temperature and transparency on photosynthesis, which results in the production of carbohydrates in the form of agar, is understandable [2]. Varied habitat types and environmental conditions may influence the populations of these seaweeds and their agar production [29].

The provision of fertilizer in the maintenance media aims to meet the nutrients needed by seaweed (*Gracilaria* sp.), so that it will increase its production. The precise process by which fertilizer dosage influences growth rate and seaweed agar content can be elucidated as follows. Nutrients are crucial in seaweed farming as they provide energy essential for growth and development, regulating cellular components. Organic fertilizer of shrimp pond solid waste contains macro and micronutrients such as nitrogen, phosphate, carbon, and potassium that can support the process of chlorophyll formation and photosynthesis. With the nature of phytoextraction, the *Gracilaria* thallus wall absorbs and stores organic materials such as nitrogen and phosphorus in the thallus cells. Moreover, organic waste contained inside seaweed cells is subsequently decomposed by photosynthesis, leading to the assimilation of energy and cellular structures, which reflects the growth of seaweed clusters and agar content [18, 67].

### 4.5 Chemical properties of organic fertilizer

4.5.1 Macronutrient composition

Conventional composting refers to the breakdown of biodegradable garbage aided by microorganisms, such as bacteria, fungi, and actinomycetes. This process converts organic waste into carbon dioxide ($CO_2$), water ($H_2O$), ammonia ($NH_3$), inorganic nutrients, and a final product referred to as compost [68, 69]. The biological breakdown of organic waste occurs in either aerobic or anaerobic conditions, with the former being more common. Thermophilic and mesophilic aerobic bacteria metabolize organic waste, converting it into mineralized chemicals such as $CO_2$, $H_2O$, $NH_4$, or stable organic matter [70]. Table 2 presents the nutritional content of $P_2O_5$, $K_2O$, and C-Organic, in relation to the carbon-to-nitrogen ratio of organic fertilizers sourced from the solid waste of shrimp pond aquaculture.

Total nitrogen content is 0.70%, phosphorus pentoxide content is 1.59%, potassium oxide content is 0.90%, organic carbon content is 10.82%, and the carbon to nitrogen ratio is 15.30. The nutritional composition is consistent with other prior investigations. The solid waste in shrimp ponds comprises the following nutrient composition: 0.14% total nitrogen (N), 5.0% phosphorus pentoxide ($P_2O_5$), 1.38% organic carbon, and a C: N ratio of 9.9 [71]. Nutrient composition of plant compost is as follows: total nitrogen (N) 1.22%, phosphorus pentoxide ($P_2O_5$) 0.32%, potassium oxide ($K_2O$) 1.70%, organic carbon (C) 12.2%, and C/N ratio of 10 [72]. Nutritional composition of pond waste combined with vermicompost. N totals 0.99%, $K_2O$ 0.45%, and $P_2O_5$ 0.24%. Organic carbon is 16.3%, and the carbon-to-nitrogen ratio is 16.46 [73]. The nutrient composition of animal waste fertilizers includes total nitrogen at 4.0%, $P_2O_5$ at 0.50%, $K_2O$ at 0.40%, organic carbon at 20.82%, and a carbon-to-nitrogen ratio of 5.21 [74]. The nutrient profile of mushroom compost contains a total nitrogen concentration of 0.98%, $P_2O_5$ 0.80%; $K_2O$ 0.28%; C 14.7%; C:N ratio 15.0 [75]. Organic fertilizers markedly enhance plant growth and yield. They function as reservoirs of nutrients during mineralization and humification, releasing macro and micronutrients essential for plant growth [9, 76].

4.5.2 Micronutrient composition

Micronutrients are vital substances that plants need in small amounts for proper growth, development, and reproduction. Despite their modest presence, they are essential for numerous physiological and metabolic functions. The name "micronutrient" refers to the quantity of these nutrients, rather than their significance [77]. Every trace element facilitates intricate processes that emphasize growth and development [78]. The complex interplay of these micronutrients with enzymes exemplifies nature's design, wherein even the minutest components play significant roles in sustaining life. Photosynthesis is not merely a technique; it is the essential mechanism that enables plants to thrive by transforming sunlight into energy. Chlorophyll is fundamental to this process, with its generation and function reliant on magnesium availability. Micronutrients are essential for sustaining the general health and vigour of plants. They participate in various physiological and biochemical processes, each enhancing plant health and productivity. An insufficiency or imbalance may result in physiological disturbances, reduced yield, and inferior plant quality [79, 80].

Table 2 presents the analysis findings on the micronutrient composition of organic fertiliser sourced from shrimp pond solid waste. Concentrations of Fe, Mn, Zn, and Cu were 8580 ppm, 778 ppm, 57.02 ppm, and 19.93 ppm, respectively. The concentrations of these micronutrients in solid organic fertilizers comply with the quality standards established by the Decree of the Minister of Agriculture of the Republic of Indonesia No. 261/ KPTS/ SR.310 /M/4 /2019 [81]. The allowable concentration of Fe is 15,000 ppm; Zn is 5,000 ppm; Mn is 5,000 ppm; and Cu is 5,000 ppm. The solid waste produced from aquaculture activities contains the following micronutrient concentrations: 3.81% iron, 0.06% manganese, 168 ppm copper, and 250 ppm zinc. The solid waste from Gracilaria extraction was discovered to include five micronutrients: copper, boron, iron, manganese, and zinc. The micronutrient concentration of manganese (Mn) was superior to that of other micronutrients, measuring 57.58 ppm, followed by boron (B) at 32.32 ppm, zinc (Zn) at 8.42 ppm, copper (Cu) at 4.85 ppm, and iron (Fe) at 0.24 ppm [79]. The red seaweed *Amphiroa anceps* demonstrated the highest iron concentration at 36 mg per 100 g, followed by the brown seaweed *Iyengaria stellata* at 6mg per 100g, and the green seaweed *Acrosiphonia orientalis* at 2 mg per 100 g [82]. *Padina pavonica* demonstrated the highest concentrations of Fe (15030 mg/kg-dw), Zn (33.46 mg/kg-dw), and Mn (443.79 mg/kg-dw). The mineral composition of cultivated seaweeds was comparatively inferior to that of wild species [83].

The vermicompost from agricultural activities had 3.64% iron, 0.55% manganese, 19 parts per million (ppm) copper, and 266 ppm zinc regarding micronutrient concentration [84]. The nutritional composition of fertilizers obtained from biogas processing (slurry). The concentrations of the following elements are: carbon dioxide (CO) at 2.35 parts per million (ppm), cadmium (Cd) at 0.11 ppm, zinc (Zn) at 295 ppm, and copper (Cu) at 36.3 ppm. Simultaneously, the nutritional composition of compost is: The concentration of Co is 1.19 ppm, Cd is 0.24 ppm, Zn is 195 ppm, and Cu is 20.4 ppm [85]. The quantities of micronutrients in processed palm oil waste are: iron (Fe) at 2.24%, zinc (Zn) at 130 parts per million (ppm), copper (Cu) at 45.05 ppm, and manganese (Mn) at 422.56 ppm. The WHO and FAO have instituted microelement standards. The established standards specify that the permissible levels are 140 parts per million (ppm) for zinc (Zn), 75.0 ppm for copper (Cu), and 500 ppm for manganese (Mn) [86]. These micro-minerals are utilized by seaweed in the process of photosynthesis and enzyme cofactors, chloroplast formation, DNA transcription, and protein and carbohydrate metabolism [87, 88]. The concentration and composition of minerals in seaweeds can fluctuate based on taxonomic classification, geographical factors, seasonal changes, physiological conditions, location, and species [89].

Seaweed requires micronutrients, including iron, zinc, manganese, and boron, for various physiological processes. These micronutrients function as catalysts and cofactors for enzymes that facilitate photosynthesis, nutrient absorption, and general metabolism, hence affecting growth rate, biomass yield, and the quality of the seaweed. Iron (Fe) is a crucial element for seaweed, necessary for chlorophyll synthesis and photosynthesis [90]. Iron is essential for various enzyme reactions. The presence facilitates the efficient operation of activities, including photosynthesis, respiration, and nitrogen metabolism [91]. Decreased concentrations can impair these activities, leading to diminished plant vitality [92]. Manganese (Mn) in the volced enzyme system and photosynthetic oxygen evolution, as well as nitrogen metabolism, influences the efficiency of nutrient absorption and utilisation. Mn is crucial for chlorophyll synthesis, functions as a coenzyme, activates many respiratory enzymes, and is involved in nitrogen metabolism and photosynthesis [79]. Zinc (Zn) is crucial for enzymatic function, protein synthesis, cellular division, and hormone production. Its roles include DNA synthesis and glucose metabolism, thereby positioning it as a vital element in biological processes [93]. Copper (Cu) contributes to enzymatic activity and photosynthesis, participating in various metabolic pathways and redox reactions. Micronutrient deficiencies can result in stunted development, diminished productivity, and compromised biochemical composition of seaweed [30, 80]. Micronutrients enhance biomass production by optimising metabolic processes and nutrient absorption. Micronutrients enhance seaweed biomass, yield, and total productivity.

4.5.3 Microstructure of pond solid waste

The results of microstructural observation of shrimp pond solid waste using SEM showed that particles before composting had a dense and smooth surface (Figure 6(A)). After composting, the outside and interior of the solid waste particles exhibited increased roughness and the emergence of many fissures (Figure 6(B)). Similar findings were published by study [94], indicating that scanning electron microscopy (SEM) revealed an increase in roughness on both the surface and inside of biochar particles post-composting. The surface morphology of the compost analysed using SEM before and during the incorporation of $Cu^{2+}$ revealed that the irregular, heterogeneously formed, and fractured surface could enhance $Cu^{2+}$ sorption on various regions of the adsorbent, rendering it an advantageous adsorption method [95].

The microstructure of the organic compost was examined using SEM. Initially, all treatments revealed the particle's surface to be heterogeneous and irregular in shape. The millicompost displayed a moderate particle size, sparse material distribution per unit area, heterogeneity with limited compaction, and small, rough structural surfaces characterised by many irregular pores indicative of fulvic acid presence. After 150 days of decomposition, millicompost displayed spores and fungal hyphae, primarily in the treatments lacking fertilisation [96]. The SEM micrograph of the sawdust biochar exhibits a coarse and extremely porous shape that is nearly uniform. The considerable porosity allows sawdust biochar to accommodate guest nutritional ions like as nitrogen, phosphorus, and potassium. Subsequent to impregnation, the formulation of the biochar-derived N–P–K slow-release fertiliser displayed aggregates on an irregular surface with a reduced number of pores relative to those in SBC [97].

SEM analysis showed that the amount of Si crystallites in the cross-sectional slices of Empty Fruit Bunches (EFB) was quite abundant, both in the fresh (pre-composted) and post-composted conditions [98]. The fermentation process modified the surface structure of *G. verrucosa*. Fermented *G. verrucosa* displays several fissures and disturbances compared to unfermented *G. verrucosa* [99]. Similarly, reported alterations in the surface morphology of *Sargassum binderi* following treatment with sulphuric acid and thermal exposure [100].

## 5. CONCLUSIONS

The solid waste from shrimp ponds can serve as organic fertilizer for seaweed production. The optimum dosage of organic fertilizer was 4 g/L, resulting in a total growth and growth rate of Gracilaria of 105.69 g and 1.31% per day, respectively, and a yield of 15.58%. The water quality throughout the investigation facilitated seaweed proliferation and agar yield. Future studies regarding the nutrient absorption of this organic fertilizer by seaweed and its physiological effect on seaweed are necessary to be explored. The practical relevance of this research for waste management is the potential use of Gracilaria as an efficient biofilter to reduce nitrate and phosphate levels in shrimp culture pond water, and its application in equalization ponds on a waste water treatment plan (WWTP) before being discharged into the aquatic environment to support sustainable aquaculture and waste management.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