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The Effect of Shrimp Pond Solid Waste Utilization on Protein and Chlorophyll Content in *Caulerpa lentillifera* Cultivation

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Abstract

Aquaculture waste is material produced by fish or shrimp farming that can pollute aquatic environments if not promptly managed. The solution to solve this problem is to utilize shrimp pond solid waste as organic fertilizer. The shrimp pond solid waste will be used as fertilizer to cultivate *Caulerpa lentillifera*. *Caulerpa lentillifera* cultivation has begun to develop in Indonesia because, in addition to being edible fresh, it also contains good nutrition for the body. This study aims to determine the effect of shrimp pond solid waste on protein and chlorophyll levels of *Caulerpa lentillifera*. This study was conducted with 4 (four) treatments in the form of different doses of shrimp pond waste (0, 2, 4, and 6 g/L) and 3 (three) replications in each treatment. The parameters tested included nutrient, protein, and chlorophyll content, as well as water quality, in *Caulerpa lentillifera* cultivation. The results obtained from this study are *Caulerpa lentillifera* is able to utilize the nutrient content in shrimp pond solid waste as fertilizer to add nutrients. Treatment C had the highest effect with protein content (8.27%) and chlorophyll-a (3.46 mg/g), chlorophyll-b (7.41 mg/g); and total chlorophyll (16.08 mg/g) in *Caulerpa lentillifera*. Based on these data, it can be seen that shrimp pond solid waste can be used as fertilizer in the cultivation of *Caulerpa lentillifera*.

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Introduction

Asia, particularly East and Southeast Asia, plays a dominant role in the global seaweed industry. Asia accounted for approximately 97.4% of total global seaweed production, with 99.1% of this production coming from the aquaculture sector in 2019 (Cai *et al.*, 2021). One promising species from Southeast Asia is *Caulerpa lentillifera*, a green seaweed widely consumed as a fresh vegetable (Noviana *et al.*, 2018). In recent years, this green seaweed has shown increasing demand in the global food sector (Syakilla *et al.*, 2022).

Caulerpa lentillifera, also known as "sea grapes" or "green caviar," is an edible green seaweed with a distinctive texture and various nutritional benefits (Stuthmann *et al*., 2023). The protein content of *C. lentillifera* ranges from 0.43 to 19.38% in various countries (Syakilla *et al*., 2022). The nutritional composition of *C. lentillifera* from Hainan, China (12.5% ± 0.70%), Shandong (14.7% ± 0.72%) (Zhang *et al*., 2020), from Thailand (4.67–12.49%) (Chaiklahan *et al*., 2020), from Vietnam (4.89–7.0%) (Hoan *et al*., 2020). The wide differences and instability of protein content can be influenced by various external factors, such as water temperature, season, geography, weather, and other factors (Kumar *et al*., 2011).

Intensive shrimp farming activities produce solid waste in the form of pond bottom sediment rich in organic matter (Syah *et al*., 2006). The accumulated waste can pollute coastal waters if unmanaged. However, it still contains macro- and micronutrients that can be reused as a nutrient source for macroalgae (Uddin *et al*., 2025). This waste reuse aligns with the principles of Integrated Multi-Trophic Aquaculture (IMTA) and the circular economy in the aquaculture sector (Troell *et al*., 2009).

Utilization of shrimp pond solid waste is expected to increase the chlorophyll content of sea grapes (Bambaranda *et al*., 2019). Increased chlorophyll content is positively correlated with better growth (Lideman *et al*., 2024). Furthermore, high chlorophyll content also affects the color of sea grapes (Stuthmann *et al*., 2022). Color is an indicator of sea grape quality. Consumers tend to prefer sea grapes with a bright green color compared to those with a faded green or white color (Hien *et al*., 2025).

The majority of shrimp pond waste research has been confined to analyzing and utilizing it for the cultivation of natural feed and other types of seaweed, such as the research of de Morais *et al*. (2023), who utilized solid shrimp pond waste in the cultivation of *Ulva ohnoi* seaweed, and Chen *et al*. (2025), who utilized waste to improve the quantity and quality of *Sarcodia suiae*. Therefore, this research is expected to produce sea grape products that have economic value and minimize the use of chemical fertilizers.

Materials and methods

Research Location

The research was conducted at the Laboratory of the Faculty of Fisheries and Marine Sciences, Fish Reproduction Laboratory (FPIK), Fishery Product Engineering Laboratory (FPIK) and Soil Chemistry Laboratory (FP) Brawijaya University, Malang.

Preparation of Shrimp Pond Solid Waste

The solid waste used in this study was pond bottom sediment, an accumulation of organic matter at the bottom of a tiger prawn (Penaeus monodon) rearing pond. It consisted of uneaten feed, shrimp feces, exuviae (moulting shells), dead plankton, and suspended organic particles. The shrimp pond solid waste was obtained from the tiger prawn farm at the Jepara Brackish Water Aquaculture Development Center (BBPBAP). The solid waste was collected by opening the central drain in the tiger prawn rearing tank for 15 minutes before feeding. The waste collected at the bottom of the cultivation tank was piped to a waste collection point. The collected solid waste was oven-dried at 105°C for 48 hours. The dried solid waste was used as the main material in this study.

Cultivation of *Caulerpa lentillifera*

Dry solid waste was weighed according to the treatment dose (0, 10, 20, and 30 g) and placed into an aquarium containing 5 L of seawater with a salinity of 32 ppt to obtain concentrations of 0, 2, 4, and 6 g/L. The waste was soaked for 48 hours with continuous aeration before planting the seeds. After soaking, initial measurements of NH4+, NO3-, and PO43- were taken in the media. Next, 300 g of *Caulerpa lentillifera* seeds were planted using the bamboo weaving method (20 × 20 cm) and maintained for 30 days. Water quality parameters (temperature, pH, salinity, and DO) were measured daily at 08.00 WIB; protein and chlorophyll levels were measured at the end of the maintenance. *Caulerpa lentillifera* used in this study were obtained from Sea Farmers in Musi Village, Gerokgak District, Bali. The source of seeds came from their natural habitat on the coast or from cultivated harvests specifically used as seeds. The light requirements that can be accepted by *Caulerpa lentillifera* range from 5400 – 6720 lux using a lux meter exposed to direct sunlight (Hiroyuki and Kadowaki, 2009).

Experimental Design

The research design used in this study was a completely randomized design (CRD). The treatments in this study were different doses with different ranges, replicated three times. The treatments were as follows:

Treatment K = No shrimp pond solid waste (Control)

Treatment A = Application of shrimp pond solid waste (Dosage 2 g/l)

Treatment B = Application of shrimp pond solid waste (Dosage 4 g/l)

Treatment C = Application of shrimp pond solid waste (Dosage 6 g/l)

Nutrient analysis

Nutrient analysis of shrimp pond solid waste includes organic C content analysis using the Walkley and Black Method (1934), total N content using the Kjeldahl Method (1883), total P content using the Bray and Kurtz Method (1945) and Olsen *et al*. (1954), Fe (iron) content using the Phenanthroline Method (SNI 19-1127-1989), Cu (copper) content using the method (SNI 19-1129-1989), and B (boron) content using the method (SNI 19-1128-1989).

Protein Analysis

Protein levels can be calculated using the Kjeldahl method (1883). This method consists of three steps: digestion, neutralization, and titration. The principle is that the sample is digested with a strong acid, releasing nitrogen, the concentration of which can be determined using an appropriate titration technique. The amount of protein present is then calculated from the nitrogen content in the sample.

Chlorophyll

Chlorophyll can be calculated using the Arnon Method (1949), using 80% acetone solvent and measuring the absorbance value of the chlorophyll solution at wavelengths (λ) 630, 645, 647, 663 and 664 nm. Before the measurement, the sample will be left in a dark place for 15 minutes. Fresh *C. lentillifera* samples are crushed using a mortar and pestle and mixed with acetone in a 1:1 ratio in a 2 ml tube. The sample is centrifuged for ± 15 minutes at a speed of 4000xg. The supernatant is transferred into a tube and mixed with acetone to reach a total volume of 10 ml. The absorbance is measured at wavelengths of 630, 645, 647, 663 and 664 nm (with the standard solution being acetone). The measurements at these wavelengths will later be used to determine the content of chlorophyll a, b and total chlorophyll as per the formula below:

Chlorophyll-a (mg/g) = 11,85 (A_{663}) – 1,54 (A_{647}) – 0,08 (A_{630})

Chlorophyll-b (mg/g) = 4,68 (A_{663}) – 22,9 (A_{645})

Total Chlorophyll (mg/g) = 20,21 (A_{645}) + 8,02 (A_{663})

Water Quality

Water quality measurements during the study included temperature, pH, dissolved oxygen, and salinity, which were carried out daily.

Data Analysis

Data were analyzed for ammonium, nitrate, phosphate, protein, and chlorophyll levels using SPSS version 21.0 with a one-way ANOVA test. The analysis began with a variance analysis, followed by a least significant difference (LSD) test using the

Duncan test if the results were significantly different or significantly different.

Results

Nutrient Content of Shrimp Pond Solid Waste

The analysis of shrimp pond solid waste shows that organic waste contains nutrients needed for the growth of *Caulerpa lentillifera*. These nutrients include macro and micro nutrients. Macro nutrients consist of C (carbon), N (nitrogen), Organic Matter, and K (potassium). Micro nutrients are used as fertilizer for *Caulerpa lentillifera*. division, and carbohydrates, as well as activating enzymes (Hasanuzzaman *et al.*, 2018). If plants experience a K deficiency, the photosynthesis process decreases, while it consists of Fe (iron), Cu (copper), Zn (zinc), Mn (manganese), B (boron). The content of these nutrients can be seen in Table 1.

From the comparative data in Table 1, it was found that the nutrients contained in shrimp pond solid waste can be used as fertilizer for *Caulerpa lentillifera*. Potassium is one of the elements that is essential for plants in the process of photosynthesis, where

Table 1. Results of Nutrient Content Analysis of Shrimp Pond Solid Waste and Comparator

Parameter	Unit	Organic Waste from Tiger Shrimp Ponds	Manure (Hadisuwito, 2007)
C organic	%	4,24	4,36
N total	%	0,70	0,81
Organic Materials	%	7,34	7,52
K	mg kg-1	14,32	2,18
Fe	ppm	2,30	76,1
Cu	ppm	3,00	41
Zn	ppm	tu	128
Mn	ppm	190,50	119
B	ppm	59,50	77,8

Description: tu = not measurable

Potassium is one of the elements that is essential for plants in the process of photosynthesis, where the potassium value is greater than that of manure at 14.32 mg kg-1 compared to 2.18 mg kg-1. Another macro nutrient is potassium (K), which functions as a catalyst in the formation of proteins, cell the potassium value was found to be greater than that of manure, at 14.32 mg kg^{-1} compared to 2.18 mg kg^{-1}. Another macronutrient is potassium (K), which functions as a catalyst in the formation of proteins, cell division, and carbohydrates, as well as activating enzymes (Hasanuzzaman

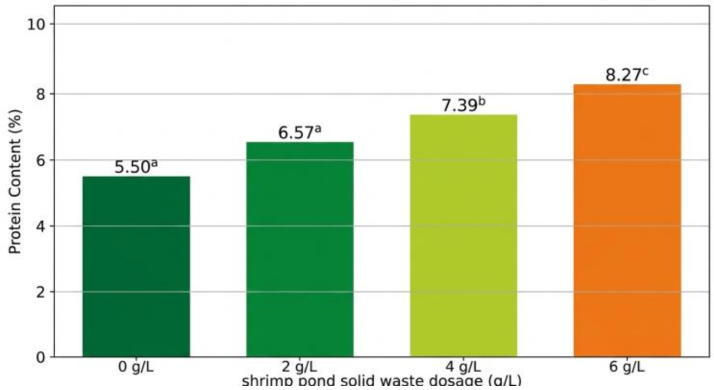

Figure 1. Protein content of *Caulerpa lentillifera* weighing 100 grams

et al., 2018). If plants experience a K deficiency, the process of photosynthesis will decrease while the process of plant respiration will increase (Tränkner *et al*., 2018).

The functions of micronutrients include influencing oxidation and reduction processes, helping regulate acid levels, acting as catalysts (stimulants), influencing osmotic values, promoting growth, and influencing nutrient absorption (Sudarmi, 2014). Meanwhile, the manganese (Mn) content is also higher, at 190.50 ppm, compared to manure's 119 ppm. According to Alejandro et al. (2020), manganese (Mn) is one of the elements that play a crucial role in photosynthesis. This element functions as an enzyme activator in the light process of photosynthesis. The higher the Mn concentration in the culture medium, the higher the rate of photosynthesis, resulting in improved product quality.

Protein Content in *Caulerpa lentillifera*

Based on the image above, it can be seen that the protein content of *Caulerpa lentillifera* planted in treatment C has the highest value compared to the other treatments with a protein content of 8.27%.

Paul's (2008) study found a protein content of 6.8% in cultivation conducted without additional fertilizer in a controlled tank. Meanwhile, Ratana and Chirapart's (2006) study found a protein content of 12.49% in the native habitat, or directly from the water. A comparison of the literature suggests that using shrimp pond solid waste in a controlled tank is superior to using commercial fertilizers. However, when taken from the native habitat, the protein content is certainly superior to that of the cultured fish in the controlled tank because the nutrients absorbed are in accordance with *Caulerpa lentillifera* needs and are natural.

Chlorophyll Quality in *Caulerpa lentillifera*

Based on the image above, it can be seen that *C. lentillifera* grown in treatment C has the highest chlorophyll quality compared to other treatments, with a total chlorophyll quality of 16.07 mg/g. Differences in chlorophyll quality are seen to differ between each treatment. Nutrient concentrations, especially N and P, are the main determining factors for chlorophyll concentration and growth rate of *C. lentillifera* in cultivation systems, including the use of aquaculture wastewater (Chen *et al*., 2019). According to Paul *et al*. (2013), the chlorophyll content of sea grapes cultivated without additional fertilizer reached 2.58 mg/g (chlorophyll a), 1.47 mg/g (chlorophyll b), with a total chlorophyll of 4.1 mg/g. Then, in the study of Guo *et al*. (2015) stated that the total chlorophyll content of sea grapes reached 10.97 mg/g using additional fertilizer and chlorophyll a of 2.25 mg/g. The quality of sea grape chlorophyll cultivated using shrimp pond solid waste produces better results than using other fertilizers.

Nitrogen is the main constituent of the porphyrin ring in chlorophyll molecules (Paine *et al*., 2021). NH4+ and NO3- ions absorbed by the thallus are assimilated into glutamate, which is then converted through the tetrapyrrole biosynthesis pathway into δ-aminolevulinic acid (ALA), the direct precursor of chlorophyll (Pozzobon *et al*., 2021). Therefore, the availability of inorganic nitrogen in the culture medium is positively correlated with the accumulation of chlorophyll-a and chlorophyll-b. (Pozzobon *et al*., 2021).

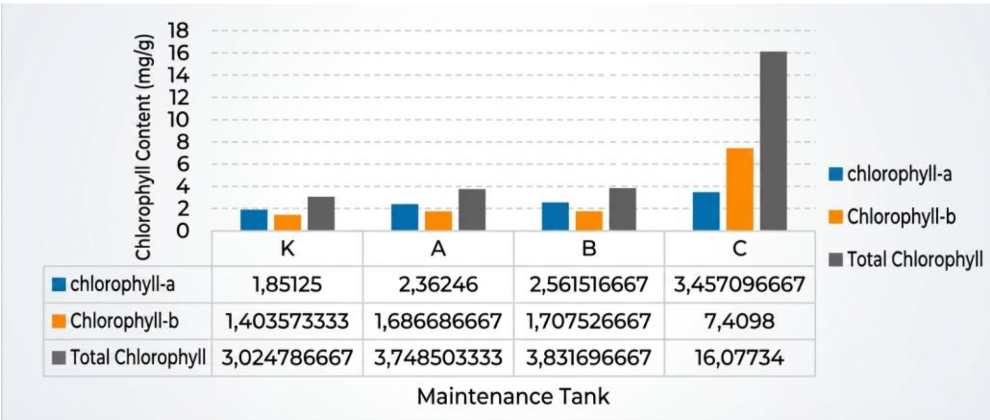

Figure 2. The Results of Chlorophyll Quality of *Caulerpa lentillifera* in Each Maintenance Container

The resulting differences are consistent with observations by Marzetz *et al*. (2020), who stated that changes in pigment content are influenced by the interaction of two factors: light intensity and nutrients. Photosynthesis can also be influenced by sunlight intensity (Simkin *et al*., 2022). General parameters for microalgae cultivation include a light intensity range of 1,000–10,000 lux, depending on the culture volume. Specifically, 1,000 lux is suitable for small Erlenmeyer flasks, while 5,000–10,000 lux is required for larger culture volumes, as light must penetrate the entire culture medium (Wang *et al*., 2022). Meanwhile, according to Yuliana and Lestari (2015), excessive sunlight can cause plants to turn white and wilt due to a lack of protein in the stems, often referred to as bleaching. Therefore, the optimal light intensity ranges from 800–3,000 lux.

Water Quality

Water quality is a key parameter that can influence phytoplankton life. Observable parameters include temperature, pH, dissolved oxygen (DO), and salinity. Each water quality parameter has a favorable range for supporting *Caulerpa lentillifera*. The range of water quality values can be seen in Table 2.

The total temperature range from the beginning to the end of the observation was 27-28 °C. Research by Sulaimana *et al*. (2021) showed that the highest specific growth rate and chlorophyll content occurred at 28°C, compared with lower temperatures such as 20°C or 24°C. Based on observations made during the study, the pH value range was 6.4–8. The difference in pH values reaching 2 was due to the duration of immersion, which depends on the ammonification and nitrification processes, as stated by Nainggolan *et al*. (2009) that the longer the immersion time, the more nitrate will decrease, which is also accompanied by a decrease in pH. Most algae thrive in slightly acidic to slightly alkaline conditions, usually ranging from pH 6.5 to 8.5. Chlorella vulgaris, a green alga closely related to Caulerpa in the Chlorophyta division, can tolerate a relatively wide pH range from 6.0 to 9.0 (Kalaji *et al*., 2018).

The optimum salinity range in a controlled tank for sea grapes is between 25-30 ppt. Research shows that although sea grapes can tolerate a wide range of salinity levels, optimal growth and metabolic health typically require levels between 30 and 40 ppt (Bambaranda *et al*., 2019; Ly *et al*., 2021). Based on observations, maintenance DO values range from 5.60 to 5.91 ppm.

Optimal dissolved oxygen conditions required by sea grapes range from 5.0 to 8.0 ppm (Eviana *et al.*, 2024).

Conclusion

The study has shown that shrimp pond solid waste contains nutrients that can be used as nutrients for *Caulerpa lentillifera* growth with varying chlorophyll qualities. As a result of the results, *Caulerpa lentillifera* has a protein content ranging from 8.27% and a chlorophyll-a quality ranging from 1.8–3.46 mg/g, a chlorophyll-b quality ranging from 1.4–7.41 mg/g, and a total chlorophyll range from 3.0 to 16.00 mg/g.

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