Original article DOI: 10.19027/jai.23.2.224-236

Production performance and water quality of *Caulerpa lentillifera* with fermented fruit vegetable fertilizer

Performa produksi dan kualitas air *Caulerpa lentillifera* dengan penambahan pupuk fermentasi limbah buah sayur

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(Received August 24, 2023; Received in revised form September 29, 2023; Accepted April 28, 2024)

ABSTRACT

The use of fermented vegetable and fruit waste (FVF) based fertilizers as an effort to reduce waste has the potential to be used to support aquaculture activities. This study aims to evaluate the effectiveness of *Caulerpa lentillifera* cultivation with the addition of fertilizer based on fruit and vegetable waste fermentation on water quality parameters and production performance. The experimental design used was a completely randomized design with three replications for four different doses of FVF fertilizer treatments, namely 0, 0.05, 0.1, and 0.2 µl/L. This study used an initial weight of 500 g which was reared in a concrete tank with a capacity of 1000 L for 30 days growth period. The results showed that the use of FVF of 0.1 µl/L gave a better net harvest weight ($p<0.05$), namely 0.08 ± 0.06 kg.

Keywords: *Caulerpa lentillifera*, fermentation, fertilizer, seaweed, waste

ABSTRAK

Penggunaan pupuk berbasis fermentasi limbah sayur dan buah (FVF) sebagai salah satu upaya pengurangan limbah memberikan potensi sebagai larutan pendukung kegiatan budidaya. Penelitian ini bertujuan untuk mengevaluasi efektivitas budidaya *Caulerpa lentillifera* dengan penambahan pupuk berbasis fermentasi limbah buah dan sayur terhadap parameter kualitas air dan kinerja produksi. Rancangan percobaan yang digunakan adalah rancangan acak lengkap dengan tiga kali ulangan pada empat perlakuan pupuk, yaitu 0, 0.05, 0.1, dan 0.2 µl/L. Penelitian ini menggunakan bobot awal 500 g yang dipelihara dalam bak beton berkapasitas 1000 L. Hasil pengamatan menunjukkan bahwa penggunaan FVF sebesar 0.1 µl/L memberikan bobot panen bersih yang lebih baik ($p<0.05$) yaitu 0.08 ± 0.06 kg.

Kata kunci: *Caulerpa lentillifera*, fermentasi, limbah, pupuk, rumput laut

INTRODUCTION

Many species of seaweed have been used since ancient times as food, animal feed, fertilizer, industrial raw materials, and biopharmaceuticals (Fouda *et al*., 2018). *Caulerpa lentillifera* is one species of green seaweed that is often consumed as fresh food in Southeast Asia region especially Japan and South Korea (Darmawan *et al*., 2022). Caulerpa has a nickname as seagrape because of its shape like a tight collection of round beads. *C. lentillifera* has a higher market demand as a fresh food because of its light, fresh, and soft taste compared to other species. This species has been cultivated in several countries such as the Philippines and Vietnam (Rabia, 2016; Anh *et al*., 2020).

Caulerpa cultivation is generally carried out in ponds with soil substrate or on the coast. Seaweed cultivation activities at PT Bulung Bali was conducted in concrete tanks with plastic net based for growth as a culture model (Cahyono, 2021). The use of tanks aims to make the cultivation process more efficient and controlled. Plantation which is done directly on the coast and tank, shows differences in quality and harvest weight. Production performance was influenced by environmental conditions (Estrada *et al*., 2020).

Observations of Anh *et al*. (2020) showed that several factors such as stocking density, water movement, cultivation methods, and nutrient supply can affect the growth rate, production, shape, and ramuli texture of *C. lentillifera*. Generally coast planted shows better quality harvest. Efforts to increase harvest weight in tank cultivation have been carried out by providing stimulation. Some efforts include adding fertilizer, substrate, and change in light intensity. According to Nurfebriani *et al*. (2015), an important factor for *C. lentillifera* cultivation is the nutrient requirements in the grow-out tank.

The addition of fertilizer is useful for increasing the growth and productivity of seaweed biomass (Susilowati *et al*., 2019). Other efforts to increase productivity in seaweed are to provide stimulation in the form of phytohormones or growth regulators (ZPT). Supriyono *et al*. (2022) showed the use of PGR along with fertilizer increased callus growth on *Eucheuma cottonii* seaweed. One alternative that has the potential to increase productivity is the use of vegetable and fruit fermented fertilizers (FVF). This fertilizer utilizes the end product of fruit and vegetable fermentation.

FVF is also commonly called as ecoenzyme or garbage enzyme solution. Made from a mixture of leftover vegetables and fruit, molasses, and water, in a ratio of 3:1:10 for three months of fermentation. Molasses functions as a carbon source for degradation microorganisms and water as a binding medium for organic acids formed during fermentation (Napitupulu, 2021). The FVF contains microorganisms, protein chains (enzymes), minerals, organic acids, and plant hormones (Azhar *et al*., 2021; Neupane & Khadka, 2019; Rasit *et al*., 2019). FVF showed different nutrient content depending on the fruits and vegetables used. This FVF test uses a mixture of various vegetables and fruits from the market leftover.

The small nutritional value makes FVF unable to be used as the sole source of nutrition for plants but it will be used to determine its effectiveness as a growth stimulant for it contains other compounds. The bioactive compounds in FVF are primary and secondary metabolites from fermented plants (Banerjee *et al*., 2017). In addition to plant metabolites, there are microbial secondary metabolites. One of its roles is to inhibit the growth of pathogens, induce plant defense, and increase growth (Pang *et al*., 2021).

It has concentrations of organic acids in the form of lactic acid (4.46 g/L), acetic acid (1.52 g/L), and microbes such as *Lactobacillus* (72.45%) and *Acetobacter* (15.23%) (Zhang *et al*., 2020). This fertilizer has been fermented for three months and contains plant hormones in the form of auxin, namely indolF3-acetic acid (IAA) (Gao & Liu, 2020). This hormone is allegedly able to stimulate the development of *Caulerpa* and increase the weight of the harvest. This liquid solution can be classified as an organic fertilizer since it is able to contribute only a small amount of nutrients compared to inorganic fertilizers (Novianto *et al*., 2020).

This research is the first study to observe the role of three months of fruit and vegetable waste fermented fertilizer in *Caulerpa* seaweed cultivation. Testing the effectiveness of liquid fertilizer based on fruit and vegetable fermentation on *Caulerpa lentillifera* cultivation media will be useful as a science to support the application of fermented fertilizers. Considering that organic waste contributes as much as 61.2% of the total waste contained in landfills (Hariyanto, 2014), this liquid can be used as a solution for organic waste treatment.

MATERIALS AND METHODS

This research took place within 30 days and was carried out from December 2021 to January 2022 at PT Bulung Bali, Gerokgak, Buleleng, Bali. Water quality tests were carried out at PT Bulung Bali, Gerokgak, Buleleng, Bali.

Experimental design

This study used a completely randomized design, containing four treatments and three replications. Treatment using a container in the form of a concrete tank. Treatments were applied as four different FVF doses, namely F1 0 µL/L, F2 0.05 µL/L, F3 0.1 µL/L, and F4 0.2 µL/L. Dose that were used are counted based on rearing media volume. Each treatment has three replicates in the form of three algae mats in each container. Determination of the dose before the study (pretest) is carried out first to determine the appropriate dose range. The pretest used a dose level of 0.08 µl/L, 0.1µl/L, and 0.3 µl/L measured for three days. The results of the pretest showed that the use of a dose that was too high with 0.3 µl/L could cause *C. lentillifera* to become yellow, wilt and wrinkle showed it was dying (Hasbullah *et al*., 2014). The treatment dose was determined using the pretest threshold.

Research procedures

C. lentillifera was reared in a concrete tank measuring 2×1×0.5 m with a transparent plastic roof and black shade fabric. The tank is also equipped with circulation for eight hours. The tank is washed and brushed before placing the algae mat. *C. lentillifera* is cultivated using plastic-based algae mats. The mat size is 30×30 cm with 1.5 cm hole size.

Each mat is filled with 500 g of seeds which are spread evenly. Seedlings come from all parts of sorted *C. lentillifera* in the form of roots, stems, and ramuli (Figure 1). *C. lentillifera* was then placed on the bottom of the culture tank. The planting medium is in the form of a concrete tank with a capacity of 1000 L with 3/5 of the water from the tank, which is 600 L. In addition to FVF, an additional form of fertilizer is used, namely AB mix fertilizer of 0.05 µl/L to meet nutritional needs.

Water circulation in the tank is turned on at 08.00 am and turned off at 16.00 pm. Seawater will go through the filter tank before entering the rearing pond. The circulation in the *C. lentillifera* was turned on for eight hours and water will stagnate for 16 hours. FVF and AB mix fertilizer were added on the 10th day of planting and added once a day in the afternoon after the water circulation stopped. Each tank received the same fertilizer treatment and a different FVF treatment.

Research parameters

Production performance parameters and water quality were measured for data collection. Production parameters were then calculated to obtain a specific weight growth rate (SWGR) and production performance. Production performance was measured based on gross and net weights to measure productivity. Net weight is the weight

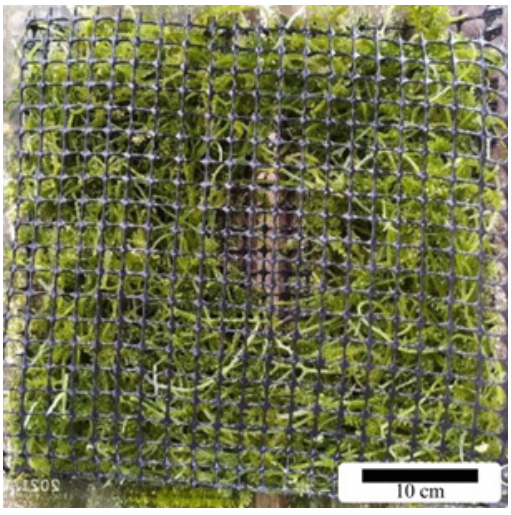

Figure 1. Grow net and seedling of *C. lentillifera.*

of *C. lentillifera* that is qualified to enter the market for sale, while gross weight is the harvest that has not been sorted. Specific weight growth rate (SWGR) was calculated with the following formula:

$$SWGR\ (\%) = \frac{(\ln W_t - \ln W_0)}{t} \times 0.1$$

Note:
SWGR = Specific weight growth rate (%)
Wo = Initial weight (kg)
Wt = Final weight (kg)
t = Growth time

Production performance was determined by the following formula:

$$W = Wt - Wo$$

Note:
W = Production of Caulerpa (kg)
Wt = Biomass of Caulerpa at the end of this study (kg)
Wo = Biomass of Caulerpa at the beginning of this study (kg)

Water physical-chemical parameters (temperature, pH, and salinity) were measured in situ in the morning and evening. Nitrate and phosphate started to be measured on the 10th day of planting. Nitrate and phosphate then were measured every seven days from first measurement. Measurement were taken before and after FVF and fertilizer treatment. Measured in the evening before and in the morning 16 hours after treatment. Biometric data was obtained by measuring total harvest weight, secondary stem length (frond), and total ramuli per 2 cm. Secondary stem length and total ramuli were measured every seven days. A total of 30 secondary stem lengths were used as samples per pond. A digital scale with an accuracy of 0.01 g and a ruler with an accuracy of 0.1 cm were used for biometric measurements.

Data analysis

Production performance parameters were analyzed by variance (ANOVA) with a 95% confidence interval and if they have a significant effect will be continued with the Duncan test. Water quality parameter data were analyzed descriptively by presenting tables or pictures. Data analysis was carried out using software in the form of IBM SPSS version 22.0 and Microsoft Excel 2019.

RESULTS AND DISCUSSION

Result

Specific weight growth rate (SWGR)

Weight-specific growth rates resulted in significant differences (P<0.05) between each treatment. F1 and F3 treatment (FVF 0 µl/L and FVF 0.1 µl/L) were significantly different from F4 treatment (FVF 0.2 µl/L) and F2 (FVF 0.05 µl/L). The highest specific growth rate of seaweed explants was in the F4 treatment of 3.72%. The lowest rate lies in the F2 treatment of 1.10% as presented in Table 1.

Production performance with gross and net harvest comparison yielded significant differences (P<0.05) between each treatment. The yields showed that there was a significant difference in treatment without FVF (F1) and FVF (F2 F3 F4). The F2 treatment experienced a significant difference because of seepage in the rearing tank.

Table 1. Specific weight growth rate of *C. lentillifera*.

Treatment	Initial weight (kg)	Final weight (kg)	specific weight growth rate (%)
F1	0.5	1.10^{b}	2.62^{b}
F2	0.5	0.70^{a}	1.10^{a}
F3	0.5	1.25^{b}	3.06^{b}
F4	0.5	1.53^{c}	3.72^{c}

Note: F1 (FVF 0 µl/L), F2 (FVF 0.05 µl/L), F3 (FVF 0.1 µl/L), F4 (FVF 0.2 µl/L). Data are mean ± SD, and the same letters over each treatment bar indicate no significant difference (P>0.05; Duncan's test). Data are mean ± SD, different letters over each treatment bar indicate a significant difference (P<0.05; Duncan's test).

In the F3 and F4 treatments, the highest gross harvest weight were 0.75 ± 0.05 kg and 1.03 ± 0.05 kg. The highest net weight values were in the F3 and F4 treatments of 0.08 ± 0.06 kg and -0.05 ± 0.05 kg. The lowest net weight was obtained in the F2 treatment, namely -0.37 ± 0.07 kg, as shown in Figure 2.

Number of Ramuli

Number of ramuli at H7, H14, H21, and H28 in Figure 3 produced significant differences (P<0.05) in some treatments. At H21 there was no significant difference in each treatment. The highest number of ramuli was in the F4 treatment in the H14 with a value of 33 ± 4.75 grains and in the H28 with 28 ± 5.22 grains. The lowest number of ramuli was found in the F2 treatment of 19 ± 3.38 grains in H7.

Secondary stem length

Secondary stem length at H7, H14, H21, and H28 produced significant differences (P<0.05). The F1 treatment gave the highest score at H7, H14, and H21, with 3.24 ± 0.94 cm, 4.60 ± 1.18

Table 2. Production performance of *C. lentillifera*.

Treatment	Initial weight (kg)	Average gross harvest (kg)	Average net harvest (kg)
F1	0.5	$0.60^{b} \pm 0.10$	$-0.13^{b} \pm 0.03$
F2	0.5	$0.20^{a} \pm 0.15$	$-0.37^{a} \pm 0.07$
F3	0.5	$0.75^{b} \pm 0.05$	$0.08^{c} \pm 0.06$
F4	0.5	$1.03^{c} \pm 0.05$	$-0.05^{b} \pm 0.05$

Note: F1 (FVF 0 µl/L), F2 (FVF 0.05 µl/L), F3 (FVF 0.1 µl/L), F4 (FVF 0.2 µl/L). Data are mean ± SD, the same letters over each treatment bar indicate no significant difference (P>0.05; Duncan's test). Data are mean ± SD, different letters over each treatment bar indicate a significant difference (P<0.05; Duncan's test).

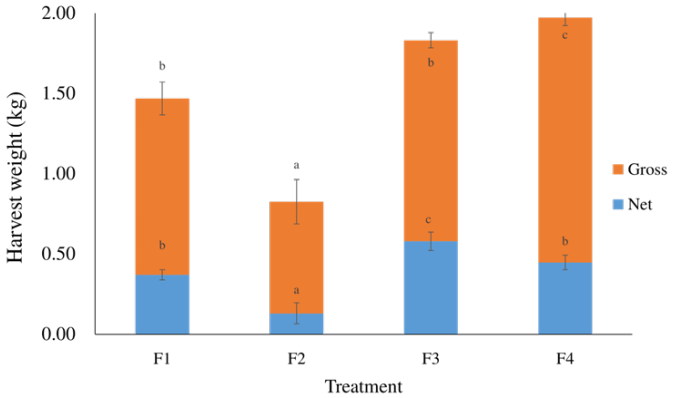

Figure 2. Total gross and net harvest weight (final weight) of *C. lentillifera.* Dose F1 (FVF 0 µl/L), F2 (FVF 0.05 µl/L), F3 (FVF 0.1 µl/L), F4 (FVF 0.2 µl/L). Data are mean ± SD, different letters over each treatment bar indicate a significant difference (P<0.05; Duncan's test).

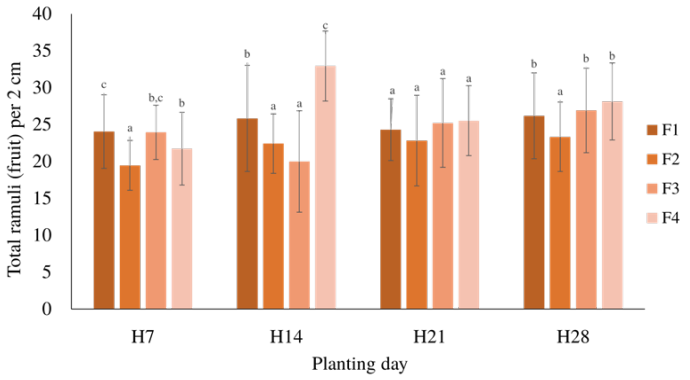

Figure 3. Total ramuli per 2 cm of *C. lentillifera.* Dose F1 (FVF 0 µl/L), F2 (FVF 0.05 µl/L), F3 (FVF 0.1 µl/L), F4 (FVF 0.2 µl/L). Data are mean ± SD, different letters over each treatment bar indicate a significant difference (P<0.05; Duncan's test).

cm, and 6.14 ± 2.00 cm. The lowest length growth was found in the F4 treatment at H7, H14, H21, and H28 with values of 2.51 ± 0.87 cm, 3.18 ± 1.06 cm, 3.84 ± 1.94 cm, and 3.97 ± 1.05 cm. The highest secondary stem length was found in the F1 treatment at H21 with a value of 6.14 ± 2.00 cm. The lowest secondary stem length was in the F4 treatment with a value of 2.51 ± 0.87 cm in H1. Based on Figure 4, there is an increase in the length of the secondary stem as it progresses.

Water quality parameters

Water quality values during the study on temperature parameters ranged from 25.1-30.2°C, salinity parameter ranged from 18-38 ppt, pH parameter ranged from 7.3-9.8, nitrate parameter ranged from 0.0-4.07 mg/L, and the phosphate parameter ranged from 0.0-0.25 mg/L. Phosphate in all treatments of the 11th day indicates an increase in phosphate concentration level. The smallest increases were from F4 (0.06 mg/L) and F3 (0.06 mg/L), then F1 (0.1 mg/L), and F2 (0.24 mg/L). On the 18th day, phosphate of F2, F3, and F4 before and after treatments decreased indicating a reduction in phosphate levels in the media. F1 phosphate still increased but in a smaller value compared to 11th day.

The 25th day showed an increase in absorption value but lower compared to the previous week in FI F3 F4 treatments. Based on Figure 5, from

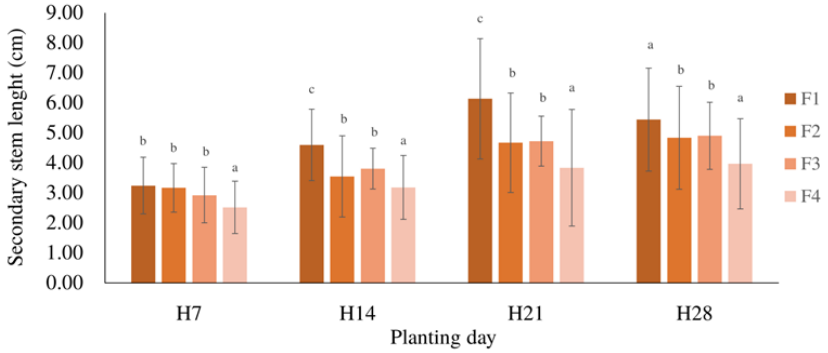

Figure 4. Secondary stem length of *C. lentillifera.* Dose F1(FVF 0 µl/L), F2 (FVF 0.05 µl/L), F3 (FVF 0.1 µl/L), F4 (FVF 0.2 µl/L). Data are mean ± SD, different letters over each treatment bar indicate a significant difference (P<0.05; Duncan's test).

Table 3. Water quality in *C. lentillifera* tank.

Parameter	Unit	Value	Optimal value	Reference
Temperature	°C	25.1-30.2	22-28	Guo *et al.* (2015)
Salinity	Ppt	18-38	25-35	Chen *et al.* (2019)
pH	-	7.3-9.8	6.8-9.6	Ginting *et al.* (2015)
Nitrate	mg/L	0-4.07	0.1-0.6	Chen *et al.* (2019)
Phosphate	mg/L	0-0.25	0.02-0.12	Chen *et al.* (2019)

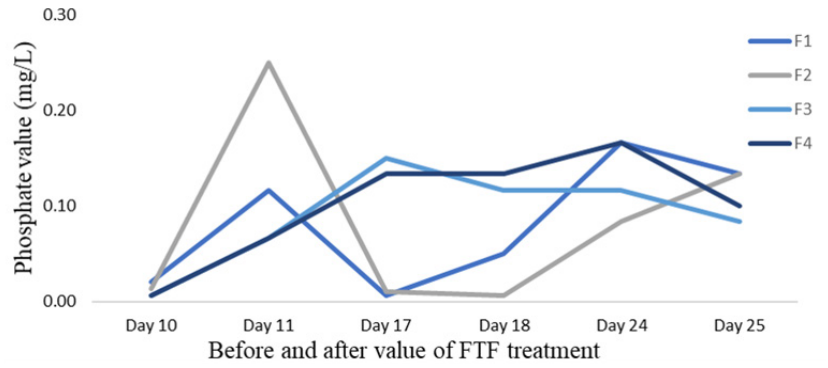

Figure 5. Phosphate values in *C. lentillifera* tank. Dose F1 (FVF 0 µl/L), F2 (FVF 0.05 µl/L), F3 (FVF 0.1 µl/L), F4 (FVF 0.2 µl/L).

10th day to 25th there was a gradual increase in phosphate absorption in all treatments. The lowest change in nitrate concentration in the 11th day of the study was found in treatments F2 and F4. Both F1 and F3 treatments indicated a reduction in nitrate levels. Nitrate values on the 18th day of all treatments showed a reduction.

On the 25th day treatments except F2 also showed nitrate reduction. The F2 treatment showed an increase in the concentration of nitrate in the media. The lowest absorption was found on the 11th day of F2 treatment, F4 (0 mg/L), and 18th day of F2 treatment. The highest absorption value was on 11th day by treatments F1 and F3. Based on Figure 6, only F1 and F3 reduced nitrate concentrations in the 11th day of the study. After all treatments indicate a reduction in nitrate levels.

Discussion

Fertilizers based on fruit and vegetable fermentation (FVF) are improving the quality of the environment for plants to grow more optimally (Gao & Liu, 2020). The principle of the fermented fertilizer process is that volatile acids are reduced from carbohydrates (Napitupulu, 2021). During the fermentation process, glucose is broken down to produce pyruvic acid. Pyruvic acid is broken down by pyruvate decarboxylase into acetaldehyde, then acetaldehyde is converted by alcohol dehydrogenase into ethanol and carbon dioxide. Acetobacter bacteria will convert alcohol into acetaldehyde and water, which will then convert acetaldehyde into acetic acid (Rohmah *et al*., 2020).

Extraction of skin and fruit flesh in FVF releases organic acids. The addition of FVF to *C. lentillifera* gave a significant difference on production performance data in the form of harvest weight, number of ramuli, and secondary stem length in the treatment. In the treatment with FVF (F3 and F4), the secondary stem length was lower than the treatment without FVF (F1). A decrease in stem length could be caused by evenly distributed due to being covered by high biomass growth.

This can be seen from the larger size of the ramuli in the F3 and F4 treatments compared to F1. The number of ramuli did not differ between treatments in H21 and H28, but there were differences in the ramuli density caused by different ramuli sizes. Although there were no differences in the number of ramuli between treatments at the end of the study, there were differences in the size thus affecting density between the treatments. The F2 treatment gave unmeasurable results because seepage occurred in the grow-out container.

Production performance, nitrate levels, and phosphate levels of the F2 treatment could not be compared with other treatments. Day 11th, of all treatments, experienced a decrease in both phosphate and nitrate values. There are two possibilities when the measured value reaches 0 mg/L, which are sufficient or limited. In the F1 and F3 treatments, there was a decrease in nitrate concentration that showed more use of nitrate than F4. On day 18th nitrate observation indicates that F3 is more optimal than F1 (non FVF) and on the 25th day the greatest decrease in nitrate by the F1 treatment compared to F4. Looking at the nitrate levels in Figure 6 there is the trend.

There's a pattern of decreasing nitrate levels in *C. lentillifera* culture tanks following its growth. This pattern can be the answer to the possibility

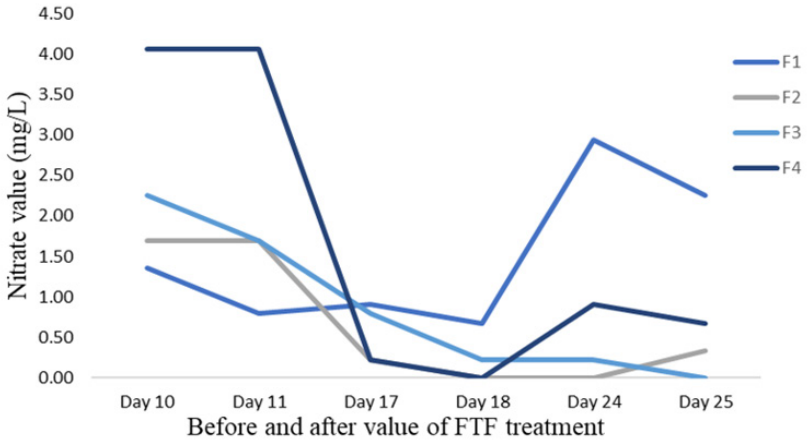

Figure 6. Nitrate values in *C. lentillifera* tank. Dose F1 (FVF 0 µl/L), F2 (FVF 0.05 µl/L), F3 (FVF 0.1 µl/L), F4 (FVF 0.2 µl/L).

that occurs at 0 mg/L when nitrate is no longer sufficient resulting in deficiency or limiting factor for growth. The limiting factor for the growth of seaweed is nutrition (Harrison & Hurd, 2001). Day 25th of the F1 treatment showed higher water nitrate levels compared to the FVF treatment (F3 F4). This can be caused by growth that is no longer optimal, unlike in the previous week.

According to Bambaranda *et al*. (2019a), *C. lentillifera* growth reached its best value on the 20th planting day. This statement is similar to the observation of nitrate and phosphate lowest values on the 18th day for all treatments. Nitrate nitrogen measurement is a form of validation of the performance of *C. lentillifera*. According to Farahdiba *et al*. (2020) changes in nitrogen in the environment can occur due to macroalgae activities. There are two forms of solubility of nutrients in water, the form utilized by seaweed is inorganic. Dissolved inorganic nitrogen ions are in the form of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) (Meirinawati, 2017).

As an essential nutrient for algal growth, nitrogen deficiency affects the growth stage, decreases protein biosynthesis, and decreases photosynthetic efficiency (Hsu *et al*., 2023). *C. lentillifera* used nitrate as the primary nitrogen source. However a study conducted by Hsu *et al*. (2023) showed biomass, growth rate, and tissue nitrogen content showed a nonlinear relationship with the total nitrogen concentration in a cultured environment. So although the uptake rate follows enviroment concentration, the molecular mechanism of *C. lentillifera* nitrogen regulation is unclear. Excess nitrogen also decreases growth rate and inhibits photosynthesis (Hsu *et al*., 2023).

Higher nitrate content on the 25th day for F1 treatment could be caused by this stress or its inability to process higher nitrogen compared to F3 F4. Since nitrate content before treatment is already higher than the previous week. Orthophosphate (PO_4^{-3}) is a source of inorganic phosphorus which plays an important role for algae growth (Han *et al*., 2016). Phosphate is required in smaller amounts than nitrate. Phosphate values in the 11th day of all treatments showed an increase and was concluded that the dosage is excessive.

There is a concentration difference in the final values of all treatments with the lowest phosphate added values the day after fertillizion by F3 and F4 treatments. This shows that FVF treatment has an impact on better absorption of phosphate by *C. lentillifera*. On the 18th day phosphate value from fertilization in the treatment without FVF (F1) was still excessive. On the 25th day, all treatments showed optimal absorption of phosphate from fertilizers because the levels before and after did not change. The greatest absorption by F4 followed by F3 and F1 with the same value. More *Caulerpa* can increase the need for phosphate resulting in a decrease in phosphate levels.

There is a trend of increasing water phosphate levels every week before fertilization. The increasing value may be caused by the rainy season which causes a mix of sediment and water. Low water phosphate levels were found on the 11th day of all treatments and the 18th day of treatment without FVF. The function of phosphate is energy metabolism, protein synthesis, regulation, formation of proteins, carbohydrates, cell structure, and cell membrane stabilization. The efficiency of using phosphate depends on the composition of the media and environmental conditions (Lee *et al*., 2015).

According to the observations of Chu *et al*. (2019), fermented fruit and vegetable fertilizers contain lactic acid bacteria (LAB). LAB has the ability to produce alkaline phosphatase (ALP) enzymes. Through this enzyme, *Caulerpa* can utilize phosphates in organic form. The enzyme from this bacterium has the property of hydrolyzing organic phosphate on the surface of *Caulerpa* to become inorganic phosphate PO_4^{-3} so it can be absorbed (Schaffelke, 2001).

ALP activity is optimal at 37°C, pH 8.5, active by Mg^{2+}, Ca^{2+}, and inhibited by Cu^{2+}, Zn^{2+}. This enzyme works by removing phosphate groups from organic molecules such as nucleotides, proteins, and alkaloids. The addition of FVF in the media allegedly contributed to the effectiveness of phosphate uptake by *C. lentillifera*. Nitrate:phosphate ratio in *C. lentillifera* according to Chen *et al*. (2019) is 5:1. Measured values in the *C. lentillifera* tank are much greater which is 16:1.

Balance growth requires a certain ratio because there are interactions between nutrients. Treatment on the 11th day showed that growth used more nitrate levels compared to phosphate. After the 11th day nitrate is a limiting factor because of its low levels. Macroalgae take up ammonium and nitrate simultaneously, but the levels are different for each nitrogen source. For example, ammonium is absorbed more quickly than nitrate with a limited absorption rate (Roleda & Hurd, 2019). Nitrate and ammonium always coexist and are found at various concentrations in

close proximity (Hachiya & Sakakibara, 2017).

Nitrate in the sea is generally much more abundant than ammonium. The concentrations of phosphate, nitrate, and ammonium affect the growth of seaweed but the use of phosphate on growth is not dependent (Xiao *et al*., 2019). The secondary stem length of *C. lentillifera* based on the observations of Estrada *et al*. (2020) varies around 3–11 cm. In this study, the secondary stem length varied around 2-8.5 cm. Temperature measurements on the rearing medium during the study were in the range of 25.1–30.2°C. This range is in accordance with the opinion of Chen *et al*. (2019) that the temperature range of 25-35°C can increase algae growth.

The temperature range measured during the study is suitable for the growth of *C. lentillifera*. Temperature affects all aspects of seaweed physiology through regulation of enzyme activity, rate of chemical reactions, and rate of diffusion of nutrients. Seaweed metabolism goes well at high temperatures and in a saturated light environment. The measured pH in the maintenance medium during the study ranged from 7.3–9.8. This range is still within a reasonable limit to support the growth of *C. lentillifera.* This is the same as the research by Ginting *et al*. (2015) that the pH range is 6.8-9.6 all algae can still live and grow.

The salinity range during the study was 18-38 ppt, this range is still tolerable and suitable for the growth of *C. lentillifera* according to the opinion of Guo *et al*. (2015), which stated that *C. lentillifera* can survive at a salinity of 20-50 ppt and thrive at a salinity of 30-40 ppt. Changes in salinity have no effect on nutrient absorption, instead, temperature has a synergistic effect up to a certain point (Mandal *et al*., 2015). At salinities of 20 ppt and 45 ppt, only stems appeared, but at salinities of 30 ppt to 40 ppt fruit stalks were produced (Chen *et al*., 2019). Sudden changes in salinity, for example, when it rains, have an impact on quality. *C. lentillifera* will look wilted, but this condition does not last long if the salinity returns to normal.

Salinity changes also affect the bacterial community in the water column. Microbes can withstand sudden changes in salinity in a short time, but if the salinity changes slowly there will be changes in the microbial community in the waters (Gaumet *et al*., 2019). This can trigger disease in *C. lentillifera*. Therefore, during the rainy season, the yields are generally poor. The microbial community indirectly affects *C. lentillifera.* The highest growth was respectively in the F4, F3, F1, and F2 treatments of 3.72%, 3.06%, 2.62%, and 1.10%.

The observed growth rate is still below the observed value by Bambaranda *et al*. (2019b) with a value of 4.29%. Growth rate values with F4 and F3 (0.2 µl/L FVF and 0.1 µl/L) were significantly different from the F1 treatment (0 µl/L FVF). In the 18th day, it was suspected that the growth rate reached its limit due to the seaweed being too dense so a limiting factor was formed between the grain surfaces. According to Hurd (2017), the dense layer creates a 'canopy boundary layer', an additional layer through which nutrients must pass to reach the sea grass surface. The movement of water can encourage the absorption of nutrients and productivity of seaweed because it contains a supply of nutrients and inorganic carbon.

Nutrients in the water currents across the surface of the seaweed are absorbed by diffusion or active transport. Light and temperature are abiotic factors that affect the capacity of seaweed to absorb nutrients (Roleda & Hurd, 2019). Biotic factors are in the form of pests, diseases, and seaweed microbial communities. The FVF fermentation process allegedly produces humus because it occurs under anaerobic conditions. According to Wang *et al*. (2022) humic acid is the main component in humus which can improve seeding and growth.

Through the first stage of anaerobic digestion, the humic acid content first decreases and after further degradation, the humic acid content will again build up. In the first 112 days of fermentation, the humic acid content formed increased by 13%, then slowed down to 5% (Wang *et al*., 2022). The use of nutrients by bacteria to produce humic acid may be the reason for the low NPK content in FVF when compared to standard liquid organic fertilizer, which is 2-6% according to PMP (2019). The bioactive compounds in FVF function to reduce biotic and abiotic stress in plants. In addition to bioactive metabolites from waste, there are also secondary metabolites from microbes.

Antimicrobial activity in FVF can interfere with the activity of gram-positive and gram-negative bacteria to help prevent biofouling activity in *Caulerpa.* The secondary metabolites contained flavonoids, quinones, saponins, alkaloids, and cardiac-glycosides (Vama & Cherekar, 2020). Secondary metabolites are multifunctional, namely as growth regulators, plant defense, and work together with primary metabolites in different functions (Erb &

Kliebenstein, 2020). Microbes in seaweed play a role in the morphogenesis and growth of seaweed directly and indirectly. Most communities found on the surface are *Proteobacteria* and *Firmicutes* (Singh & Reddy, 2014).

The association of bacteria with seaweeds results in growth-stimulating substances, quorum-sensing signaling molecules, bioactive compounds, and active molecules for morphology. This molecule also helps seaweed get rid of harmful microbes. There are differences in the structure and diversity of the microorganism community in healthy and diseased *C. lentillifera* (Liang *et al*., 2019). There was increased prolific biofouling activity in diseased *C. lentillifera* by *Bacillariophyta, Ulvales,* and *Tetraselmis*. Biofouling is a threat because it can increase the hydrodynamic resistance of stems and leaves resulting in an increase in other fouling organisms; nutritional competition; gas exchange inhibitors; and close the light. Parts of seaweed with fertile biofouling activity will indirectly cause disease attacks (Failu *et al*., 2016).

Another common disease encountered in *C. lentillifera* cultivation is bleaching. Bleaching in *C. lentillifera* does not only occur as a result of disease but can be caused by an excessive oxidative response when facing a pathogen attack (Kopprio *et al*., 2021). The bacteriocin metabolite from lactic acid bacteria (LAB) in FVF is toxic to microbes. Glycolipids play an important role in preventing bacterial attachment and eradicating biofilms. LAB is halotolerant and able to survive in a minimal environment with high salinity water. Currently, the effectiveness of biological fertilizers based on LAB and *Bacillus* has been validated for use (Raman *et al*. (2022).

Safitri *et al*. (2023) showed that microorganism-based fertilization from bean sprouts increased growth and production performance in *C.lentillifera.* This finding correlates with microorganisms in FVF role to enhance performance. FVF has other components in the form of natural plant hormone auxins namely indolF3-acetic acid (IAA) (Gao & Liu, 2020). The composition of IAA in FVF matches that of biological fertilizers in the range of 2.5-0.05 mg/L. Siderophore is another FVF composition with an amount of 27.2 mg/L which is useful for resisting stress and protecting plants from pathogens.

The same thing was said by Raman *et al*. (2022), that LAB in FVF exhibits plant growth-promoting properties by producing auxin indolF3-acetic acid (IAA) and dissolving minerals. Microbial communities play an important role in helping host plants develop immunity, suppress disease, supply nutrients, and protect against environmental stresses (Pang *et al*., 2021). In *Caulerpa* the associated bacteria are endosymbiotic bacteria. The bacteria associated with this seaweed have a role in fixing atmospheric nitrogen (Chisholm *et al*., 1996). Fixation helps nourish the waters. In general, the best organic carbon sources for nitrifying microbes are acetate, fornate, and pyruvate (Poughon, 1997).

FVF has abundant acetate as its final ferment product. The enzymes in FVF are thought to be able to help *C. lentillifera* ramuli optimally absorb light without being hindered by pollutants in the water. Lipase can cut the ester bonds of triacylglycerols in oils and fats which helps reduce the contaminant of oils and fats in water (Chandra *et al*., 2020). Extracellular proteases have the function of hydrolyzing proteins in the environment into forms that can be absorbed and used by microbial cells (Hamza, 2017).

Even though it has hydrolytic enzymes, the mechanism of enzymes added to *C. lentillifera* cultivation media still needs to be studied because of the high salinity of the water. Braham *et al*. (2021) concluded that it is difficult to determine the effect of salinity on enzymes because the mechanisms are complicated with regard to pH, salt concentration and type, and enzyme protocols. The production of FVF is expected not only to contribute to reducing the greenhouse effect of waste but also to inspire every individual to process household waste into useful liquids. Fertilizer based on fruit and vegetable fermentation with its role as a stimulant gives significance to the quality of *C. lentillifera.*

CONCLUSION

Fermented fruit and vegetable-based fertilizers had effects on harvest weight, water quality, and secondary stem length on *C. lentillifera* cultivation. The best production performance counted by net harvest was obtained by F3 with a value of 0.08 ± 0.06 kg. The highest specific growth rate of explants was in the F4 treatment of 3.72% but performed lower in net production value by -0.05 ± 0.05 kg. Future study is recommended to observe microbial parameters for FTF addition to *C. lentillifera* cultivation media.

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